Biodiversité et fonctionnalités de la flore lactique autochtone des produits laitiers fermentés de chèvre au Liban

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Biodiversité et fonctionnalités de la flore lactique autochtone des produits laitiers fermentés de chèvre au Liban

Biodiversity and functionalities of native lactic acid bacteria of Lebanese fermented goat milk products

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ÉCOLE DOCTORALE
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Titre : Biodiversité et fonctionnalités de la flore lactique autochtone des produits laitiers fermentés de chèvre au Liban

Mots clés : Bactéries lactiques, biodiversité, lait de chèvre fermenté, métagénomique, propriétés technologiques

Résumé : Au Liban, la production de produits fermentés traditionnels à partir de lait cru de chèvre est réalisée par un petit nombre de producteurs, avec un vrai risque de disparition de ces produits et de perte de leur microflore. Cette thèse vise à étudier la biodiversité et les fonctionnalités des bactéries lactiques isolées des trois produits : Ambriss, Serdaleh et Labneh El Darff. 34 échantillons ont été collectés. Leur composition a été déterminée par des analyses culture-dépendantes et à l'aide d'outils métagénétiques et métagénomiques. Les résultats diffèrent selon les produits. Le Serdaleh révèle une codominance de *Lb. kefiranofaciens* et *Lc. lactis* qui se trouve dans un état viable mais non cultivable. Cette composition évoque celle des grains de kéfir, bien que *L. kefiranofaciens* se différencie par les gènes codant pour la production d'EPS. Les souches de *Lc. lactis* possèdent un plasmide portant des gènes de résistance aux antibiotiques. Le Labneh El Darff démontre une dominance de *L. delbrueckii*, provenant probablement du Laban. Plusieurs pathogènes zoonotiques ont aussi été détectés. Les propriétés technologiques de 40 isolats ont été étudiées. L'activité acidifiante des entérocoques, lactocoques et des souches de *L. rhamnosus* et *P. acidilactici* est similaire, alors qu'elle est plus variable au sein des autres espèces. L'étude des propriétés physiques des laits fermentés montre que certaines souches conduisent à une forte fermeté (*E. durans* LD50, *Lb. diolivorans* LD47), tandis que d'autres génèrent des gels très cohésifs (*L. paracasei* LP12, *L. kisonensis* LKN28, *Lev. brevis* LB36, *E. durans* ED65). Une faible viscosité apparente a été observée pour tous les isolats. Le degré de synérèse varie beaucoup selon les espèces (faible chez *Lb. diolivorans* mais élevée chez *L. plantarum*). 17 composés d'arômes ont identifiés dans les cultures des souches: 6 acides gras volatils, 4 alcools, 3 cétones, 2 furanes , un composé carbonylé et un ester. Leur production diffère selon les souches, les isolats *Lc. lactis* LL01 et *Lb. diolivorans* LD46 générant les plus fortes quantités de composés d'arôme. Enfin, la plupart des isolats sont résistants au stress gastro-intestinal, les espèces *Lb. kefiranofaciens* et *Lev. brevis* étant les plus résistantes. Finalement, ces travaux ont permis de mettre en valeur les souches *L. plantarum* LP14 et LP15 pour être sélectionnées comme ferments pour la production de Serdaleh ou d'Ambriss, et les souches *Lb. diolivorans* LD44 et LD45 qui pourront être valorisées pour la fabrication de fromages de chèvre traditionnels Libanais.

Title : Biodiversity and functionalities of native lactic acid bacteria of Lebanese fermented goat milk products

Keywords : Lactic acid bacteria, biodiversity, fermented goat milk, metagenomic, technological properties

Abstract: In Lebanon, the production of traditional fermented goat milk products is carried out in a limited number of production units, with a real risk of extinction of these products and loss of their microbial resources. This thesis aims to study the biodiversity and functionalities of native lactic acid bacteria isolated from three products: Ambriss, Serdaleh and Labneh El Darff. 34 samples were collected and characterized by culture-dependent analyses and using metagenetic and metagenomic tools. Serdaleh revealed a co-dominance of *Lb. kefiranofaciens* and *L. lactis,* in a viable but not cultivable state. This composition is evocative of that of kefir grains, even if *Lb. kefiranofaciens* differed considering EPS producing genes. *Lc. lactis* strains possessed a plasmid with an antibiotic multi-resistance island. Labneh El Darff displayed a dominance of *Lb. delbrueckii,* probably due to the addition of Laban. The presence of zoonotic pathogens was also detected. The technological properties of 40 isolates have been studied. Acidification activity of *Enterococcus*, *Lactococcus genera,* and *L. rhamnosus* and *P. acidilactici* species was quite similar, by considering the other species. . Low apparent viscosity was observed for all isolates. Syneresis strongly varied according to the species (low for *Lb. diolivorans* but high for *L. plantarum*). Up to 17 aromas were produced by the isolates, including 6 volatile fatty acids, 4 alcohols, 3 ketones, 2 furans, one carbonyl compound and one ester. Their production varied according to the isolates, *L. lactis* LL01 and *L. diolivorans* LD46 being the most productive in aroma compounds. Finally, most of the isolates were resistant to gastrointestinal stress, *L. kefiranofaciens* and *Lev. brevis* species being the most resistant ones. Finally, most of the isolates were resistant to gastrointestinal stress, *Lb. kefiranofaciens* and *Lev. brevis* species being the most resistant ones. In conclusion, these results allowed to highlight the strains *L. plantarum* LP14 and LP15 that can be retained for the production of Serdaleh or Ambriss, and the strains *Lb. diolivorans* LD44 and LD45 that are good candidates for traditional Lebanese goat cheese production.

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Résumé

Au Liban, la production de produits fermentés traditionnels à partir de lait cru de chèvre est principalement assurée par de petites entreprises, souvent familiales. Cependant, cette tradition est aujourd'hui menacée, ce qui risque d'entraîner la disparition de ces produits uniques ainsi que la raréfaction de leur microflore. Dans cette thèse, notre objectif est d'étudier la biodiversité et de caractériser les fonctionnalités et les propriétés technologiques des bactéries lactiques autochtones isolées à partir de trois produits traditionnels spécifiques : l'Ambriss, la Serdaleh et le Labneh El Darff.

1- Enquête sur les producteurs de produits fermentés traditionnels au Liban

Dans le but de sélectionner les produits pour cette étude, une enquête auprès de 50 producteurs a été réalisée, portant sur trois aspects (i) le profil socio-démographique (ii) les activités d'élevages, et (iii) la production et la commercialisation. Les résultats montrent que 25% des enquêtés exercent à la fois en tant que producteurs et éleveurs, tandis que 75% se concentrent uniquement sur la production. La production familiale est assurée principalement par des femmes au foyer (60%) âgées entre 40 et 70 ans, avec un accès limité au marché régional (38%). Pour la majorité des producteurs (88%), le lait cru de chèvre est utilisé comme matière première, tandis que seulement 4% fermentent leurs produits à partir d'un mélange de lait de chèvre et de vache. Les produits Ambriss, Serdaleh et Labneh El Darff sont fabriqués à partir de lait de chèvre de race Baladi dans les régions de Bekaa, du Chouf et du sud du Liban, respectivement (Figure IV.1A). La production s'effectue pendant la période de lactation des chèvres, de juin à novembre, de manière artisanale et à petite ou moyenne échelle (Troisième quartile = 216,5 kg/saison).

2- Analyse métagénétique et métagénomique de la biodiversité microbienne dans les produits étudiés

La composition et la diversité macrobiotique de 34 échantillons prélevés auprès de 18 producteurs ont été déterminées grâce à des analyses dépendantes et indépendantes ainsi qu'à des outils métagénétiques et métagénomiques. L'analyse de la diversité alpha a révélé une richesse spécifique plus faible dans les échantillons de Labneh El Darff ($p<0,05$) par rapport aux deux autres produits (Figure V.3A). De plus, les indices Chao1, Shannon et InvSimpson, qui mesurent l'uniformité, ont confirmé cette faible diversité. L'analyse des composantes principales (ACPo) a clairement distingué les échantillons de Labneh des échantillons de

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Serdaleh et d'Ambriss, qui ne pouvaient pas être différenciés et qui partageaient probablement un microbiote bactérien similaire (Figure V.3B).

Les analyses de la composition taxonomique (Figure V.3C et Annexe IV.2) ont révélé que l'espèce prédominante était *Lactobacillus delbrueckii* dans les échantillons de Labneh El Darff, représentant 55 à 82% des dénombrements, tandis qu'il n'était pas détecté ou présent à des niveaux inférieurs à 0,4% dans l'Ambriss et le Serdaleh. Les échantillons de ces derniers produits contenaient *Lc. lactis, Lb. kéfiranofaciens* et *Lentilactobacillus kefiri* comme espèces dominantes, ainsi que, dans une moindre mesure, le groupe de *Lb. diolivorans*. L'analyse différentielle des OTU a révélé une plus grande abondance de *Lc. lactis* dans les échantillons de Serdaleh par rapport à Ambriss (padj < 0,01).

L'outil MetaPhlAn, utilisé pour comparer les lectures métagénomiques à une base de données génomique, a identifié 98 espèces bactériennes (Annexe IV.4). La composition des espèces obtenue était relativement cohérente avec celle obtenue par séquençage de l'ARNr 16S. De manière intéressante, il a permis de résoudre la plupart des ambiguïtés d'attribution taxonomiques pour les bactéries lactiques telles que *Lentilactobacillus kefiri, Lentilactobacillus diolivorans, Lactiplantibacillus plantarum, Lactobacillus helveticus* et *S. thermophilus*, ainsi que pour les contaminants tels que *S. parauberis* et *M. agalactiae.* Il a également détecté la présence de la levure *Saccharomyces cerevisiae* dans trois échantillons mais à des niveaux faibles.

L'analyse des MAG (Metagenome-Assembled Genome) par gènes marqueurs, a identifié 104 espèces bactériennes et 15 espèces fongiques (Annexe IV.5). La composition est illustrée à la figure V.4. Bien que cette analyse taxonomique ait révélé des espèces cohérentes avec l'analyse précédente, certaines différences méritent d'être soulignées. Alors que MetaPhlAn a attribué 35% des lectures d'A4 à *S. parauberis*, le gène marqueur n'a détecté que 6,9% de cette espèce, ainsi que *S. parasuis*, qui a représenté 55% des lectures.

2.1 Détection d'espèces pathogènes dans les produits étudiés

L'analyse du gène marqueur a signalé la présence d'espèces pathogènes dans plusieurs échantillons d'Ambriss et de Serdaleh. Tous les échantillons de Serdaleh prélevés en 2019 contenaient de l'espèce pathogène caprine *M. agalactiae* mais à des niveaux faibles (1-4%). De plus, des espèces de streptocoques zoonotiques, comme *S. parauberis* et *S. parasuis*, ont été détectées à des niveaux variant de 0,5% à 2% dans plusieurs échantillons d'Ambriss et Serdaleh,

allant de 7% à 55%. Des entérobactéries telles que *E. coli* et *K. pneumoniae* ont été détectées dans environ la moitié des échantillons à des niveaux inférieurs à 1% (à l'exception de *K. pneumoniae* présent à 2,7% dans Ambriss A1). Une analyse approfondie du profil taxonomique dans les échantillons de métagénome a été effectuée pour détecter une faible abondance d'espèces pathogènes potentiellement présentes dans les produits laitiers (Annexe IV.6). Aucune *Brucella abortus, Listeria monocytogenes, Mycobacterium vaccinae* ou *Staphylococcus agalactiae* n'a été détectée. Cependant, *S. aureus* a été détecté à de faibles niveaux dans cinq échantillons de Serdaleh.

2.2 Détection des gènes de résistance aux antibiotiques

Une approche de comparaison par carte génomique contre une collection de gènes de résistance aux antibiotiques a été réalisée. Au total, 18 gènes ABR appartenant à sept classes différentes d'antibiotiques ont été détectés dans tous les échantillons (Figure IV.9). Les échantillons de Serdaleh présentaient des niveaux plus élevés de gènes de résistance et une plus grande diversité par rapport à ceux d'Ambriss. Les classes d'antibiotiques les plus fréquemment détectées étaient les aminoglycosides, la MLS (Macrolide, Lincosamide, Streptogramine), les nucléosides et la tétracycline. Les échantillons de Serdaleh contenaient les niveaux les plus élevés d'aminoglycoside (aad (6) et APH (3’’-IIIa), de MLS (ermB), de nucléosides (sat-4) et de tetracyline (teS).

Les résultats des analyses dépendantes et indépendantes de la culture étaient radicalement différents, en particulier pour Ambriss et Serdaleh. Ce dernier révélait la co-dominance de *Lb. kefiranofaciens* et de *Lc. lactis* qui se trouvait dans un état viable mais non cultivable. Cette composition rappelle celle des grains de Kefir. Cependant, les analyses phylogénomiques et fonctionnelles des génomes des espèces clés ont révélé des différences dans les gènes codant pour les EPS chez *Lb. kefiranofaciens*. En revanche, le produit Labneh El Darff a montré une dominance de *Lb delbrueckii*, probablement provenant du Laban.

Plusieurs pathogènes zoonotiques ont été détectés, notamment *Streptococcus parasuis,* qui était dominant dans un échantillon de Serdaleh. L'analyse MAG a révélé qu'il avait acquis des gènes de lactose par transfert horizontal. Le troupeau de chèvres dans la région du Chouf était également contaminé par *Mycoplasmopsis agalactiae.* De plus, les souches de *Lc. lactis* possèdent un plasmide portant des gènes de résistance aux antibiotiques.

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3- Caractérisation des propriétés technologiques et fonctionnelles de 40 espèces de bactéries lactiques

Les propriétés technologiques de 40 souches de bactéries lactiques ont été étudiées. L'activité acidifiante des entérocoques, des lactocoques et des souches de *L. rhamnosus* et *P. acidilactici* est similaire, tandis qu'elle varie davantage au sein des autres espèces. L'étude des propriétés physiques des laits fermentés montre que certaines souches entrainent une fermeté élevée *(E. durans* LD50, *Lb. diolivorans* LD47), tandis que d'autres produisent des gels très cohésifs (*L. paracasei* LP12, *L. kisonensis* LKN28, *Lev. brevis* LB36, *E. durans* ED65).

3.1 Activité d'acidification

Dans cette étude, les caractéristiques d'acidification ont été établies pour la première fois pour les espèces *Lb. diolivorans, Lb. kisonensis, Len. kefiri, Pediococcus acidilactici, Lpb. plantarum et Lb. Rhamnosus*. Une diversité significative a été observée parmi les différentes souches, tandis que les Enterocoques, les Lactocoques et les espèces *L. rhamnosus et P. acidilactici* présentaient des caractéristiques communes. En revanche, les caractéristiques des autres espèces étaient dépendantes des souches et variables en termes de vitesse d'acidification, et de temps nécessaire pour atteindre les pH 5,5, 4,5 et final.

En comparant les descripteurs d'acidification avec les bactéries lactiques natives du Laban traditionnel Libanais, des taux d'acidification plus élevés et des temps plus courts pour atteindre le pH 4,5 ont été observés pour *Lb. bulgaricus* (0,0111 × 0,0017 u.pH / min; 337 min) et *Streptococcus thermophilus* (0,0085 × 0,0009 u.pH / min; 297 min). Bien que toutes les souches isolées soient considérées comme des acidifiants lents (Vm < 0,0067 upH / min) avec un temps long pour atteindre tpH4,5 (> 936 min), certaines comme *Lc lactis* ssp *cremoris* LC50 et *L. rhamnosus* LR21 ont pu atteindre des pH bas de 3,96 et 3,33 respectivement à la fin de processus de fermentation

Les vitesses maximales d'acidification ont été attribuées à *Lc. lactis* et *Lc. lactis ssp cremoris*, variant de 0,0061 à 0,0067 upH / min en valeurs absolues. Ils ont également montré les temps les plus courts pour atteindre le pH 5,5. Les *Lc. lactis* sont connus comme des acidifiants lents (Wouters *et al*., 2002) et les espèces *cremoris* sont préférées en tant que starters en raison de leurs propriétés induisant une faible amertume (Li *et al*., 2020).

La chute rapide du pH pendant la fermentation du lait améliore l'hydrolyse des protéines, ce qui entraine une augmentation des acides aminés libres et qui peut influencer la

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qualité sensorielle du produit (Ayad *et al*., 2004). Les souches d'*E. durans* ont montré une faible activité acidifiante, similaire aux résultats obtenus par Malek *et al*.,(2012), avec des valeurs variant de 0,0018 à 0,0041 upH / min pour *E. faecium.* Ceci est confirmé par les valeurs élevées de tpH4,5 (2205 -3389 min) pour *E. durans*, qui sont associés à une longue période de latence. Ces valeurs sont supérieures à celles de *L. rhamnosus* (969-2224 min) ou de souches de lactocoques (1009-1329 min).

3.2 Propriétés physiques et rhéologiques

Les propriétés rhéologiques et physiques des produits laitiers fermentés jouent un rôle essentiel dans les caractéristiques sensorielles perçues par les consommateurs (Mende *et al*., 2016). Ces caractéristiques sont influencées par la composition du lait, le processus de fabrication et le type de culture utilisée (Sodini *et al*., 2004), et dépendent largement de l'arrangement structurel de la microstructure du réseau de protéines du lait fermenté (Nguyen *et al*., 2018).

Tous les laits fermentés par les souches isolées ont présenté un taux élevé de fermeté, qui était positivement corrélée à la fracturabilité. Ce paramètre a varié entre 1,14 N pour *E. durans* ED65 et 4,37 N pour le *Lb. diolivorans* LD46. Ces valeurs étaient supérieures à celles obtenus pour le Laban traditionnel (Chammas *et al*., 2006b), qui rapportait une valeur de fermeté de 0,529 N pour *Lb. Bulgaricus*. De plus, nos résultats étaient également supérieurs à ceux obtenus par Mituniewicz-Małek *et al*., (2017) pour le yaourt fabriqué a partir du lait de chèvre (0,26 N).

La cohésion est un paramètre qui caractérise l'intégrité structurelle des laits fermentés (Prajapathi *et al.*, 2020). De nombreux auteurs ont suggéré qu'une cohésion élevée est liée à la capacité de liaison à l'eau des exopolysaccharides (EPS), ce qui réduit la séparation du lactosérum du produit fermenté et la déformation de la structure protéique du gel (Prajapathi *et al.*, 2020; Patel et Prajapati, 2013). Les valeurs de cohésion dans cette étude ont varié entre 0,69 et 0,92, montrant ainsi une faible variabilité. Ces valeurs sont en accord avec celles indiquées par Chammas *et al.*, (2006) pour les laits fermentés par des souches isolées du Laban. De plus, ces auteurs ont signalé des valeurs de cohésion plus élevées pour les lactobacilles (1,09) et des valeurs plus faibles (0,676) pour les souches de streptocoques. En considérant l'espèce *Lpb. plantarum,* Zhao et Liang (2022) ont démontré qu'un yaourt fermenté par avec la souche Lpb. *plantarum* MC5 avait considérablement amélioré la cohésion du produit. Dans notre étude, les souches Lpb. *plantarum* LP12, LP13 et LP15 ont conduit à des gels hautement

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cohésifs. Nos valeurs étaient également supérieures à celles récemment rapportées par Szopa et Pawlos, (2023), où les valeurs ont atteint 0,58, 0,67 et 0,76 lorsqu'elles sont inoculées par L. *paracasei, L. rhamnosus* ou *Lb. acidophilus,* respectivement.

Les résultats obtenus pour les produits fermentés ont montré une faible viscosité apparente (< 1,73 Pa.s) pour tous les souches analysées, caractérisant ainsi des gels visqueux faibles. Ces observations sont cohérentes avec celles rapportées par Chammas *et al*., (2006b) qui ont présentés des viscosités apparentes (VA) comprises entre 0,01 Pa.s et 1,28 Pa.s pour *S. thermophile* et entre 0,29 Pa.s et 0,82 Pa.s pour *Lb. bulgaricus*. La valeur moyenne de la viscosité pour *E. durans* (0,80 Pa.s) était légèrement supérieure à celle observée par Malek *et al*., (2012) pour 35 souches d'*E. faecium* isolées des fromages égyptiens traditionnels (0,05 à 0,41 Pa.s). La viscosité d'un lait fermenté est généralement attribuée à la production d'exopolysaccharides (EPS) par des souches productrices de polymères (Marshall et Rawson, 1999; Béal *et al*., 1999). Ces EPS influencent les propriétés physicochimiques et rhéologiques des produits laitiers fermentés, améliorant ainsi leur viscosité et réduisant la synérèse (Mende *et al*., 2022). Récemment, Zhao et Liang, (2022) ont démontré l'inoculation du lait de chèvre par *Lpb. plantarum* et une culture du yaourt thermophile a considérablement augmenté la viscosité apparente du produit fermenté, passant de 1,90 Pa.s à 12,24 Pa.s. Ces valeurs de viscosité sont cependant considérablement plus élevées que celles observées dans notre étude.

La synérèse (SYN) des laits fermentés par les 40 souches variait largement, allant de 1,1% à 73,1%. Les souches *Lb. diolivorans, Len. kefiri* et *E. durans* ont présenté les valeurs les plus faibles. En revanche, la plupart des souches de *Lpb. plantarum* ont montré une synérèse élevée (73,07% pour LP15). On peut observer que les souches isolées dans notre étude ont conduit à une synérèse plus importante par rapport à celles obtenues par Szopa et Pawlos, (2023). Dans leur étude, la synérèse dépendait de la souche et atteignait 45,6% pour *L. paracasei*, 47,0% pour *L. casei*, 56,6% pour *Lb. acidophilus* et 52,36% pour *L. rhamnosus* dans les produits de lait de chèvre fermentés. De plus, étant donné que la cohésion et la synérèse sont des propriétés intéressantes pour la fabrication des produits traditionnels fermentés à base de lait de chèvre tels que l'Ambriss ou le Serdaleh, certaines souches de *Lpb. plantarum* se révèlent prometteuses en tant que ferments grâce à leur capacité à réduire la capacité de rétention d'eau et à augmenter la productivité par saison de production en accélérant la synérèse.

3.3 Analyse des arômes

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Dans cette étude, les principaux composés volatils produits par les 15 souches de bactéries lactiques inoculées dans les laits fermentés sont les aldéhydes, les alcools, les acides, les cétones, les esters et les hydrocarbures. Le nombre total de composés volatils variait de 7 à 13, avec une prédominance des acides gras volatils tels que l'acétique, le benzoïque, le butyrique, le n-décanoïque, l'hexanoïque et l'octanoïque. Les alcools tels que l'éthanol, le butanol, le 2,3-butanediol, et le n-propanol, ainsi que les cétones comme l'acétone, l'acétoïne, le diacétyle étaient également présents en quantités significatives. Les composés carbonylés tels que l'acétaldéhyde, les furanes tels que le furfural et le 2-furanméthanol, ainsi que les esters tels que l'acétate d'éthyle complétaient le profil aromatique. Ces résultats sont cohérents avec ceux rapportés par Huang *et al*., (2020a), qui ont pu identifier jusqu'à 19 composés dans du yaourt de chèvre préparé à partir d'un mélange de culture de 1: 1 de *S. thermophilus* et *Lb. bulgaricus*. La production d'arômes dépendait plus des souches que des espèces. Plus précisément, des variabilités intra-espèces ont été observées, notamment chez *Len. kefiri* (LK07 et LK10) en ce qui concerne la teneur en furfural, et chez *Lb. diolivorans* (LD40, LD44, LD45 et LD46) en ce qui concerne l'éthanol et le 2,3-butanedione (diacétyl).

La plupart des acides volatils, qui sont produits pendant le processus de fermentation, dérivent principalement de la décomposition de la chaine C4-C20, et en partie par la fermentation par des bactéries lactiques (Cheng, 2010b). L'acide butyrique contribue à la saveur du fromage tandis que l'acide octanoïque est associé à des notes à des notes de cire, de savon, d'arôme caprin, rance et fruité. Les acides hexanoïque et octanoïque confèrent une odeur « caprine » caractéristique aux produits fermentés (De Santis *et al* 2019; Salles *et al*., 2002) et contribuent à la saveur des fromages à pâte molle (Shu *et al*., 2016; Bao et al., 2016). Les acides butanoïque et hexanoïque sont responsables des perceptions douces et lipolyzées du Parmigiano (Qian et Reineccius, 2003). Dans notre étude, l'acide hexanoïque était prominent sur les acides décanoïque (caprique) et octanoïque (caprylique) dans tous les laits fermentés.

L'acétaldéhyde, l'acétoïne et le diacétyle contribuent également de manière significative à la saveur des laits fermentés (Ott *et al.* 2000). L'acétaldéhyde présente une saveur de pomme verte ou de noisette (Cheng, 2010b) tandis que les cétones ont des arômes fruités, végétaux voire même floraux. Ces derniers sont considérés comme des composés intermédiaires et peuvent être réduits à des alcools secondaires (Bezerra *et al.*, 2017b). Les concentrations optimales d'acétaldéhyde dans le yaourt variaient de 14 à 20 mg/kg, tandis que des concentrations inferieures à 8,0 mg/kg ont donné une saveur faible, et des quantités élevées

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d'acétaldéhyde (> 200 mg/kg) ont conduit à un yaourt au gout astringent (Chen *et al*., 2017b). Dans une étude similaire portant sur un produit fermenté de lait de chèvre semblable au kéfir, Buran *et al.* (2021) ont identifié une concentration d'acétaldéhyde de 19,02 mg/L et de 2,3-butanedione de 3,18 mg/L. Cependant, les valeurs obtenues dans notre étude étaient plus élevées, avec la souche *Lb. diolivorans* LD44 présentant une concentration d'acétaldéhyde de 43,6 mg/kg et la souche *Lb. diolivorans* LD40 présentant une concentration de diacétyle de 43,6 mg/kg.

L'acétoïne est connue pour sa saveur crémeuse, vanillée et beurrée, et a un impact sur la qualité des arômes des laits fermentés. Les valeurs d'acétoïne variaient entre 1,8 mg.kg-1 (*Lpb. plantarum* LP15) et 33,8 $mg.kg^{-1}$ (*Lb. diolivorans* LD 44). Cette dernière valeur était supérieure à celle obtenue par Erkaya et Şengül (2011) dans le yaourt de chèvre traditionnel, qui présentait une aire de pic de 1,25% par rapport à tous les autres composants détectés. Une étude menée par Muelas *et al*. (2022) a également signalé une faible teneur en acétoïne, avec une aire de pic de 1,7%, dans un produit fermenté de chèvre alimentée avec du brocoli et de l'artichaut.

L'éthanol est l'un des principaux composés aromatiques du yaourt, résultant du métabolisme du glucose ou de la dégradation des acides aminés (Dan *et al.*,2019). Il contribue de manière positive à la perception de l'arôme doux et éthéré, mais uniquement à des concentrations faibles (Tian *et al*.,2019). Dans notre étude, des différences significatives ont été observées entre les échantillons concernant ce composé. Des niveaux élevés d'éthanol (295,2 à 917,3 $mg.kg^{-1}$) ont été trouvés dans des échantillons fermentés par *Lb. diolivorans* (D40 et LD45).

À partir de cette analyse, les composés aromatiques synthétisés par les bactéries lactiques isolées à partir des produits laitiers traditionnel de chèvre fermentés ne sont pas si différents de ceux identifiés dans d'autres laits fermentés. Cependant, certaines différences ont été observées entre les espèces bactériennes, ce qui peut aider à la sélection de souches présentant des caractéristiques sensorielles spécifiques. Par conséquent, en choisissant des souches spécifiques de bactéries lactiques, il est possible de produire des produits avec des profils aromatiques distincts, répondant ainsi aux préférences des consommateurs ou aux exigences du marché.

3.4 Résistance au stress gastro-intestinal

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Dans cette étude, le taux de résistance de certaines espèces de bactéries lactiques isolées des produits laitiers de chèvre traditionnels Libanais face au stress gastro-intestinal a été démontré pour la première fois pour les espèces suivantes: *Len. kefiri, Lb. kéfiranofaciens, Lev. brevis, Lb. diolivorans, Lb. kisonensis et P. acidilactici.* Nos résultats ont indiqué que certaines des bactéries lactiques étaient capables de survivre dans le tractus gastro-intestinal. Les souches *Lb. Kefiranofaciens, Lev.brevis, P. acidilactici et Lpb. plantarum* ont montré un taux de survie élevé dans des conditions gastro-intestinales défavorables. Il est important de noter que cette résistance est spécifique à la souche, en raison de la grande diversité observée au sein de la même espèce. En comparant nos résultats avec ceux obtenus par Shehata *et al*., (2016) sur deux laits fermentés égyptiens traditionnels (Boza et Rayeb), le taux de survie de *Lc. lactis* (66,3 %) était proche de celui observé dans leur étude (68 – 88,3%). De plus, le taux de survie de *L. rhamnosus* (85,4%) était légèrement supérieur à celui de la même espèce isolée du Boza traditionnel égyptien (76,2%). Le taux de survie de *Lpb. plantarum* face au stress gastro-intestinal (90%) était supérieur à ceux rapportés par Zhang *et al*., (2016) avec des souches isolées de Qula tibétain, un produit à base de yak fermenté (71% - 82%).

Enfin, il convient de souligner que le type de lait peut également affecter cette résistance, car le lait de chèvre influence l'environnement gastro-intestinal et sa communauté microbienne différemment du lait de vache (Prosser, 2021).

4- Conclusion

Les produits laitiers traditionnels fermentés à base de lait de chèvre ont été étudiés grâce à des approches métagénétiques et métagénomiques. Les résultats obtenus par ces deux méthodes étaient sensiblement différents, notamment pour les produits Ambriss et Serdaleh. Bien qu'aucune étude n'ait encore prouvé cela, ces produits laitiers apporteraient des avantages pour la santé, et il serait donc intéressant d'étudier cet avantage. Ces produits sont également intéressants en tant que sources de biodiversité notamment en ce qui concerne les souches de *Lb. kéfiranofaciens* et *Len. kefiri*, qui sont génétiquement très différentes de celles présentes dans le Kéfir.

Les souches Lpb. plantarum LP14 et LP15, caractérisées par une faible vitesse d'acidification et un taux élevé de synerèse, montrent un intérêt particulier pour la production traditionnelle de Serdaleh ou d'Ambriss. En revanche, les souches *Lb. diolivorans* LD44 et LD45 peuvent améliorer la fermeté et incorporer une saveur appréciée lors des étapes de

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maturation des fromages traditionnels Libanais. Les souches de *Lb. Kefiranofaciens* et *Lev. brevis* se sont révélées les plus résistantes au stress gastro-intestinal, ce qui les positionne comme des candidates potentielles en tant que cultures probiotiques. Les produits fermentés testés présentent une faible viscosité apparente pour toutes les souches, caractérisant ainsi des gels peu visqueux attribuables à une faible production d'EPS par les souches.

En outre, certain mesures doivent être mises en place pour garantir la sécurité de ces produits tout au long de la chaîne d'approvisionnement, afin de prévenir la contamination par des bactéries pathogènes potentielles et la propagation de gènes de résistance aux antibiotiques. De plus, cette étude fournit des éléments clés liés au fonctionnement de la microbiologie de ces produits, qui faciliteront le développement des stratégies de préservation de leur identité. À cet égard, plusieurs perspectives peuvent être proposées.

En ce qui concerne la conservation de ces produits, deux approches peuvent être envisagées en fonction de la caractérisation génomique et des propriétés fonctionnelles des bactéries lactiques identifiées. La première approche repose sur le développement et la durabilité du système de production traditionnel, tandis que la seconde vise à développer une industrie laitière locale en préparant des ferments spécifiques.

Pour mettre en œuvre la première approche, il est nécessaire d'adopter une stratégie regroupant des activités simultanées et multidimensionnelles. Certaines études doivent être menées par l'IRAL (Institut de Recherche Agronomique Libanais) pour l'établissement d'une banque de gènes d'ADN et de souches indigènes impliquées dans le processus de fabrication. Après cette phase, un plan de développement et de surveillance devra être mis en place pour suivre les différentes étapes de production de ces produits artisanaux. Une assistance technique sera fournie aux producteurs, notamment en ce qui concerne l'accès à des outils tels que des jarres en terre cuite ou des barils en inox, ainsi qu'aux ferments lactiques appropriés. L'aspect socio-économique de cette approche nécessite la formation spécialisée des producteurs ou producteurs / agriculteurs afin de promouvoir l'autonomisation des femmes. Les jeunes seront également ciblés dans ce processus grâce à la création de politiques de commercialisation et de coopératives spécialisées. Des services complémentaires de vulgarisation peuvent inclure les bonnes pratiques de production, les bonnes pratiques d'hygiène, la gestion d'élevage de cheptel et des services vétérinaires. Parallèlement, il est essentiel de promulguer des décrets réglementaires pour ces productions, tels que des normes nationales, des programmes de contrôle qualité et des projets de loi, afin de garantir leur mise en œuvre.

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La deuxième approche dépend du développement de cultures pures ou mixtes à partir des souches sélectionnées. Cette stratégie implique la compréhension complète des cinétiques de croissance microbienne, ainsi que des conditions défavorables probables, de la viabilité des souches et de l'infestation des phages pendant la préparation. Il est également important de prendre en compte le stockage, les conditions de post-stockage et de revivification des cultures. Cet aspect comprendra une évaluation économique de cette innovation liée à la commercialisation et aux préférences des consommateurs. Enfin, il est nécessaire d'envisager l'adoption de différents programmes de dissémination de ces cultures parmi les producteurs traditionnels ou les associations industrielles laitières.

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Communications:

1- I. AMMOUN, C.I. KOTHE, C. BEAL, R. YAACOUB and P. RENAULT, Revealing the microbial heritage of traditional Lebanese goat milk fermented products. Copenhagen Bioscience Conferences- Microbial Foods, Copenhagen, may 8 – 12, 2022.

2- I. AMMOUN, C.I. KOTHE, C. BEAL, R. YAACOUB and P. RENAULT, Metagenomics reveal the specificities of the microbiota of three traditional Lebanese goat milk fermented products. International Symposium on Lactic Acid Bacteria. Copenhagen, august 21-23, 2021.

3- I. AMMOUN, C.I. KOTHE, C. BEAL, R. YAACOUB and P. RENAULT, Biodiversity of native lactic acid bacteria in three artisanal Lebanese fermented goat dairy products: Ambriss, Serdaleh and Labneh El Darff. La conférence du Club des bactéries lactiques. Caen, 12-14 Juin 2019.

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LIST OF ABBREVIATIONS

AA: Acetic acid

ABR: Antibiotic Resistance

ACT: Acetone

ACH: Acetaldehyde

ACO: Acetoin

ANI: Average Nucleotide Identity

AV: Apparent Viscosity

BA: Benzoic acid

BLAST: Basic Local Alignment Search Tool

BTA: 2,3-Butanediol

BTD: 2,3-Butanedione

BUT: Butanol

BUTA: Butyric acid

CAP: n-Decanoic acid

CARD: Comprehensive Antibiotic Research Database

CEP: Cell Envelope Proteinase

CFt: Curd Firmness time

CFU: Colony Forming Unit

COH: Cohesiveness

E.: *Enterococcus*

EMP: Embden-Meyerhof-Parnas glycolytic pathway

EPS: Exopolysaccharides

ETA: Ethylacetate

ETH: Ethanol

FASTA: Fast Adaptive Shrinkage Threshold Algorithm

fastq: fast adaptive shrinkage quality file

FastQC: Fast Adaptive Shrinkage Threshold Algorithm Quality Control

FCA: 2-Furancarboxaldehyde

FIR: Firmness

FM: 2-Furnamethano

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FRA: Fracturability

FROG: Find Rapidly OTU with Galaxy solutions

GC-MS: Gas Chromatography coupled to Mass Spectrometry

GRAS: Generally Regarded As Safe

HCA: Hierarchical Cluster Analysis

HCT: Heat Coagulation Time

HA: Hexanoic acid

HePS: Hetero-exopolysaccarides

IgA: Immunoglobulin A

K20: Curd firming time

L.: *Lacticaseibacillus*

Lb.*: *Lactobacillus

Lc.*: *Lactococcus

Len.*: *Lentilactobacillus

Lev.*: *Levilactobacillus

Lpb.*: *Lactiplantibacillus

MAG: Metagenome Assembled Genome

MCP: Milk Coagulation Parameters

MRS: De Mann, Rogosa, Sharpe medium

NCBI: National Center for Biotechnology Information

NSLAB: Non Starter Lactic Acid Bacteria

OA: Octanoic acid

OTU: Operational Taxonomic Units

P.: *Pediococcus*

PA: Plasminogen Activator

PAI: Plasmiogen Activator Inhibitor

Pa.s: Pascal-second

PATRIC: Pathosystems Resource Integration Center

PCA: Principal Component Analysis

PDO: Protected Designation of Origin

PGI: Protected Geographical Indication

PG: Plasminogen

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pHm: pH at which maximum acidification rate occurred

PI: Plasmin Inhibitors

PL: Plasmin

ppm: part per million

PRO: n-Propanol

RCT: Rennet Coagulation Time

rpm: Revolutions per minute

S.: *Streptococcus*

SNP: Single Nucleotide Polymorphism

SPME: solid-phase micro-extraction

SSFD: Slow Food Foundation for Biodiversity

ssp.: subspecies

SYN: Syneresis

THF: Tetrahydrofuran

Tm: Time to reach maximum acidification rate

tpH5.5: Times to reach pH 5.5

tpH5.0 : Times to reach pH 5.0

tpH4.0: Times to reach pH 4.5

VA: Valproic acid

VBNC: Viable But non Culturable

Vm : Maximum Acidification rate

VOC: Volatile Organic Compound

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I. INTRODUCTION

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In recent years, goat milk has been the focus of interest due to the nature of its composition, organoleptic characteristics and healthy attributes (Nayik *et al.*, 2021). Particularly in Lebanon, this sector has increasingly received attention and it continues to grow (Kayouli, 2015). Goat milk and fermented goat milk products are highly appreciated by local consumers (Balaa and Marie, 2008), especially for their specific "goaty taste" (Semaan *et al.*, 2011). Compared to cow's milk, it has a higher digestibility and less allergic, as well as a higher content of short-chain fatty acids in milk fat (Shu *et al.*, 2016; Zenebe *et al.*, 2014) and minerals such as magnesium, calcium and phosphorus (Abbas et al., 2014).

These goat products are traditionally made from heat treated milk, such as Laban (Béal and Chammas, 2012), Labneh (Serhan *et al.*, 2016), Shankleesh (Toufeili et al., 1995a), Kishk (Salameh et al., 2016a) or from raw milk, such as Darfiyeh cheese (Hosri and El Khoury 2004) and fermented from row goat milk such as Serdaleh (Tabet et al., 2019a), Ambriss (Dimassi *et al.*, 2020) and Labneh El Darff (Semaan Hajj *et al.*, 2011). The diversity of these products not only enriches the Lebanese culinary culture and pantry (Massaad, 2017), but also represents a main source of women empowerment in rural poor areas. Women-headed households contribute effectively in the development of goat rearing sector and the manufacturing of goat's milk products (Kayouli, 2015). The market share of this sector is not well characterized. However, 70% of all small ruminant industries are located in rural areas with limited market access (Saadeh, 2016). The manufacturing process of such traditional goat fermented products is carried out at a small scale and only in a limited number of isolated production units. As a result, there is a real risk of extinction of these products and loss of corresponding microbial resources (Dimassi *et al.*, 2020; Serhan *et al.*, 2016; Serhan and Mattar, 2013).

The majority of Lebanese medium and large-scale dairy industries use selected imported cultures in their production process (El Khoury *et al.*, 2011; Dib *et al.*, 2008). However, due to the limited number of these commercial strains, it results in a homogenous and uniform production (Wouters *et al.*, 2002). Besides, these cultures are not adapted for manufacturing local dairy products and don't meet consumer's requirements or expectations when they are compared to the artisanal dairy products. In addition, they endangers the sustainable production of these traditional cheeses and milk fermented products (Dib *et al.*, 2012). All these practices result in a severe reduction in variability in dairy microflora originated from the local milks and milk fermented products. It also present the risk of extinction of these products and the loss of

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corresponding microbial resources, as reported by many studies (Dimassi *et al.*, 2020; Serhan and Mattar, 2018; Marino *et al.*, 2003).

During the past few decades, importance has been given to the isolation and characterization of LAB (lactic acid bacteria) strains usually collected from traditional dairy products (Ayad *et al.*, 2004;Chammas *et al.*, 2006b). Previous studies have reported important technological characteristics and applications of indigenous LAB strains. They include the acidifying, proteolytic, lipolytic, texturizing, aroma-producing and probiotic attributes (Frau *et al.*, 2016; Kirmaci, 2016; Malek *et al.*, 2012). More recently, some studies intend to isolate the indigenous microbial community in some dairy goat products such as Darfyieh *(*Abu Saliba *et al.*, 2014; Serhan *et al.*, 2009), Ambriss (Abiad *et al.*, 2022), and raw goat milk (Saliba *et al.*, 2021) in order to characterize their probiotic potential by using conventional and genetic based methods.

Nevertheless, knowledge about the Lebanese traditional milk fermented products is currently limited, especially by considering their microbial composition and their technological properties except those reported for the cow milk Laban by Chammas *et al.*, (2006a; 2006b). Therefore, once these autochthonous LABs are isolated, they may contribute to numerous interesting applications in dairy sector and in the sustainable and controlled production of these traditional products (García-Cano *et al.*, 2019; Agostini *et al.*, 2018; Viana de Souza and Silva Dias, 2017).

The objective of this thesis is to promote some traditional Lebanese goat fermented products by revealing their microbial specificity and characteristics as well as their technological properties. This exploration will possibly lead to the development of a Protected Designation of Origin (PDO) for each product under investigation. In this regards this thesis is divided into four main chapters.

The literature review consists of one chapter entitled “State of the Art’. Its first part presents the current Lebanese dairy sector, the characteristics of goat production and local consumption habits. The second part introduces the different properties, technological and sensory characteristics, texture and safety characteristics of goat milk fermented products. The third part overviews the manufacturing process and the specific characteristics of the main Lebanese traditional goat milk products such as Laban, Labneh, Shankleesh, Labneh El Darff, Ambriss, Serdaleh, Darfyieh and Ariche cheeses, and Kishk. In the fourth part of this chapter,

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reliable tools for the isolation and identification of LAB that remain a crucial point for any further investment as new industrial cultures are surveyed, by considering different discriminatory methods like culture dependent and culture independent meta-omics approaches. The diversity and composition of microbial communities in different goat milk products produced locally or from Mediterranean origin are presented in the 5th part. The last part depicts the main technological characteristics of LAB isolated from milk fermented products such as acidification activity, antagonistic and physical properties, metabolic activities, resistance to gastro-intestinal stress and the importance of volatile organic compounds as detrimental in final flavor and consumer acceptance.

In response to the tackled problematic issues, the second chapter of the thesis set the main as well as the specific objectives. The global experimental approach is presented, to take into consideration the rationale behind the selection of these endangered traditional products and the methods needed to reveal the characteristics of their isolated LAB strains.

In the third chapter, the materials and methods are described. The first part clarifies the selection process of the products according an elaborated producer's survey. The conditions for sample selection and collection are explained in the second part. The physico-chemical tests and the culture dependent techniques are presented in the third and fourth parts, respectively. The culture independent analyses are described in the fifth part of this chapter. The sixth part of this chapter focuses on the methods used to quantify the technological parameters, such as the acidification activity, the metabolic properties, the physical and textural properties and the volatile organic compounds synthesized by the bacteria in goat milk. This chapter concludes with the statistical analyses.

The fourth chapter is dedicated to the results and discussion of the thesis. It firstly introduces the data results from the questionnaire on goat farmers and dairy goat producers. The second part deals with the culture dependent and independent data results. It includes notably the results obtained from metagenetic and metagenomic analyses. This chapter constitutes an article entitled "Lebanese fermented goat milk products: from tradition to meta-omics" that was submitted.

Chapter V focuses on the results concerning the technological properties of the isolated strains, by considering their acidification activity, proteolytic and lipolytica activities, physical and textural properties in terms of texture (firmness, cohesiveness), syneresis and apparent

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viscosity, and ability of the strains to produce volatile organic compounds. A general discussion concludes this chapter, by considering all the results.

Finally, a conclusive section summarizes the main findings of the present thesis and offers a general discussion and some perspectives for future research.

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II. LITERATURE REVIEW

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II.1.Lebanese dairy sector

Lebanese dairy sector constitutes an important socio-economic support representing about 9% of agricultural activists in terms of the number of stakeholders (Lebanese Miinistry of Agriculture and FAO, 2012). According to (Saadeh, 2016), this sector is clustered into small, medium or traditional enterprises of which most of them (70%) are established and scattered in the rural regions of the country. These capacities are added to only 20 main modern dairy plants barely contributing to 25 % of the local market demand for these products.

II.1.1. Production and consumption of dairy products

The consumption of dairy products is still the most preferable in the Lebanese culinary culture. It's estimated that the annual consumption per inhabitant is about 58 Kg (Saadeh, 2016), which is close to the levels of European Mediterranean countries (90 Kg /inhabitant in France and Spain, 70 Kg in Greece) and much higher than those in Iran and Egypt (33 Kg and 25 Kg /inhabitant respectively) (CNIEL, 2016).

Small ruminant sector plays an important role in this production, not only economically by its share of 45% (28.440 farms) of all Lebanese livestock farms, but also socially by the preservation of a patrimonial heritage of farming and related traditional product manufacturing which is revealed specifically in goat farming.

II.1.2. Characteristics of the Lebanese goat production sector

Goat production sector has increasingly received attention in recent years and it continues to grow and develop (Lebanon Recovery Fund, Uplands 2015).

Dairy products from goat milk represent between 12 and 20% of food products total groceries sales, which is considered as a high amount. Goat milk and goat milk products such as, Kishk, Shankleesh, and Ariche. Darfiyeh and Baladi Cheese are highly appreciated by the local consumers (Balaa and Marie, 2008), especially for their specific "goaty taste"(Semaan *et al*., 2011). These products are locally known for their typical production characteristics and preservation techniques (Serhan and Mattar, 2018).

Local goat population counts 550,000 heads, with 96.8 % belonging to the indigenous "*Black*" or "*Baladi*" breed (Aw-Hassan, *et al*., 2018). The extensive production systems used with this breed are well adapted to the semi-arid environmental constraints of the country (Dick *et al*., 2008). Goat milk production is seasonal, from March to August, and milk yield is low,

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estimated at 189 kg per head for the 180 days of milking period (Serhan and Mattar, 2018). However, in spite of many sanitary, biological and socio-economic constraints, the production has increased between 2008 and 2010 from 21.2 to 34 thousand tons of milk (Lebanese Ministry of Agriculture, 2010).

Moreover, due to the scarcity of intensive production system, producers tend to commercialize their product (87.6 %) as a whole milk for processing in the dairy industries (Saadeh, 2016; Serhan and Mattar, 2018. The rest is intended for direct consumption and processed into traditional dairy products to be sold to consumers and distributors. In fact, the trend in Lebanon is to sell these local dairy products in about 25,000 traditional and modern stores or groceries called *Dukkaneh*, which represent the remaining portion of the food distribution market. These *Dukkaneh* present a good opportunity for farmers to sell their products beyond their geographic area since they are less demanding when it comes to quality and marketing, even if some artisanal dairy products are still marketed on the spot of production (Balaa and Marie, 2008). In total, only 10 supermarket chains spread all over the country control over 35% of the Lebanese distribution market (MercyCorps, 2014).

II.1.3. Milk fermented products

As a method of milk preservation, fermented milks have been prepared for a very long time in Central Asia and in Mediterranean countries thanks to pH reduction but also to their much-appreciated flavor (Eurostat, 2015) and more recently for their biogenic metabolites. As a result of the multitude of food-microbe combinations, there are thousands of different types of fermented milk products, such as yoghurts and yogurt-like products with proven health benefits (Teneva-Angelova *et al*., 2018; Tamime and Robinson, 2007). The nature of these products depends on the type of milk used, the pre-treatment of the milk, the microorganisms involved in the transformation, and the conditions of fermentation and subsequent processing. They mainly involve lactic acid bacteria (LAB), but micrococci, coryneform bacteria, yeasts, and molds can also occur (Zamfir *et al*., 2006a). The different nutritive, health and microbiological components are also implicated in the variability of milk fermented types **(Figure II.1)**.

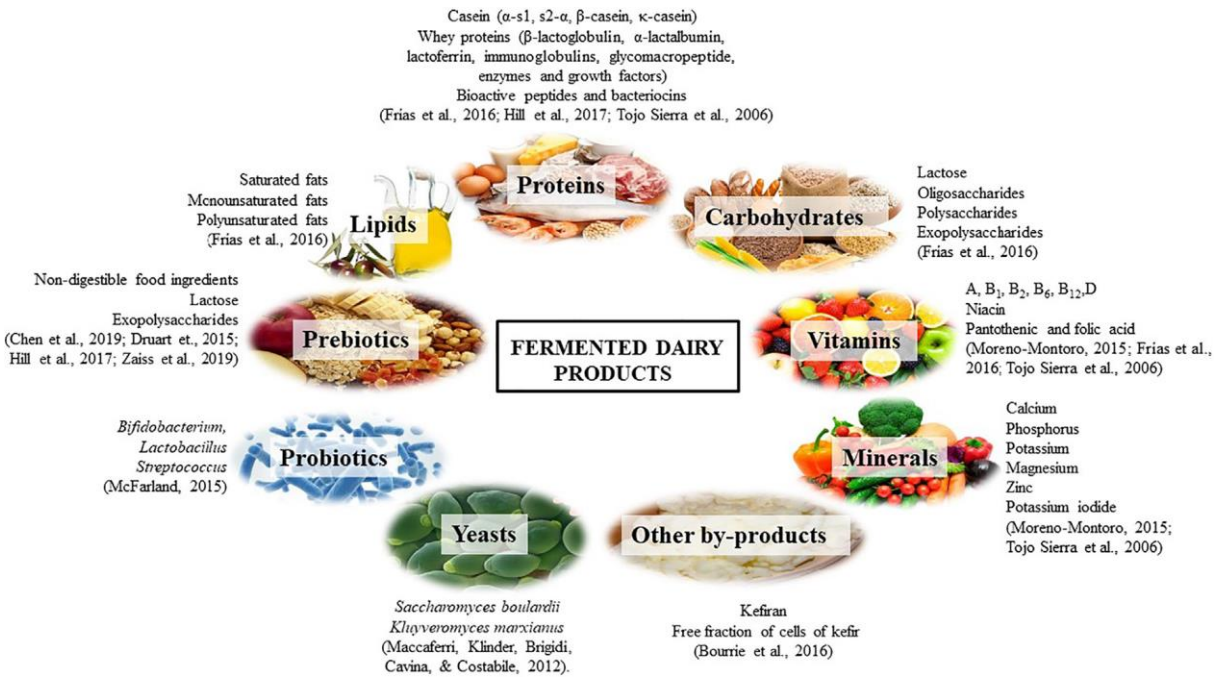

FIGURE II-1. MAIN COMPONENTS OF FERMENTED MILKS, (ADAPTED FROM García-Burgos *et al*., 2020)

II.1.3.1. Definitions

Fermented milks are dairy products obtained with milk fermentation thanks to suitable and harmless microorganisms. The cultures of microorganisms shall be viable, active and abundant in the product. Each fermented milk has a specific culture (or specific cultures) used for fermentation. This fermentation results into milk acidification and consequently gel formation without the involvement of rennet (Béal and Helinck, 2019).

At early times, fermented milk products were produced spontaneously by the action of indigenous microorganisms present in the raw milk or from the environment. Subsequently, spontaneous fermentations were replaced by the "back slopping" technique, which consists in inoculating milk with a small amount of a precedent successful fermentation (Shrivastava and Ananthanarayan, 2015). Even nowadays and with traditional and artisanal methods, several naturally fermented milk products are produced by back slopping. Currently, most of commercialized fermented milk products are prepared in large factories that employ selected dairy starters on frozen or freeze-dried forms.

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The microbial ecology of these products are complex and dynamic resulting in numerous biochemical reactions. Bacterial community composition is dominated by Lactic Acid Bacteria (LAB), which metabolically convert lactose into lactic acid in a phenomenon called fermentation (Dal Bello and Cocolin, 2011; LAB involved in milk fermentation comprise a wide range of genera which include *Lactococcus, Leuconostoc, Streptococcus, Lactobacillus* and *Enterococcus* (Hamama, 1992).

II.1.3.2. Types of milk fermented products

Classification of these products is based on the type of fermentation they undergo (**Table II.1**), such as lactic, yeast-lactic, and mold-lactic (Teneva-Angelova *et al*., 2018). Products produced by lactic fermentation could be classified depending on the features of LAB, such as mesophilic, thermophilic, and probiotic (Robinson *et al*., 2002). However, the most commonly used is the one proposed by Tamine and Robinson (2007) based on the product's final texture and its physical characteristics:

1- Liquid and viscous fermented product
2- Semi-solid fermented product
3- Solid milk fermented product
4- Milk/cereals fermented mix product

TABLE II-1. MAIN FERMENTED MILKS, TOGETHER WITH THE CORRESPONDING CULTURE TYPES (FROM CODEX ALIMENTARIUS, 2011).

Product type	**Culture type**
Yogurt	Symbiotic cultures of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*
Yogurt based on alternative cultures	Cultures of *Streptococcus thermophilus* and all species of Lactobacillus
Acidophilus milk	*Lactobacillus acidophilus*
Kefir	Culture prepared from kefir granules, *Lactobacillus kefiri*, species of the genus Leuconostoc, Lactococcus and Acetobacter that grow in a close specific relationship. The kefir granules constitute lactose fermenting yeast (*Kluyveromyces marxianus*) and lactose-free fermenting yeasts (*Saccharomyces unisporus, Saccharomyces cerevisiae*, *Saccharomyces exiguus*)
Koumys	*Lactobacillus delbrueckii subsp. bulgaricus, Kluyveromyces marxianus*

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In the Mediterranean countries, another classification based on the total solids (TS) content of the product has been adopted (Abd El-Salam, 2011).

1- Fermented milks with normal milk composition (12–18% TS), which can be either liquid or set gel. This group includes Laban, Laban rayeb, Laban kad, and Gariss.
2- Concentrated fermented milks (20–40% TS). This group includes Labneh and Laban Zeer.
3- Dried fermented milks (>85% TS), which include Kishk, Tarhana and related products.

II.1.3.3. Main traditional fermented milk products and cheeses in the Mediterranean countries

Based on EU regulation 2082, The European Food International Resources (EuroFIR) defined traditional food, as "a food with a specific feature or features, which distinguish it clearly from other similar products of the same category in 3 terms. The use of 'traditional ingredients' (raw materials of primary products) or 'traditional composition' or 'traditional type of production and/or processing method'' (Trichopoulou *et al*., 2007). However, the opinion of consumers is not necessarily connected to this definition. In six European countries (Italy, Spain, Norway, Belgium, France, and Poland) a survey of TRUEFOOD was carried out, which has examined how consumers perceive the traditional foods. The TRUEFOOD concluded the definition on the base of local production, commercial availability, authenticity, and gastronomic heritage (Vanhonacker *et al*., 2008).

From consumer's perceptions, Guerrero *et al*., (2009) defined a traditional food product as "a product frequently consumed or associated with specific celebrations and/or seasons, normally transmitted from one generation to another, made accurately in a specific way according to the gastronomic heritage, with little or no processing/manipulation, distinguished and known because of its sensorial properties and associated with a certain local area, region or country".

Fermented dairy products and cheeses have been produced to prolong the shelf-life of milk (Hamama, 1992). They have persisted over centuries and they have often evolved from traditional home manufacture to large-scale industrial production using specific starter cultures and modern equipment (Zamfir *et al*., 2006b).

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Egypt is one of the largest producers of milk in Africa and the Middle East, accounting for 16.3% of the dairy product market value. Different raw milks (cow, buffalo, sheep, goats and camel) are consumed. Egyptian dairy products are generally produced under artisanal conditions from raw milk without using industrial starter cultures (Ayad *et al*., 2004). The main traditional cheeses are **Ras** (hard type), **Domiati** (soft type), **Mish** and the main fermented milk products are **Zabady** and **Laban Rayeb** (Phelan *et al*., 1993; El-Gendy, 2001; Abou-Donia, 2002). Traditional **Zabady** is characterized by a cooked flavor and a consistency as firm as that of yogurt. **Zabady** is thought to be the oldest fermented milk known in the world, especially in the Middle East (El-Gendy, 2001). The best **Zabady** product is mainly made from buffalo's milk; it can be also made from cow's milk or from a mixture of both (Abou-Donia, 2008). There are promising perspectives for traditional Egyptian dairy products (particularly for the **Ras/Romi** cheese and milk-based desserts) on Middle Eastern and African markets (LACTIMED, 2014).

In Algeria, the goat population is very high (4.9 million heads), just after sheep population (23 million heads) according to Statistiques Agricoles,(1999). Goat milk plays a vital role in human consumption, most being consumed by the rural community, while little is available on the market. Commercially, goat's milk is processed into products such as **Raib**, **Lben** (local traditional fermented milk) and **Jben** (local traditional fresh cheese) (Badis *et al*., 2004).

In Morocco 20 to 30% of all milk produced is still processed by private individuals. These dairy shops and farmers manufacture traditional Moroccan dairy products such as **Lben** and **Raib** (fermented milks), **Zabda** (farm butter), and **Jben** (fresh cheese). These products are made from raw milk, and their physical properties are similar to those of commercially produced buttermilk, yogurt, butter, and fresh cheese. Although they are usually made from cow's milk, milk from sheep, goats, and camels also can be used. These products are very popular in Morocco mainly because of their refreshing qualities (Hamama, 1992).

Basically, all these traditional dairy products are prepared by simply allowing the raw milk to ferment spontaneously at room temperature (15° to 25°C) for 1 to 3 days depending on the season. The coagulated milk is called **Raib**. It can be consumed as such or churned in a clay jar to separate the liquid phase **Lben** from fat. .**Jben** is prepared by placing the coagulated milk

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in a cloth at room temperature and draining the whey. Salt is added to **Jben** made in northern Morocco.

Moroccan soft white cheese is a traditional dairy product well known and highly appreciated by consumers for centuries. It is widely manufactured and consumed in Morocco, especially in the ‘‘Ramadan’’ fasting month (Ouadghiri *et al*., 2005). The technology transfer aspects of Moroccan traditional dairy products **Lben** (fermented skimmed milk), **Jben** (soft white cheese), and **Smen** (fermented butter) were well reviewed by Benkerroum and Tamime, (2004).

Cheese is by far the largest segment of the dairy market in Turkey, with traditional white, semi-soft, feta type cheese accounting for the majority of sales. This type has roughly 33% market share, dominating the market in sales, followed by “Kashar”. Yogurt and **Ayran** present the third highest share of the dairy sector, at 18% in total. According to (Turkish Statistics, 2017), currently 1,350 processors are involved in the Turkish dairy markets, with the majority engaged in traditional-style production methods (Mandiras) and concentrated mostly on **Ayran** and **Feta** type white cheese products. The quality and food safety of traditional processing methods vary and largely remain outside the supervision of the government (Kýrda and Karaca, 2017).

More than 50 varieties of cheese can be found in Turkey. However, 3 of them (Beyaz, Kasar, and Tulum cheeses) are the most popular (A. Hayaloglu *et al*., 2007). According to Kurt *et al*., (1991), **Tulum cheese** has a white or cream color, a high fat content, and a crumbly and semi-hard texture; it is dispersible in the mouth and has a buttery and pungent flavor. Different types of milk (cow’s, ewe’s or goat’s milk) can be used in the manufacture of **Tulum cheese**. The name tulum means “goat’s or sheep’s skin bag” in Turkish, which is the bag used for packaging and ripening. According to consumers, the cheese ripened in **Tulums** has a superior quality in comparison with the cheese ripened in plastic barrels.

Urfa cheese which is a brined type cheese produced in South-eastern Anatolia region of Turkey. It is an artisanal cheese made from either raw milk of Awassi sheep breed or mixture of caprine and ovine milks at appropriate ratios. In traditional **Urfa cheese**-making practices, the milk is not pasteurized and no starter cultures are used. The milk is coagulated with animal rennet at 30–32^{o}C for about 90 min, cut into small cubes and drained by gravity drainage in special molds of triangular shape called “parzin”. Afterwards, the cheese blocks are dry salted

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and/or kept in brine (~16 g/100 g NaCl, w/v) for 5–6 months (Ozer *et al*., 2002). From a sensory point of view, it has significantly higher aroma and flavor scores than cheeses made from pasteurized milk and lactic acid starters (Kirmaci, 2016).

Trachanas is a dry mixture of fermented milk and crushed grain of wheat. Trachanas is produced in Greece (Georgala, 2012) and Cyprus (Bozoudi *et al*., 2017) and is the most known and characteristic traditional product of the country, while also being a very common meal (consumed at least once a week in most households and exported for specialty artisanal product stores). Trachanas-like products are also known under different names in many countries such as **tarhana** in Turkey (Tangüler, 2014; Daglioğlu, 2000), **kishk** in Egypt (Gadallah and Hassan, 2019), Jordan, Palestine, Syria (Tamime and O'Connor, 1995), and Lebanon (Chedid *et al*., 2018; Salameh, 2016); **kushuk** in Iraq and **tarkhineh** in Iran (Tamime *et al*., 2011). The diversity of the amount and type of ingredients, as well as the processing techniques, affect chemical composition, nutritional content and sensory attributes of the products (Tamime *et al*., 2000).

Table II.2 and **Table II.3** summarize the main dairy products from goat milk that are elaborated within the Mediterranean region by considering fermented milks **(Table II.2)** and cheeses **(Table II.3)**.

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TABLE II-2. ARTISANAL FERMENTED GOAT MILK (TOTALLY OR PARTIALLY) PRODUCTS IN THE MEDITERRANEAN REGION

Product name	Countries	Characteristics	References
Yogurt	Turkey, Greece	Cow, ewe or goat milk	Funda Sömer, 2012; Tamime *et al*., 2011; Şenel *et al*., 2011; Şanlidere Aloğlu and Öner, 2013
Tuzlu yoghurt	Turkey	Goat milk, PGI	Abd El-Salam, 2011; Güler, 2007
Kishk, Kushuk	Lebanon, Egypt, Jordan, Palestine, Syria, Iraq	Cow, goat or a mixture of milks Dry mixture of fermented milk and crushed grain of wheat	Bozoudi *et al*., 2017; Salameh, 2016; Georgala, 2012
Tarhana, Trachanas	Turkey, Greece, Cyprus	Cow, goat or a mixture of milks Dry mixture of fermented milk and crushed grain of wheat	Bozoudi *et al*., 2017; Salameh *et al*., 2016
Lben	Morocco	Cow or goat milk	Ouadghiri *et al*., 2009
Laban, Ayran	Lebanon. Syria, Jordan, Palestine	Cow or goat milk	Béal and Chammas, 2019; Serhan and Mattar, 2018; Tamime *et al*., 2011
Raeb, Zabady	Egypt	Cow, buffalo or goat milk	Ayad *et al*., 2004
Labneh	Lebanon, Syria, Jordan, Palestine	Cow, goat or mixture of milks	Serhan and Mattar, 2018; Serhan *et al.*, 2016; Kaaki *et al*., 2012; Nsabimana *et al*., 2005
Suzme yoghurt, Torba	Turkey	Cow or goat milk	Tamime *et al*., 2011
Ambriss	Lebanon	Raw goat milk	Dimassi *et al*., 2020; Semaan *et al*., 2011
Serdaleh	Lebanon	Raw goat milk	Tabet *et al*., 2019
Labneh El Darff	Lebanon	Raw goat milk	Semaan *et al*., 2011

PGI (Protected Geographical Indication)

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TABLE II-3. ARTISANAL CHEESES PRODUCED FROM GOAT MILK (PARTIALLY OR ENTIRELY) IN THE MEDITERRANEAN REGION

Product name	Type of milk	Product characteristics	Country	Label	References
Anevato	Goat or mixture of goat and ewe milk,	Soft brined cheese	Greece	PDO	Michailidou *et al*., 2021
Ariche	Raw goat milk	Whey cheese, soft consistency	Lebanon	ND	Serhan and Mattar, 2013
Armada	Unpasteurised goat or cow milk or a mixture of both	Semi hard to hard depending on ripening	Spain	Non-PDO	Herreros *et al*., 2003
Batzos	Goat or a mixture of both ewe and goat milk,	Semi-hard to hard brined cheese	Greece	PDO	Meng *et al*., 2018; Michailidou *et al*., 2021; Psoni *et al*., 2006
Bouhezza	Raw goat, Soft cheese	Soft texture	Algeria	ND	Boudalia *et al*., 2020
Carra	Cow or mixture of cow and goat milks	Slightly hard or soft consistency	Turkey	ND	Kamber, 2008
Chefchaouens	Goat milk	Soft texture	Morocco	PGI	Association Nationale Ovine et Caprine-Maroc
Çimi	Goat milk	Soft texture, off white, close to Tulum cheese	Turkey	ND	Kamber, 2008
Darfiyeh	Raw goat milk	Semi-hard texture	Lebanon	Presidium Quality by SFFD	Hosri and El Khoury, 2004
Feta	Goat or ewe milk	Semi-soft texture, brined cheese, white color	Turkey, Greece	PDO	Bozoudi *et al*., 2016; Michailidou *et al*., 2021
Flor de Guía	Ewe (50 %), ewe (40 %) and Goat (10 %),	Semi hard, off white color	Spain	PDO	Pulina *et al*., 2018
Fresh goat cheese	Goat milk	Soft texture	Turkey, Lebanon	ND	Kamber, 2008
Galotiri	Ewe or mixture of ewe and goat milk	Soft creamy brined cheese	Greece	PDO	Michailidou *et al*., 2021
Helim (Hallaoumi)	Ewe and goat milk or a mixture of both milks or cow milk	Semi-soft texture "squeaky", brined cheese, white to off white	Turkey, Cyprus, Greece, Lebanon, Syria, Palestine, Jordan	PDO	Kamber, 2008; Milci *et al*., 2005
Ibores	Raw Goat milk,	Semi-hard cheese	Spain	PDO	Meng *et al*., 2018; Pulina *et al*., 2018
Juben Balady	Ewe, goat or a mixture of both milks	Soft white-brined cheese	Jordan	ND	Haddad and Yamani, 2017

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Product name	Type of milk	Product characteristics	Country	Label	References
Kalathaki	Ewe or mixture of ewe and goat milk (up to 30%)	Soft white-brined cheese	Greece	PDO	Zoumpopoulou *et al*., 2020; Michailidou *et al*., 2021
Maiorchino	Ewe milk or mixture of ewe and goat milks	Hard ripened cheese	Italy	ND	Conte *et al*., 2015
Melichloro	Ewe or mixtures of ewe and goat milk (up to 30%)	Hard cheese	Greece	Non-PDO	Zoumpopoulou, *et al*., 2020
Mish	Buffalo, cow , goat milk or a mixture of both milks	Hard, ripened yellowish - brown, sharp taste cheese	Egypt	ND	Ayad *et al*., 2004; Abou-Donia, 2002
Murcia	Unpasteurised goat milk	hard, ripened off white cheese	Spain	PDO	Pulina *et al*., 2018
Murcia al Vino	Goat milk	Semi-hard texture	Spain	PDO	Pulina *et al*., 2018
Otlu	Raw ewe milk or ewe milk mixed with cow or goat milks	Semi hard and salty texture	Turkey	ND	Hayaloglu and Fox, 2008
Palmero	Unpasteurised goat milk	Semi hard ripened cheese	Spain	PDO	Pulina *et al*., 2018
Parmak (Finger) Cheese	Ewe, goat, or cow milk	Soft texture	Turkey	ND	Kamber, 2008
Robiola di Roccaverano	Raw goat milk or a mix with ewe milk	Soft white cheese	Italy	PDO	Biolcati *et al*., 2022
Semicotto Caprino	Goat milk	Semi-hard cheese	Italy	ND	Suzzi *et al*., 2000
Sepet	Goat milk, Semi-hard cheese	Fermented milk	Turkey	ND	Meng *et al*., 2018
Shankleesh	Goat or cow or mixture of both, cheese/Fermented milk	Soft to hard texture and from mild flavor to pungent odor	Lebanon	ND	Toufeili *et al*., 1995
Sünme	Sheep, goat, or cow milk	Semi soft texture in rope shape, white color and law fat	Turkey	ND	Kamber, 2008
Surk	All types of milk	Soft to hard texture, red to brown color	Turkey	ND	Kamber, 2008
Sütçüler Tortusu	Ewe or goat milk	fresh soft cheese	Turkey	ND	Kamber, 2008
Sütlü (Milky)	Goat milk	Semi-hard texture	Turkey	ND	Kamber, 2008
Tenerife and Babia	Goat milk	Soft white cheese	Spain	Non-PDO	Meng *et al*., 2018
Testi (Pitcher)	Goat milk	Semi-hard cheese	Turkey	ND	Kamber, 2008

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Product name	Type of milk	Product characteristics	Country	Label	References
Tulum	Cow, ewe or goat milk	Semi hard texture, brined cheese	Turkey	ND	Hayaloglu *et al* 2007
Urfa	Raw ewe, or mixture of both milks	Fresh soft cheese	Turkey	ND	Kirmaci *et al* 2016
Xinotyri	Unpasteurised goat or ewe milk	Whey cheese, Hard consistency	Greece	ND	Meng *et al*., 2018; Pappa *et al*., 2017
Yörük	Ewe or goat milk	brined white cheese-similar to tulum	Turkey	ND	Kamber, 2008

SFFD Slow Food foundation for biodiversity, POD: Protected Designation of Origin, ND: Not Determined

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II.2.Characteristics of goat milk

Previously considered as “The poor man’s cow” the goat —and goat milk— began in recent years gaining attention (Clark and Mora García, 2017a), due to its specific composition, organoleptic characteristics and healthy attributes (Monteiro *et al*., 2019; Silanikove *et al*., 2010). Moreover, it has compositional similarities with human milk and displays nutraceutical properties, thus being recommended to be used as an alternative source of milk for infants (Sonu HN and Basavaprabhu KS, 2020; Reeta *et al*., 2015).

From a health perspective, goat milk was suggested to show very beneficial and useful for people suffering from stomach acidity, asthma, migraine, colitis, stomach ulcer, digestive disorder, liver and gallbladder diseases, stress-related symptoms, constipation, and neurotic indigestion (Jandal, 1996).

II.2.1. Characteristics of goat milk compounds

Several factors affect the biochemical composition, technological properties and microbiological quality of goat’s, milk depending on genetic, physiological, environmental and handling factors like farming practices and feeding systems (Yangilar, 2013). In addition, high variability among individual animals within the same breed is recorded (Yangilar, 2013; Mayer and Fiechter, 2012; Park *et al*., 2007).

Milk secretion and seasonality of goat is explained by the activation of plasmin system, including the native serine proteinase plasmin (PL), Plasma zymogen plasminogen (PG), Plasminogen Activators (PA), Plasmin Inhibitors (PI), Plasminogen Activator Inhibitors (PAI) in milk (Albenzio *et al*., 2006). This effect is well pronounced through lactation in a synchronous manner; therefore, all animals are at the same stage of lactation at a given time. The consequence to the increase in plasmin activity can lead to casein degradation and reduction in cheese yield (Haenlein, 2017; Yangilar, 2013; Haenlein, 2004).

II.2.1.1. Nutritional composition of goat milk

On average, goat milk contains 12.2% total solids, where 3.8% are fat, 3.5% protein, 4.1% lactose, and 0.8% ash. Fat, protein and lactose make up approximately 50, 25, and 25% respectively of the total energy (Hilali *et al*., 2011). The general proximate analysis of goat milk is compared to that of other species in **Table II.4**.Variation in the chemical composition of goat milk is seasonal. The major constituents are high in early lactation, decline rapidly, then remain

low for a variable length of time, and increase again towards the end of lactation. Only lactose remain independent of stage of lactation (Guo *et al*., 2004).

TABLE II-4. BASIC COMPOSITION OF DIFFERENT MILKS (MEAN VALUES IN G PER 100 G) (ADAPTED FROM YADAV AND SINGH, 2006).

Composition	Goat milk	Cow milk	Human milk
Energy (kcal)	70	69	68
Water (%)	87.5	87.7	86.7
Total solids (%)	12.2	12.3	12.3
Protein (%)	3.2	3.3	1.3
Fat (%)	4.0–4.5	3.8	4.1
Lactose (%)	4.6	4.7	7.2
Ash (%)	0.8	0.7	0.2

Goat milk is an adequate to excellent source of protein, calcium, phosphorus, potassium, niacin, pantothenic acid, riboflavin, thiamin, and vitamin A. It contains minerals such as magnesium, calcium and phosphorus (Abbas *et al*., 2014). Neither cow nor goat milk are good sources of iron, vitamin C, or vitamin D. In contrast to cow milk, goat milk contains inadequate levels of vitamin B6, vitamin B12, and folic acid (Ziegler *et al*., 2005; Basnet *et al*., 2010). However, goat milk lacks folic acid, which can be compensated either by the direct addition of folic acid (Slačanac *et al*., 2010) or by the integration of folate-producing bacteria during fermentation (Mahara *et al*., 2021). In addition, it contains a higher content of short-chain fatty acids in milk fat (Shu *et al*., 2016; Zenebe *et al*., 2014).

Goat milk fat is mainly composed of triacylglycerol (~98%), with minor amounts of phospholipids, cholesterol, free fatty acids, and mono and diacylglycerols (Taylor and MacGibbon, 2011). Compared to cow milk, goat fat milk globules are much smaller in diameter (3.49 compared to 4.55 μm in cow's milk) which is one of the factors that increase milk digestibility (Monteiro *et al*., 2018). Differences in fatty acids composition also contribute in better digestibility **(Table II.5)**. The capric, caprylic and caproic fatty acids represent about 15–18% of all fatty acids present, instead of 5–9% in cow's milk (Monteiro *et al*., 2018; Clark and Mora García, 2017b). Differences in the polymerization of acetate produced by the rumen microbiota in the goat's stomach are responsible for this feature, and this particular composition

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is associated with the characteristic odor and flavor of goat's milk (Verruck *et al*., 2019; Amigo and Fontecha, 2011). In addition, the proportion of unsaturated fatty is slightly higher (25.8%) than in cow milk (24.7%).

TABLE II-5. FATTY ACIDS COMPOSITION (IN % OF TOTAL FATTY ACID) OF GOAT AND COW MILK (ADAPTED FROM AMIGO AND FONTECHA (2011) AND Tylor and MacGibbon (2011).

Fatty acid	**Goat milk**	**Cow milk**
Butyric (C4:0)	2.18	3.70
Caproic (C6:0)	2.39	2.40
Caprylic (C8:0)	2.73	1.50
Capric (C10:0)	9.97	3.20
Lauric (C12:0)	4.99	3.60
Myristic (C14:0)	9.81	11.10
Myristoleic (C14:1)	0.18	0.90
Pentadecanoic (C15:0)	0.71	1.20
Palmitic (C16:0)	28.00	28.30
Palmitoleic (C16:1)	1.59	1.60
Stearic (C18:0)	8.88	11.80
Oleic (C18:1)	19.3	18.80
Linoleic (C18:2)	3.19	1.40
Linolenic (C18:3)	0.42	0.90
Linoleic conjugated (C18:2)	1.10	1.10
Minor acids	3.19	4.40

Concerning protein content, like in cow's milk, caseins are classified as κ-, β-, αs1-, αs2-, and γ-caseins and the whey proteins as β-lactoglobulin, α-lactoalbumin, serum albumin, and immunoglobulins **(Table II.6)**. Regarding the soluble protein portion, differences occur in the charged amino acids of α-lactoalbumin and β-lactoglobulin between caprine and bovine milks, and two genetic variants having Lys or Ala as N-terminal residues (Amigo and Fontecha, 2011).

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TABLE II-6. COMPARISON OF THE PROTEIN COMPOSITION OF GOAT MILK FROM THOSE OF COW MILK (ADAPTED FROM AMIGO AND FONTECHA (2011) AND (Swaisgood, 2008)

Component	**Goat milk**	**Cow milk**
Total caseins (g/100 mL)	2.33–4.63	2.40–2.80
αs1 - Casein (%)	0**a**–28.0	42.86–53.57
αs2 - Casein (%)	10.0–25.0	10.71–14.28
β - Casein (%)	0**a**–64.0	32.14–39.28
κ - Casein (%)	15.0–29.0	10.71–14.28
Total whey proteins (g/100 mL)	0.37–0.70	0.50–0.70
α - Lactalbumin (%)	17.8–33.3	14.28–21.42
β - Lactoglobulin (%)	39.2–72.1	28.57–57.14
Serum albumin (%)	5.1–21.5	1.43–5.71
Immunoglobulin (%)	4.6–21.4	8.57–14.28

The letter **(a)** indicates the absence of αs1-casein or β-casein in milk from animals carrying the respective null alleles.

The casein content in goat's milk represents 74% of total milk proteins, whey proteins amount is close to 17% and the proportion of non-protein nitrogen compounds is 9% (Campos *et al*., 2022; Al-Saadi *et al*., 2014). The differences in protein content among species are related to genetic polymorphisms, mainly regarding αs1-casein (Raynal-Ljutovac *et al*., 2008). The casein micelles size is smaller in goat's milk than in cow's milk, thus being more friable and attacked more rapidly by the stomachs proteases. Theese properties helps enhancing the digestibility of goat's milk and corresponding dairy products (Clark and Mora García, 2017b; Haenlein, 2004).

Goat milk confers beneficial health effects due to the presence of health-promoting compounds. Among them, bioactive peptides and lipids such as conjugated linoleic acids and others like hormones, cytokines, oligosaccharides, nucleotides and minor components can be emphasized (de Assis *et al*., 2016; Abeijón Mukdsi et al., 2013; Salva *et al*., 2011). Their role affects contributes to the development of functional dairy products (Campos *et al*., 2022; de Assis *et al*., 2016; Granier *et al*., 2013). During food processing and/or gastrointestinal ingestion, some bioactive peptides are formed from inactive protein precursors. They exhibit specific biological activities, such as antimicrobial, antihypertensive, antioxidant, immunomodulatory or mineral-binding (Balthazar *et al*., 2017). In this regard, pepsin

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hydrolysates and/or isolated peptides of goat protein fractions offer great promises for their potential application as bioactive and anti-oxidative properties (Ahmed *et al*., 2015).

Furthermore, goat's milk has been recognized as a functional food since it holds potential as a natural source of lactose-derived oligosaccharides, together with a high content of vitamin (A and complex B) and calcium content (Haenlein and Anke, 2011; Silanikove *et al*., 2010; Park *et al*., 2007).

II.2.1.2. Prebiotic role of goat milk

Goat milk is an attractive natural source of oligosaccharides to be used in human nutrition. Oliveira *et al*. (2011) reported that oligosaccharide-rich fractions from goat's milk whey, collected after cheese making, had suitable functionality and promoted gut health. A product enriched with these oligosaccharides was obtained following a two-stage tangential filtration process (Martinez-Ferez *et al*., 2006). These authors also stated that the concentrated oligosaccharides show potential applications in the development of innovative goat's milk functional products. In fact, prebiotics improve the survival of probiotics in the gut (Buran *et al*., 2021). Moreover, they improve host immunity by enhancing immunoglobulin A (IgA) and regulating cytokine production (Speranza *et al*., 2018). Although new functional substances are being evaluated and entering the value chain, there is a lack of prebiotic substances employed in goat milk products as prebiotics. (Scott *et al*., 2020; Verruck *et al*., 2019).

II.2.1.3. Tolerance to milk proteins

Allergy towards a given food may be developed, particularly in the first years of life, for any food (Noimark and Cox, 2008). Symptoms of allergy to goat milk appeared at a much later age than cow-milk allergy (Ah-Leung *et al*., 2006), which may benefit younger infants who are milk dependent. In fact, allergy-related symptoms to goat milk may develop in individuals who have already developed it to cow milk (Bellioni-Businco *et al*., 1999). However, about 50% of the human population who is allergic to bovine milk tolerates caprine milk (Park *et al*., 2007). Goat milk proteins present a reduced level or even a lack of αs1 casein, as well as structural differences in α-lactoalbumin and β-lactoglobulin. These characteristics make it less allergenic when compared to bovine milk (Albenzio and Santillo, 2011). However, Ballabio *et al*., (2011) suggested that caution must be taken about this topic before milk substitution to subjects, as they indicated that goat milk from particular αS1-CN genotypes could serve as protein sources for hypoallergenic formulas. These findings are consistent with

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the work of (Lisson *et al*., 2014), who confirmed that, although genetic variants of caseins differ in their allergenicity, cross-reactivity of IgE antibodies of goat and buffalo milks with cow milk caseins limit feeding goat or buffalo products to cow milk-allergic patients.

Proteome analyses showed that some goat's milk proteins comprise components that are precursor of peptides involved in potential hypotensive effects (angiotensinogen), in the normal mammary gland development (i.e. dystroglycan). In addition they may contribute to fetal immunological protection (factor H) and may be classified as defense factors (complement components C2 and C6) (Cunsolo *et al*., 2015).

II.2.2. Technological properties of goat milk

Goat milk's transformation into cheese or fermented milk differs from that of cow milk, depending on the functionality traits for the milk used (Slačanac *et al*., 2010)**.** Heat stability of goat milk is influenced by environmental and genetic factors such as the effect of α_{s1}-casein genotypes which is highly variable under heat treatments. Similarly, temperature also affect heat stability since a higher heating temperature lead to a poor heat stability (Tafes, 2020). Milk heat treatment with temperatures of 110, 120, 130, and 140 °C decreased the heat stability of both goat's milk and bovine's milk (Chen *et al*., 2012). In general goat milk at its natural pH has a lower heat stability than that of bovine. However, the addition of sodium citrate improve heat stability of goat milk better than bovine milk (Chen *et al*., 2012; Manfredi *et al*., 2002). At a temperature of 140 °C, Heat coagulation time (HCT) is the lowest at 11 min when compared to 53, and 41 min for bovine milk and buffalo milk respectively (Prajapati *et al*., 2017). When goat milk is treated at 95 °C for 30 min, It significant impact on the physiochemical properties of the goat yogurt produced resulting in higher dynamic viscosity values, lowest whey separation and strong curd compared to treatments at 63 °C, 85 °C for 30 min and 72 °C for 15 sec (Desouky and El-Gendy, 2017).

The coagulation properties of goat milk is effected by its nutritive composition, environmental conditions and individual factors (Vacca *et al*., 2018). Compared to other species, goat milk coagulates later than sheep milk but earlier than bovine milk whereas curd firming and curd syneresis instant rate constants are greater in small ruminants (Pazzola *et al*., 2018). According to Stocco *et al*., (2018), milk with high fat content (6.40 %) was associated with better coagulation properties, high protein content (4.30 %) was correlated with delayed coagulation and a large quantity of casein with improved ability of gel formation. The work of

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Vacca *et al*., (2018) showed a high variability in the different goat Milk Coagulation Parameters (MCP): Rennet Coagulation Time (RCT, min), Curd Firming time (K_{20}, min) which is the time interval between gelation and curd firmness attainment of 20 mm and Curd Firmness (CFt) at times 30, 45 and 60 min after rennet addition (a_{30}, a_{45} and a_{60} respectively in mm). These are influenced by the management (37% of variability) and also by RCT and CFt a_{60} (68% of the total variability). The incidence of farm effect on the MCPs was mainly linked to the rearing techniques, (e.g., extensive *vs*. intensive ones). Its variable for goats (27 to 48% of total variance), whereas it was very limited for cows (7 to 16%) and intermediate for sheep (16 to 43%). The average value of RCT recorded by the authors was 13.2 min, similar to the Mediterranean buffalo and intermediate to sheep and cattle. The mean CFt (a_{30}) was 36 min, and the prolongation of the analysis up to 60 min revealed a marked syneresis starting at 45 min after rennet addition.

Other traits that significantly influence MCPs are parity and breed type (Pazzola *et al*., 2018). Milk from goats belonging to the first class of parity reaches a faster RCT and a greater curd firmness than goats with 3 or more parities, but with similar Curd Firming time (Vacca *et al*., 2018). Significant differences were observed between the specialized Italian breeds (Saanen et al.) and the autochthonous Mediterranean breeds, with values ranging from 13.6 % for Saanen to 17.8% for the Sarda breed. Interestingly the curd firmness was higher for the native Sarda goat, with a mean value of a30 at 40 mm which suggest a higher dairy efficiency per liter of milk for native breeds (Pazzola, 2019).

The titratable acidity of fresh, as well as heat-treated, caprine milk has been consistently higher than that of bovine milk. (Park *et al*., 2007). Goat's milk present a faster acidification process than cow's milk (Costa *et al*., 2016). This behavior was explained by the enhancement of the microbial growth, acidity progress and peptidase activity of *Lb. delbrueckii ssp. bulgaricus* in goats' milk (Tamime, 2006). In fact, Rogelj and Perko, (1998) pointed out that the activity and growth rate of the starter cultures are strictly strain dependent.

In a comparable study of goat's milk collected from different small and medium enterprises of Greece, Portugal and France, whey drainage measured after rennet coagulation at pH 6.30 or direct acidification at pH 4.5 was similar between the three countries ranging between 71.1 % and 82.1 %. However, the highest level of whey drainage was negatively

correlated to fat and protein milk content in all collected samples (Morgan *et al*., 2003). A trait which result in a weaker gel formation in the coagulated milks (Remeuf *et al*., 2003).

II.2.3. Sensory characteristics of goat milk

Goat's milk has stronger flavor and aroma than other kinds of milk or milk products. This characteristic that may be explained by the higher amount of short-chain fatty acid contents (Verruck *et al*., 2019; Amigo and Fontecha, 2011). Among them, are caprylic, capric and caproic acids, as well as 4-methyloctanoic acid and 4-ethyloctanoic acid that have been found to be the most responsible for the "goat milk flavor" (Clark and Mora García, 2017a). The known "goaty flavor" is attributed to the short-branched chain (less than 11 carton atoms) of these fatty acids (Amigo and Fontecha, 2011). However, this specific taste becomes less noticeable and the milk nutritive value increases during goat's milk fermentation (Slačanac *et al*., 2010).

Goat's milk shows a white color, since all of the β-carotene content is converted into retinol, and it is devoid of that pigment. In the CIELAB color coordinates, coordinates a* and b* depend on natural pigment concentration of goat's milk (R Muelas *et al*., 2018). Sant'Anna *et al*. (2013) observed lower a* values in goat's fresh Minas cheese after 14 days of storage, indicating a higher amount of greener tones than that of cheeses made with cow's milk, or a mixture of cow's and goat's milk. Meira *et al*., (2015) also observed low values of a* for goat's ricotta cheese. The b* values (yellow component) were higher in cow's fresh Minas cheese when compared to cheeses made with goat's milk, or a mixture of both.

II.2.4. Texture characteristics of fermented goat milk products

A regular intake of fermented goat products significantly improves the body weight gain, mineralization of skeleton, increased blood serum vitamin D, mineral and hemoglobin levels (Getaneh *et al*., 2016). These authors reported that the weak consistency of the curd formed during the coagulation of goat's milk is beneficial for human digestion. This behavior is influenced by calcium and inorganic phosphorus contents, a lower solvation, lower heat stability, and more readily loss of β-casein than cow's milk (Al-Saadi *et al*., 2014).

The studies of Vargas *et al*., (2008) on yoghurt made with different cow and goat milk mixtures demonstrated that pH and titratable acidity values were significantly influenced by the culture used. Yoghurt containing 75% and 100% goat's milk reached after 1 day of cold storage

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the lowest pH of 4.1, which remained constant during a storage period of 14 days. However, a proportion of 50% or less of goat's milk induces a slower pH decrease to reach a value about 4.0 at the end of storage.

Fermented goat milk which are stored for four weeks present the highest post-acidification rate (1.45 g/100 g) when compared to cow's milk (1.26 g/100g) or a mixture (1:1) of cow and goat milk (1.35 g/100 g). This trend may be attributed to the lower buffering capacity of goat milk (Dimitrellou *et al*., 2019). The post-acidification effect of goat's milk yoghurt could be inhibited by the addition of natural milk calcium, the best inhibition was reached at the level of 5.4 mmol/kg and 7.2 mmol/kg of calcium addition. A possible explanation of this behavior could be that milk calcium increased the content of citrate in the yogurt system, thus enhancing the synergistic effect of citrate and adenosine triphosphate (ATP), which together with calcium, inhibited the key enzyme of glucose metabolizing glycolysis pathway (EMP) of lactic acid bacteria.

Technological characteristics such as firmness, consistency, cohesiveness and viscosity are significantly low in goat milk fermented products compared to cow's milk (Miocinovic *et al*., 2016). Reduced adhesiveness, extension forces and higher susceptibility to syneresis are also observed (Domagała, 2009; Vargas *et al*., 2008). The difference in rheological and textural properties of the acid gels obtained are a result of milk's different composition and properties (da Silva and da Costa, 2019). The low proportion or absence of a_{s1}-casein in goat's milk and its great genetic variability in individual animals , are the most important factor for protein gel structure (Costa *et al*., 2016; Küçükçetin *et al*., 2011). Seasonal changes in the composition of caprine milk may also influence the consistency of fermented caprine milk products (Guo 2003). Miocinovic *et al*., (2016) reported that goat milk yoghurt had significantly lower G' (elastic modulus at 1 Hz), G" (viscous modulus at 1 Hz) and delta values than a fortified goat milk with protein isolate (5%) and cow's milk yoghurts. Because of these textural attributes, the authors concluded that goat milk yoghurt could not be classified as set type yoghurt. Stirred yoghurt prepared with half and half cow's /goat's milk had a higher viscosity than that only containing goat's milk and also a lower number of grains, mean perimeter of grains and roughness than that containing cow's milk (Küçükçetin *et al*., 2011).

To improve textural characteristics of fermented goat's milk product different approaches were adopted, such as enhancing the non-fat solids content, either by milk

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concentration using membrane processes, by addition of stabilizers like pectin or inulin (Karimi *et al*., 2015), by using EPS producing lactic acid bacteria (Badel *et al*., 2011), by complementation with skim milk powder or whey protein concentrates (Herrero and Requena, 2006) or even by milk pretreatment with transglutamase (Farnsworth *et al*., 2006).

II.2.5. Safety of goat milk and its fermented products

Zoonotic disease, when present in goat milk, might be transmitted to consumers even in fermented goat milk products or goat cheeses. This is more evident when goats are a major property in countries where veterinary services are not optimal or even available. The key technological barrier that reduces the risk of spreading zoonoses is the widely adopted heat treatment. However, this is not the case of raw goat fermented products. The most common zoonoses known to be involved with goat milk are brucellosis, Q-fever and toxoplasmosis, though some others are also found (Rahman *et al*., 2020; Acha and Szyfres, 2003).

The most prevalent problem with goat milk-borne bacterial contamination is alimentary toxicosis and the most prevalent cause is the presence of *Staphylococcus aureus* and its enterotoxin in milk and milk products (Ertas *et al*., 2010). Other reported outbreaks of alimentary toxicosis involve major pathogens, such as *Escherichia coli* (Shabana *et al*., 2017), coliforms producing Shiga Toxin (Nichols, 2021; Picozzi *et al*., 2005), *Salmonella enterica* serotype paratyphi B (Bzdil *et al*., 2017; Desenclos *et al*., 1996) and *Streptococcus equi* ssp *zooepidemicus* (Steward *et al*., 2017; Kuusi *et al*., 2006). In addition, there are several reports on the presence of *Listeria monocytogenes* in raw milk (Tahoun *et al*., 2017).

As for cow milk that can suffer from the presence of somatic cells associated to mastitis, goat milk is can be strongly affected by these kinds of infections (Rupp *et al*., 2019; Raynal-Ljutovac *et al*., 2008). According to LIBNOR (Lebanese Standard Institution, 2011) and US FDA (Department of Health and Human Services, Public Health Service, 2015), the basal level of somatic cells in bacteria-free udders of goats (~300,000 cells/mL) is higher than that of sheep (~200,000 cells/mL) and dairy cows (~70,000 cells/mL). This is probably due to the presence of the apocrine secretary system in mammary gland (Raynal-Ljutovac *et al*., 2008). Hence, the level of somatic cells associated to udder infections is usually higher in goats and sheep than in cows (Rupp *et al*., 2019). Many non-infectious factors can also cause considerable variation in somatic cells in goat milk.

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Food poisoning cases from goat milk products have been reported, which highlights the need to monitor the manufacturing process of traditional dairy products in order to avoid microbiological hazards (Lebanese Ministry of Agriculture, 2010; Serhan and Mattar, 2018). Along with enterococci, (Serhan *et al*., 2009) detected non-lactic acid bacteria in Darfiyeh artisanal cheese like *Staphylococcus haemolyticus*, group D *Streptococcus sp*. (*S. bovis, S. suis, S. alactolyticus*, or *S. equinus*), *E. coli*, *Clostridium bifermentans* and *Eubacterium tenue*. In another study, Semaan *et al*. (2011) reported that samples collected from 8 different types of traditional goat milk products were alarming regarding total and fecal coliforms (50 and 58% of collected samples respectively).

II.3. Types of traditional Lebanese goat cheese and goat milk fermented products

In Lebanon, traditional fermented milk products are made from either standardized milk, such as Laban (Chammas *et al*., 2006), Labneh (Serhan and Mattar, 2018; Serhan *et al*., 2016), Shankleesh (Toufeili *et al*., 1995b), Kishk (Salameh *et al*., 2016a) or artisanally made from raw goat milk, such as Darfiyeh (Hosri and El Khoury, 2004a), Aricheh (Serhan and Mattar, 2013), Serdaleh (Tabet *et al*., 2019b), Ambriss (Dimassi *et al*., 2020) and Labneh El Darff (Semaan Hajj *et al*., 2011). Only a small amount of small ruminant milk production is processed into dairy products such as laban or Shankleesh to be sold to consumers or distributors. Producers usually sell the largest part of goat milk to manufacturers as whole milk. This account to 87.6% of goat milk and 92.4% of sheep milk sold (Haddad *et al*., 2007).

The diversity of these artisanal dairy products not only enriches the Lebanese culinary culture and pantry, but also represents a main source of women empowerment in poor rural areas (Massaad, 2017). Women-headed households contribute effectively in the development of goat rearing sector and the manufacturing of goat's milk products, according to the project of Recovery of the Dairy Sector in the Bekâa Valley and Hermel-Akkar Uplands (Kayouli, 2015). The market share of this sector is however not well characterized. About 70% of all small ruminant industries are located in rural areas with limited market access (Saadeh, 2016). The production processes of Ambriss, Serdaleh and Labneh El Darff are documented but not well defined (Tabet *et al*., 2019; Saadeh, 2016).

In addition, these products are real candidates to award the PGI (Protected Geographical Origin) label according to the SRVA (Service Romand de Vulgarisation Agricole) Report and

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the Ministry of Lebanese Economy (IDEAS Centre, 2005). They are also announced to be in danger of disappearing, mainly due to commercial, regulatory and food safety concerns (Semaan Hajj and Dib, 2011; Serhan and Mattar, 2018), Food Heritage Foundation (Shakar, 2021) and the electronic Lebanese examiner journal (Examiner Staff, 2021). The most important Lebanese fermented goat milk products are described below.

II.3.1. Laban

Laban is a fermented milk product produced in Lebanon and some Arab countries, using traditional and industrial manufacturing processes (Surono and Hosono, 2011). It is usually consumed as such, or transformed into Labneh by draining or centrifugation, or cooked in the preparation of many dishes that makes a part of the Lebanese cuisine. Laban is processed with cow, sheep or goat milk, but most of goat Laban is dedicated to the production of traditional Labneh (Serhan and Mattar, 2018).

The manufacturing process of Laban involves a heat treatment of milk at 80 to 85 °C for 30 min or at 90 to 95 °C for 5 to 10 min that is submitted to lactic acid fermentation by the inoculation of a starter mainly composed by *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* (Béal and Chammas, 2012) It leads to casein aggregation and gel formation, thus attaining the norm NL33 (Lebanese Standard Institution, 1999). Lactic acid rate shall not excess 1.5 g per 100 g in the finished product.

Artisanal Laban is made from raw milk that is inoculated with a starter consisting of an unknown number of undefined and indigenous thermophilic strains, thus resulting in a variable product (Béal and Chammas, 2012). This procedure generates diversified and typically flavored products that are generally preferred by consumers (Chammas *et al*., 2006a). This is the case of goat Laban, a home-made product elaborated in artisanal way and difficult to find on the market (Massaad, 2017). There are no exact figures concerning its consumption rate in Lebanon, however according to Nasreddine *et al*., (2006), the consumption of cow Laban, the most consumed dairy product, is estimated at about 25 Kg per year per person.

II.3.2. Labneh

Well known in the Middle East, Labneh is considered as a semisolid concentrated Laban. It is knows as a popular product for breakfast, its consumption is estimated at about 10 Kg per year per person, the second after Laban (Béal and Chammas, 2012). It is produced by

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removing part of the whey from Laban, either by draining (artisanal mode) or by centrifugation or ultrafiltration (industrial modes) **(Figure II.2)**. The final total solids level reaches between 23 and 25 g/100 g of which 8–11 g/100 g is fat (Tamime and Robinson, 2007).

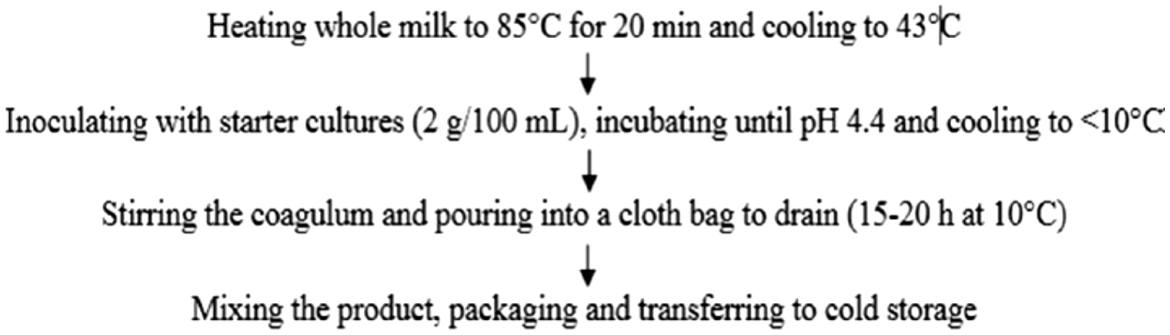

FIGURE II-2. FLOW CHART OF TRADITIONAL LABNEH MANUFACTURING PROCESS (SALAMEH ET AL., 2016B)

In the traditional process, the Laban is not subjected heat treated, therefore, Labneh displays higher counts of lactic acid bacteria and a more-complex flavor as compared to the industrial products (Haddad *et al*., 2007). According to the old practice, Laban is drained in cloth bags for 12–18 hours at refrigerated temperature, until the desired total solid level is attained. *Streptococcus thermophilus* and *Lb. delbrueckii* sp. *bulgaricus* are the starter cultures used in its production (Serhan and Mattar, 2018). Labneh is commonly produced from cow's milk but also from ewe and goat milk, but in a more limited availability (Kaaki *et al*., 2012). It can also be produced from a mixture of goats' milk and cows' milk, thus leading to more complex physicochemical, microbiological, and sensory characteristics.

Due to goat production seasonality, goat Labneh is produced from April to September. It has a short shelf-life about 10 days, thus being consumed fresh (Serhan and Mattar, 2018; Abou Jaoude *et al*., 2010). Labneh could also be dried to be shaped into balls, sometimes covered with herbs or spices to be preserved with olive oil in glass jars for about one year. It is served as a popular mezze dish and sandwich ingredient (Balaa and Marie, 2008).

II.3.3. Shankleesh

Shankleesh is the only mould-ripened product in the Middle East (Serhan and Mattar, 2013) and regarded as a functional food (Hamad, 2017). Regarding its manufacture process, it is obtained by heating defatted Laban, thus leading to a precipitate. The formed coagulant is filtered in dried bad for about 48 hours then is seasoned with salt and powdered with pepper

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before being shaped into balls. The balls are then sun-dried for 3 days, placed into jars and left to ripen for 20 to 30 days at ambient temperature (Dagher, 1991). The mould-covered balls are washed with water, rolled in olive oil and finally covered with thyme (*Thymus vulgaris*). The diversity of microbiota involved during ripening transform the coagulum into a highly flavored product of unique textural properties (Toufeili *et al*., 1995b).

The low levels of fat (5.6%), coupled with the low water activity of the system and the inhibitory effects of thyme confer a good microbial stability to the product thus allowing safe storage for long period of time. Although Shankleesh is typically prepared from ewes' milk, cow and goat Shankleesh products are also produced and marketed by local dairy processors (Serhan and Mattar, 2018; Guizani *et al*., 2006; Haenlein, 2004).

II.3.4. Labneh El Darff

Labneh El Darff is consumed in the South part of Lebanon, where goat milk is abundant, particularly in Rmeich and around. Its preparation is preferably made from goat Laban, in September-October. The skin of a goat (Darff) is used for the preparation and serves as a ripening chamber. All the orifices are well sewed except on the top, and the skin is rubbed regularly with salt. Labneh El Darff is made from goat Laban which is prepared from goat milk "Backsloped" from the previous homemade inoculum. The produced Laban is poured into a brine treated "Darff". Every two days and for a period of one to two months, additional Laban (1- 2 Kg/ day) is filled into the "Darff" to replace the drained whey and about 200g of salt is surface dressed till it the Darff is completely filled and then permanently sealed. During draining and ripening period, the outer skin is daily scrapped to remove any excess exudates **(Figure II.3).** Finally, the product is divided into dumplings and stored in olive oil for one year (Semaan *et al*., 2011).

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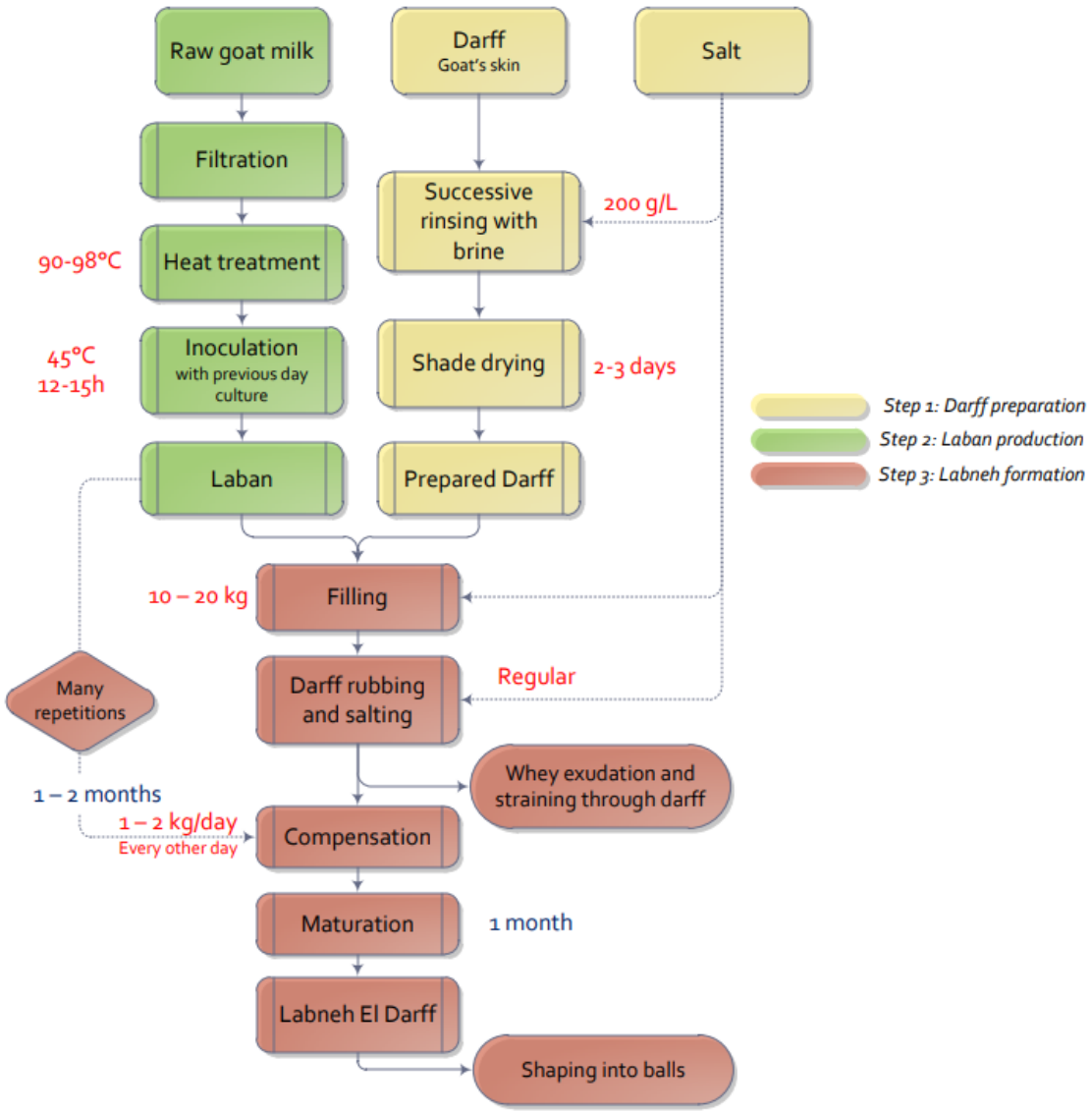

FIGURE II-3. FLOW CHART OF LABNEH EL DARFF MANUFACTURING PROCESS

II.3.5. Ambriss and Serdaleh

Ambriss and Serdaleh are both fermented goat milks obtained by fermenting and draining goat milk into jars. The manufacturing processes are similar, but Serdaleh is produced in Chouf region in Mount Lebanon (Semaan Hajj *et al*., 2011) whereas Ambriss is produced in the Bekaa Valley of Lebanon (Dimassi *et al*., 2020).

The production process is still completely manual and traditional (including tools and materials), with a low production capacity (20 – 60 Kg per producer). The production is seasonal as it depends on goat milk production and availability, i.e. from May/June to November (Tabet *et al*., 2019).

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As shown in **Figure II.4**, Ambriss and Serdaleh are produced in terracotta jars that are first cleaned with "Baladi" soap made from olive oil. Raw goat milk is introduced and mixed with coarse salt, then the jar is covered with a cloth for 15 days and stored at ambient temperature (kitchen or cellar). After 2 weeks, the whey is evacuated from a hole at jar bottom, and fresh and salted goat milk is added on the top of the jar. This procedure is repeated every 8 days, for a period of 4 to 5 months. Ambriss is collected and dried in cloth bags for 24 to 48 hours (Dimassi *et al*., 2020). Serdaleh can be collected after 12- 15 days to be consumed fresh or dried by placing on canvas bags up to 24 hours (Serhan and Mattar, 2013).

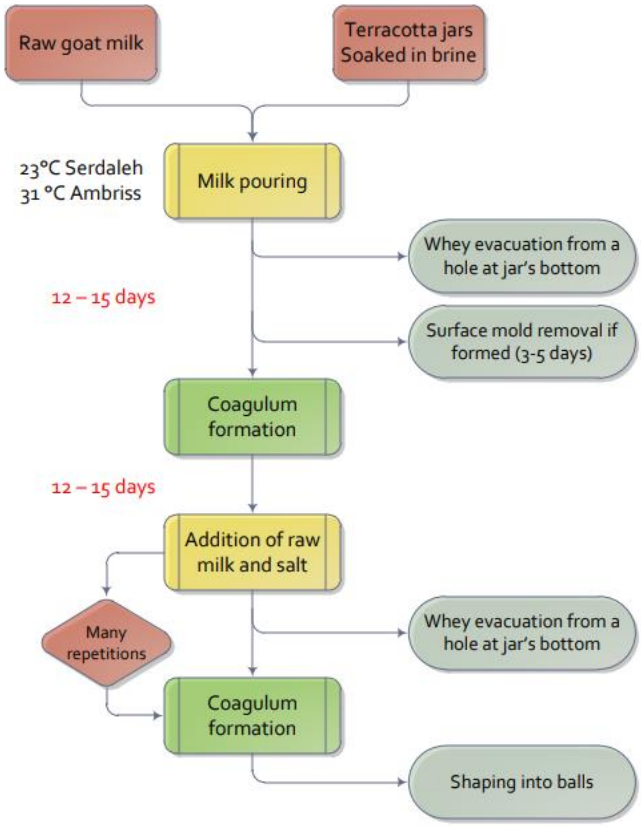

FIGURE II-4.FLOW CHART OF AMBRISS AND SERDALEH MANUFACTURING PROCESSES.

Serdaleh is thought to reach its peak of ripeness and flavor after a fermentation of four months. It has a creamy texture and a slightly sour taste. It is considered as one of the oldest methods of preserving food. The formed coagulum of Serdaleh can be stored as pellets in glass bottles, with oil, or in the form of a compact block with olive oil on the surface (Semaan Hajj *et al*., 2011).

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II.3.6. Darfyieh and Aricheh cheese

Darfyieh cheese is a semi-hard goat cheese, produced in the mountains of North Lebanon, especially in Ehden (Abu Saliba *et al*., 2014). As for Labneh El Darff, its maturation is carried out in goat's skins.

The manufacturing process involved raw goat milk that is first filtered then kept at room temperature for 24 to 48 hours, depending on the season. After adding rennet and coagulating the milk, the resulting coagulum is pressed by had to give the parallelepiped shape (12 cm x 9 cm x 9 cm), salted and then poured into the goat skin to be drained. The collected whey is heated at about 90 ºC for 30 min, then newly raw goat milk is added (1 L) to the whey. The coagulated solid formed at the surface (serum proteins) "Aricheh" is then collected and salted (100 g). Salted Ariche will be dressed on the molded cheese already present in the skin "Darff". In a process of alternating a layer of Darfiyeh with another outer layer of Ariche (Serhan and Mattar, 2018) . The skin is then hermetically sealed, salted from the its surface and placed in a humid natural cellar at 10 to 12ºC for a ripening period of 1 to 6 months, during which periodic salting is carried out (Hosri and El Khoury, 2004).

II.3.7. Kishk

Kishk is a dried fermented milk-cereal mixture. It is prepared from Laban and cereals which are allowed to ferment together, shaped into balls and sun-dried (Tamime and O'Connor, 1995). Kishk is a traditional product widely consumed from eastern Mediterranean countries to Indian sub-continent and very popular in Syria, Palestine, Jordan, Egypt (Gadallah and Hassan, 2019) and Lebanon (Chedid *et al*., 2018). It is known as Tarhana in Turkey (Tangüler, 2014), Trachanas in Cyprus and Greece (Georgala, 2012) and Xinohondros in Crete (Bozoudi *et al*., 2016).

Traditional Lebanese Kishk is typically prepared from Laban (goat, cow or a mixture of them), parboiled Burghol (cracked wheat) at a ratio 1:4 (Burghol:Laban) and salt (Chedid *et al*., 2018). Kishk ingredients are kneaded daily for up to 6 days at 35 °C in order to complete the fermentation and conditioning period. Afterwards, the dough is shaped into balls, placed on trays, and dried in the sun for up to 7 days. The dried mixture is finally milled at granaries. Dried kishk is not hygroscopic and can be stored in an open jar for two years without spoilage (Salameh, 2016).

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The final product can be consumed as a soup after reconstitution with boiling water and additions of sliced meat. One of the crucial steps in the traditional production is sun drying, where a high risk of microbial and physical contamination exists (Sengun and Karapinar, 2012). Some authors reported an unacceptable amount of contaminants in traditional kishk samples such as yeast and its ability to possible transmission of antimicrobial-resistant *Staphylococcus* spp. (Zouhairi *et al*., 2010). This indicates that strict Good Manufacturing Practices have to be implemented (Salameh *et al*., 2016).

II.4.Identification of native microorganisms in traditional dairy products

The natural biodiversity in artisanal products can offer new possibilities when explored and applied in practice (Dal Bello *et al*., 2010). Isolation and characterization of indigenous LAB from fermented products has been considered in the search for new industrially important cultures (Dolci *et al*., 2015; Temmerman *et al*., 2004). In this context, reliable identification of LAB remains a point of crucial importance. Over the past decades, the scientific community has paid special attention to the correct identification of bacteria (Ercolini *et al*., 2001; Hamilton-Miller *et al*., 1999), and a broad range of identification techniques is available, with different discriminatory power, reproducibility and work load.

II.4.1. Taxonomy and classification of lactic acid bacteria

LAB group belongs to the phylum of firmicutes, class Bacilli, and order Lactobacillae (Quinto *et al*., 2014). Six families were described, *Aerococcaceae, Carnobacteriaceae, Enterococcaceae, Lactobacillaceae, Leuconostocaceae,* and *Streptococcaceae* and include more than 20 genera (Dal Bello and Cocolin, 2011).

Various classification schemes were recognized. The first classification of Orla-Jensen (1919) was based on the following criteria: cellular morphology, growth temperature and mode of glucose fermentation. Kandler and Weiss (1986) classified the LAB according to carbohydrate fermentation pathways in: obligate homofermentative, facultative heterofermentative, and obligate heterofermentative.

i) Obligate homofermentative lactobacilli by the metabolism of hexoses via the Emden–Meyerhoff pathway; this group uses the classical pathway of glycolysis to convert one molecule of glucose into two lactate, under optimal growth conditions. The genera belonging to this group

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are Streptococcus, Lactococcus, Pediococcus, and the majority species of Lactobacillus such as *Lb. salivarius, Lb. bulgaricus, L. casei, Lcb. lactis, Lb. acidophilus*.

ii) **Facultative heterofermentative lactobacilli**, producing lactic acid, and under certain conditions acetic acid, formic acid and ethanol. This group includes the following species: *L. casei, Lb. curvatus, Lpb. plantarum,* and *Lb. sakei*.

iii) **Obligate heterofermentative lactobacilli,** producing lactic acid, acetic acid, CO2 and/or ethanol. This group includes the following species: *Lev. brevis*, *Lb. buchneri*, *Lb. fermentum* and *Lb. reuteri.*

Lactobacillus was the largest genus within LAB, which are extremely diverse at phenotypic, ecological and genotypic levels. However, all are Gram-positive, rod-shaped, catalase-negative, facultative anaerobic and non-spore forming bacteria (De Angelis and Gobbetti, 2016).

However, Zheng *et al*., (2020) proposed to reclassify it into 25 genera including the emended genus *Lactobacillus*, which includes the host-adapted organisms that were referred to as the *Lactobacillus delbrueckii* group, *Paralactobacillus* and 23 novel genera for which the names are *Holzapfelia*, *Amylolactobacillus*, *Bombilactobacillus*, *Companilactobacillus*, *Lapidilactobacillus*, *Agrilactobacillus*, *Schleiferilactobacillus*, *Loigolactobacilus*, *Lacticaseibacillus*, *Latilactobacillus*, *Dellaglioa*, *Liquorilactobacillus*, *Ligilactobacillus*, *Lactiplantibacillus*, *Furfurilactobacillus*, *Paucilactobacillus*, *Limosilactobacillus*, *Fructilactobacillus*, *Acetilactobacillus*, *Apilactobacillus*, *Levilactobacillus*, *Secundilactobacillus* and *Lentilactobacillus*.

Methods to investigate the microbiota from a given ecosystem can be either culture-dependent or culture-independent. Culture-dependent methods as those which consist of isolating and culturing strains prior to their identification according to either phenotypic or genotypic characteristics. In fact, knowledge of the microbial diversity of dairy products and the reservoirs involved was at first derived from culture-dependent methods using different culture media (Montel *et al*., 2014). Methods based on the analysis of direct DNA extractions from samples of fermented food products is presented as culture- independent methods. They are nowadays frequently used s to profile both culturable and non-culturable microbial populations (Ferrocino and Cocolin, 2017; Dolci *et al*., 2015; Alegría *et al*., 2012). However,

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the diversity of the methods applied sometimes makes it difficult to quantitatively compare microbiota from one study to another.

Both types of methods have advantages and drawbacks and were suggested to be considered as complementary when investigating on microbial ecology of dairy ecosystems (Yeluri Jonnala *et al*., 2018; Tamang *et al*., 2016).

II.4.2. Culture-dependent approaches

II.4.2.1. Culture enumeration methods

The classical plate count approach is based on bacterial culture enumeration on a medium. The standard media for Lactobacillus, which is MRS was first developed by De Man *et al*., (1960), as a selective cultivation of all *Lb*. spp. Since Streptococci cannot grow well in MRS, M17 was developed for Streptococcus. spp. (Terzaghi and Sandine, 1975) and also for the enumeration of Leuconostoc. spp. with or without lactose supplementation (Karimi *et al.*, 2012). These media are now widely used for most genera of LAB including also *Pediococcus, Enterococcus, Weissella, Aerococcus, and Oenococcus* (Stiles and Holzapfel, 1997). The drawback of these standard media is that they are largely undefined and not optimum for high cell density growth. Consequently, a Chemically Defined Media (CDM) targeted towards the elimination of unrequired nutriment has gain success for metabolism research experiment for *Lactiplantibacillus plantarum* (Wegkamp *et al*., 2010), for *Lactococcus lactis* (Cocaign-Bousquet *et al*., 1995), *Enterococcus faecalis*, *Streptococcus thermophilus* (Zhang *et al*., 2009) and *Lactobacillus delbrueckii subsp lactis* (Hébert *et al*., 2004).

LAB have fastidious nutritional requirements that differ among species. Bacterial counts are known to be biased because bacteria can only be cultivated if these metabolic and physiological requirements can be reproduced in vitro (Nadkarni *et al*., 2009). Since such standard media cannot provide optimum growth conditions, a large number of media types was proposed for cultivation. For example ST agar and LAE (Lactic Agar Elliker) for Streptococcus theromophilus or LBS agar for Lactobacilli (Hayek *et al*., 2019). LAB have high demand for Nitrogen source such as peptone, tryptone, beef and yeast extract which make form these media more expensive than others. In this regards commercial MRS broth such as Difco, from bovine and porcine protein, was developed as an alternative nitrogen source in dehydrated culture media to accommodate various nutritional requirements of LAB. Other non-animal protein

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sources such as phytone peptone have also been shown to support the growth of LAB (Atilola *et al*., 2015).

LAB characteristics like the ability to produce acid and antimicrobial compounds have added additional limitations and challenges to the type of media used (Hayek and Ibrahim, 2013). These limitations include the availability of certain essential molecules, for cell metabolism, the production of organic acids that cause a drop in media pH resulting in antimicrobial effects, lack of nutrients during exponential growth. Lack of essential minerals is also recorded such as Fe2+ and Ca2+ that are required by some LAB strains (Burns *et al*., 2008; Djeghri-Hocine *et al*., 2007).

Apart from selectively allowing growth of some species and suppressing growth of others, the community composition of the cultural fraction is distorted during culturing because replication times vary, with fast-growing species efficiently outcompeting others (Nocker *et al*., 2007).

Research studies related to LAB cultivation media have also been done using Lactococcus. lactis ssp. lactis IL1403 or ssp. cremoris MG1363 that were sometimes endowed with plasmid(s) that enabled growth in a milk-based medium (Steele *et al*., 2013). The use of a milk-based medium or food grade medium in such research could help to bridge the gap between laboratory research and the dairy industry. As a result, it might be appropriate to develop a medium based on milk or dairy byproducts for dairy research applications.

Above all, culturing methods can only measure viable culturable or replicating cells and discard non-culturable microorganisms (Tamang *et al*., 2016). In fact, during milk fermentation or cheese processing, a part of LAB population may enter into a Viable But Non-Culturable (VBNC) state, in which cells are dormant but metabolically active (Davis, 2014). It has become evident that, culture-independent methods for microbiological analysis of dairy products such as flow cytometry (Geng *et al*., 2014;Díaz *et al*., 2010) and other genomic methods like polymerase chain reaction (Boyer and Combrisson, 2013) will overcome all these barriers. These methods offer the potential to enumerate both culturable and VBNC bacteria.

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II.4.2.2. Characterization of culturable species

A rapid and accurate identification of bacterial isolates is crucial in subsequent characterization (Woo *et al*., 2008). In general, phenotypic methods are cheaper compared to genotypic methods. In this regard, once LAB are isolated, they are identified based on the strain carbohydrate profile using commercially available and non-sophisticated miniaturized systems such as API (BioMérieux) or BIOLOG (Temmerman *et al*., 2004). Although the application of such phenotypic techniques has proven to be useful for only certain LAB, however there is a general awareness that similar phenotypes displayed by strains do not always correspond to similar or even closely related genotypes (Moraes *et al*., 2013). Moreover, Sakaridis *et al*., (2014) considered that all LAB isolates, initially identified by API and not further sequenced as unknown samples since API were found to be relatively unreliable. For these reason, it has become necessary to refer to genetic tools to identify isolated probable LAB strains.

The 16S rRNA (~1500 base pair gene) sequencing of the isolated strain, have been used as standard phylogenic universal technology that, theoretically yielding unambiguous data, even for unusual and slow-growing isolates, often within 48 h, which are reproducible among laboratories (Randazzo *et al*., 2009). The information on rRNA sequences has proved to be effective for comparative identification of microorganisms, leading to recognition of thousands of microbial species, including LAB species. However, DNA sequencing is generally expensive and requires a high degree of technical competence to perform (Ndoye *et al*., 2011). The phenotypic typing of LAB provide evidence of the metabolic capabilities of strains, whereas the advantages of genotyping include the stability of genomic DNA as its composition is independent of culture conditions or preparation methods (Beresford *et al*., 2001).

The 16S rRNA gene sequences are used in two ways in microbial systematics: (i) the gene sequence similarity between two strains which provides a simple yet very robust criterion for the identification of newly isolated strains and (ii) the phylogenetic analyses to elucidate the overall evolutionary relationships between related taxa. The first is a critical checkpoint for species-level identification, while the second is dedicated for genus or suprageneric classification (Kim and Chun, 2014).

In some cases, the 16S rRNA sequence poorly discriminate closely related species, although it is rarely the cae for LAB. Nevertheless, LAB differentiation by protein-coding genes has been previously explored to determine and identify the most appropriate genes for bacterial

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taxonomy and phylogeny (Coppola *et al*., 2008). In fact to distinguish close related species and when compared to 16S rRNA methods from PCR amplification, the use of markers provides more details and detect minor groups among microbial assemblages (Roux *et al*., 2011).

The use of single-copy target genes that shows sequence differences was sufficient enough for improving phylogenetic resolution within species (Coppola *et al*., 2008). These target genes have proven to be more discriminative than the 16S rRNA, from these like rpoB (Martín-Platero et al., 2009b) and pheS (phenylalanyl-tRNA synthase) for *Lacticaseibacillus paracasei* (Van Hoorde *et al*., 2010). Others like the elongation facture Tu gene (tuf) for Propionibacterium (Falentin *et al*., 2010) and Enterococcae (Ventura *et al*., 2003), the DNA repair recombinase gene (recA) (Felis and Dellaglio, 2007), the RNA polymerase B subunit gene (rpoB) (Rantsiou *et al*., 2004) and the chaperonin HSP60 gene (cpn60), have been well exploited for the differentiation of LAB species (Ventura *et al*., 2003). Sequence determination is now possible with available programs like BLAST or FASTA based on homology searches are commonly available on the net to determine the closest relative of the DNA sequence.

Culture-based microbiology techniques were used to gain an understanding of the microbial ecology of dairy products. However, it has become increasingly clear that this approach fails to reproduce the ecological niches in complex natural environments. This limitation is explained by the ability to detect "difficult-to-culture" or sub-dominant microorganisms, thereby drastically underestimate both microbial number and composition in the samples under study (Cao *et al*., 2017; Carraro *et al*., 2011).

As a result, culture-independent approaches have become increasingly popular. These approaches involve DNA, and occasionally RNA, based on molecular methods, with High Throughput Sequencing (HTS) gaining particular attention (Yeluri Jonnala *et al*., 2018).

II.4.3. Culture-independent approaches

Molecular methods have several advantages, above all, that of allowing workers to distinguish between different species with similar phenotypic characteristics and also between strains belonging to the same species (Martín-Platero et al., 2009b). DNA-based molecular methods use as a template a pool of DNA deriving both from live, VBNC and dead bacterial cells. This intrinsic characteristic was alternatively presented as an advantage (detection of microbiological contamination in raw materials) and as a disadvantage (overestimation of the risk of microbiological contamination) of the methods. The application of High throughput

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Sequencing to characterize different cheese microbiota could provide valuable information relating to the influence of geography, manufacturing processes, climatic conditions, seasonal variation, use of raw or pasteurized milk, and a variety of other factors on these ecosystems (Yeluri Jonnala *et al*., 2018). It involves mainly 2 approaches (1) Amplicon sequencing, whereby a fragment of highly conserved gene, ideally with variable regions therein, is used for sequencing, with comparison to databases allowing taxonomic assignment. The 16S rRNA gene is most frequently used to provide an insight into the bacterial composition of samples. (2) Shotgun metagenomics sequencing, which involves non-targeted sequencing of the DNA in a sample, on the basis of comparison with databases, and can be used to classify not only the microorganisms but also information regarding the functional potential of the whole community.

II.4.3.1. Metagenetic analyses

According to Esposito and Kirschberg, (2014) metagenetic is considered to be a specific type of metagenomic studies involving PCR methods that target just one gene. Targeted metagenomics utilizes gene fragment DNA sequencing to determine identities of microbiota such as bacteria, yeast and mold. In most cases, a conserved segment of a hypervariable region (V1-V3 or V3-V4) of the 16S rDNA gene is used (Salazar *et al*., 2018). And more recently full-length 16S intragenomic copy variants, with appropriate treatment, has the potential to provide taxonomic resolution of bacterial communities at strain level (Johnson *et al*., 2019).

Identifying the prokaryotic operational taxonomic units (OTUs) based on the DNA sequence of the 16S rRNA gene (16S) has long been a method for taxonomically profiling microbial communities (Beiko and Hsiao, 2018). It is a promising tool to identifying key microorganisms in the curd and cheese samples without the limitations imposed by culture-dependent methods (Rasolofo *et al*., 2010). PCR-amplified sequences can be directly cloned and sequenced for species identification, composition, or structure of the community members (Delbès *et al*., 2007). During the studies of microbial population of Montasio cheese cheesemaking and ripening, clone libraries were constructed from 16S rRNA genes amplified from DNA extracted and cDNA retrotranscribed from RNA extracted from raw milk, curd, at five days and cheese at 30 and 60 days. The comparison of results obtained from cDNA (Reverse transcribed RNA) and DNA samples led to distinguishing viable from non-viable cells. (Carraro *et al*., 2011). Lastly, the importance of adopting the Quantitative PCR (qPCR)

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analysis targeted on corresponding bacterial genera was pointed out in order to validate the metagenetic approach (Cauchie *et al*., 2017).

An upmost interest of molecular-based taxonomy methods, such as metagenetics, is that data can be compared in different laboratory, while results of culture dependent methods are more experience-environment dependent. Thanks to the extensive amount of 16S rRNA gene data generated by amplicon-based food microbiota descriptions, there currently the possibility of data sharing. Sequence data are available through public databases, e.g. the Sequence Read Archive of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/ Traces/sra) or the European Nucleotide Archive of the European Bioinformatics Institute (http://www.ebi.ac.uk/ ena). In this way, researchers an easily access datasets, re-analyse and use them for meta-studies (De Filippis *et al*., 2017).

The metagenetic methodology has a few disadvantages, such as biases associated with PCR, overestimation of community diversity or species abundance, and inability to describing biological functions Escobarr –Zepeda (2015).

II.4.3.2. Metagenomic shotgun analyses

Shotgun sequences can be processed in different ways. There are broadly two main principles, one based on the assembly of the resulting sequences, and the other based on the use of references. In the assembly-based method, the sequences are first assembled and then annotated, which then allows them to be classified to deduce to which species they belong. In the reference-based method, the sequences obtained are compared with those in a reference database, which allows them to be classified directly. The MetaPhlAn3 (Beghini *et al*., 2021) is an extensively used software for metagenome profiling. It aligns reads to a marker gene database and normalizes the counts for each gene for a given sample. From such genome-scale information, the abundance of each MAG (Metagenome Assembled Genome) is estimated. In contrast, PanPhlAn3 software maps the obtained reads against the pangenome of a species and offers gene presence or absence information.

The advantage of the assembly-based method is that it allows the characterization of species for which no reference currently exists. On the other hand, it requires more computing time, and only the majority micro-organisms with sufficient DNA can be assembled and thus can be characterized. For reference-based methods, there are various software packages

available, some of which are very fast, but the assignments are sometimes less precise. And above all, important species may not be characterized if there is no reference in the database.

High-throughput metagenomic sequencing technology has transformed food microbial study in all its approaches **(Figure II.5).** In recent years, metagenomics has flourished enough to discover microbiomes insight of many traditional products such as kefir (Kazou *et al*., 2021; Korsak *et al*., 2015), milk fermented products (de Melo Pereira *et al*., 2020) and cheeses (Kothe *et al*., 2022; Kothe *et al*., 2021; Escobar-Zepeda *et al*., 2016) and contributed in the creation of a dairy reference genome catalog (de Almeida Júnior *et al*., 2015).

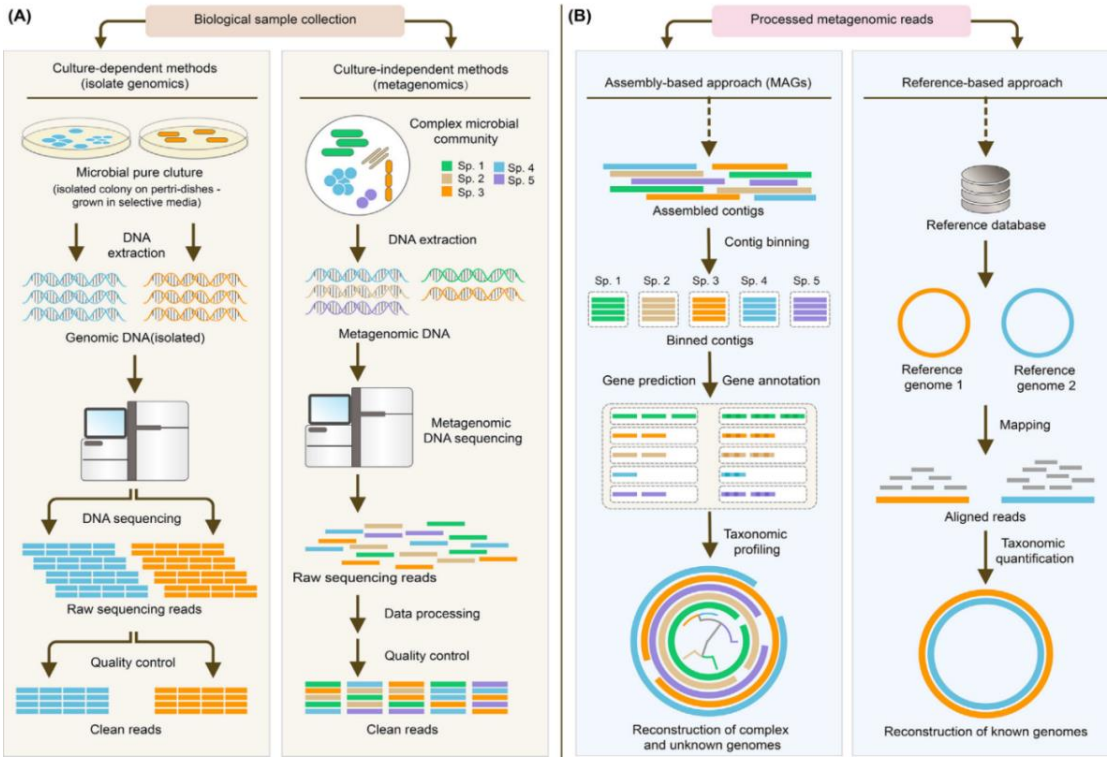

FIGURE II-5. SCHEMATIC REPRESENTATION OF THE DIFFERENT APPROACHES USED IN THE METAGENOMIC RESEARCH FIELD.

(A) A schematic contrast between culture-independent (metagenomics) methods, and culture-dependent methods. The process for generating sequencing data for the two strategies have been illustrated. (B) A schematic contrast between assembly-based and reference-based approaches on metagenomic sequencing data (from Yang *et al*., 2021).

II.4.3.3. Amplicon-based Metagenomic and shotgun analyses

The metagenetic methodology has a few disadvantages, such as biases associated with PCR, overestimation of community diversity or species abundance and inability to describing biological functions (de Melo Pereira *et al*., 2020). As an alternative, shotgun metagenomic

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sequencing can be used to fulfill lacks and provide a better understanding of the microbiome, especially taxonomic analysis (who is there?), functional analysis (what are they doing?), and comparative analysis (how to compare them?) (Escobar-Zepeda *et al*., 2015). It offers several advantages over amplicon-based approach **(Table II.7)**.

Although PCR-dependent, the amplicon-based HTS is considered quantitative: the number of reads obtained for each operational taxonomic unit (OTU) is proportional to the abundance of that OTU in the sample and the higher sensitivity allows identifying also sub-populations previously difficult to detect. Data which are generated need to be interpreted with the awareness of culture- independent PCR biases that have been reviewed elsewhere (Sipos *et al*., 2010), such as the possibility of preferential amplification, due to the different efficiency of the primer towards selected species, that may result in the under-representation of some clades (De Filippis *et al*., 2017; Pinto and Raskin, 2012).

Despite of recent advances in amplicon sequencing, whole Genome shotgun sequencing (WGS) is the preferred tool to explore microbial ecology. In this approach shotguns sequencing are applied to millions of random genomic fragments sampled from a microbial community.

The construction of shotgun libraries has the potential to discriminate strains of common species by gene content and the detection of novel microorganisms like *Pseudoalteromonas haloplanktis* and *Psychrobacter immobilis* in cheese (Almeida *et al*., 2014). Besides, it offers the possibility to provide information on genome assembly, species level taxonomy abundance, gene predication, metabolic pathway reconstruction and functional pathways that define the microbiome under study (Moreno-Indias and Tinahones, 2020). However, each stage of the analysis is complicated by incomplete coverage and is limited the processing of huge amounts of data, complex outputs with difficult gene tracking, length of sequencing fragments, depth of sequencing effort, and bias on DNA extraction and amplification technics used (Kergourlay *et al*., 2015).

TABLE II-7. SUMMARY OF ADVANTAGES AND DISADVANTAGES OF THE DIFFERENT HTS APPROACHES (Forbes et al., 2017).

Targeted-amplicon sequencing		Shotgun metagenomics sequencing	
Advantages	**Disadvantages**	**Advantages**	**Disadvantages**
Target is specific to a particular microbial group (e.g., 16S rRNA common for bacteria, archaea, 18S rRNA for eukaryotes, ITS for fungi, RdRP for RNA viruses).	Requires a priori knowledge for microbial group target.	Can sequence all DNA in a given sample (e.g., bacteria, archaea, eukaryotes, parasites, and viruses).	Virome assays require complex sample and nucleic acid work-ups.
Can use relative abundance changes to compare microbiomes across different samples or treatments.	Universal target may be present in varying copy numbers across different taxa (e.g., 16S rRNA). PCR amplification bias, primer bias and errors.	Universal markers can be inferred from metagenomics datasets.	High abundance of host DNA can make it challenging to sequence low abundance microbial DNA.
Can capture abundance of rare taxa provided that sequencing depth is sufficient.	Absolute abundance difficult to impute.		Low abundance taxa difficult to identify. Difficult to accurately bin each sequence to a genome.
Relatively easy to taxonomically classify sequences using a variety of validated tools and curated databases.	Databases can be self-limiting and have the potential to exclude novel microbes.	Plethora of software using phylogenetically informative gene markers.	High proportion of taxonomically uninformative sequences are discarded
	Universal targets within microbial groups can give variable taxonomic classifications.		Availability and access to comprehensive and curated databases across all microbial groups limited.
	Taxonomic resolution variable –species level identification should be interpreted with caution.		
Low cost		Can be carried out on most bench-top sequencers and sequencing platforms.	Can be cost prohibitive depending on the sequencing depth, sample type, and microbe(s) of interest.
Can be carried out on most bench-top sequencers and sequencing platforms.			If high host DNA is expected or interest is in the low-abundance microbes or rare taxa, use of a higher throughput sequencer (Illumina HiSeq), may be required.
Most analysis steps can be carried out on a modern desktop.	Large datasets (high sample number and/or sequencing coverage) may require access to a high performance computing cluster dependent on analytical pipeline chosen.	Cloud computing services are available for metagenomics data analysis for those without access to a high performance computing cluster.	High performance computing environment absolutely necessary.
			Cloud computing – potentially cost-prohibitive and might not have all available pipelines and/or software.
			Data privacy and sensitivity may prohibit the use of commercial cloud computing services.
Moderate to high technical expertise is required depending on the analytical pipeline chosen.			High technical expertise required

II.4.4. Metagenome Assembled Genomes (MAGs)

A MAG refers to a microbial genome, a group of scaffolds with similar characteristics binned from a metagenome assembly. It enables to identify novel species and understand their

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potential functions in dynamic and complex microbial communities. In this context, sequencing reads are assembled into scaffolds which are grouped into candidate MAGs based on Tetra Nucleotide Frequencies (TNFs), abundances, complimentary marker genes, taxonomic alignments and codon usage. The MAGs with high completeness and low levels of contamination are then used for further taxonomic annotation and gene prediction (Yang *et al*., 2021).

When annotating MAG's, it is important to determine their taxonomic classification. In prokaryotic communities, traditional methods based on 16S rRNA small subunit genes have been successfully established to understand the diversity of isolated strains, but it cannot be applied to MAGs or only with limited resolution and poor representation due to the difficulty to assemble it in metagenomes (Yang *et al*., 2021). In contrast, methods using single-copy marker genes have gained popularity due to their improved resolution, for example GTDB-Tk uses HMMER to identify marker genes in the reference genomes from the Genome Taxonomy Database (GTDB). These marker genes are concatenated for MSA and then used to construct a reference tree using a likelihood-based phylogenetic inference algorithm GTDB-Tk performs taxonomic classification for a queried MAG.

Once MAGs are banned from the metagenome assembly, the second step is gene identification and annotation. In this regards Model-based prediction tools are used, the most common are derived from Hidden Markov Model (HMM) with also a series of tools under this category such as MetaGeneMark and FragGeneScan to optimize the probabilistic inference.

More recently, MAGs has been intensively used in a large-scale genome-wide analysis (publicly available:15740 and newly sequenced metagenomes: 666) to prove that *Streptococcus thermophilus* and *Lactococcus lactis* being most prevalent LAB in human stool samples and identify links between gut and food microbiomes (Pasolli *et al*., 2020). The authors demonstrated also that closely related LAB strains occur in both food and gut environments and provided evidence that fermented foods can be regarded as a source of LAB for the gut microbiome.

In a holistic approach, both classical microbiological and molecular methods have been used to achieve an appropriate identification and quantification of different bacterial species present in a complex matrix and specifically LAB in dairy ecosystem.

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II.5.Current microbial ecology of different goat milk products

II.5.1. General overview

Microbial composition in traditional fermented milk products and cheeses is well documented (Macori and Cotter, 2018; Vahabzadeh *et al*., 2017). The diverse microbial ecology pertains to complex sensory, technological, and nutritional characteristics and stems from different sources (O'Sullivan and Cotter, 2017). These sources include the environment and location, traditional practices, type of animal feed, type of milk and stage of lactation, and eventual/final milk inoculation/inoculum, and collection vessels, which might contaminate the product at any point of its manufacture (McInnis *et al*., 2015; Quigley *et al*., 2012; Beresford *et al*., 2001;Béal *et al*., 1999) Microbiota distribution is also affected by sampling location within the product (i.e., core or rind of cheese) (Irlinger *et al*., 2015; Montel *et al*., 2014; Wolfe *et al*., 2014).

Bacterial identification in a milk product ecosystem is important for understanding their individual and combined contribution during manufacturing (Ogier *et al*., 2002). In fact, acidification and fermentation of starter free raw milk fermented products rely on the activity of the indigenous Lactic Acid Bacteria (also called Non Starter Lactic Acid Bacteria or NSLAB) (Delavenne *et al*., 2012; Wouters *et al*., 2002). NSLAB confer a high diversity and typicity to traditional products by contributing to their typical properties and flavors (Malek *et al*., 2012; Chammas *et al*., 2006b). However, due to commercialization, the uniqueness of these products is being with biodiversity depletion (Morandi *et al*., 2019; Wouters *et al*., 2002). With the limed number of commercial strains, and the subsequent homogeneous production, studies have reported the tendency to scarcity and depletion of the beneficial native microflora of milk in the recent decades (Marino *et al*., 2003; Michel *et al*., 2001). Thus, investigating the microbiota will enable regulating the microbial activities that meet consumer's demands while maintaining and improving product quality and safety.

In the Mediterranean region, traditional dairy fermented milk products are closely related to the territory of production and the cultural history and heritage (Boyazoglu and Morand-Fehr, 2001). This fact draws the attention of many studies to their unique microbial diversity (Rhaiem *et al*., 2016; Abdalla and Hussain, 2010; Ayad *et al*., 2004; El Soda *et al*., 2003). Numerous genera such as *Lactobacillus, Lactococcus, Enterococcus, Leuconostoc* and *Streptococcus* are generally involved in these transformations.

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In the course of studying microbial community of raw goat milk, 725 LAB isolated from raw goat milk of four local races in Algeria were characterized (Badis *et al*., 2004). These were phenotypically classified as *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Streptococcus*, and *Pediococcus*. The phenotypic and biochemical analyses revealed a diversity of species with the most abundant being *Streptococcus thermophilus*, *Lactobacillus helveticus*, *Lactobacillus plantarum*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Levilactobacillus brevis*, and *Lactococcus lactis* subsp. *lactis*. Another study identified 11 strains out of 233 isolates exhibiting antimicrobial activity with 5 among them serving as potential probiotics (Benreguieg *et al*., 2013). The latter are *Lactococcus lactis* subsp. *cremoris*, *Lactobacillus casei* subsp. *casei*, *Lactobacillus casei* subsp. *rhamnosus*, *Streptococcus salivarius* subsp. *thermophilus* and *Lactococcus lactis* biovar *diacetylactis*.

Kefir constitutes a unique symbiotic association, comprising mainly LAB, yeasts, and occasionally acetic acid bacteria (Wszolek *et al*., 2006). Classical microbiological analysis on kefir grains and beverages from different geographical regions in Greece revealed varying populations of LAB and yeasts in both homemade and industrial samples. The richness estimation, according to observed species, indicated that bacterial communities did not differ significantly among the samples although also being from different types of milk (cow, goat, and buffalo) (Kazou *et al*., 2021). A combined classical microbiological and amplicon based metagenomics analyses revealed that *Listeria monocytogenes*, *Salmonella* spp. and *Staphylococcus* spp. were absent from all the samples analyzed, whereas *Enterobacteriaceae* were detected in one of them. From 91 isolates, *Lentilactobacillus kefiri*, *Leuconostoc mesenteroides*, and *Lactococcus lactis* were the mostly identified bacterial species in the home-made samples. On the contrary, *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, and *Lacticaseibacillus rhamnosus* were the main bacterial species isolated from the industrial beverages. These results were concomitant with those of amplicon-based metagenomics analysis in terms of genera abundance.

A microbiological survey performed on 28 fresh raw goat's milk cheeses from Northern Morocco determined that LAB were the dominant microbiota followed by yeasts and total coliforms which were also in considerable numbers. *Salmonella* spp. was not detected in any of the cheese samples while *Listeria monocytogenes* was detected in four cheeses. A total of 294 LAB isolates were identified by PCR as *Enterococcus* spp. mainly, then *Lactococcus lactis*, and only some *Lactiplantibacillus plantarum* and few *Lacticaseibacillus paracasei* (El Galiou

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et al., 2015). *Lacticaseibacillus paracasei* and *Lacticaseibacillus rhamnosus* are common in several cheeses types from the Mediterranean region. The cheeses are Ibores, Tenerife, and Babia from Spain, Batzos and Xinotyri from Greece, and Sepet and Tulumn from Turkey (Meng *et al*., 2018). In Iran, traditional Liqvan cheese, which is made from raw goat and ewe milk, reveals dominance of *L. paracasei* and *Lpb. plantarum* followed by *Leuconostoc* and *Lev. brevis* (Barouei *et al*., 2008; Abdi *et al*., 2006).

Lactococcus lactis subsp. *lactis* and *Enterococci durans* were identified as the main cocci species in traditional Greek cheeses Anevato (Hatzikamari *et al*., 1999) and Batzos (Psoni *et al*., 2003) both made from raw goat's milk. The studies of (Nieto-Arribas *et al*., 2009, 2010; 2011). on Spanish Manchego cheese showed an important diversity in *Enterococcus, Leuconostoc* and *Lactococcus* population. In Italy, the LAB community was studied in traditional goat cheese, produced from raw milk and without starter bacteria, at different steps of the manufacturing process. As 332 isolates were characterized, *Lactococci* were the dominant lactic acid bacteria in raw milk (Colombo *et al*., 2010). The growth trend of enterococci was comparable to that of lactococci although their counts were lower. During goat cheese manufacturing, the most frequently isolated species were *Lactococcus lactis* subsp. *cremoris*, *Lactococcus garviae*, and *Enterococcus faecalis*, whereas from ripened cheese samples, *Enterococcus* (*E. faecium, E. durans, E. gilvus,* and *E. casseliflavus*) were isolated with the greatest frequency. Occasionally, *Leuconostoc mesenteroides*, *Leuconostoc lactis*, and *Lactobacillus paraplantarum* also were isolated.

II.5.2. Traditional Lebanese goat milk products

Only a limited number of studies have clarified the native microbiota in the Lebanese fermented milk products. The first attempts were recorded on cow milk Laban where 96 strains of *S. thermophiles* and *Lb. bulgaricus* were isolated and characterized using phenotypic and biochemical analysis (Chammas, *et al*., 2006a.). Studying the Lebanese Baladi goat milk, the LAB counts were rather variable and less than those reported in other raw goat milk (Saliba *et al*., 2021). The *lactobacillus* strains isolated were all identified as *Lactobacillus rhamnosus* thus presenting a poor LAB biodiversity. They exhibited diverse susceptibility to commonly used antibiotics and showed different antimicrobial activity. From North Lebanon, the Darfiyeh cheese was shown to have *Streptococcus thermophilus, Enterococcus faecium, Enterococcus durans, Enterococcus faecalis, Enterococcus malodoratus,* group D *Streptococcus* sp.,

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Lactococcus lactis subsp. *Lactis, L. lactis* subsp. *cremoris, Lactobacillus plantarum, Lactobacillus curvatus, Staphylococcus haemolyticus, Escherichia coli, Clostridium* sp. *Eubacterium tenue*. (Serhan *et al*., 2009a) *Leuconostoc mesenteroides* spp. *dextranicum* 2 (Dib *et al*., 2012) and *Lacticaseibacillus paracasei* (Meng *et al*., 2018). However, Dib *et al*. (2012) did not succeed to isolate LAB from Ambriss and Labneh El Darff.

Microbiological analyses on Serdaleh from the mountainous areas of Lebanon, showed that samples were free from *Salmonella* spp. and *Staphylococcus aureus* but not from fecal coliforms in samples ripened in terracotta jars (Tabet et al., 2019). Cheese samples ripened in jars exhibited higher numbers of thermophilic LAB than those ripened in goat-skin bags after 20 days of ripening. The high counts were observed at the beginning of ripening before decreasing significantly after 1 month. This trend was similar during the ripening of many Mediterranean cheeses such as Feta (Manolopoulou *et al*., 2003), Tulum (Hayaloglu *et al*., 2007) and Halloumi (Milci *et al*., 2005).

When the four artisanal cheeses Darfiyeh, Aricheh, Shankleesh and Serdaleh were analyzed, thermophilic cocci growing on M- 17 medium were markedly present at higher levels than mesophilic and thermophilic lactobacilli growing on De Man Rogosa and Sharpe medium (Serhan and Mattar, 2013). The results also showed that most of the samples fall within the accepted national microbiological standards for total aerobic bacteria, *Listeria monocytogenes, Shigella*, and *Salmonella* whereas *Escherichia coli*, *Campylobacter* and *Staphylococcus aureus* were contaminating them with different percentages. Kishk was highly contaminated with yeast where 16 isolates were identified. *Candida krusei* was the most frequent followed by *Candida lusitania*, *Candida famata*, *Saccharomyces cerevisiae* and other unidentified species. Conversely, no or minimal LAB growth was recorded after storage while earlier during the product's fermentation, the LAB species identified by API were *Lactobacillus plantarum*, *Lactobacillus pentosus*, and *Streptococcus thermophilus* (Salameh *et al*., 2016; Tamime, 2006)

Goat milk may exhibit a larger number of regionally defining taxa compared to regional cow milk (Bokulich *et al*., 2015). Considering the close traditional practices within the Mediterranean countries and the resulting microbial diversity of LAB, it is interesting to plot the microbial biogeography of traditional fermented milk in these regions. In addition to the milk type, another segregating and sometimes more important factor is the starter culture type.

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With LAB being well characterized, their important technological properties are investigated in order to improve dairy products.

II.6. Technological characteristics of lactic acid bacteria isolated from milk fermented products

Lactic acid bacteria are extensively used as starter cultures, probiotics or in the production of interesting compounds (i.e. nutraceuticals), due to their versatile metabolism (Ruiz Rodríguez *et al*., 2019; De Souza and Silva Dias, 2017). Technological characteristics of LAB, such as sugar and citrate catabolism, proteolytic and lipolytic activities are important traits for acidification, and adding texture and aroma in fermented foods (Viana de Souza and Silva Dias, 2017; Domingos-Lopes *et al*., 2017). Thus the selection of LAB is commonly based on technological and sensory parameters (Hébert *et al*., 2000).

II.6.1. Acidification activity

The acidifying activity of LAB is a key driver of the fermentation process and the subsequent technological characteristics of the final product. Lactose, the main carbohydrate source in milk, is fermented to organic acids, essentially lactic acid, leading to acidification of milk (Hati *et al*., 2013). Rapid milk acidification is one of the most important properties of LAB that are used as starter cultures, but it is not desirable in the adjunct cultures, since it could negatively affect the quality of the final products (Kask *et al*., 2003). Therefore, strains with poor acidifying activity but with interesting technological or functional properties, can be used as adjunct cultures and combined with more acidifying strains (Araújo-Rodrigues *et al*., 2021).

The kinetic parameters of native microorganisms are important for biotechnological scaling. According to their acidification rate, acidification intensity, and total acidity level, LAB can be classified as slow (pH decrease < 0.34 after 24 h and acidification rate $\leq 40^0$D within 18h), medium (pH decrease ranging between 0.35 and 2.12 after 24 h and acidification rate between 40 and 60^0D within 18h) and fast acidifiers (pH decrease > 2.26 after 24 h and acidification rate $\geq 60^0$D within 18h) (Giraffa *et al*., 2004; Xanthopoulos *et al*., 2001).

The acidifying activity of 71 *Lactobacillus* strains isolated from Algerian goat milk was determined after 6, 12 and 24 h of growth in sterile skim milk by the team of Marroki and Bousmaha-Marroki (2014). In their studies, milk pH varied between 4.36 and 5.65 after 24 h of incubation in all cultures. The isolated *Lb. plantarum* strains showed a high acidifying

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activity after 24 h of incubation with a pH change between 2.08 and 2.48, a similar result to this activity was also reported for the *Lb. plantarum* strains in artisanal Turkish white pickled cheese (Dagdemir and Ozdemir, 2008). However, a variability in the acidifying activities was observed for this species, similarly as in Feta cheese (Xanthopoulos *et al*., 2000). On the other hand, the acidifying activity by *L. rhamnosus* strains was more homogeneous and remained higher, whereas *Lb. fermentum* strains showed the least acidifying capacity. An interesting result was obtained in a previous study on raw goat Algerian milk where a pH change ranging between 1.87 and 1.96 pH was observed after 24 h of incubation with 2 isolates of *L. rhamnosus* (Marroki *et al*., 2011). Generally, *Lb. plantarum* strains produce acid more rapidly, when compared to other *lactobacilli* (Dagdemir and Ozdemir, 2008).

From raw goat milk of four Algerian races (Makatia, crossed Makatia-Chamia, Kabyle and Arabia), 725 lactic acid bacteria have been isolated (Badis *et al*., 2004). From these isolates, 38.6% of them showed a fast acidifying rate (more than 60^0D during 18 h of incubation). These stains belong to *Lactococcus* (44.3%) and *Lactobacillus* (36.1%), whereas *Leuconostoc* strains showed a low acidifying activity, due to their heterofermentative metabolism and their sensitivity to low pH. The speed of acidification of mesophilic isolates was slow compared to that of the thermophilic isolates. The authors concluded that cheese prepared with isolates *Lactococcus lactis* subsp *lactis* biovar *diacetylactis* 19 MCA and *Lactococcus lactis* subsp. *lactis* 10 MCM had a very good sensory quality and a pleasant taste.

A total of 755 wild lactic acid bacteria have been isolated from Egyptian raw milk and its products, and their acidification activity was tested after 3 and 5 h (Ayad *et al*., 2004). However, only 2% of the studied Lactococcus stains mainly *Lc. lactis* subsp. *lactis* were considered as a fast acidifying species, the other majority of lactococci 60% of *Lc. lactis* subsp. *lactis* and 70% of *Lc. lactis* subsp. *cremoris* strains showed a medium acidification activity. These results are in agreement with Ayad (2004), who found that the overall acidifying activity of several wild *Lactococci* strains was rather low. Among *Lactobacillus isolates*, 13% were fast acidifying strains. The ones having the highest acidification activity included 43% of the isolated *Lb. helveticus*, 32% of the *Lb. delbrueckii* subsp. *bulgaricus*, 27% of *Lb. fermentum* and 26% of the *Lb. delbrueckii* subsp. *lactis*. On the other hand, all strains of *Lb. pentosus* and most strains of *Lb. plantarum*, *L. rhamnosus* and *Lb. fermentum* showed a slow acidification rate, which is consistent with their facultative heterofermentative metabolism (Kandler and Weiss, 1986). Only 1% of *Enterococci* were fast producers of lactic acid while the other ones

showed medium and slow acidification rates. These results are in agreement with those of Sarantinopoulos *et al*., (2001) who indicated *Enterococci* strains were poor acidifiers in milk. *E. faecium* and *E. faecalis* have been reported to degrade lactose in milk more slowly than *L. paracasei*, which is itself a slow acidifier (Freitas *et al*., 1999). The two tested *S. thermophilus* strains showed fast acidification activity and the four *Leuconostoc* strains were slow acidifiers. The *Pediococcus pentosaceus* strain had a slow acidification activity.

More recently, the potential of 25 *Lactobacillus* isolates from 8 semi hard artisanal goat cheeses manufactured in 4 different Mediterranean areas was examined for use as nonstarter lactic acid bacteria (Meng *et al*., 2018). Most of the lactobacilli displayed low acidification activity; 16 strains, including 9 of *L. paracasei* and 7 of *L. rhamnosus*, presented change in pH ≤0.4 U after 6 h of growth.

In Lebanese Laban, the acidification activity of 96 strains has been determined by using the Cinac system (Corrieu *et al*., 1988; Béal *et al*., 2001). *Lb. bulgaricus* strains displayed a high acidifying activity compared to *Streptococcus thermophilus* strains that were less acidifying. By comparing the mean results corresponding to the two species, the absolute value of the maximum acidification rate of *Lb. bulgaricus* was higher than that of *S. thermophilus* (0.0111 ± 0.0017 instead of 0.0085 ± 0.0009 u.pH/min).

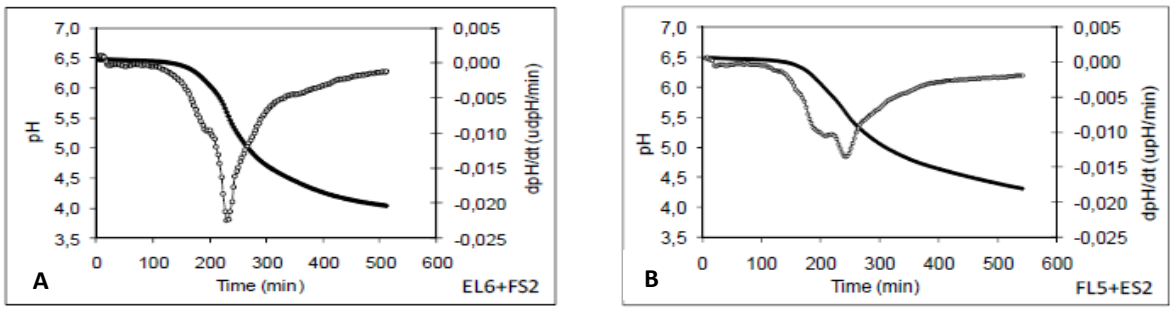

FIGURE II-6. TYPICAL ACIDIFICATION KINETICS OBTAINED DURING MANUFACTURE OF TWO LABAN SAMPLES (BÉAL AND CHAMMAS 2012).

. These results allowed the authors to select various mix of strains that displayed different acidification results, as illustrated in **Figure II.6**. In this figure, the strain mix A was highly acidifying as pH 4.5 was obtained after 334 min, while the strain mix B needed 485 min to reach the same pH.

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II.6.2. Antagonistic properties

Some LAB are used as probiotics due to their health benefits. An important characteristic of probiotics is their ability to defend the host from pathogens by producing antimicrobial molecules (Atanasova *et al*., 2014a). LAB produce various antimicrobial substances during fermentation, such as lactic acid, volatile acids, hydrogen peroxide, carbon dioxide, diacetyl, low-molecular-weight antimicrobial substances, and bacteriocins (Powell *et al*., 2007; Cotter *et al*., 2005).

Bacteriocins play a considerable role in food preservation. Most bacteriocins from Gram-positive bacteria are produced by LAB (Ennahar *et al*., 2000). Bacteriocins are a group of potent antimicrobial peptides. Although they display antibiotic properties, they differ from antibiotics in that they are synthesized ribosomally, exhibit a narrow spectrum of activity and the organisms responsible for their production have immunity against them (Parada *et al*., 2007; Cleveland *et al*., 2001). Therein, they may replace antibiotics (Richard *et al*., 2006) or be used in combination with them (Todorov *et al*., 2010; Minahk *et al*., 2004), thus limiting the development of antibiotic resistance.

Bacteriocins are active against Gram-positive foodborne pathogens such as *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus*, and vegetative cells and spores of *Clostridium botulinum* (Deegan *et al*., 2006; Chen and Hoover, 2003) or even against other LAB from the same species (Ayad *et al*., 2004). Strains of *Enterococcus faecium* isolated from Italian goat milk produced bacteriocins active against *Listeria monocytogenes*, *Clostridium butyricum*, and 6 *Salmonella* spp. isolates (Schirru *et al*., 201212; Cocolin *et al*., 2007). The bacteriocins produced by *Lb. sakei* GM3, isolated from goat milk, showed antagonistic activity against *Pseudomonas aeruginosa*, *S. aureus*, *L. monocytogenes*, *Salmonella typhi*, *S. enterica*, *Klebsiella pneumonia*, *E. coli*, *Candida albicans*, and *Candida tropicalis* (Devi Avaiyarasi *et al*., 2016). *Lc. lactis* subsp. *lactis* strain isolated from goat milk obtained from Sao Paulo expressed bacteriocin showing remarkable activity against different strains of *L. monocytogenes* (Furtado *et al*., 2014). *E. faecium* MMT21 isolated from a Tunisian dairy product produced two enterococcins active against closely related LAB and also *L. monocytogenes* and *S. aureus* (Ghrairi *et al*., 2008). During the manufacturing and ripening stages of five traditional starter-free cheeses, 17 strains of *Lc. lactis* expressed nisin A, nisin Z, lactococcin 972, and lactococcin G-like (Alegría *et al*., 2010).

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Several factors affect the mode of action of bacteriocins and thus may present limitations in implementation as biopreservatives. Temperature, pH, presence of lipids, proteins, food additives, NaCl, and preservatives may influence bioavailability of bacteriocins, their stability, and their adsorption to the microbial contaminates (Parada *et al*., 2007). Combination of traditional and new approaches of biopreservation may be the leading approach (Todorov, 2018).

II.6.3. Physical Properties

The physical properties of fermented milks are affected by milk composition, heat treatment, processing technologies (Love *et al*., 2014), types of stabilizers and types of LAB used as starters (Zhao and Liang, 2022). The main parameters that characterize the physical properties of yogurt are syneresis, texture and viscosity, which is strongly related to the ability of the strains to produce exopolysaccharides.

Yoghurt texture is also related to its sensory parameters (Béal and Helinck, 2019). The most common are thickness /viscosity, smoothness (opposite to lumpiness, graininess, grittiness), and sliminess (or ropiness) (Sodini *et al*., 2004; Walstra *et al*., 2006).

II.6.3.1. Syneresis

Syneresis is often considered as a quality defect, as it can reduce product shelf life and consumer acceptance (Tamime, 2006). It corresponds to the shrinkage of the gel, which induces whey formation on the surface of fermented milks (Lucey *et al*., 1998). The problem can be solved by increasing the hydrophilicity and water-binding capacity of the casein–lactoglobulin complex. Addition of stabilizers (gelatin, starch, pectin, …), increasing the protein content, or maintaining a low mineral content may prevent syneresis in yogurt (Korcz and Varga, 2021; Lesme *et al*., 2020; Sodini *et al*., 2004).

The stability of gel formation towards syneresis or whey drainage can be evaluated either under the influence of regular gravity or by applying additional gravitational forces by centrifugation, whereas the procedure based on centrifugation requires the milk fermentation in special containers which is the case of set yoghurt (Jaros and Rohm, 2020; Szczesniak, 2002). Lucey *et al*. (1998) developed a method by producing gels (220 g) in volumetric flasks, with the advantages of sloping walls it appeared to encourage spontaneous whey separation. Whey is then easily collected and poured-off through the neck of the flask to be measured.

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Measurement of syneresis is expressed as the percentage of expelled liquid compared to the initial volume of the product (% v/v).

In Lebanese traditional fermented milk "Laban", syneresis range is highly variable between species (Gisele Chammas *et al*., 2006) and among the same strain. For *S. thermophilus*, syneresis ranged from 0% to 13.2%, whereas for *Lb. bulgaricus*, it ranged from 1% to 17.5%. Seven streptococci and seven lactobacilli strains among 96, produced gels with very high syneresis values (more than 6%). Set-type probiotic yogurts made from caprine milk with an EPS-producing starter culture showed a high apparent viscosity, a low level of syneresis, and improved sensory properties (Madhubasani *et al*., 2020).

II.6.3.2. Texture

The acid gelation of milk is the most important factor in the formation of structure and consistency of the product. Milk fermentation destabilizes the entire casein complex as a result of acidification, thus leading to protein aggregation and gel formation (**Figure II.7**). During the fermentation, lactic acid which is excreted by lactic acid bacteria thus lowering the pH of milk. This acidification causes a demineralization of the casein micelles which disintegrate, destabilize, then gradually precipitate until the pH reaches their isoelectric point (Robinson *et al*., 2007); Béal and Chammas, 2012). This phenomenon, known as coagulation or gelation, which strongly modifies the texture of the product (**Figure II.7**).

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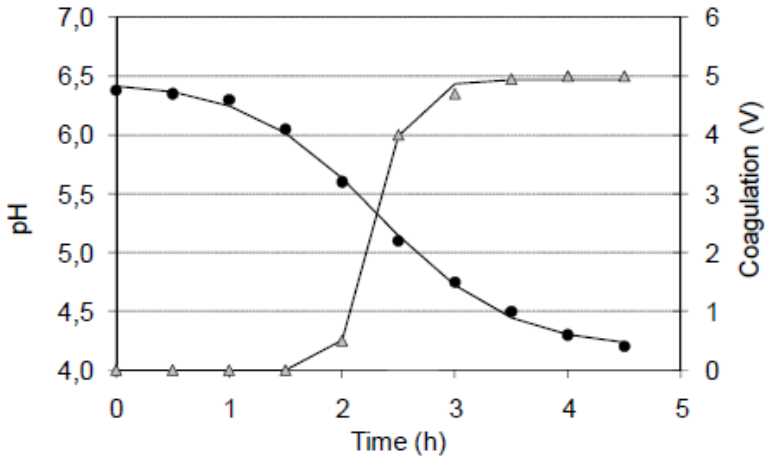

FIGURE II-7. TIME COURSE OF ACIDIFICATION AND MILK COAGULATION DURING YOGURT PROCESSING INOCULATED BY ST. THERMOPHILUS AND LB. BULGARICUS (BÉAL AND HELINCK, 2019)

(▲Coagulation, ●pH)

In fermented milk products, texture quantification is mostly analyzed by measuring firmness, cohesiveness and fracturability of the product (Béal and Chammas, 2012). It is evaluated by using a Texture Profile Analysis (TPA) instrument (Maccaferri *et al*., 2012; Foegeding *et al*., 2011). The force of penetration of the equipment probe into the sample at a well-defined speed and at a given temperature allows determining the texture profile of the sample. From the resulting force-time curve, obtained from two successive compressions of the sample, the force (F1, in N) required to disrupt the sample is used as a measure of fracturability, the maximum force (F2, in N) recorded during the first compression is a measure of firmness.

Fracturability is defined as the force with which a material (Product) fractures: a product of high degree of hardness and low degree of cohesiveness (Nishinari and Fang, 2018).

Cohesiveness was first calculated as the ratio of the areas under the force: time curve of the second bite (A2) compared to the first (A1). According to (Rosenthal and Thompson, 2021), this value is attributed to Friedman *et al*., (1963) definition of cohesiveness, "the strength of the internal bonds making up the body of the product". Which is related to consumer acceptability.

Hardness is the most important parameter for evaluation of yogurt texture. As a physical parameter it is regarded as the force (N) required to attain a certain deformation and is

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considered also as measure of firmness. As a sensory parameter, it is the force required to compress a substance between tongue and palate (in the case of semi-solids) (Szczesniak, 2002).

Adhesiveness is regarded as the force required to remove the adhered material in the mouth while eating. It is considered as a measure of stickiness of yogurt and is inversely related to eating quality Szczesniak, 2002). A typical example of profile texture of fermented milk is shown in **Figure II.8.**

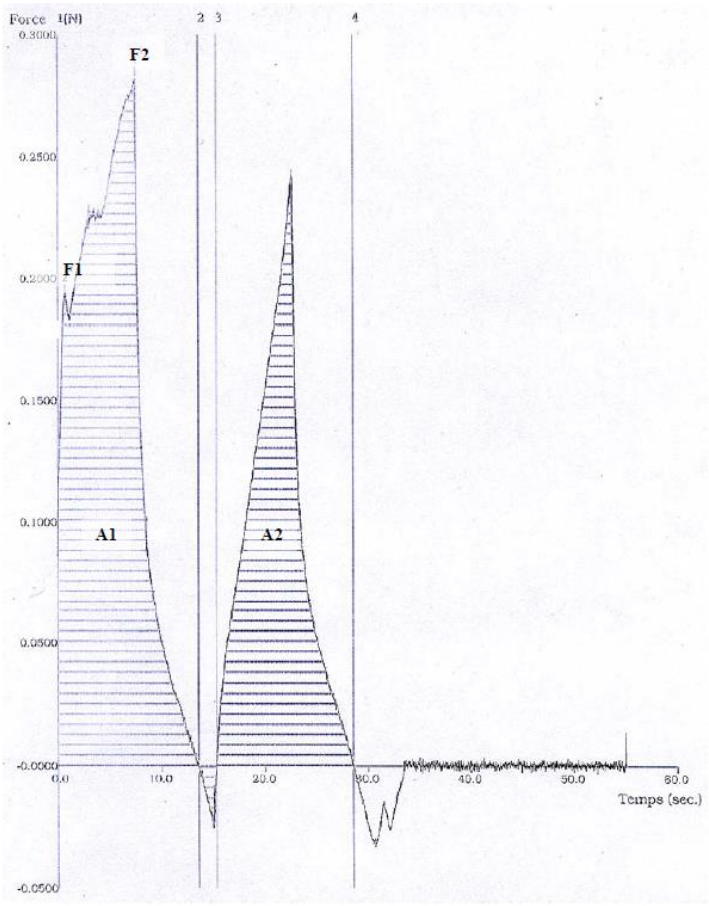

FIGURE II-8. TEXTURE PROFILE OF FERMENTED COW MILK INOCULATED WITH *S. THERMOPHILUS* (CHAMMAS ET AL., 2006)

II.6.3.3. Viscosity

Most fermented milk products exhibit a variety of non-Newtonian behaviors, such as shear-thinning, yield stress, viscoelasticity, and time dependency (Miocinovic *et al*., 2016). Their rheological characterization needs at least two kinds of measurements to define

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viscoelasticity and flow properties. Consistency as viscosity, and viscoelasticity as elastic moduli (G') and viscous moduli (G") (Vélez-Ruiz and Barbosa Canovas, 2009).

Viscosity is a physical property of fluids to resist their flow or pouring (Szczesniak, 2002). It corresponds to the informal concept of "thickness" (Park *et al*., 2007). It is influenced by kinetics of gel formation, the casein structural arrangement and by the presence of exopolysaccharides, EPS composition and EPS molecular mass (Priyashantha *et al*., 2021; Nguyen *et al*., 2018).

Conventional methods used to measure the physical properties of fermented milks are by using the Plummet device, the falling ball apparatus and also the Posthumus funnel (Lewis, 1996). According to Hellinga *et al*., (1986), the later was considered to determine the viscosity value for Yoghurt by measuring the product flow in the cylindrical part of the instrument. Both authors, Béal *et al*., (1999) and Martin *et al*., (1999) developed this method by introducing the flowing time coefficient (FTC), a parameter proportional to apparent viscosity defined by recording the weight of stirred yogurt flowing down (w, in grams) and the flow rate (dw/dt, in grams per minute). However these two viscosity measures are not correlated in sensory data (Janhøj *et al*., 2006).

To investigate the structure of a viscoelastic dairy product, dynamic analysis is used which involves an oscillatory applied stress. The sample is subjected to a sinusoidal oscillating strain that generates a sinusoidally oscillating stress response. These responses include the elastic or storage modulus (G'), which is a measure of the energy stored per oscillation cycle, the viscous or loss modulus (G"), which is a measure of the energy dissipated as heat per cycle, and the loss tangent (tan a), which is the ratio G"/G'(Lucey and Singh, 1997). According to Ozcan, (2013), for fermented milk gels, there is a small frequency dependence in the linear-viscoelastic region. This means that the moduli (G' and G") become non-linear at smaller strains as the frequency is increased. Beyond this linear region, the elastic structure begins partially to break down. When G' < G", the product undergoes transition from a predominantly elastic structure to a viscous structure. The main parameters that quantify the gel formation in a fermented milk are type of culture and milk composition (Marshall and Rawson, 1999).

Viscosity is influenced by fat and casein contents more than lactose and whey proteins. As a consequence, the factors that can change the fat and casein contents of goat milk modulate

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the rheological features of goat milk and its derivatives (McCarthy, 2011). The smaller sizes of the fat globules and casein particles in goat's milk (Silanikove *et al*., 2010), and in particular αs1-casein contents (Li & Guo, 2006) lead to a weaker texture and a lower viscosity of gel formation when compared to cow's milk fermented product (da Silva and da Costa, 2019; Vargas *et al*., 2008). In addition, it has been also reported that goat α-s1 casein is more favorable to precipitation in the presence of calcium compared with cow α-casein (Haenlein and Anke, 2011). Thus, the reported low viscosity by Gomes *et al*., (2013) of the goat fermented beverages made from 70 g/100 g goat milk and 30 g/100 g goat whey when compared to cow fermented beverages made from 70 g/100 g cow milk and 30 g/100g cow whey was possibly caused by the higher precipitation of α-s1 casein present in such milk during heat treatment. This cause to lose some of the gel formation ability that is required to increase the viscosity of the product(Jacob *et al*., 2011).

Cold storage also increased significantly the viscosity of milk fermented product (Kavas *et al*., 2003). Küçükçetin *et al*., (2011) measured the apparent viscosity in a comparison study between fermented milks of cow, goat and a mix of cow and goat. Values varied significantly from 0.79 to 1.12 Pa.s for goat yoghurt and from 1.66 to 2.38 Pa. s for cow yoghurt at day 1 and day 15 of refrigeration at 4ºC respectively.

The work of Miocinovic *et al*., (2016) showed that acid gel produced from fermented pasteurized goat milk had a very low G', compared to cow milk which indicates a weak gel structure. The addition of milk protein isolates (80% protein) significantly increased the G' value in the fortified goat yoghurt. This is probably due to the lower number of bonds in the formed network and lower protein content in comparison with cow milk (Peng *et al*., 2009).

The production of exopolysaccharides by lactic acid bacteria which are used as starter culture significantly improve the viscosity of the produced fermented product (Han *et al*., 2016). In this regard, the indigenous strain *Lactobacillus plantarum* MC5 which is isolated from the traditional fermented Yalk yoghurt improved the water holding capacity and apparent viscosity. When it is cultured with commercial starter strains (*Streptococcus thermophilus and Lactobacillus delbrueckii* subsp. *bulgaricus*) in different formulations groups of 1:1, 2:1, and 1:2 during storage, high apparent viscosity was recorded. It attained in the 1:1, 2:1, and 1:2 samples was 19.69 Pa.s, 21.76 Pa.s, and 20.23 Pa.s, respectively was significantly higher than that of the commercial group 12.24 Pa.s after 21days of storage (Zhao and Liang, 2022).

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Traditional fermented milks differ in their characteristic and texture types. Nordic traditional sour types such “Tatmjôlk’ in Sweeden or ‘Tettemelk’ in Norway are noted for their high viscosity and a ropy consistency among all traditional milk fermented products. A character which is strongly related to the growth of *Lactococcus lactis* subsp. *cremoris* strain (Narvhus, 2014; Fondén *et al*., 2007).

Type of milk also affects the textural properties and viscosity index of kefir beverage. Viscosity of Turkish kefir prepared from goat milk and kefir culture is lower than kefir obtained from cow milk (Buran *et al*., 2021). The work of Sarica and Coşkun (2020) showed that the general mean viscosity value of cow kefir samples was 64.80 mPa.s significantly higher than that of goat kefir samples which attained 9.46 mPa.s.

In the characterization of 96 lactic acid bacteria isolated from Lebanese traditional fermented cow Laban. These strains displayed varied low measurements of apparent viscosity ranging from 0.01 to 1.28 Pa.s and from 0.29 to 0.82 Pa.s for the group of *Streptococcus thermophilus* and the group of *Lactobacillus delbrueckii* subsp. *Bulgaricus* respectively. Consequently, they were considered as non-ropy strains similar to the set style character of Laban (Chammas *et al*., 2006a). In other Mediterranean milk fermented product such as Labneh, higher values of viscosity were recorded. According to Abu-Jdayil *et al*., (2002) there was no significant difference between the apparent viscosity of ‘mechanical Labneh” that reached 3000 mPa.s and traditional Labneh that attained 2800 mPa.s at 10 s^{-1} of shear rate, however the latter is more sensitive to temperature which decrease when milk total solids increase.

II.6.3.4. Exopolysaccharide production

Exopolysaccharides (EPS) produced by LAB **(Figure II.9)** contribute to functional properties of fermented milks. They are recognized in the improvement of the appearance, the stability, and the rheological properties in several fermented foods (Patel and Prajapati, 2013). EPS act as thickeners and texturizers by increasing the viscosity of the fermented products and as stabilizers by binding water and interacting with other milk constituents, such as proteins and micelles, thus improving the firmness of the casein network. Moreover, EPS can decrease syneresis (Daba *et al*., 2021; Mende *et al*., 2016; Hassan, 2008).

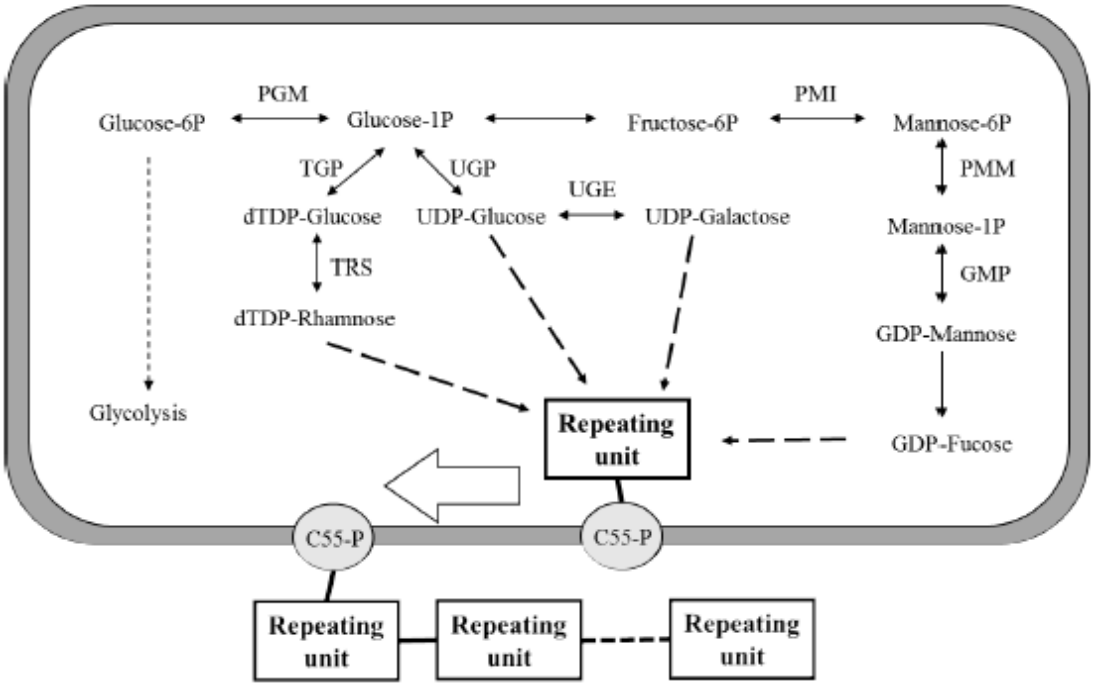

FIGURE II-9. BIOSYNTHESIS OF HETERO-EXOPOLYSACCARIDES (HEPS)

PGM: α-phosphoglucomutase, UGP: UDPglucose pyrophospholyrase, UGE: UDP-galactose 4-pimerase, TGP: dTDP-glucose pyrophospholyrase, TRS: dTDP-rhamnose synthetic enzyme system, PMI: phosphomannoisomerase, PMM: phosphomannomutase and GMP: GDP-mannose pyrophospholyrase (from Sanalibaba and Cakmak, 2016).

Exopolysaccharides are distinguished by their location, their functionality, and their molecular composition and structure. Most LAB synthesize free EPS that are liberated into the medium from the cell wall of bacterial cells. Some strains, like *Streptococcus thermophilus*, produce a second type, namely capsular EPS, which are attached to the bacterial cell wall (Khanal and Lucey, 2017). In fermented dairy products, the presence of capsular EPS improves water binding properties, whereas free EPS are often associated with an increased viscosity and improved texture of fermented milk (Teixeira, 2014). The presence of linear, stiff, and anionic EPS from *Streptococcus thermophilus* increases the elastic modulus in set and stirred yoghurt conditions, possibly owing to electrostatic interactions with caseins. Higher viscosity values and elastic modulus and reduced syneresis values were obtained with stiff and linear or slightly branched EPS from the *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* strains in yoghurt (Xu *et al*., 2018; Gentès *et al*., 2016).

With respect to their molecular characteristics, EPS can be distinguished into homopolysaccharides (HoPS) and heteropolysaccharides (HePS) (Mende *et al*., 2022).

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Branched HePS from *S. thermophilus*, *Lb. delbrueckii* subsp. *bulgaricus*, *Lc. lactis* spp., *L. helveticus* display high variety in structure. Charged HePS from *Lc. lactis* subsp. *cremoris* B40 and *L. rhamnosus* RW9595M which have a defined structure have rheological applications in fermented milk. Finally, kefiran, a HePS from *L. kefirgranum*, *Len. parakefiri Len. kefiri*, and *Lb. kefiranofaciens* plays a role in kefir rheology (Moradi and Kalanpour, 2019).

The structural diversity of EPS is largely due to the presence of different sugar monomers, variations in the modes of linkage, substitution and branching and even differences in molecular mass (Jurášková *et al*., 2022). The different polysaccharides may be recognized by a number of receptors, these include C-type lectin receptors, Toll-like receptors (TLR) and dendritic cell-specific intercellular adhesion molecule-3- grabbing nonintegrin receptors (Riaz Rajoka *et al*., 2020)

Finally, EPS composition and yield by LAB depend on temperature, incubation time, source of carbohydrate and growth phase (Patel *et al*., 2012). Due to their characteristic, they might be used as thickeners and stabilizers in other types of foods (Patel and Prajapati, 2013).

II.6.4. Metabolic activities

II.6.4.1. Proteolytic activity

The low concentrations of free amino acids and peptides in milk can't efficiently support the growth of LAB. In response to this limitation, LAB have developed a complex system of proteinases and peptidases, which enable them to use casein as a source of organic nitrogen (Donkor *et al*., 2007). The proteolytic activity in fermented dairy products is also involved in the development of their organoleptic properties (García-Cano *et al*., 2019). Proteolysis is the most complex metabolic activity and the most important in enhancing aroma, flavor, and texture (Savijoki *et al*., 2006). Moreover, the proteolytic activity of LAB is advantageous because proteolysis is known to improve the digestibility of fermented products and to enhance their nutritional quality (El-Ghaish *et al*., 2011).

The proteolytic system of most LAB consists of three major components: extracellular proteinases such as cell envelope proteinases (CEPs) that cleave the casein into oligopeptides, oligopeptides transporters, and intracellular peptidases that convert oligopeptides into smaller peptides and amino acids (Liu *et al.*, 2010). These amino acids are the substrates for various metabolic reactions, with aminotransferases being the first enzymes in the subsequent catabolic

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cascade. Diverse and numerous aromas are released in these reactions (aldehydes, ketones, carboxylic acids and volatile sulfur compounds) (Zheng *et al.*, 2021; Marilley and Casey, 2004).

Previous studies reported that the proteolytic activity of *Lactobacillus* strains is greater than that of *Lactococci, Enterococci, Streptococci, Leuconostoc* and *Pediococci* (Ayad *et al*., 2004). In particular, many strains of *S. thermophilus* do not express or express low level of proteinases (Fernandez-espla *et al*., 2000).

Among the LAB family, *Lb. helveticus* strains have the highest proteolytic activity (Savijoki *et al*., 2006) . The vast majority of LAB have only one CEP, but for certain strains of *Lb. helveticus*, it has been demonstrated through multiplex PCR analysis, that at least four different proteinases exist (Broadbent *et al*., 2011). The presence of a higher number of proteinases with different substrate and cleavage specificities could explain the efficiency of the *Lb. helveticus* proteolytic system (Stefanovic *et al.*, 2017). Literature data revealed that the highest proteolytic activity in lactococci was found in traditional Manchego ewe milk cheeses (Nieto-Arribas *et al*., 2009) while in lactobacilli, significant proteolytic activity was reported from traditional Pecorino ewe milk cheese and from goat milk from different Algerian breeds (Badis *et al*., 2004; Madrau *et al*., 2006).

II.6.4.2. Lipolytic activity

The lipolytic activity of LAB relies on their ability to produce various lipolytic enzymes, such as lipases and esterases. Lipases are active against emulsified substrates with long-chained fatty acids and they hydrolyze mono, di, and triglycerides whereas esterases are active against short-chained fatty acids and lead to the liberation of volatile fatty acids (Chen et al., 2017a). The lipolytic activity of LAB is important for the fermentation process in the dairy production since short and intermediate chain fatty acids contributes to cheese flavor (Collins et al., 2003)llins *et al*., 2003) thus improving quality and maturation of the product (Ortiz de Apodaca *et al.*, 1993) as they represent the starting molecules for catabolic reactions resulting in the production of numerous flavor and aromatic compounds

The early studies reporting the lipolytic activity of LAB is weak and varies between species. For example, that of *Lactococcus* is better than that of *Streptococcus thermophilus* and *Lactobacilli* (Béal *et al*., 2006). However, some LAB species have high lipolytic activity such as *Enterococci* that have the highest activity among the LAB family (Akabanda *et al.*, 2013) . The appropriate combination of *Streptococcus lactis* ACCC 11093 and three *Lactobacilli*

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strains and the addition of lipase efficiently improved the quality of yogurt-flavored bases. Lipase significantly increased the production of amino acids associated with sweet and bitter flavors, and resulted in rougher milk globule surface and tighter network structure compared with fermentations without the addition of lipase (Huang *et al.*, 2020a).

II.6.4.3. Autolytic activity

LAB can induce cell-autolysis through the enzymatic degradation of cell wall peptidoglycan (Zambonelli *et al.*, 2002). This phenomenon takes place in response to a change in the environmental conditions or to cell starvation (Lortal and Chapot-Chartier, 2005). The ability of LAB to lyse and the subsequent release of their intracellular enzymes is a desirable trait during the ripening of cheese. These enzymes promote the fat and protein hydrolysis which favors rapid development of flavor (Ramírez-Nuñez, *et al*., 2011). Factors that influence the cell autolysis commonly include pH, salt concentration, water activity or temperature (Wilkinson and LaPointe, 2020; Thiboutot *et al*., 1995). Huang *et al*. (2020b) provided evidence for a relationship between proteolysis, cell wall synthesis pathways and resultant autolytic phenotype of *Lc. lactis* subsp. *cremoris* MG 1363. However, gaps in the knowledge still remain especially in the area of starter non-viability responses during prolonged ripening (Wilkinson and LaPointe, 2020).

Autolytic properties have been studied in eight semi hard artisanal cheeses produced in the Mediterranean countries, Spain, Greece, Turkey, and Lebanon. From 25 isolates, 9 strains of *Lacticaseibacillus paracasei* and 5 of *Lacticaseibacillus rhamnosus* showed autolysis values ranging between 25 and 65% (Meng *et al*., 2018). Isolated from two Egyptian cheeses, 35 strains of *E. faecium* showed autolysis properties: 11% of them displayed poor autolysis (40–60% of residual absorbance), 49% of the strains were considered as being moderately autolytic (20–40% of residual absorbance) and 40% of the strains were highly autolytic (4–20% of residual absorbance) (Malek *et al*., 2012).

II.6.4.4. Gaz production

The gas formation by lactic acid bacteria has been well studied as it may promote the development of undesirable flavor and body defects. This property is mainly related to the obligatory heterofermentative lactobacilli. *Lactococcus spp.* performs homolactic fermentation, while *Lactobacillus* and *Leuconostoc spp.* perform heterolactic fermentation (Wang *et al*., 2021). In the process of pure lactic acid fermentation, lactic acid bacteria use glucose as a

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carbon source to produce pyruvate through glycolysis, and then produce lactic acid under the action of lactate dehydrogenase (Eiteman and Ramalingam, 2015). In theory, 1 mole of glucose produces 2 moles of lactic acid In lactic acid bacteria of the heterolactic fermentation type, glucose can be decomposed into lactic acid, ethanol, CO_2 (in *Leuconostoc*, etc.) Through the pentose phosphate (PP) pathway, glucose 6-phosphate was converted into carbon dioxide, ribulose 5-phosphate and NADPH (Stincone *et al*., 2015).

It was observed that LAB isolated from the traditional Himalayan Ethnic fermented Milks, such as *Leuconostoc* spp. and *Lev. brevis*, produced gas during the degradation of glucose, while *Lb. plantarum* and *Lc. lactis* did not (Dewan and Tamang, 2007). These result were coherent to the recent work of Kamarinou *et al*., (2022) who isolated lactic acid bacteria from traditional Greek fresh goat cheese. In this study, 33 strains were able to produce CO2 from glucose from which 28 isolates belonging to *Lactobacillus mesenteroides*, *Lactobacillus pseudomesenteroides*, *Leuconostoc* sp., and *Levilactobacillus brevis*.

II.6.5. Volatile organic compounds production

In fermented dairy products, the sensory properties largely depend on the relative amount of flavor compounds derived from carbohydrate, protein or fat in milk. The flavor components of yogurt include the volatile and non-volatile compounds already present in the milk and specific compounds produced from milk fermentation (Cheng, 2010a; Ott *et al.*, 1999). More than 90 different volatile organic compounds (VOC) have been identified in yogurt, including alcohols, aldehydes, ketones, acids, esters, lactones, sulphur-containing compounds, pyrazines, and furan derivatives (Ott *et al.*, 1999). Only volatile compounds whose concentrations are above the threshold are said to be of sensory importance. The aroma threshold values vary by several orders of magnitude which influences their perception by the human senses. Although no final conclusion has yet been drawn, the major compounds commonly reported as being responsible for imparting desirable flavor to yogurt are lactic acid, acetaldehyde, diacetyl, acetoin, and 2-butanone (Routray and Mishra, 2011; Cheng, 2010a; Tamime and Robinson, 2007). Good flavored yogurt are obtained when proper levels of these compounds are produced. For example, the optimal concentrations of acetaldehyde in yogurt ranges from 14 to 20 mg/kg, while less than 8 mg/kg results in weak flavor and too much acetaldehyde leads to an “astringent” off-flavor yogurt (Ott *et al*., 2000).

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Furthermore, like many other dairy products, yogurt is prone to deterioration, especially under improper storage conditions. Generation of volatile by-products leads to off-flavors and makes the product unsatisfactory for consumers (Rychlik *et al*., 2006). Accordingly, a desirable flavor means presence of major flavor compounds in proper levels without off-flavors.

A great majority of the flavor compounds produced in yogurt results from the activity of LAB. During fermentation, the biochemical conversions done by LAB lead to the production of various flavor and off-flavor compounds for yogurt catalyzed by microbial enzymes (Smid and Kleerebezem, 2014; Steele *et al*., 2013). The level of flavor compounds is speculated to be much greater in mixed cultures of *S. thermophilus* and *Lb*. *bulgaricus* than either of the two single cultures due to their associative growth and mutual stimulation (de Bok *et al*., 2011; Tamime and Robinson, 2007).

By considering other fermented milk products, the volatile compounds composition of fermented dairy products differs. A specific composition is observed by considering goat milk products (**Table II.8**), which include volatile compounds issued from raw goat milk. In particular, kefir made from goat milk has a higher acetaldehyde amount compared to cow milk kefir (Buran *et al*., 2021; Beshkova *et al*., 1998; Rysstad and Abrahamsen, 1983).

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TABLE II-8. LIST OF IDENTIFIED VOLATILE ORGANIC COMPOUNDS IN GOAT MILKS AND DIFFERENT TYPES OF MILK FERMENTED PRODUCTS.

Product type	Volatile organic compounds	Literature
Yogurt (cow milk)	High impact on desired flavor: Acetaldehyde, acetone, diacetyl, 2-butanone, acetoin, ethanol Compounds identified in plain yogurt: 38 carbonyl compounds, 19 heterocyclic compounds, 18 alcohols, 12 acids, 11 aromatic, 6 sulfur, 5 esters, 3 nitrogen, 4 hydrocarbon	(Chen et al., 2017b) (Cheng, 2010a)
Yogurt with 4 probiotic strains (*Lacticaseibacillus. casei, Lacticaseibacillus. rhamnosus, Lactoplantibacillus. plantarum,* and *Lactobacillus. acidophilus*)	Aldehydes (7), alcohols (14), ketones (13), organic acids (8), and sulfur compounds (3). Ethanol, cyclobutanol, 1-hexanol, 2 ethyl 1 hexanol, 3 pentanol, 3-hexanol, 2-butanol, 2-propanol, 1-heptanol, 2-Nonanol, heptanol, hexanal, butanal, Nonanal, benzaldehyde, decanal, 3-methylbutanal, acetone, 2-butanon, diacetyl, 2.3-pentanedione, 2-pentanone, 2-heptanone, 2-octanone, acetoin, methyl heptanone, 2-undecanone, acetic, hexanoic, pentanoic, heptanoic, octanoic, butanoic, decanoic, butyric acid, dimethyl sulfone, 3-4 dimethylthioquinoline. *Lactobacillus plantarum* and *Lacticaseibacillus rhamnosus* increase acetaldehyde and ketones. *Lactobacillus casei* generates cyclobutanol, 2-ethyl-1-hexanol, 2-butanone, 2-pentanone, 2-heptanone, 2-undecanone, and 2-tridecanone as well as organic acids (hexanoic and butanoic acid. 2-Butanone and 3-methyl-1- butanol were identified in *Lactococcus lactis* subsp. *lactis* and *Lactococcus cremoris* subsp. *cremoris*	(Tian *et al*., 2017) (Gallegos et al., 2017a)
Yogurt (goat, ewe, buffalo and cow milk) during storage	Acetaldehyde and hexanal are found in all products but their levels are low in goat milk. Diacetyl, acetoin , 2-3 pentadione, 2-heptanone, 2-nonanone, 2-undecanone, ethanol, ethyl acetate and octanoate, diethyl phthalate, acetic acid, butyric acid, caproic acid, caprylic acid, capric acid, benzoic acid, limonene, alfa pineen, p-cymene, p-xylene, alfa-xylen, toluen, styrene, ethyl benzene, 1-ethyl-3benzen, 1.3 trimethyl benzene, heptane, octane, nonane, undecane, dimethyl sulphone. Capric acid is found only in goat yoghurt with an increasing amount during storage.	(Erkaya and Şengül, 2011a)
Bulgarian yoghurt	Acetaldehyde, acetone, 2-butanone, diacetyl, ethyl acetate, and ethanol: Formation of typical Bulgarian yogurt flavor	Gyosheva, 1985; Kondratenko and Gyosheva, 1978
Lebanese Laban	Acetaldehyde, acetone, diacetyl, 2,3-pentanedione, acetoin, furancarboxaldehyde, butanoic acid, furanmethanol, hexanoic acid, butanone, dimethyl disulfide, acetic acid	Chammas *et al*., 2006b

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TABLEI II.8. LIST OF IDENTIFIED VOLATILE ORGANIC COMPOUNDS IN GOAT MILKS AND DIFFERENT TYPES OF MILK FERMENTED PRODUCTS

Product type	Volatile organic compounds	Literature
Fermented milk (*Lactobacillus. delbrueckii ssp bulgaricus and/or S. thermophilus*)	Acetic acid, 4-chlorobutanoic acid, 3-methylbutanoicacid, hexanoic acid, octanoic acid, and *n*-decanoic acid. Acids produced by one pure strain or a mix of them: acetic acid, butanoic acid, 3-methylbutanoic acid, hexanoic acid, and octanoic acid. Aldehydes: mainly acetaldehyde, benzaldehyde in milk fermented by *S. thermophilus*, ketones, 16 acids, 10 esters, 25 alcohols and 13 hydrocarbons.	Dan *et al*., 2017
Fermented milk by 17 strains (*Lactobacillus delbrueckii ssp bulgaricus)*	85 VOC, 17 carboxylic acids, 14 aldehydes, 13 ketones, 29 alcohols, 8 esters, and 5 aromatic hydrocarbon compounds. Key contributors to the flavor: acetaldehyde, 3-methyl-butanal, (E)-2-pentenal, hexanal, (E)-2-octenal, nonanal, 2,3-butanedione, acetoin, 2-heptanone, 2-non-anone, and ethenyl ester formic acid) with Odor Activity values (OAVs) > 1.	Dan *et al*., 2019
Turkish fermented Kefir from goat milk and kefir culture (GC), and GC with *Lactobacillus acidophilus* La-5 and fructooligosaccharide (GA) and GC with *Bifidobacterium bifidum*Bb-11 and fructooligosaccharide (GB).	Acetaldehyde: 16.51 ppm in goat kefir significantly lower than in cow kefir samples Diacetyl: ranged from 1.85 to 4.52 ppm without any significant difference aomg the goat and cow milk samples	Buran *et al*., 2021
Kefir fermented (from cow milk)	Alcohol: Ethanol, ketones: 3-hydroxy-2-butanone (acetoin and 2-butanone), ester: ethyl acetate and aldehyde: acetaldehyde. Mediated by *Saccharomyces cerevisiae*: isopentyl acetate, ethyl hexanoate, ethyl octanoate, phenethyl acetate, and ethyl decanoate	Farag *et al*., 2020
Kefir fermented (from skimmed milk powder)	Ethanol (39.3%), 2-butanone (31.6%), ethyl acetate (8.9%), ethyl butyrate (5.5%), acetone (3.6%), 3-hydroxy-2-butanone (acetoin, 3.3%), 2,3-butanedione (diacetyl, 2.9%) and acetaldehyde (1.7%). In addition to acetone, diacetyl, ethanol, acetaldehyde and ethyl acetate in lower quantities.	Farag *et al*., 2020
Fermented milk (*L. delbrueckii*)	Acetaldehyde, 3-methyl-butanal, (E)-2-pentenal, hexanal, (E)-2-octenal, nonanal, 2,3-butanedione, acetoin, 2-heptanone, 2-non-anone, formic acid ethenyl ester.	Dan *et al*., 2019
Goat milk (from grazing animals)	Dominant terpens β-caryophyllene, α-copaene, 3-carene; camphene; limonene α-pinene; β-pinene; sabinene; α and γ Terpineol, 4-Terpineolerpineol; p-cymene; β-phellandrene; tricyclene., α-copaene, alloaromadendrene	Fedele *et al.*, 2004

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Raw goat milk (from Saanen breed in Brazil)	Hexanol (9,5 mg/L), pentanoic acid (3,0 mg/L), 2-pentanone (1,6 mg/L), methyl 9-octadecenoate (1,6 mg/L), methyl hexadecanoate (1,0 mg/L) and 2-pentanol (1,7 mg/L) Responsible for goat milk aroma: decanal, (*E*)-2-hexenal, hexanol, octanal, ethyl hexanoate, pentanal, nonanal, 2-pentanone, heptanal, methyl hexanoate, γ-dodecalactone, 2-heptanone, heptanol, ethyl octanoate, 2-pentadecanone, β-ionone, α-pinene, 2-methylthiophene, octadecanal. First time reported: Isopropyl butanoate, isobutyl acetate, ethyl tridecanoate, butyl 10-undecenoatea, isoamyl cinnamate, benzyl benzoate, butyl dodecanoate, isopropyl tetradecanoate, methyl (*Z*)-9-hexadecenoate, methyl 9-octadecenoate, octadecanol acetate, butyl heptadecanoate, 2-methyl-1-hexanol, tridecanol, 1,2-dodecanediola, Cumenea, camphor, *β-ionone* , β-farnesene, γ-cadinene, δ-cadinenea, cubenol, α-cadinol, farnesol, 2,3-hexanedione, butanethiol, 2-methylthiophene, goaty short fatty acids (C6:0 to C9:0).	Queiroga *et al*., 2019

II.6.6. Resistance to gastrointestinal stress

Passage of LAB through the gastrointestinal tract (GIT) is a stressful journey, with stress stages, which may affect cell viability. These stresses are encountered in the stomach, where the bacteria face high acidity (up to pH 2) and in the duodenum, where they are affected by bile salts. The detergent property of bile confers potent toxicity, primarily through the dissolution of bacterial membranes (Tambekar and Bhutada, 2010). Hence, tolerance to bile salts is a prerequisite for colonization and metabolic activity of bacteria in small intestine of the host. LAB acid tolerance is important not only for withstanding this stresses, but also a prerequisite for their use as dietary adjuncts and enables strains to survive for longer period of time in high acid carrier food without larger reduction in humans (Ji *et al*., 2015; Prasad *et al*., 1998).

In vitro resistance to bile salts and acidity, as based on survival and growth studies, has become necessary in screening potential probiotic strains (Corcoran *et al*., 2005). LAB represent different bile response mechanisms **(Figure II.10)**, however *Lactobacilli* are considered to be more resistant to both acid and bile salts, with differences among species. Usually, sensitivity increases with a pH below pH 3. Acid resistance is attributed to the presence of a constant gradient between extracellular and cytoplasmic pH. Bile resistance is related to the presence of bile salt hydrolase (BSH), as bacteria that possess BHS are able to counteract the toxicity of bile salts (Menconi *et al*., 2018). Two studies demonstrated that *Lactococcus lactis* ssp *lactis* and *Lacticaseibacillus paracasei* strains, respectively isolated from Egyptian Boza beverage (Shehata *et al*., 2016) and Italian raw goat milk (Pisano *et al*., 2019) were able to support bile salt at 0.3% and reached a viability rate of 88.3%.

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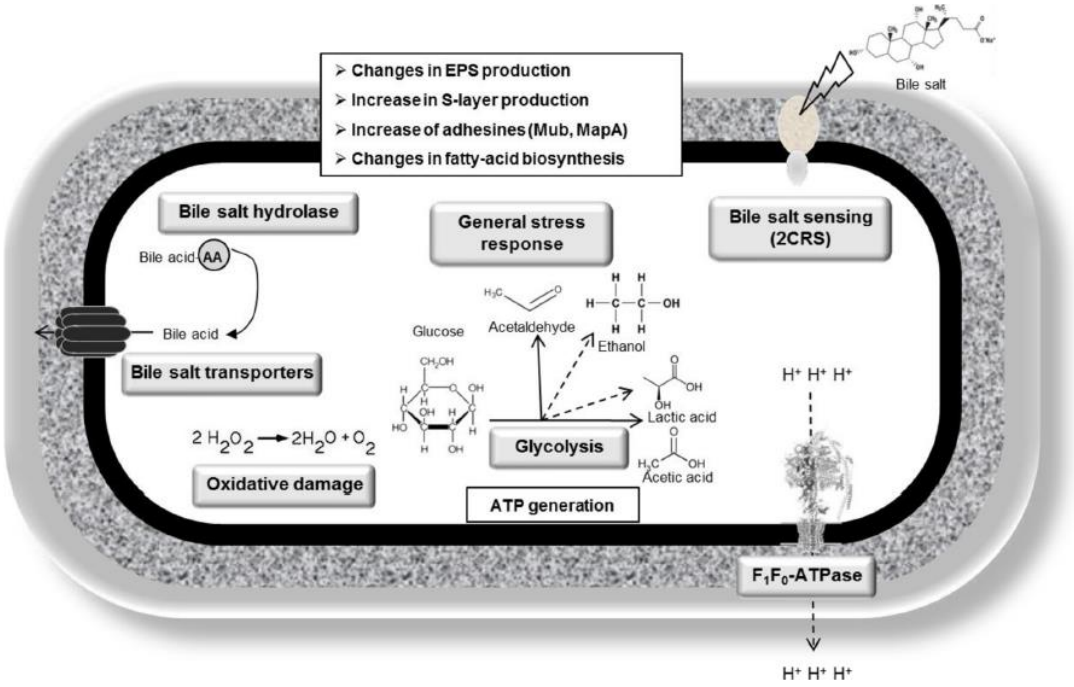

FIGURE II-10. DIFFERENT BILE RESPONSE MECHANISMS IDENTIFIED AND CHARACTERIZED IN LACTOBACILLI (FROM RUIZ ET AL., 2013)

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III. OBJECTIVES AND EXPERIMENTAL APPROACH

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III.1. Thesis objective

III.1.1. General Objective

The general objective of this thesis is to promote traditional and artisanal fermented goat milk products in Lebanon, as they make a part of the culinary Lebanese heritage but are endangered from disappearing. To reach this goal, this work aims at studying three different Lebanese artisanal products by exploring their production processes, identifying their microbial specificities and characterizing their technological properties, to further help for the development of a Protected Designation of Origin for each product under inquiry. We hypothesized that LAB characteristics, the geographical origin of raw materials (nutritional and non-nutritional) and the home-made manufacturing process by itself play a role in the originality of these fermented products.

III.1.2. Specific Objectives

Three scientific questions have been defined to answer the previous objectives:

1. Identify the native microflora of lactic acid bacteria in three fermented goat milk products namely: Ambriss, Serdaleh et Labneh El Darff, artisanally produced across three Lebanese regions.
2. Characterize the functionalities and technological properties of lactic acid bacteria isolated from the fermented products.
3. Select of some LAB isolates according to their technological functionalities as candidate starter strains to be used and adopted in the Lebanese dairy industry.

III.2. General experimental approach

To answer these scientific questions, an experimental approach associating four main steps has been defined:

1. Three artisanal fermented goat milk products were first selected to conduct the study: Ambriss, Serdaleh and Labneh El Darff, originating from three different regions of Lebanon. The studied products were collected from only experienced producers following traditional manufacturing methods according to a producer's survey conducted on 50 producers;

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2. The microbial composition and diversity of these three products was identified by using conventional culture-dependent and culture-independent methods including metagenetic and other meta-omics approaches;
3. About 40 isolates were selected and characterized by considering their main metabolic activities and technological properties;
4. Some specific isolates were selected according to their interesting functionalities.

According to these approaches, this study is considered to be the first attempt that combines both conventional culture-dependent and culture-independent methods including metagenetic and other meta-omics approaches to discern the microbial diversity and identify representative LAB in Lebanese fermented goat milk products.

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IV. MATERIALS AND METHODS

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IV.1. Selection of the producers of fermented dairy products

IV.1.1. Elaboration of a producer's survey

A producers' survey was conducted over 50 breeders/producers from three Lebanese production regions: Bekaa Valley, Chouf Mountains and South Lebanon. They are producing fermented goat milk products Ambriss, Serdaleh and Labneh El Darff respectively. The questionnaire consisted of 34 questions, covering three main parts Appendix III.1.

i) Socio-demographic aspects,
ii) Breeding activities,
iii) Production activities including commercialization.

The questions included general and socio-economic information about producers, information regarding livestock size, goat breed, breeding type and practices, milk production volumes as well as the type of dairy product produced, season of production, raw material used, production volumes, marketing practices and selling prices.

In addition, a detailed observation of the processing was conducted in order to construct a common production flow chart for each product type, showing also the existing difference between the producers.

IV.1.2. Producers selection

Producers of the fermented products from the three regions were first selected from the survey according to two main criteria: fitting an artisanal production level and being an experienced producer for more than 15 years. The selected producers will be the resource for further sample collection.

In total, 34 artisanal goat milk fermented milk producers were pre-selected among the 50 producers initially identified. The complete list of producers and production locations is given in **Appendix III.2.**

IV.2. Samples collection, preparation and preservation

Samples were collected from each producer during two consecutive production seasons, 2018 and 2019, by considering the 3 fermented products (Ambriss, Serdaleh or Labneh El Darff) and their corresponding raw products (raw goat milk in the case of Ambriss, Serdaleh or laban in the case of Labneh El Darff). The fermented products were freshly taken from the surface and the middle part of the jars for Ambriss (n=11) and Serdaleh (n=19) once the product

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is formed and also during the production period. The sampling of Labneh El Darff was done directly from the Darff bag at the end of the manufacturing process (n=4).

All samples were collected in duplicate and filled into 100 mL sterile plastic containers that were well sealed and transported in a vehicle-mounted refrigerator kept at 5°C during a two hours' journey to the Microbiology Laboratory of LARI (Lebanese Agricultural é Institute). At reception of the containers in the laboratory, samples (15 g) were diluted in 15 mL of Phosphate Buffer Saline (PBS 15X, pH 8.7) in conical Falcon tubes. Final pH reached is between 5.15 and 5.70. Tubes were kept for a long-term storage at -20°C and -80°C until further analyses.

IV.3. Physico-chemical analyses

At the moment they were collected, the 3 products were characterized by considering their pH and salinity level. Each sample was homogenized for 60 s in a laboratory blender (Blender LB20E, Waring, Torrington, USA) with respectively 90 mL and 20 mL distilled water for pH and salinity measurements.

IV.3.1. Measurement of product's pH

The pH of the three products was measured using a digital pH meter (GLP 21, Crison, Barcelona, Spain) at the moment they were received in the Laboratory at LARI. All measurements were performed in triplicate.

IV.3.2. Measurement of product's salinity

Since salt is commonly used in the manufacturing process of Ambriss, Serdaleh and Labneh El Darff, salinity content was measured using chloride test strips, in the range of 30 to 600 mg/L (QuanTab Titrator, Hach, Loveland, Colorado USA). All measurements were performed in triplicate.

IV.4. Culture dependent analyses

IV.4.1. Sample preparation

For culture dependent analyses, samples were rapidly diluted in sterile buffered peptone water (Oxoid, Cambridge, UK) at the rate of 1:10 (10 g of the fermented product in 90 mL of the buffer). Samples were then homogenized in a stomacher (BagMixer 400 CC, Interscience, and Hoboken, USA) for 2 min at a speed of 300 rpm.

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IV.4.2. Bacterial enumeration, isolation and preservation

Enumeration of total mesophilic microflora and of lactic acid bacteria was carried out by spreading the diluted samples, in triplicate, onto the following specific media: PCA (Plate Count Agar, Scharlab, Barcelona, Spain), MRS (De Man, Rogosa and Sharpe agar, pH 6.2, Sigma-Aldrich, Saint-Louis Missouri, USA), M17 (pH 6.5, Sigma-Aldrich, Saint-Louis Missouri, USA), BEA (Bile Esculine Agar, Sigma-Aldrich, Saint-Louis Missouri, USA), and KF (Kenner-Faecal Streptococcal Agar Base, HiMedia Laboratorie, India). Plates were incubated anaerobically and aerobically according to specific culture conditions **(Table IV.1).** Colonies were counted by considering 2 successive dilutions in triplicate. Results were given in Colony Forming Unit per mL of sample (CFU/mL).

TABLE IV-1. LIST OF CULTURE CONDITIONS ON DIFFERENT SELECTIVE MEDIA TYPES FOR THE ISOLATION OF LAB STRAINS

Targeted microorganisms	Types of media	Conditions			References
		Temp (°C)	T (h)	Incubation	
Mesophilic *Lactobacilli*	MRS Agar amended with cyclohexamide 0.01% (v/v)	30	74	Anaerobic	Wang *et al.*, 2016; Lima *et al.*, 2009
Thermophilic *Lactobacilli*	MRS Agar amended with cyclohexamide 0.01% (v/v)	42	48	Anaerobic	Wang *et al*., 2016
Mesophilic *Lactococci*	M17 Agar	30	48-72	Aerobic	Atlas, 2004; Terzaghi and Sandine, 1975
Lactic *Streptococci*	M17 Agar amended with 0.04 g/L of nalidixic acid	37	48-72	Aerobic	Atlas, 2004; Terzaghi and Sandine, 1975
Enterococcus ssp	BEA Agar	37	48	Aerobic	Facklam and Moody, 1970
Streptococcus faecalis	KF Agar	37	48	Aerobic	Kenner *et al.*, 1961

In total, 404 isolates were isolated. They were all Gram positive, catalase and oxidase negative.

In order to preserve the isolated strains for long term storage, each colony was purified by successive streaking on its selective agar medium. One colony from each of the purified cultures was transferred into 10 mL broth of MRS, M17 or BAE in Falcon tubes and incubated

for 48 hours in the convenient conditions. After incubation, tubes were centrifuged at 4000 rpm for 5 min. For Lactobacilli isolates, 1 mL from the formed pellets from each tube was supplemented with glycerol (20% v/v) and transferred into 1.8 mL cryotubes containing MRS broth for Lactobacilli or M17 broth for Lactococci, Streptococci and Enterococci supplied. For long term preservation, the cryotubes were kept at -20°C and -80°C.

From the 404 isolates, 331 were then identified by morphological, physiological and biochemical tests. Strains were taxonomically verified by genetic 16S rRNA analyses.

IV.4.3. Morphological examination and phenotypic characterization

Microscopic and macroscopic examination of the presumptive LAB strains was done on the basis of distinct colony morphologies (shape, color and size). Four to six colonies with different morphologies were selected from each plate and purified by repetitive cultures streaking on the same medium.

Isolates were assessed by Gram staining, catalase activity and gas production tests. The Gram staining of the isolates was determined as described by (Harigon and McCane, 1998). The catalase activity was measured by using hydrogen peroxide solution 3% (Merck, Maharashtra, India). Gas (CO2) production tests were conducted according to the method described by (Schillinger, 1987). Tested isolates were incubated in citrate omitted MRS broth or M17 broth supplemented with glucose at a concentration of 20 g/L (Merck, Darmstadt, Germany) along with inverted Durham tube for 48 to 72 h. Gaz formation was assessed according to presence of broth turbidity and Druham tubes elevation compared to control groups.

Phenotypic identification of the isolates was determined by the establishment of their carbohydrate fermentation patterns using API 50 CH and API 20 STREP test kits (BioMérieux Inc., Marcy l'Etoile, France) for *Lactobacilli,* and *Lactococci* and *Streptococci* strains respectively.

IV.4.4. Extraction of genomic DNA

In order to identify the different isolated morphotypes, bacterial DNA was extracted as the following: A pool of 4 to 5 colonies originating from one colony were first suspended in 400 µL Tris-EDTA buffer (TE 1X, pH 8.0) into a 2 mL tube. DNA was then lysed using a bead beater homogenizer BeadBlaster 24 Microtubes, Thomas Scientific, New Jersey, USA), with

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100 mg glass beads of 0.1 and 100 mg of 0.5 mm diameters. The extracted DNA was then recovered by centrifugation for 20 s at of 4.5 m/s.

IV.4.5. Identification of genomic DNA by 16S rRNA analyses

Bacterial isolates were genetically identified by 16S rRNA analysis according to the universal method described by (Muyzer *et al.*, 1993**).** The extracted DNA was amplified by Polymerase Chain Reaction (PCR) that was processed on a thermocycler (BioRad thermocycler T100, Hercules, CA, USA). for 30 cycles were applied on a final mix of 25 µl containing the following universal primers: Bact-27F (5′-TGA ATT GTA ATA C GA CTC ACT ATA GGG GTT TGA TCC TGG CTC AG-3′) and 1492R (5′-CGG CTA CCT TGT TAC GAC-3′). The expected amplicon size was close to 1400 bp, using a polymerase enzyme (TaKaRa ExTaq DNA polymerase Cat nb RR001, Takara Bio. Inc., Shiga, Japan). The PCR product was finally purified using a specific rapid purification kit (GenElute PCR clean-Up kit NA1020, Sigma-Aldrich, MO, USA). Genotypic identification is carried out by blasting the different genomic sequences of the isolated strains using the National Center for Biotechnology Information (NCBI) database.

IV.5. Culture independent analyses

To characterize the microbial composition of the traditional fermented goat milk products, different metagenetic and metagenomic analyses were carried out at the Laboratory of Food Microbial Ecology, Micalis Institute, INRAE, Jouy-en-Josas. In total 34 collected samples were studied by metagenetic (11 Ambriss, 19 Serdaleh, 4 Labneh El Darff). From the results of these analyses, 16 fermented products (6 Ambriss, 6 Serdaleh and 4 Labneh) were selected for more in-depth studies such as taxonomic composition and profiling with MetaPhlan and ychF gene marker by the Shotgun analysis. The selected products are listed in **Table IV.2**.

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TABLE IV-2. LIST OF ALL SAMPLES STUDIES FOR METAGENETIC AND FROM WHICH THOSE SELECTED FOR METAGENOMIC ANALYSES. A; AMBRISS, S: SERDALEH, L: LABNEH EL DARFF.

Sample's code	Producer	Collection date	Collection from the Jar or Bag	Metagenomic analyses
A1	P1	May-2019	Surface	X
A1-b	P2	July-2019	Surface	X
A2	P2	May-2019	Surface	X
A2-b	P2	July-2019	Surface	
A3	P3	May-2019	Surface	
A3-b	P3	July-2019	Surface	
A4	P4	May-2019	Surface	X
A4-b	P4	July-2019	Surface	X
A5	P5	May-2019	Surface	
A5-b	P5	July-2019	Surface	
A6	P6	May-2019	Surface	X
S7	P7	May-2019	Surface	
S7-b	P7	July-2019	Surface	X
S8	P8	May-2019	Surface	X
S8-b	P8	July-2019	Surface	X
S9	P9	May-2019	Surface	
S9-b	P9	July-2019	Surface	X
S10	P10	May-2019	Surface	
S10-b	P10	July-2019	Surface	X
S11	P11	May-2019	Surface	
S11-b	P11	July-2019	Surface	X
S12	P12	May-2019	Surface	
S13	P13	May-2019	Surface	
S14	P14	May-2019	Surface	
S19-b	P19	July-2018	Surface	
S19-b-c	P19	July-2018	Middle	
S19-c	P19	May-2018	Middle	
S20	P20	May-2018	Surface	
S20-c	P20	May-2018	Middle	
S20-c-b	P20	July-2018	Middle	
L15	LP1	Nov-2019	Surface	X
L16	LP2	Nov-2019	Surface	X
L17	LP17	Nov-2019	Surface	X
L18	LP18	Nov-2019	Surface	X

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IV.5.1. Sample DNA extraction

Product samples (0.5 g) were first diluted twice (w/v) in guanidinium thiocyanate 4 M solution (Sigma-Aldrich, St. Louis, MO, USA) with Tris HCl 0.1 M and proceeded as for DNA extraction. The mixture was then added with a 10 % N-lauryl sarcosine (Sigma-Aldrich) in a proportion of 1:0.08 (v/v), vortexed and centrifuged at 4°C, 14,000 g for 30 min. Supernatant was discarded and extraction was continued on the emerged pellet as described by (Almeida *et al.*, 2014). DNA was visually evaluated on 0.8% agarose gel and quantified by a fluorometer (Qubit 2.0, ThermoFisher Scientific, Waltham, USA) supplied with Qubit dsDNA HS Assay Kit (ThermoFisher Scientific, Waltham, USA).

IV.5.2. Sample metagenetic analyses

To analyze microbial diversity in the sample product, the amplified region V3-V4 of the 16S rRNA gene was amplified using primers V3F (5'-ACGGRAGCWGCAGT-3') and V4R (5'-TACCAGGGTATCTAATCCT-3'). The PCR was performed with MTP Taq DNA Polymerase enzyme (Sigma-Aldrich, St. Louis, MO, USA). Cycling conditions were: 94°C for 1 min, followed by 30 cycles of amplification at 94°C for 1 min, 65°C for 1 min, and 72°Cfor 1 min, with a final extension step of 10 min at 72°C. Sequencing was performed with V3 Illumina MiSeq kit, as described in Poirier *et al.*, (2018) and fastq files were generated at the end of the run (MiSeq Reporter software, Illumina, USA).

The quality of the raw data was evaluated with FastQC (Wingett and Andrews, 2018) and the sequences were imported into the FROGS (Find Rapidly OTUs with Galaxy Solution) pipeline (Escudié *et al.*, 2018) to obtain the Operational Taxonomic Units (OTUs). The sequences were filtered by length (150–500 bp) and then pooled into OTUs with SWARM algorithm (Mahé *et al.*, 2014) with the distance parameter of 1. Chimeras were removed with VSEARCH (Rognes *et al.*, 2016) and OTUs with at least 0.0054% in the whole dataset were retained (Bokulich *et al.*, 2013) OTUs were affiliated with SILVA 138.1 databases (Quast *et al.*, 2013). Alpha-diversity and beta-diversity analyses were performed in R Studio v.3.6.1 using the phyloseq and ggplot2 packages v1.30.0 (Poirier *et al.*, 2018; McMurdie and Holmes, 2013).

Statistical analyses were performed on alpha-diversity using Kruskall-Wallis and linear model regression. DESeq2 was applied to characterize statistically significant differentially abundant OTUs in the different products (Love *et al.*, 2014). The PCoA was plotted with ward D2 method and Permanova tests were carried out between samples.

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In total 30 samples were collected and analyses from the three goat milk fermented products (11 Ambriss, 15 Serdaleh and 4 Labneh El Darff).

IV.5.3. Taxonomic composition and metagenomic Shotgun analyses

Sample product DNA was sequenced using Illumina HiSeq2500 technology at GATC-Biotech (Konstanz, Germany), which yielded between six and eight million paired-end reads of 150-nucleotide length. Metagenomic reads corresponding to the *Capra hircus* genome were filtered with Bowtie2 (Langmead and Salzberg, 2012) which are visualized with Sam tools flagstat (Li *et al.*, 2009). From the fastq file generates, microbial composition was estimated by mapping the samples reads against the representative clade-specific marker catalog contained on the MetaPhlAn tool v. 3.0.4 (Truong *et al.*, 2015).

As an additional profiling method, gene marker prediction was used to allow binning of assembled metagenomics contigs without the need for reference sequences. Therefore, the reads were merged and *de novo* assembly was performed using SPAdes v. 3.9 (Bankevich *et al.*, 2012). Genes were then predicted using Prodigal (v.2.6.3) and marker genes were extracted using fetchMG, v.1.0 (Sunagawa *et al.*, 2013; Ciccarelli *et al.*, 2006). Profiling was carried out with the COG0012 ychF gene and Blasts (Basic Local Alignment Tool) were performed on available sequences in the National Center for Biotechnology Information (NCBI) for characterization of closet homologues.

IV.5.4. Metagenome Assembled Genome (MAG) analyses

Genome binning assembled from three products, was performed using MetaBAT2-2.12.1 (Kang *et al.*, 2015) with minimum contig size of 1,500 nucleotides and the default settings. The quality of the resulting prokaryotic bins were assessed with CheckMnd (Parks *et al.*, 2015) and MAGs < 80% completeness and/or > 10% contamination were excluded.

In addition and as part of LAB strain-level analysis, several MAG assemblies were refined by additional binning, such as blastn analysis with reference genomes of related species. Contigs were then filtered by percentage of the length covered, identity and coverage levels. Finally, manual curing of questionable contigs (new, low coverage of homology on references, etc.) were performed by blastn and blastx analysis against nr/nt and nr NCBI databases, respectively. Annotations were performed using the Rapid Annotation using Subsystem Technology server.

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IV.5.5. Phylogenomic and functional analyses

The Automatic Multi-Locus Species Tree web server (Alanjary *et al.*, 2019) was used to determine closely related genomes based on core gene alignments of the recovered MAGs. The closest species were inferred based on the percentage of average nucleotide identity (ANI) calculated using FastANI, v.1.31(Jain *et al.*, 2018).

The single nucleotide polymorphism package kSNP (v.3.0) was used to perform the phylogenomic analysis for the most representative species present in MAGs (Gardner *et al.*, 2015) with the maximum likelihood option and kmer size of 21. Genomes of the corresponding species were downloaded from Genbank and combined with our MAG to compute this analysis.

Gene content analysis and metabolic reconstruction was performed on SEED using sequence and function based comparison tools (Aziz *et al.*, 2012). The search for antibiotic resistance (ABR) genes was performed by read mapping against the CARD database (Jia *et al.*, 2017: McArthur *et al.*, 2013) using the PATRIC web server (Antonopoulos *et al*., 2018).

IV.6. Study of technological properties of the isolated strains

The acidification activity of 40 strains isolated from the fermented products Ambriss, Serdaleh, Labneh El Darff and from laban and raw milk were evaluated at UMR SayFood (AgroParisTech-INRAe, Palaiseau). These isolates are listed in **Table III.3**. The physiological characteristics, textural properties, viscosity and syneresis were analyzed at the Milk Department of Fanar Station (LARI, Lebanese Agricultural Research Institute, Beirut). The ability of the isolated strains to produce different volatile organic compounds (VOC) was determined at the Analytical Testing Laboratory (ATL) of the American University of Sciences and Technology (AUST, Beirut Campus, Lebanon).

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TABLE IV-3. LIST OF LAB STRAINS REPRESENTING 9 SPECIES ISOLATED FROM AMBRISS, SERDALEH, LABNEH EL DARFF, LABAN (RAW MATERIAL OF LABNEH), RAW GOAT MILK USED FOR THE PRODUCTION OF AMBRISS (MA) AND RAW GOAT MILK USED FOR THE PRODUCTION OF SERDALEH (MS).

Isolate code	Strain taxonomy	Strain code	Product type	Producer code	Region
S01	*Lactococcus lactis*	LL01	MS	P3	Chouf
S03	*Lactococcus lactis*	LL03	MS	P3	Chouf
S04	*Pediococcus acidilactici*	PA04	Ambriss	P1	Bekaa
S05	*Pediococcus acidilactici*	PA05	Ambriss	P1	Bekaa
S06	*Lentilactobacillus kefiri*	LK06	Serdaleh	P4	Chouf
S07	*Lentilactobacillus kefiri*	LK07	Serdaleh	P3	Chouf
S08	*Lentilactobacillus kefiri*	LK08	Serdaleh	P3	Chouf
S09	*Lentilactobacillus kefiri*	LK09	Serdaleh	P3	Chouf
S10	*Lentilactobacillus kefiri*	LK10	Serdaleh	P4	Chouf
S11	*Lactiplantibacillus plantarum*	LPP11	Ambriss	P2	Bekaa
S12	*Lactiplantibacillus plantarum*	LP12	MA	P1	Bekaa
S13	*Lactiplantibacillus plantarum*	LP13	MA	P3	Chouf
S14	*Lactiplantibacillus plantarum*	LP14	Ambriss	P2	Bekaa
S15	*Lactiplantibacillus plantarum*	LP15	Labneh	P5	South
S17	*Lactiplantibacillus plantarum*	LP17	Laban	P6	South
S18	*Lactiplantibacillus*	LP18	Laban	P6	South
S19	*Lactiplantibacillus plantarum*	LP19	MA	P1	Bekaa
S20	*Lacticaseibacillus rhamnosus*	LR20	Ambriss	P1	Bekaa
S21	*Lacticaseibacillus rhamnosus*	LR21	Ambriss	P2	Bekaa
S22	*Lacticaseibacillus rhamnosus*	LR22	Ambriss	P1	Bekaa
S24	*Lactobacillus kisonensis*	LKN24	Labneh	P6	South
S26	*Lactobacillus kefiranofaciens*	LKF26	Ambriss	P2	Bekaa
S28	*Lactobacillus kisonensis*	LKN28	Laban	P5	South
S31	*Levilactobacillus brevis*	LB31	Labneh	P6	South
S33	*Levilactobacillus brevis*	LB33	Labneh	P6	South
S34	*Levilactobacillus brevis*	LB34	Labneh	P5	South
S35	*Levilactobacillus brevis*	LB35	Labneh	P5	South
S36	*Levilactobacillus brevis*	LB36	Laban	P5	South
S37	*Levilactobacillus brevis*	LB37	Laban	P5	South
S40	*Lactobacillus diolivorans*	LD40	Serdaleh	P3	Chouf
S43	*Lactobacillus diolivorans*	LD43	MS	P3	Chouf
S44	*Lactobacillus diolivorans*	LD44	MS	P3	Chouf
S45	*Lactobacillus diolivorans*	LD45	Serdaleh	P5	Chouf
S46	*Lactobacillus diolivorans*	LD46	Labneh	P5	South
S52	*Enterococcus durans*	ED52	Ambriss	P2	Bekaa
S53	*Enterococcus durans*	ED53	Ambriss	P1	Bekaa
S55	*Enterococcus durans*	ED55	Ambriss	P2	Bekaa
S57	*Enterococcus durans*	ED57	Ambriss	P2	Bekaa

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S65	*Enterococcus durans*	ED65	Ambriss	P1	Bekaa

IV.6.1. Acidification activity analyses

IV.6.1.1. Activation of the isolated strains

By streaking method, the stored lactic acid bacteria (-80 ºC) were recovered from the cryovials to be cultured on MRS or M17 agar and then, were incubated anaerobically at 37°C for 48 h and aerobically at 42 ºC for Lactobacilli and Enterococci respectively. After 2 successive cultures, pure strains from each plate were transferred into 15 mL Falcon tubes containing 10 mL of the corresponding broth. Tubes were then incubated for 24 hours at 37 ºC or at 42 ºC for MRS or M17 broth to yield a concentration of approximately 10^7 - 10^8 CFU/ml.

IV.6.1.2. Samples preparation

Samples were prepared in 250 mL sterilized Erlenmeyer flask each containing 150 mL of UHT half skimmed (1.5 % fat content) goat milk (Lactel, Auchan, France). Flasks containing milk were preheated at 37°C for 30 min. Each flask was inoculated with the activated strains at the rate of 1 % (v/v) which were used as single cultures. All flasks were then incubated at 37°C for 68 h until pH reached 4.2 (except for *Pediococcus acidilactici* at pH 6.4).

IV.6.1.3. Measurement of acidification activity by the Cinac system

Spinnler and Corrieu (1989) developed an automatic technique based on *on-line* measurement of the pH of lactic acid cultures at short intervals (30-300 s) and calculation of some key kinetic parameters.

The acidification activity of the 40 lactic acid strains isolated from the three products and their raw materials was quantified using the Cinac system (CINétique d'ACidification). This system proved to be effective with fermented milk samples even when cultured with mixed LAB strains (**Figure IV.1**). The system automatically records pH measurement at 3 min intervals and calculates the maximum acidification rate (in absolute value), the time and pH at which maximum acidification rate occurred and the times to reach determined pH values (Corrieu *et al.*, 1998; Béal *et al.*, 2001). **Figure IV.1** shows an example of acidification kinetics obtained with mixed cultures of lactic acid bacteria. A significant difference was observed between the two conditions, the strain mix (A) being highly acidifying as pH 4.5 was obtained after 334 min, while the strain mix B needed 485 min to reach pH 4.5

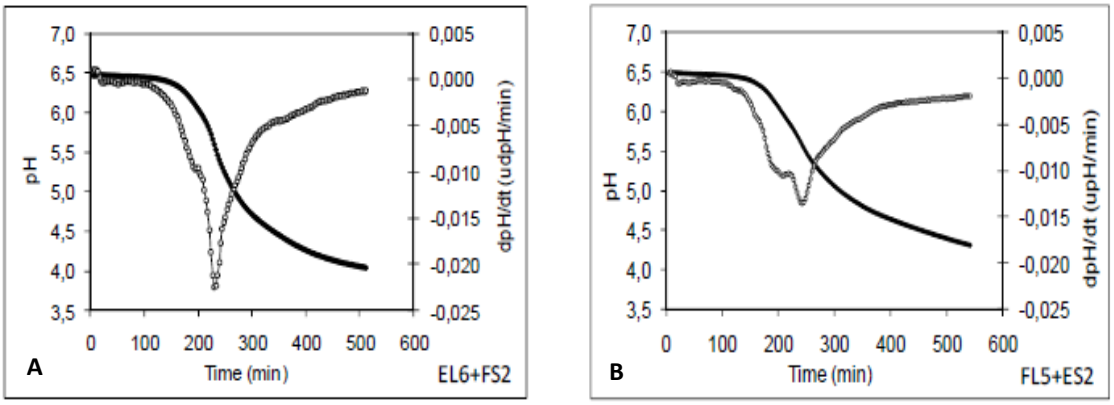

FIGURE IV-1. TYPICAL ACIDIFICATION KINETICS OBTAINED DURING THE MANUFACTURE OF TWO LABAN SAMPLES (BÉAL AND CHAMMAS, 2012)

(A) Strain mix: Strains *S. thermophilus* FS2 and *L. bulgaricus* subsp. *bulgaricus* EL6. (B) Strain mix: *S. thermophilus* ES2 and *L. bulgaricus* subsp. *bulgaricus* FL5.

In this study, half-skimmed heat-treated goat milk samples were inoculated at 37°C for 68 hours and pH was measured using WCidus Software (Béal and Corrieu, 1994). All measurements were made in triplicate and coefficients of variation (CV) were lower than 10%.

Acidification kinetics for each strain **(Figure IV.2)** were quantified according to the method described by Fonseca *et al.*, (2000) and the following descriptors were recorded:

- Vm: Maximum acidification rate (u. pH/min)
- Tm: Time to reach maximum acidification rate (min)
- pHm: pH at which maximum acidification rate occurred
- tpH5.5, tpH5.0 and tpH4.0: Times (in min) to reach pH5.5, pH5.0 and pH 4.5, respectively.

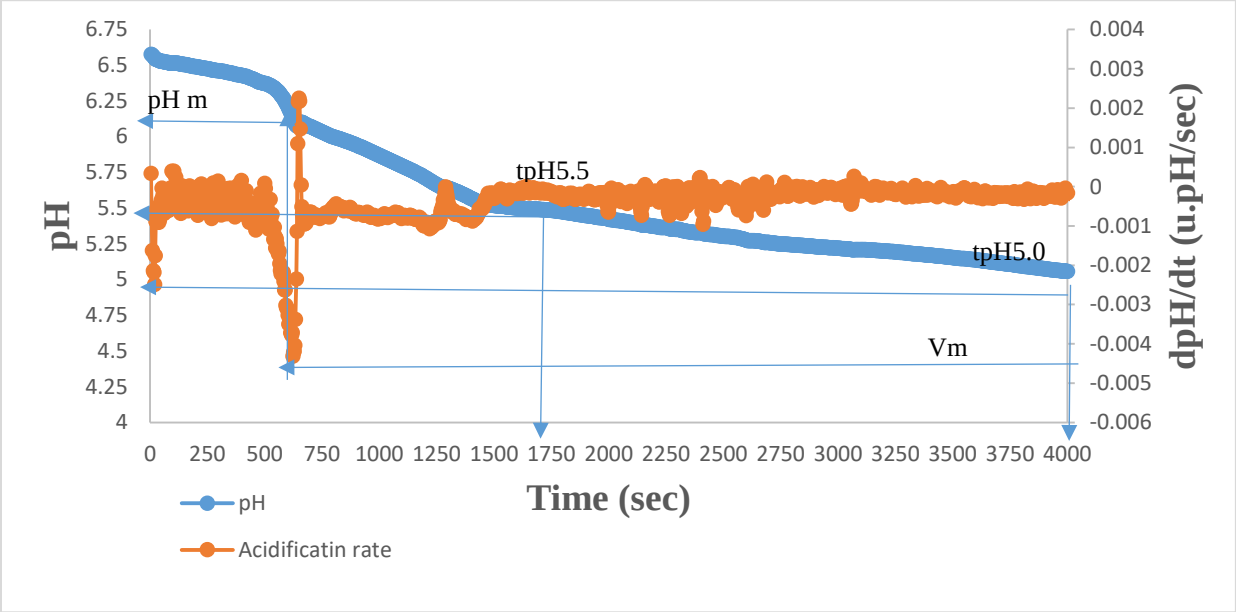

FIGURE IV-2. ACIDIFICATION KINETICS OF STRAIN LK07 *LACTOBACILLUS KEFIRI* THAT WAS ISOLATED FROM SERDALEH PRODUCT AND INOCULATED IN GOAT MILK AT 37°C.

IV.6.2. Determination of metabolic properties

Various metabolic properties have been characterized in this study. They concern the proteolytic activity, the lipolytic activity and the resistance to gastro-intestinal stress.

IV.6.2.1. Activation of bacterial strains

The preserved lactic acid strains were activated in 15 mL tubes containing 10 mL MRS or M17 broth (Oxoid, Basingstoke, UK) for *Lactobacilli* and *lactococci*, respectively. They were incubated at 37 ºC for 24 hours to reach a concentration of approximately 10^8 CFU/mL.

IV.6.2.2. Evaluation of proteolytic activity

The extracellular proteolytic activity of the strains was evaluated qualitatively following the method described by (Guillemard, 1986) Culture plates were prepared with Milk Plate Count Agar (Oxoid, Basingstoke, UK) containing 1 g/mL antibiotic free skim milk. Sterile Whatman paper discs (6 mm) were deposited on the plate surface. Bacterial aliquot of 20 µL culture of approximately 1-2.10^7 cells were spotted on each disc and plates were incubated at 37ºC for 24 h. Proteolysis was indicated by clear zones or halos around the discs. Proteolytic activity was expressed by the diameter in mm of the clear zone. All cultures were made in triplicate for each tested strain.

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IV.6.2.3. Evaluation of lipolytic activity

The lipolytic activity of the strains was evaluated qualitatively by agar spot test using glycerol plates according to the modified method described by Katz *et al.,* (2002). Tested strains were cultured overnight at 30 ºC and 42 ºC (*Lactobacillus, Lactococcus* and *Leuconostoc*) and 37 ºC (Enterococcus) in their corresponding media. Bacterial aliquots of 5 µL of the cultures at a concentration of ~10^8 CFU/mL were spotted on MRS agar containing 1% (v/v) glycerol and incubated at for 48 h at their corresponding culture temperatures. Lipolytic activity was observed by the detection of a clear zone around the colonies. All cultures were made in triplicate for each tested strain.

IV.6.2.4. Resistance to gastro-intestinal stress

Evaluation of the tolerance of the LAB to a simulated gastrointestinal digestion was conducted in duplicate, following the static method proposed by (Zárate *et al.*, 2000) with minor modifications to match human body conditions. Two successive stress conditions were applied, to simulate the gastric compartment and the duodenal compartment.

Suspensions of the activated strains were centrifuged at 4000 g for 20 min at 4°C. Bacterial cells were washed twice-using Phosphate Buffer Solution (PBS, Merck, Darmstadt, Germany) and 1.75 mL of cell pellet were transferred to 2.25 mL of a reconstituted gastric juice solution composed of NaCl 125 mmol/L, $NaHCO_3$ 45 mmol/L, KCl 7 mmol/L, pepsin 3 g/L and HCl in variable concentrations to adjust the final pH to 3. A control gastric juice was prepared by adding 5 N NaOH to reach pH 7. After incubation at 37°C for 3 h, the suspension was centrifuged and washed twice with PBS and 1.75 mL of the cell pellet was suspended in 2.25 mL of intestinal juice, composed of: pancreatin 2 mg/mL (CN Biomedicals Inc, California, USA) and bile salts solution 0.3% (w/v) (Oxgall Difco, Maryland, USA at pH 8) and incubated at 37 ºC for 3 hours. The bacterial concentration was then quantified by plate counts and compared to the initial concentration measured before the stress.

The ability of bacterial cells to tolerate gastrointestinal conditions was calculated as the survival rate (in %) after 3 h with pepsin/HCl at pH 3 and 3 h in the presence of bile salt and pancreatin as follows:

Survival rate (%) = ($\log_{10}$ N1 / $\log_{10}$ N0) x 100

Where $\log_{10}$ N0 is the log number of bacterial cells measured before the stress and $\log_{10}$ N1 is the log number of bacterial cells that survived after the gastrointestinal stress. The

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experiment was repeated after 15 days and the standard deviation of the repeatability was calculated.

IV.6.3. Analyses of textural and physical properties

The characterization of the physical properties of the fermented goat milk products with the selected strains was done according to different methods: Using texture analysis, the firmness, the cohesiveness and the fracturability were quantified. The syneresis of the products was measured statically. The viscosity was assessed using a rotary viscometer.

IV.6.3.1. Samples preparation

Full fat (4%) goat milk (Goût Blanc, White Farm SARL, Jbeil-Lebanon) was pasteurized at 75 ºC for 15 sec to be used in the texture profile analyses, viscosity, syneresis measurements and the production of volatile organic compounds. The prepared milk was then cooled and stored at 4 ºC till the next day of analysis. Prior to inoculation, milk was heated in water bath for one hour at 37 ºC. Then 1% (w/v) from each activated strain was added as single starter culture. The inoculated milk was swirled on a heating stirring plate for about 15 min and then fractioned as follows:

- 120 mL tapered sterile pots (12 pots / strain), each containing 100 mL for texture and viscosity measurements
- 25 g in 50 mL conical Falcon tubes (3 tubes / strain) for syneresis measurements
- 7 g in 12 mL glass vials for volatile organic compounds analyses.

All samples were incubated at 37ºC for 70 hours. Afterwards, fermentation was stopped when pH reached 4.2 by rapidly placing samples in ice without shaking them for one hour.

IV.6.3.2. Principle of texture analysis

The Texture Profile Analysis (TPA) of the fermented milk samples was carried out using the Texture Measurement System (TMS-Pro, Food Technology Corporation, Sterling, VA, USA) fitted with the V1.18 408 software package for data analysis (**Figure III.3**). The principle of the analysis consists of installing suitable probes to mimic the human chewing process. The device performs two successive compressions on each tested sample to determine three parameters: Firmness, Fracturability and Cohesiveness rate. Descriptors of force, distance and time data are collected and can be graphically represented and quantified.

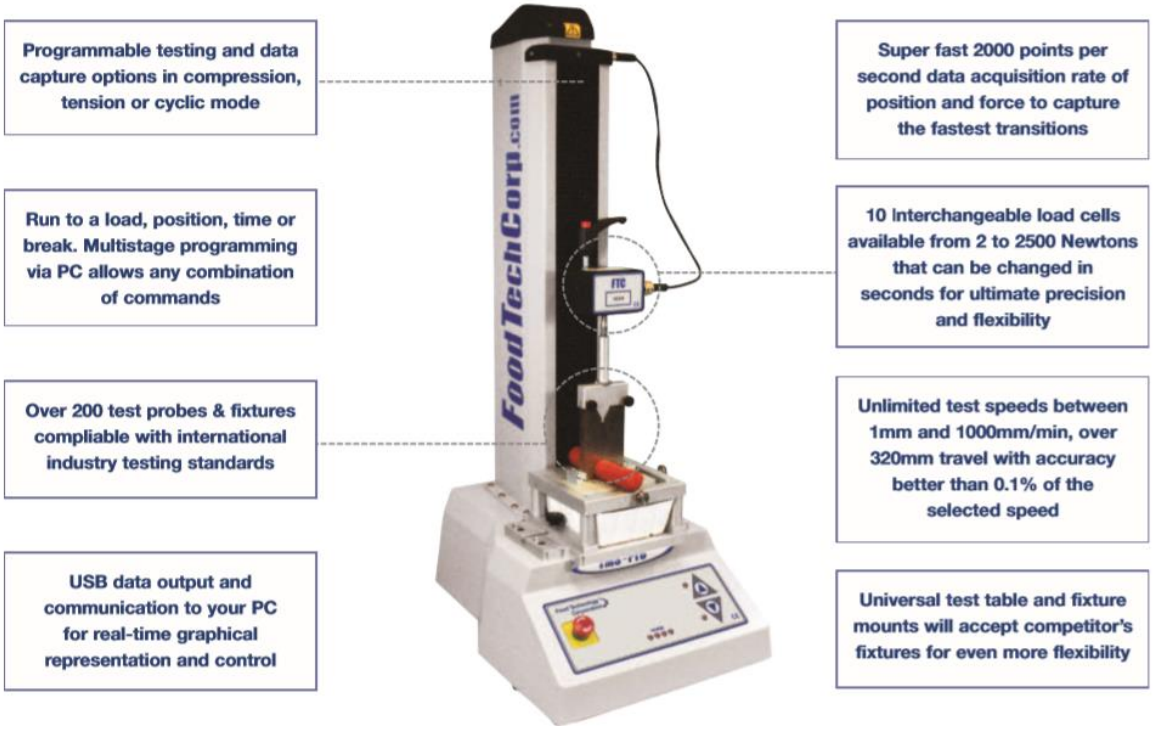

FIGURE IV-3. PICTURE OF THE TMS-PRO EQUIPMENT USED TO MEASURE TEXTURE PARAMETERS OF FERMENTED GOAT MILK SAMPLES INOCULATED WITH LACTIC ACID STRAINS AS SINGLE STARTER CULTURES AT 37°C

Before texture analyses, all fermented milk samples were stabilized at approximately 25°C, then placed on the plate (fixed support) of the analyzer, the axis of which being perpendicular to the moving part of the device. Measurements were carried out at about 5 °C, in triplicate, and coefficients of variation (CV) were lower than 10%.

IV.6.3.3. Firmness test

The firmness of a gel refers to the maximum force (F1) required for the cylinder probe (d=4.5 cm, h = 4cm) to penetrate the sample over a distance of 30 mm, at a constant penetration speed of 25 mm/min, at a temperature of 5 °C. It is given by the value of the first peak of force and is expressed in Newton (N). **Figure III.4** shows an example of a firmness graph obtained from testing a commercial Labneh sample.

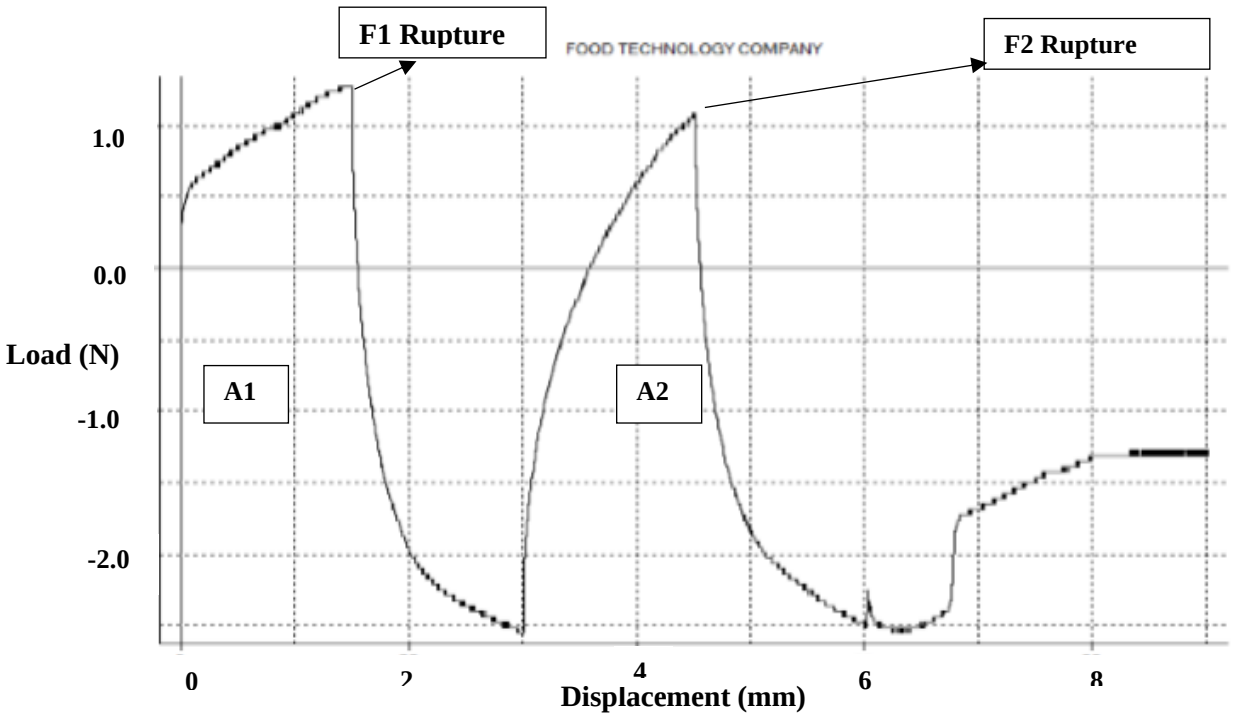

FIGURE IV-4. GRAPHICAL REPRESENTATION OBTAINED FROM A TEXTURE ANALYSIS. FORCES APPLIED TO IDENTIFY THE RUPTURE POINTS (F1AND F2) AND AREAS UNDER THE CURVE (A1 AND A2) WERE OBTAINED DURING TWO SUCCESSIVE COMPRESSIONS ON A COMMERCIAL LABNEH "BONJUS".

Forces applied to identify the rupture points (F1 and F2) and areas under the curve (A1 and A2) were obtained during two successive compressions on a commercial Labneh sample "Bonjus".

IV.6.3.4. Cohesiveness test

Cohesiveness reflects the internal agglomeration resistance constituting the body of the product. It refers to how a food product resists a second compression, relative to how it behaved upon the first compression. The measurement is carried out using a spherical probe with a diameter of 24.7 mm, with a penetration speed of 60 mm / min, at a temperature of 5°C. Cohesion is a dimensionless parameter which is represented by the ratio of the area measured under the curve of the second compression (A2) to the area measured under the curve of the first compression (A1), as illustrated in **Figure III.4**.

IV.6.3.5. Fracturability test

The fracturability (or brittleness) of a product represents its ability to crack or crumble in response to a weak force. It is related to its ability to deform due to the compression imposed by that force. Using the resulting graph **(Figure III.4)**, fracturability was determined as the

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distance (in mm) at the rupture point during the compression of the product by the probe, when a force of 0.4 N was applied to the sample at a temperature of 25 °C.

IV.6.3.6. Measurement of syneresis

Syneresis is defined as the appearance of whey on the surface of milk gel, as a result of the shrinkage of the gel formed simultaneously with liquid expulsion (Lucey *et al.*, 1998). It is generally expressed as the percentage of expelled liquid compared to the initial volume of the product (% v/v). In this study, the used method was based on sample centrifugation and whey collection according to the method described by Gentès et al., (2011).

The conical flacon tubes which contained 25 g of fermented milk products underwent high-speed centrifugation (Hermle z383k, LaborTechnik, Wehingen, Germany) at 210 g for 20 min at 4 °C. Then the mass of the separated whey in the supernatant was weighed and the syneresis (SYN) index was calculated according to the following equation:

SYN (%) = (Mass of collected whey / Mass of fermented milk) x 100

Results corresponded to the average of three independent measurements and coefficients of variation were comprised between 1% and 14%.

IV.6.3.7. Measurement of viscosity

The viscosity of fermented milk is evaluated using a rotational viscometer. This apparatus aims to measure the apparent viscosity and the shear stress of the product when it is subjected to a constant or increasing shear rate (Benezech and Maingonnat, 1994; Lewis, 1996). Apparent viscosity is an important evaluation factor for the fermented milk as is informs on its flow behavior, however these measurements are sample destructive (Li *et al.*, 2012). In contrast, complex viscosity informs on the viscoelasticity behavior of the sample which remains relatively untacked (Szczesniak, 2002). Viscosity is expressed in poise (P) or centipoise (cP) when using these viscometers which are relatively easy to use, however their measurement inaccuracy is at least ±10% (Morris and Langari, 2016).

The apparent viscosity (in Pa.s) of fermented milk samples was assessed using a Brookfield viscometer (DV-II + PRO, Brookfield Eng. Lab, Middleborough, MA, USA), equipped with a cylindrical spindle LV-2, according to the method described by Damian (2013). Samples were subjected to a shear rate of 100 rpm for 1 min which allowed to measure viscosities lower than 260 mPa.s. Samples temperature was stabilized at about 25°C before each

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measurement. Results were the average of three independent measurements. Coefficients of variation were comprised between 1% and 11%.

IV.7. Quantification of volatile organic compounds

The volatile organic compounds (VOC) of the milk fermented samples were extracted by using solid-phase micro-extraction technique (SPME) and analyzed by Gaz chromatography coupled to mass spectrometry (GC-MS). This technique was first introduced in 1989 by Arthur and his coworkers (Arthur and Pawliszyn, 1990) as a solvent-free extraction technique. It is cheap, easy to use, relatively fast, and needs a small amount of sample to permit the isolation of volatile compounds from both solid and liquid matrices (Iranmanesh *et al.*, 2018; Delgado *et al*., 2009). The SPME technique combines an absorption of the VOC on siliceous fiber coated with an appropriate stationary phase (Povolo and Contarini, 2003) to a static analysis of the head-space to (Harmon, 1997). These analyses were conducted on a selection of 15 strains that were previously chosen by considering their other technological properties (acidification activity and physical properties) to cover their diversity. They are listed in **Table III.4**.

TABLE IV-4. LIST OF LAB SELECTED FROM THE ISOLATED STRAINS ACCORDING TO THEIR DIFFERENT TEXTURAL PARAMETERS AND ANALYZED FOR THEIR PRODUCTION OF VOLATILE ORGANIC COMPOUNDS WHEN INOCULATED IN GOAT MILK.

Species	Number of strains	Isolate ode	Strain code	Origin
Lactococcus lactis	1	S03	LL03	Serdaleh milk
Lentilactobacillus kefiri	2	S07	LK07	Serdaleh
		S10	LK10	Serdaleh
Lactiplantibacillus plantarum	2	S14	LP14	Ambriss
		S15	LP15	Labneh El Darff
Lacticaseibacillus rhamnosus	1	S21	LR21	Ambriss
Lactobacillus kisonensis /parakefiri/buchneri	1	S28A	LKN28	Laban
Levilactobacillus brevis	2	S31	LB31	Labneh El Darff
		S35	LB35	Labneh El Darff
Lactobacillus diolivorans	4	S40	LD40	Serdaleh
		S44	LD44	Serdaleh milk
		S45	LD45	Serdaleh
		S46	LD46	Labneh El Darff
Lactococcus lactis ssp cremoris	1	S50	LC50	Serdaleh milk
Enterococcus durans	1	S50	ED55	Ambriss

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IV.7.1. Samples preparation

The fermented milks (7 g) were transferred into 20 mL vials and added with a mixture containing 2 g of NaCl (BioXtra, Merck, Darmstadt, Germany), 0.4 mL of ultra-pure water, 0.4 mL internal standard of tetrahydrofuran (Merck, Germany) (50 g/L) and 0.2 internal standard of valproic acid (Merck, Germany) (50 g/L). In this preparation, the salting-out method is carried out by the addition of NaCl, to increase the distribution constant between the fiber and the analytes since it minimizes the interaction of analytes with water and facilitate their passage into the headspace (Manousi *et al*., 2019). The first step consists of partitioning of analytes between the extraction phase and the sample matrix. The vials were then sealed with a polytetrafluoroethylene (PTFE) faced silicone septum (Supelco).

IV.7.2. Extraction by SPME

The extraction was carried out using a CAR⁄PDMS (carboxen⁄polydimethyl siloxane) fiber (Supelco, Bellefonte, PA, USA) of 75 µm film thickness coating the glass fiber silica. The vials were heated to 60° C and shaken at 500 rpm for 15 minutes to allow the volatile compounds to settle and to concentrate on the solid phase of the fiber. The analytes were adsorbed from the liquid sample on to the coated fused silica fiber, which is part of the syringe needle. The fiber is then inserted directly into a GC injection port for thermal desorption.

IV.7.3. Analysis of volatile organic compounds by GC-MS

The second step consists of the thermal desorption of the volatile compounds adsorbed on the fiber and their analysis by gas chromatography (GCMS-QP 2010 SE, Shimadzu, Kyoto, Japan). The Mass spectrometry (MS) is a highly-sensitive detector that separates and detects ions in the gaseous phase. When coupled to a GC, it immediately ionizes the gaseous eluted compounds, separates the ions in vacuum based on their mass-to-charge ratios (m/z) and eventually measures the intensity of each ion. These intensities are recorded to produce a series of mass spectra.

The volatile compounds were separated on a capillary column (DB 624 122-1334E, Agilent, Santa Clara, California, USA) of 30 m, 0.25 mm internal diameter, 1.4 µm film thickness. One microliter of the concentrated volatile extract was injected in the column in a splitless mode. The carrier gas used was helium and the column head pressure was maintained at 52.5 kPa. The flow rate was fixed at 1 mL/min and the linear velocity at 36.1 cm/sec. The

oven temperature was programmed as followed: after an initiation at 40°C for 4 min, the temperature was increase at 10°C/min until 260°C, then maintained at 260°C for 6 min.

Mass spectra of different treated samples were recorded with a mass range of 19 to 450 m/z at a 0.3 sec cycle time. The ion source was set at 200 °C and spectra were obtained by electron impact (0.91 kV). Compounds detected were identified by comparison with the FFNSC4 (Flavor and Fragrance Natural and Synthetic Compounds) series mass spectra database Library that is convenient for the identification of unknown components (Shimadzu, Kyoto, Japan). As for example, **Figures IV.5 and IV.6** show the resulting aromagrams of the quantified aroma peaks obtained by the GC-MS from a commercial fermented milk sample (Khoury) and a fermented milk inoculated with the strain *Lactococcus lactis* (S03) respectively.

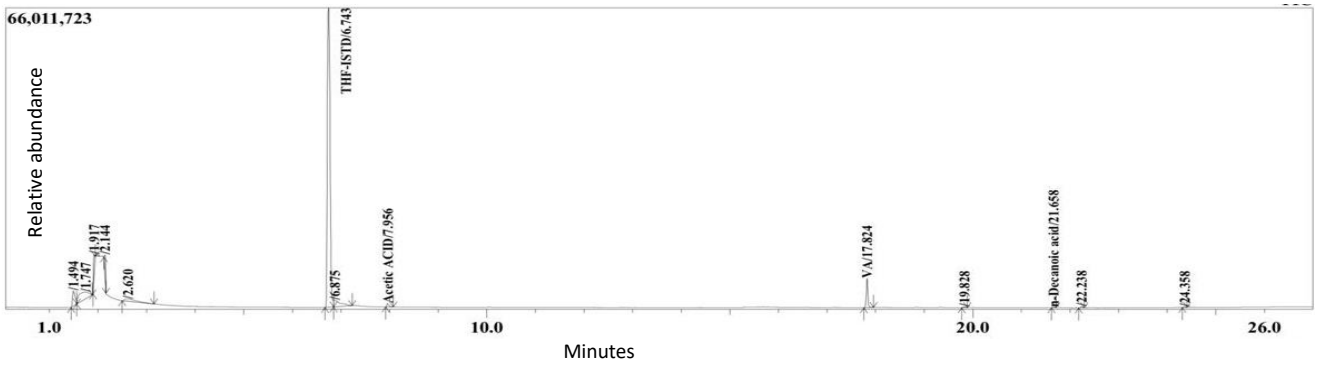

FIGURE IV-5. AROMAGRAM OF VOLATILE ORGANIC COMPOUNDS OBTAINED FROM A COMMERCIAL LABAN SAMPLE "KHOURY').

Identified peaks were: acetaldehyde, ethanol, acetone, 2,3-butanedione (diacetyl), acetoin, furfural, butyric, hexanoic, octanoic, benzoic, n-decanoic and acetic acids. Internal standards are: tetrahydrofuran (THF) and valproic acid (VA).

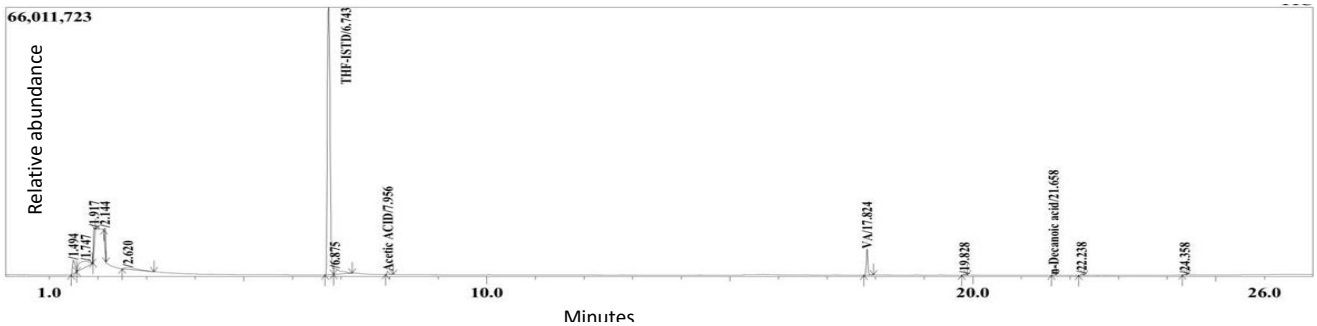

FIGURE IV-6. AROMAGRAM OF VOLATILE ORGANIC COMPOUNDS OBTAINED FROM FERMENTED MILK SAMPLE INOCULATED WITH THE COLLECTED STRAIN *LACTOCOCCUS LACTIS* (LL03).

Identified peaks were: acetaldehyde, ethanol, acetone, butyric, hexanoic, octanoic, benzoic, n-decanoic and acetic acids. Internal standards are: tetrahydrofuran (THF) and valproic acid (VA).

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IV.8. Statistical Analyses

Microbiological culture results were subjected to one-way variance analyses. Pearson correlation coefficients between the textural, flavor, viscosity and acidification activity parameters were carried out. Hierarchical cluster analysis was applied by using the Ward's method and the Euclidean distance measurement. In addition, PCA (Principal Component Analysis) was used to classify the studied strains according to their different functional and technological parameters. XLSTAT 2021.4 software (Addinsoft, Paris, France) was used to conduct the analyses of variance, the correlations, the hierarchical cluster analysis and the principal component analyses. Summary species for taxonmic composition plots were created in R v. 3.6.1 softwear (R Foundation for statistical computing, Vienna, Austria) using the package ggplot2 v. 3.3.2.

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V.RESULTS AND DISCUSSION: Lebanese fermented goat milk products, from tradition to meta-omics

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V.1. Preamble

The results and discussion of this study are presented in two major parts.

The first part (Chapter IV) aims to define and reveal the microbial composition and diversity of the three traditional fermented goat milk products: Ambriss, Serdaleh and Labneh El Darff using culture dependent and independent methods such as metagenetic and metagenomic tools. This part is presented as a submitted article for publication in the Journal of Food Research International (under revision).

The second part (Chapter V) is dedicated to the presentation of the results of the different functional and technological properties of the lactic acid bacteria isolated from these products.

Lebanese fermented goat milk products, from tradition to meta-omics

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Highlights

- Detailed analysis of the production chain of three emblematic Lebanese fermented goat milk products
- Ambriss and Serdaleh processes yield products with a composition similar to that of kefir
- Adaptation of the zoonotic agent *Streptococcus parasuis* to milk fermentation through the acquisition of lactose utilization genes
- Evidence of herd contamination by *Mycoplasmopsis agalactiae* through MAG analysis

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Abstract

Ambriss, Serdaleh and Labneh El Darff are traditional Lebanese fermented goat milk products from the Bekaa Valley, the Chouf Mountains and Southern Lebanon, respectively. A questionnaire completed by 50 producers of these products showed that they are prepared by periodic percolation either by milk or Laban in amphora or goatskin during the lactation season. Production is carried out on a small scale and in a limited number of production units, often by elderly people, entailing a real risk that these products may disappear and that their corresponding microbial resources will be lost. In this study, 34 samples from 18 producers were characterized by culture-dependent and -independent analyses. The results obtained by these two methods were radically different, in particular for Ambriss and Serdaleh, the latter revealing the co-dominance of *Lactobacillus kefiranofaciens*, a fastidious-growing species, and of *Lactococcus lactis* in a viable but not culturable state. Overall, their composition is reminiscent of that of kefir grains. However, phylogenomic and functional analyses of the genomes of the keystone species *L. kefiranofaciens* show that they differed from those of kefir, for example, in terms of their polysaccharide genes, explaining the absence of grains. Labneh El Darff displays dominance of *Lactobacillus delbrueckii*, probably due to the addition of Laban. In addition, several zoonotic pathogens were found, including *Streptococcus parasuis*, which was dominant in one sample and which MAG analysis revealed has acquired lactose utilization genes by horizontal gene transfer. The contamination of the herd with *Mycoplasmopsis agalactiae* in the Chouf region was also revealed by MAG analysis of the Serdaleh samples. Antibiotic resistance genes were detected in most of the samples, particularly in the Serdaleh samples, where the dominant *L. lactis* strains possessed a plasmid with a multi-resistance island. Finally, this study opens the way for further analyses to shed light on the resilience of these ecosystems established in amphora or in goatskins and to improve hygiene practices for milk production.

Keywords: Microbial diversity, kefir, metagenomics, genomics, ecology, VBNC, metagenome-assembled genomes, LAB, *Lactobacillus kefiranofaciens*, *Streptococcus parasuis*, *Mycoplasmopsis agalactiae*

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V.2. Introduction

The Mediterranean region is well known for its goat farming, providing about 21% of the world's goat milk supply (Miller & Lu, 2019; Dubeuf *et al*., 2016). Due to the nature of its composition, organoleptic characteristics and healthy attributes, goat milk has been the focus of interest in recent years (Prosser, 2021; Clark and Mora García, 2017). This sector has received increasing attention, particularly in Lebanon, and continues to grow (Kayouli, 2015). Goat milk products are highly appreciated by local consumers (Balaa and Marie, 2008), especially for their specific "goaty taste" (Semaan *et al*., 2011). Compared to cow milk, its casein proteins are more easily digested and it is better tolerated by people with mild lactose intolerance. It has a higher content of short-chain fatty acids in milk fat (Shu *et al*., 2016; Zenebe *et al*., 2014) and contains minerals such as magnesium, calcium and phosphorus (Abbas *et al*., 2014).

These goat milk products are made either from standardized milk at the industrial scale, such as Laban (Chammas *et al*., 2006a), Labneh (Serhan *et al*., 2016), Shankleesh (Serhan & Mattar, 2013: Toufeili *et al*., 1995), Kishk (Salameh et al., 2016a), or artisanally from raw milk, such as Darfiyeh (Hosri and El Khoury, 2004b), Aricheh (Serhan and Mattar, 2013), Serdaleh (Tabet et al., 2019c), Ambriss (Dimassi *et al*., 2020) and Labneh El Darff (Semaan *et al*., 2011). The diversity of these dairy products not only enriches the Lebanese culinary culture and pantry (Massaad, 2017), but also represents an important source of empowerment for women in poor rural areas. Female-headed households effectively contribute to the development of the goat rearing sector and the manufacturing of goat milk products (Kayouli, 2015). The market share of this sector is not well characterized; however, 70% of all small ruminant industries are located in rural areas with limited market access (Saadeh, 2016). Among these fermented goat milk products, Ambriss, Serdaleh and Labneh El Darff, made with milk from the Black Baladi race that grazes in the Bekaa Valley, the Chouf Mountains and Southern Lebanon, respectively (**Figure V.1A**), are real candidates to be awarded the PGI (Protected Geographical Origin) label according to the report of the SRVA (Service Romand de Vulgarisation Agricole; IDEAS Centre, 2005; Ministère de l'Economie et du Commerce de la République Libanaise, 2006). However, it appears that these products are in danger of disappearing, mainly due to commercial, regulatory and food safety concerns (Serhan & Mattar, 2018; Semaan *et al*., 2011). In fact, these products are made on a seasonal basis, at small or medium production scales and according to traditional methods (**Figure**

V.1B), by successive fermentation of raw goat milk in amphorae for Ambriss (Dimassi *et al*. 2020) and Serdaleh (Serhan and Mattar, 2013) and Laban in goatskins for Labneh El Darff (Serhan *et al*. 2016; Nsabimana *et al.*, 2005).

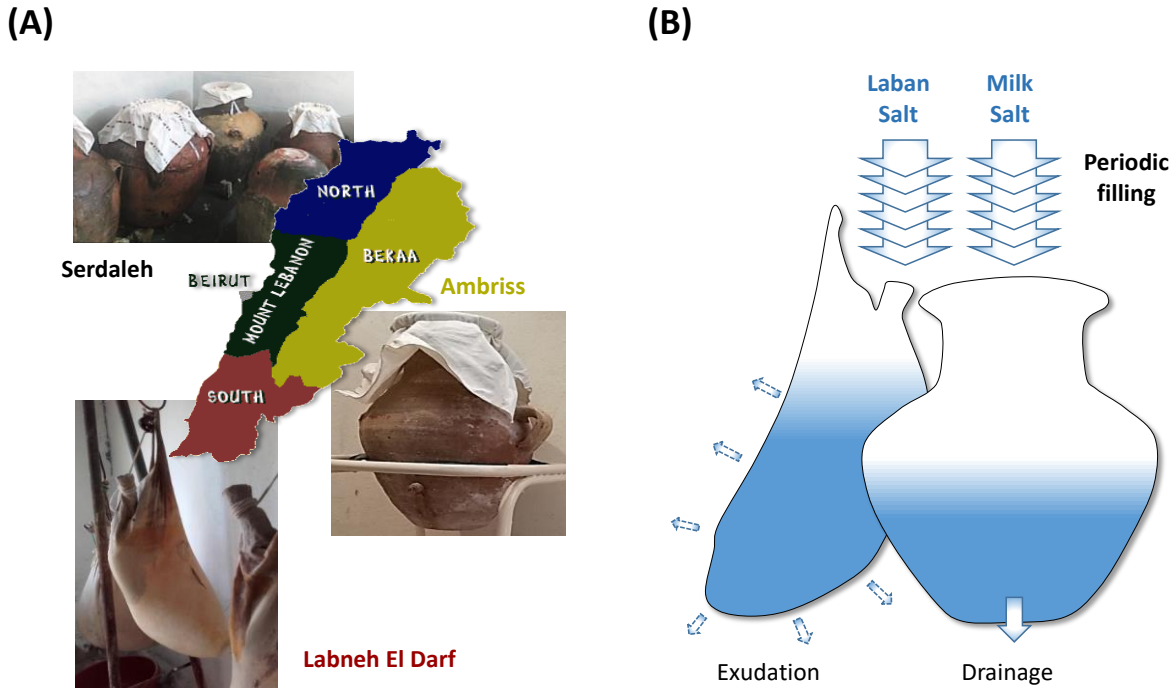

FIGURE V-1.GEOGRAPHICAL ORIGIN (A) AND SCHEMATIC PROCESSING DIAGRAM (B) OF AMBRISS, SERDALEH, AND LABNEH EL DARFF.

In the Mediterranean region, traditional fermented milk products are closely related to the area in which they are produced and the cultural history and heritage (Boyazoglu and Morand-Fehr, 2001). This fact has drawn the attention of many studies due to the unique microbial diversity of these products (Rhaiem *et al*., 2016; Abdalla & Hussain, 2010; Ayad *et al*., 2004; El Soda *et al*., 2003). Numerous genera of lactic acid bacteria (LAB) such as *Lactobacillus*, *Lactococcus*, *Enterococcus*, *Leuconostoc* and *Streptococcus* are generally involved in these transformations. However, only a few studies have described the native microbiota in the Lebanese fermented milk products, and the characterization of the isolates has been restricted to phenotypic and biochemical analyses. The first attempts were recorded on Laban, where 96 strains of *S. thermophilus* and *L. bulgaricus* were characterized (Chammas *et al*., 2006b). Serhan *et al*. (2009) then revealed the

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presence of the LAB *Streptococcus thermophilus*, *Enterococcus* spp., *Lactococcus lactis* subsp. *lactis* and *cremoris, Lactiplantibacillus plantarum*, *Lactobacillus curvatus* in Darfiyeh cheese using culture-dependent methods. Moreover, Dib *et al*. (2012) isolated 25 different strains in Serdaleh, Laban and Darfiyeh cheese belonging to the species *L. plantarum, L. paracasei, L. casei* and *Leuconostoc mesenteroides.*

Until now, these traditional Lebanese products have only been characterized using cultural methods, which provide a patchy view of the microbiota, whereas new methods for characterizing food ecosystems are now available, based on techniques such as 16S and shotgun metagenomic analyses (Walsh *et al.*, 2022). Approaches based on these techniques have already been used in a study on traditional Brazilian cheeses and provided a global picture of their microbiota, with taxonomic resolution close to the strain level for dominant populations and functional data at the gene level (Kothe *et al*., 2021). We therefore decided to develop a better understanding of the microbiota of Ambriss, Serdaleh and Labneh El Darff using these new methods to improve our knowledge of their processes, and to thus develop strategies to preserve them and boost their development. To do this, 33 samples of fermented goat milk products from 18 producers were characterized using a combination of culture-dependent and culture-independent analyses, including genomic analyses of isolated strains and metagenomic assemblies, as well as functional analyses based on deep metagenomic profiling. The results were interpreted with regard to the manufacturing processes and physico-chemical characteristics of the products.

V.3. Materials and Methods

V.3.1. Development of a producer's survey

A producers' survey was conducted over 50 breeders or producers from the three selected production regions – the Bekaa Valley, the Chouf Mountains and Southern Lebanon – for the artisanal products of Ambriss, Serdaleh and Labneh El Darff, respectively. The questionnaire consisted of 34 questions, covering three main areas: (i) socio-demographic aspects; (ii) breeding activities; and (iii) production activities, including commercialization. The questions included general and socio-economic information about producers, information regarding livestock size, goat breed, breeding type and practices, milk production volumes, as well as the type of dairy product produced, the production season, the raw materials used, production volumes, prices and

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marketing practices. In addition, a detailed observation of the processing was carried out in order to build a common production flow chart for each product type, showing the existing differences between the producers as well.

V.3.2. Sample collection, physico-chemical and microbiological analyses

Producers were first selected on the basis of the survey at the different production sites according to two main criteria: their production was small-scale and they had been producing their product for more than 15 years. In total, 34 artisanal samples from 18 producers were collected during two consecutive production seasons in 2018 and 2019.

Samples of fermented goat milk products (10 g) were taken aseptically and homogenized for 60 s in a laboratory blender (Blender LB20E, Waring, Torrington, CT, USA) with 90 mL and 20 mL of distilled water, for pH and salinity tests, respectively. The pH was measured using a digital pH meter (GLP 21, Crison instrument, Barcelona, Spain). The salinity content was determined using chloride test strips (30-600 mg/L; QuanTabTitrator, Hach, Loveland, CO, USA). All measurements were performed in triplicate. Samples were preserved in phosphate-buffered saline (PBS 15X, pH 8.7), and stored at -20°C for further analysis.

For culture-dependent analyses, samples were diluted and homogenized in buffer peptone water (2%). They were spread in triplicate on MRS (pH 6.2, Sigma-Aldrich, St. Louis, MO, USA) and M17 (pH 6.5, Sigma-Aldrich, St. Louis, MO, USA) agar plates. Plates were incubated for 48 h, anaerobically for MRS at 30°C and 42°C to allow mesophilic and thermophilic *Lactobacillus* counts, respectively, and aerobically for M17 at 30°C for *lactococci*.

Specific procedures were applied to isolate *Lc. lactis* and *Lb. kefiranofaciens* strains. Culture media were supplemented with 2% salt and 10% goat milk for *Lc. lactis*, or with 25% goat milk and adjusted at pH 5.3 for *Lb. kefiranofaciens*, and incubated anaerobically at 30°C for 10 days.

Based on their distinct morphology (shape, color and size), colonies were selected and purified by repetitive streaking on the same medium. Isolates were assessed for their catalase activity and Gram coloration according to the criteria described by Kandler and Weiss (1986), as well as for their ability to produce gas from glucose by inverted tubes (Zúñiga *et al*., 1993).

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Biochemical identification and carbohydrate fermentation patterns were determined by API 50 CH and API 20 STREP test kits (BioMérieux Inc., Marcy l'Etoile, France) following the manufacturer's recommendations.

In order to identify the different morphotypes, isolates were suspended in 400 µL Tris-EDTA (TE 1X, pH 8.0) buffer in a 2-mL tube. DNAs was lysed using a bead-beater (24 Microtubes BeadBlaster, Thomas Scientific, NJ., USA), with 100 mg zirconium beads of 0.1 and 0.5-mm diameters and homogenized for 20 s at 4.5 m/s. Once extracted, amplification of the coding gene for 16S rRNA was carried out for 30 cycles on a final mix of 25 µL containing the following universal primers: Bact-27F and 1492R, using Taqara DNA Taq polymerase (Almeida *et alet al*., 2014). Products were purified using a purification kit (Gen Elute PCR clean up, Sigma, St. Louis, MO, USA). Genotypic identification was carried out by blasting the different sequences of the isolated strains using the NCBI database.

V.3.3. Genomic DNA extraction and sequencing

For some isolates, DNA was extracted from the bacterial cells according to the protocol described by Kothe *et al*., (2021). DNA sequencing was carried out on an Illumina HiSeq at GATC-Biotech (Konstanz, Germany) to generate about 5-6 million paired-end reads (150 bases in length). For each strain, the paired-end reads were merged and *de novo* assembly was performed using SPAdes 3.9 (Bankevich *et al*., 2012). Only contigs with length > 300 bp and coverage > 100 were considered for further study. Annotations were performed with Rapid Annotation using the Subsystem Technology server (Aziz e*t al*., 2012).

V.3.4. DNA extraction from the products

Samples (0.5 g) were diluted 1:1 (w/v) in guanidinium thiocyanate 4M solution (Sigma-Aldrich, MO, USA) with TrisHCl 0.1M and processed in the same way as for the isolate DNA extraction. The mixture was then supplemented with 10% N-Lauryl sarcosine (Sigma-Aldrich, MO, USA), vortexed and centrifuged at 4°C and 14,000 *g* for 30 min. Supernatant were discarded and extraction was continued on the emerged pellet, as described by (Almeida *et al*., 2014). DNA was visually evaluated on 0.8% agarose gel and quantified by a fluorometer (Qubit 2.0, ThermoFisher Scientific) supplied with the Qubit dsDNA HS Assay Kit (ThermoFisher Scientific).

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V.3.5. Metagenetic analysis

Bacterial diversity was analyzed by sequencing the amplified region V3-V4 of the 16S rRNA gene using primers V3F and V4R. The PCR was performed with MTP Taq DNA Polymerase (Sigma-Aldrich, St. Louis, MO, USA), and cycling conditions were: 94°C for 1 min, followed by 30 cycles of amplification at 94°C for 1 min, 65°C for 1 min, and 72°C for 1 min, with a final extension step of 10 min at 72°C. Sequencing was performed with the V3 Illumina MiSeq kit, as described in Poirier *et al*. (2018), and fasta files were generated at the end of the run (MiSeq Reporter software, Illumina, USA).

The quality of the raw data was evaluated with FastQC (Wingett & Andrews, 2018) and the sequences were imported into the FROGS (Find Rapidly OTUs with Galaxy Solution) pipeline (Escudié *et al*., 2018) to obtain the Operational Taxonomic Units (OTUs). The sequences were filtered by length (480-580 bp) and then pooled into OTUs with SWARM (Mahé *et al*., 2014) with the distance parameter of 1. Chimeras were removed with VSEARCH (Rognes *et al*., 2016) and OTUs with at least 0.005% in the whole dataset were retained (Bokulich *et al.*, 2013). OTUs were affiliated with the 16S SILVA 138.1 database (Quast *et al*., 2013). Alpha-diversity and beta-diversity analyses were performed with R Studio v.3.6.1 using the phyloseq and ggplot2 packages (v1.30.0). Statistics were performed on alpha-diversity using Kruskal-Wallis and linear model regression. DESeq2 was applied to characterize statistically significant differentially abundant OTUs in the different products (Love *et al*., 2014). The PCoA was plotted with the ward. D2 method and Permanova tests were performed between samples.

V.3.1. Taxonomic composition by shotgun metagenomics study

Sixteen fermented products (six Ambriss, six Serdaleh and four Labneh) were selected on the basis of the metagenetic results for more in-depth analysis. The metagenomic DNA was sequenced using Illumina HiSeq2500 technology at GATC-Biotech (Konstanz, Germany). Metagenomic reads corresponding to the *Capra hircus* genome were filtered out by reads mapping against the *Capra hircus* reference genome (LWLT00000000) using Bowtie2 (Langmead and Salzberg, 2012). The proportion of goat reads in the samples was then assessed using samtools flagstat (Li *et alet al*., 2009). On the basis of the fastq file generated after host read filtration, we first estimated microbial composition by mapping the samples. Reads were mapped against the

representative clade-specific marker catalogue contained in the MetaPhlAn tool v. 3.0.4 (Truong *et al*., 2015).

In addition, we performed taxonomic profiling using an assembly-based marker gene analysis, which allows non-supervised binning of metagenomes using metaSPAdes v.3.9 (Bankevich et al., 2012). Genes were then predicted using Prodigal (v.2.6.3) and marker genes were extracted using fetchMG, v.1.0 (Sunagawa *et al*., 2013). Taxonomic assignation was carried out with the COG0012, *ych*F gene. Summary species composition plots were created in R v.3.6.1 using the package ggplot2 v.3.3.2.

V.3.2. Metagenome-assembled genome (MAG) analyses

Genome binning was performed using MetaBAT2-2.12.1 (Kang *et al*., 2015), with a minimum contig size of 1,500 nucleotides and the default settings. The quality of the resulting prokaryotic bins was assessed with CheckM (Parks *et al*., 2015), and MAGs < 80% completeness and/or > 10% contamination were excluded. Several MAG assemblies were refined by additional binning, such as blastn analysis with reference genomes of related species. Contigs were then filtered by percentage of the length covered, identity and coverage levels. Finally, manual curing of questionable contigs (new, low coverage of homology on references, etc.) were performed by blastn and blastx analysis against the nr/nt and nr NCBI databases, respectively. Annotations were performed using the Rapid Annotation with Subsystem Technology server.

V.3.3. Phylogenomic and functional analyses

The Automatic Multi-Locus Species Tree web server (Alanjary et al., 2019) was used to determine closely related genomes based on core gene alignments of the sequenced genomes and recovered MAGs. The closest species were inferred based on the percentage of average nucleotide identity (ANI) calculated using FastANI, v.1.31 (Jain *et al.*, 2018). The kSNP (v.3.0) was used to perform the phylogenomic analysis using distances based on single nucleotide polymorphism variations for the most representative species present in MAGs (Gardner *et al*., 2015) with the maximum likelihood option and kmer size of 21. Genomes of the corresponding species were downloaded from Genbank and combined with our MAG to compute this analysis.

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Gene content analysis and metabolic reconstruction was performed on SEED using sequence and function-based comparison tools (Aziz *et al.*, 2012). The search for antibiotic resistance (ABR) genes was performed by read mapping against the CARD database (Jia *et al*., 2017; McArthur *et al*., 2013) using the PATRIC web server (Antonopoulos *et al*., 2018) . The presence of particular genes in reference genomes, ABR and genes of technological interest was determined using the PATRIC web server, the FoodMicrobiome tool (https://migale.jouy.inra.fr/foodMicrobiome/, and blast analysis. Additionally, FoodMicrobiome was used to detect the subdominant populations of given species with high sensitivity and reliability and to discriminate between subspecies.

V.3.4. Data availability

Raw sequences were deposited on the European Nucleotide Archive (ENA) under the BioProject ID PRJNA860091 for shotgun metagenomic, amplicon and genomic reads. The MAGs are available in doi: 10.17632/7w4rd76ndj.2.

V.4. Results

V.4.1. Goat farmer and dairy producer's survey

According to the results of the questionnaire, 25% of the respondents were identified as both producers and farmers, and 75% as producers only. Production depended on female housekeepers (60%) aged between 40 and 70 years old, and had limited access to the local market place (38%). Most of producers (88%) used only goat milk compared to 4% who used a mixture of goat and cow milk, while the rest used goat Laban in addition to goat milk as the primary dairy material. Producers who reared their own goat herd were able to process larger milk quantities for the three fermented products (135 to 7,500 L/season) than producers who purchased their goat milk (140 to 1,890 L/season). Farming and production parameters for each product are summarized in **Table V.1**.

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TABLE V-1. PRODUCER'S SURVEY RESULTS OF PRODUCTION PARAMETERS, REARING PRACTICES, AND PRICE INDICATORS RELATED TO AMBRISS, SERDALEH AND LABNEH EL DARFF.

	Ambriss (n= 24)	**Serdaleh (n= 21)**	**Labneh El Darff (n= 5)**
Producers dedicated to one dairy product (%)	80	51.6	100
Milk processed by producers (L/season)	135-1890	110-1250	135-850
Milk processed by farmers-producers (L/season)	160-3200	220-7500	135-1350
Range of production (Kg/season)	20-500	25-1500	20-100
Mean production yield (Kg/100 L milk)	18.9 ± 4.1	19.2 ± 3.5	16.4 ± 3.2
Producers (%) with 1–3-month processing period	0	15	55.6
Producers (%) with 4–6-month processing period	100	75	44.4
Extensive goat farming system (%)	50	66.7	66
Feed concentrates added to goat rations (g/d)	200-250	0-300	200-400
Market price (€/kg)	8	9.4	18

Ambriss, Serdaleh and Labneh El Darff were made with milk from the Black Baladi race that grazes in the Bekaa Valley, the Chouf Mountains and Southern Lebanon, respectively (**Figure IV.1 A**). All production phases were completely manual, the production scale was small to medium (Third Quartile = 216.5 kg/season) and both tools and materials were artisanal. Production takes place during goat lactation period, i.e., from June to November. The manufacturing process of Serdaleh and Ambriss lasts up to 6 months and consists of fermenting raw goat milk on a daily basis in terracotta jars, except for Labneh el Darff in gaot skin. The jars were first soaked with salt, periodically refilled with fresh raw milk and salt, with periodic whey evacuation from a hole at their bottom (**Figure IV.1**).

The manufacturing of Labneh El Darff is conducted in a three-stage process consisting of: (i) Laban production, which is prepared from goat milk inoculated with a previously homemade preserved inoculum produced by backslopping; (ii) filling the "Darff" (bag formed by the whole goatskin, but sometimes cloth fabric) with the produced Laban; and (iii) straining of the whey from the suspended Darff during a period of 6 weeks (**Figure IV.1B**). The manufacturing processes of these products are carried out at ambient temperature.

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V.4.2. Physico-chemical and culture-dependent analyses

V.4.2.1. Temperature, pH and salinity

Temperature, pH and salinity levels were measured at the time the samples were collected (**Appendix IV.1**). The ambient temperature at which the products were developed varied between 28°C and 33°C for Ambriss and Labneh El Darff, but was lower ($p < 0.05$) for Serdaleh (between 20°C to 25°C). The pH values showed little variability within the same product type, unlike salinity, which displayed greater intra-group variability (**Figure V.2A)**.

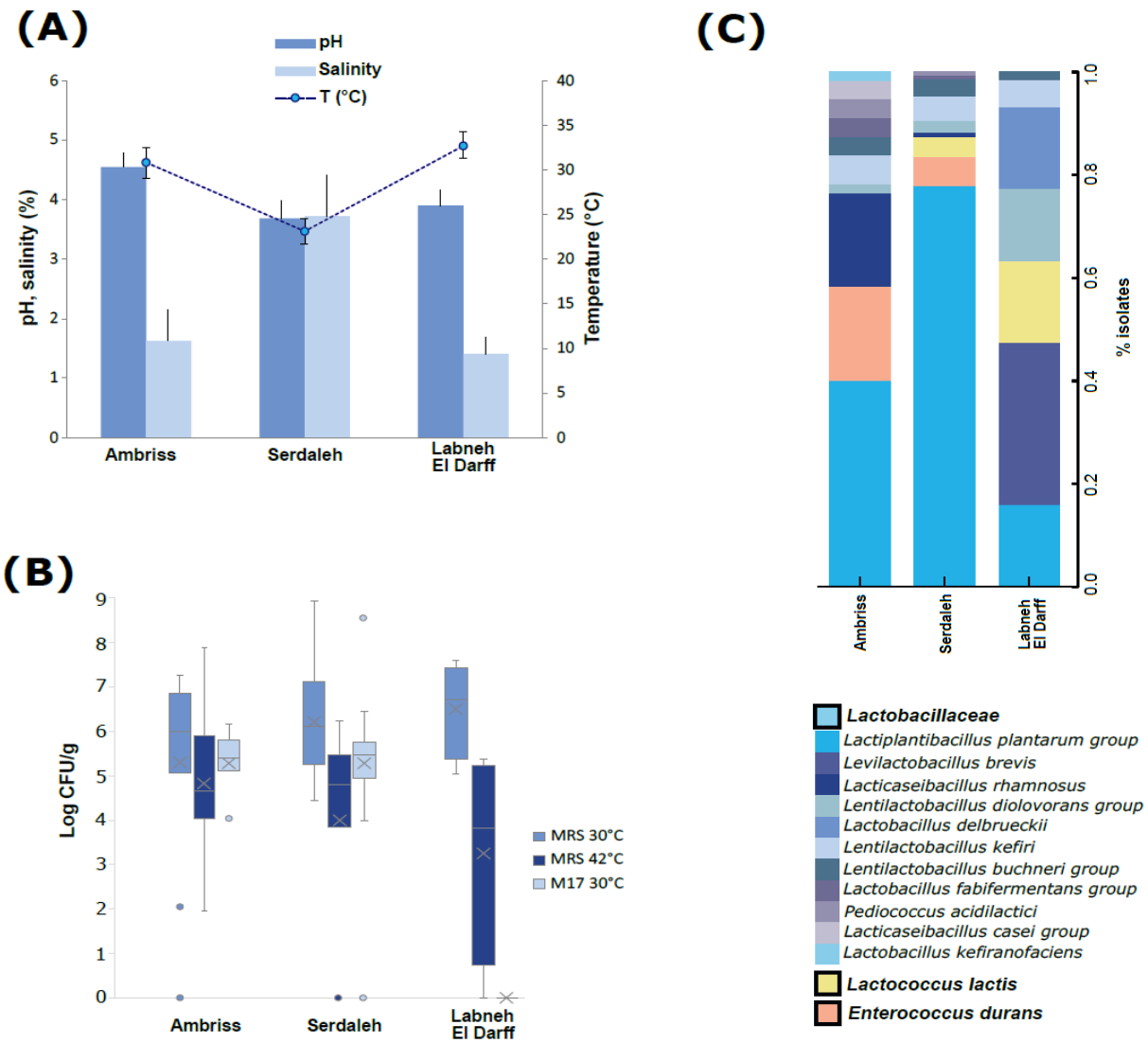

FIGURE V-2. PYHSICO-CHEMICAL AND MICROBIOLOGICAL CHARACTERISTICS OF AMBRISS, SERDALEH AND LABNEH EL DARFF: TEMPERATURE, PH AND SALINITY (A); BOXPLOT REPRESENTATON OF MICROBIAL COUNTS ON M17 AND MRS (B); AND HISTOGRAM OF ISOLATED SPECIES (C).

The pH values were comprised between 3.01 and 4.80. They were higher ($p < 0.05$) in Ambriss (4.08 to 4.80) when compared to Serdaleh (3.01 to 4.12) and Labneh El Darff (3.62-4.62).

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The Serdaleh product had the highest level ($p < 0.05$) of salinity (2.69 to 5.04%) compared to Ambriss (0.87 to 2.24%) and Labneh El Darff (1.10 to 1.75%).

V.4.2.2. Culture-dependent analyses

The examination of culture results shows a significant variability in the different microbial enumerations (**Figure V.2B**). We observed a dominance of mesophilic lactobacilli (MRS 30°C, 5.4-6.8 Log CFU/g) over lactococci (M17 30°C, 0.5-5.20 Log CFU/g) in the three products. Thermophilic lactobacilli counts were highly variable in Labneh el Darff (MRS 42°C, 1-6.2 Log CFU/g) compared to its other counterparts.

Overall, 279 strains belonging to 13 species groups were genetically identified by 16S rRNA from 331 isolates. The most commonly cultivated species in the three fermented milks was *Lactiplantibacillus plantarum*. This species appeared to be dominant in Ambriss (38% of isolates in this product) and Serdaleh (77%), and co-dominant with other species in Labneh El Darff (16%). In Ambriss, we also identified a strong prevalence of the species *E. durans* and *Lacticaseibacillus rhamnosus* (18% of each), in Labneh El Darff, *Levilactobacillus brevis* was the most frequently obtained cultured species (32% of isolates), followed by *L. delbrueckii* and *Lc. lactis* (16% of each) (**Figure V.2C).**

V.4.2.3. Taxonomic composition using amplicon sequencing

The 16S rRNA amplicon-sequencing analyses were clustered in 209 OTUs, whose abundance was over the threshold of 0.005% in the whole dataset. The rarefaction curves of most of the analyzed samples reached the saturation plateau, indicating that the sequencing depth was sufficient (**Figure V.3**).

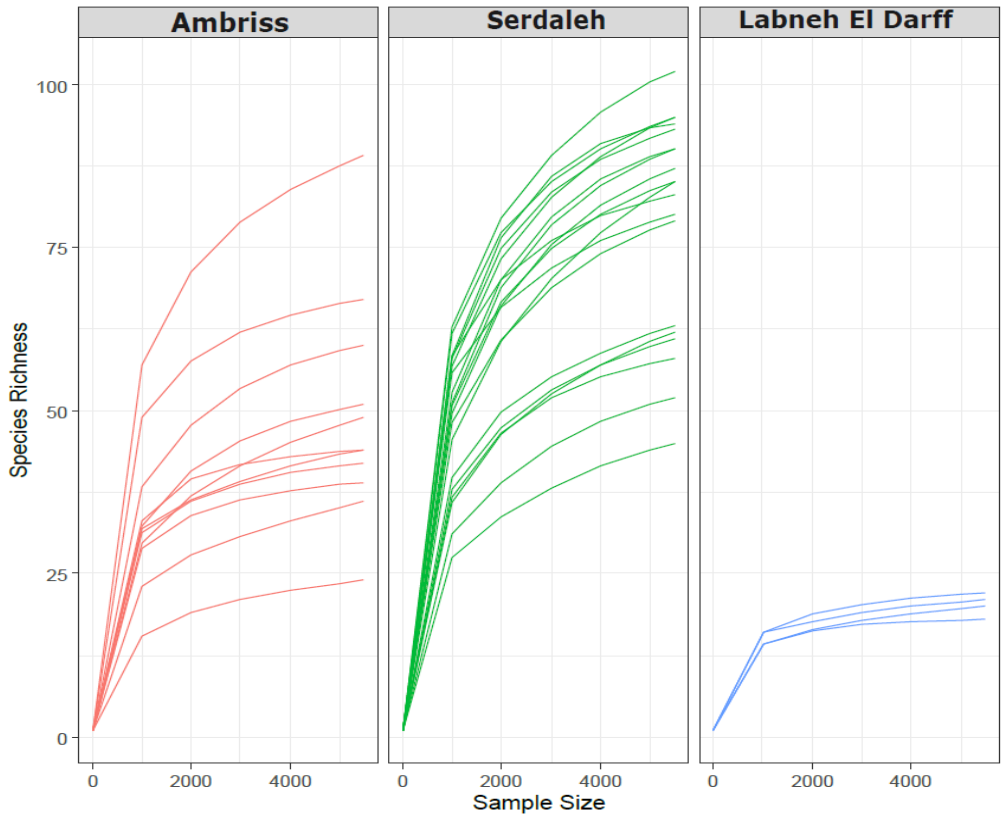

FIGURE V-3. RAREFACTION CURVES DEPICTING THE DEPTH OF 16S SEQUENCING AND SPECIES RICHNESS FOR THE DATA OBTAINED FROM AMBRISS, SERDALEH AND LABNEH EL DARFF. THE X-AXIS REPRESENTS THE SEQUENCING DEPTH (READS) AND THE Y-AXIS REPRESENTS THE ESTIMATED OUT RICHNESS DETECTED AT THE SPECIES LEVEL.

The alpha-diversity analysis revealed lower richness of species in Labneh El Darff ($p < 0.05$) compared to the other two products (**Figure V.3A**). Moreover, the Chao1, Shannon and InvSimpson indices, which measure evenness, confirmed this low diversity. The Principal Coordinates Analysis (PCoA) showed that while Labneh El Darff samples were clearly differentiated, samples of Serdaleh and Ambriss could not be clearly separated and thus likely shared a similar bacterial microbiota (**Figure V.3B**). Indeed, the analysis of their taxonomical composition (**Figure V.3C** and **Appendix IV.2**) showed that *Lactobacillus delbrueckii* was the main species present in Labneh El Darff samples, representing 55 to 82% of the counts, whereas it was undetected or at levels below 0.4% in the samples of Ambriss and Serdaleh. Moreover, the samples of the latter products contained *Lc. lactis*, *Lb. kefiranofaciens* and *Lentilactobacillus kefiri*

as the major species and, to a lesser extent, *Lb. diolivorans*-group. Differential analysis of OTUs showed that Serdaleh samples had greater *Lc. lactis* abundance than Ambriss ($p_{adj} < 0.01$).

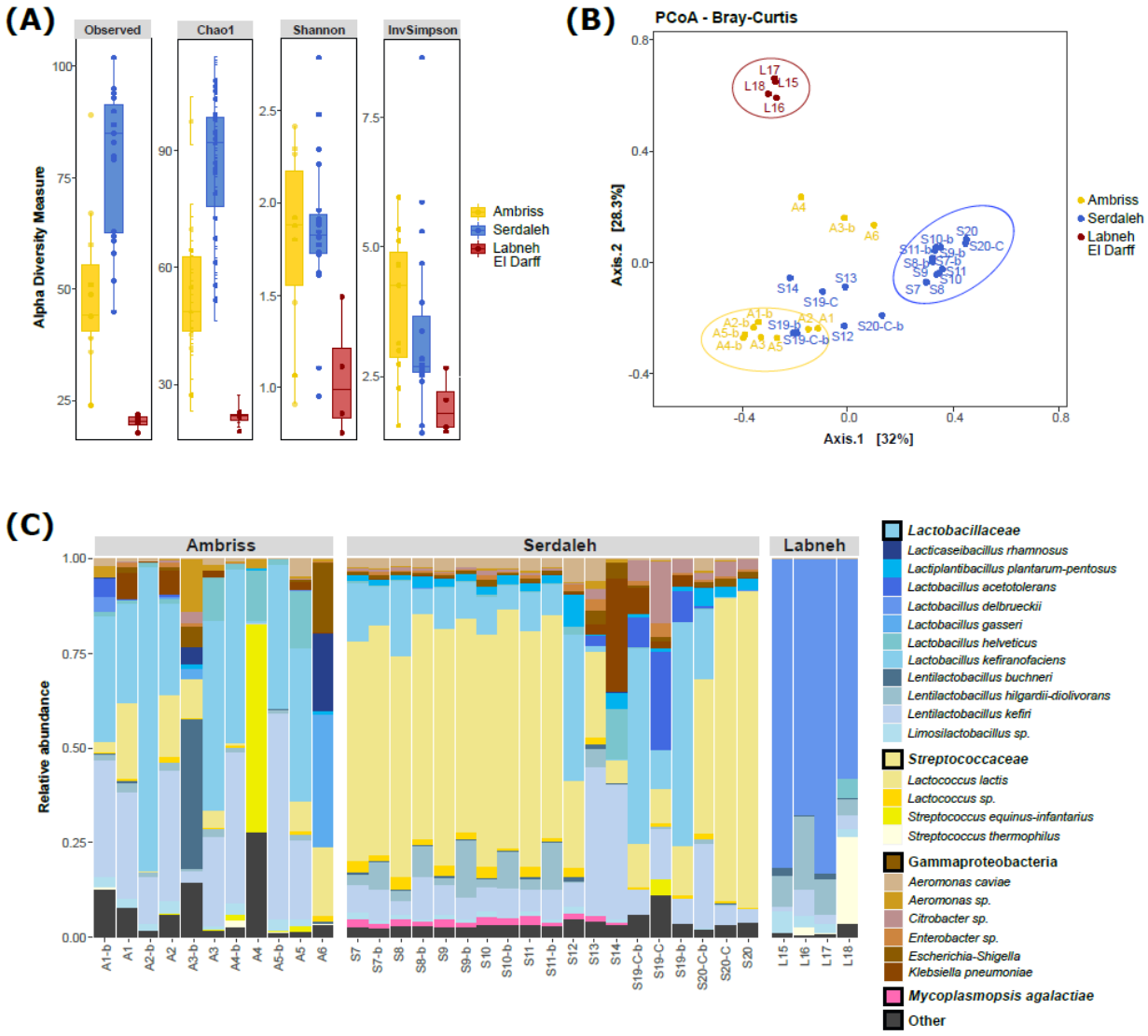

FIGURE V-4. METAGENETIC ANALYSIS BASED ON OTU DATA OF AMBRISS, SERDALEH AND LABNEH EL DARFF: BOXPLOTS OF ALPHA-DIVERSITY INDICES (A); PRINCIPAL COORDINATE ANALYSIS AMONG BACTERIAL COMMUNITIES IDENTIFIED BY OTUS (B); BACTERIAL COMPOSITION PLOT DEPICTING THE RELATIVE ABUNDANCES OF THE 20 MAIN OTUS (C). COLORS IN (A) AND (B) REFER TO THE DIFFERENT FERMENTED MILK PRODUCTS, I.E., YELLOW FOR AMBRISS, BLUE FOR SERDALEH AND RED FOR LABNEH EL DARFF.

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Remarkably, in the PCoA (**Figure V.4B**), these two products appeared to be spread along Axis 1 as a function of the proportion between Lactococcaceae and Lactobacillaceae species. In particular, a group of 12 Serdaleh samples contained *Lc. lactis* at levels of 58-83%, while the other samples were intermixed on the other part of this axis. Moreover, the 13 samples of Serdaleh collected in 2019 contained low levels (0.67-2.2%) of *Mycoplasmopsis agalactiae* or *M. bovis* (**Appendix IV.2**). Finally, three Ambriss samples showed divergent compositions compared to the others: A3-b was dominated by *Lentilactobacillus buchneri*, A4 by the *S. parasuis-sanguinis* group (> 53% of the reads), and A6 by high amounts of species poorly present in other samples such as *Lb. gasseri* (~36%), *Lacticaseibacillus rhamnosus* (~23%) and Enterobacteriaceae (> 18% of reads). The other species, usually detected at low levels in the various samples, mainly belonged to the Gammaproteobacteria class, such as *Aeromonas sp*., *Citrobacter sp., Klebsiella sp.* and *Escherichia-Shigella.*

V.4.3. Refined microbiota by metagenomics

For a refined taxonomical analysis, 16 samples were selected to perform shotgun metagenomic approaches. Such analyses allow a taxonomic identification up to the species-strain level and the simultaneous assessment of the level of reads corresponding to bacteria and fungi. Moreover, these analyses make it possible to detect eventual virulence factors in pathogenic species, antibiotic resistance genes and genes of technological interest.

Food metagenomic samples may contain animal reads, which may significantly bias the analysis when they are in high abundance. Since the present products are manufactured from goat milk, we tested for the presence of *Capra hircus* reads that were detected at levels ranging from 2.3 to 72% (**Appendix IV.3**). They were notably abundant in Labneh El Darff samples where they accounted for more than 50%, as a consequence of the process mode where the product is drained in a goat skin.

V.4.3.1. Species-level assignments by shotgun metgenomic

In a first approach, the MetaPhlAn tool, which identifies species by mapping the metagenomic reads against a genomic database, was used to determine the taxonomic composition of the samples. It yielded 98 bacterial and one eukaryotic species (**Appendix IV.4**). Species composition obtained by this analysis was relatively in accordance with those obtained with the

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16S rRNA amplicon. Interestingly, it resolved most taxonomical assignation ambiguities for lactic acid bacteria such as *Lentilactobacillus kefiri, Lentilactobacillus diolivorans, Lactiplantibacillus plantarum, Lactobacillus helveticus* and *S. thermophilus,* and also for contaminants such as *S. parauberis* and *M. agalactiae*. It also detected the yeast *Saccharomyces cerevisiae,* but at low levels and in only three samples.

In order to characterize microbiota independently of fixed references, we applied a marker gene analysis from the assembled metagenomes, which yielded 104 bacterial and 15 fungal species (**Appendix IV.5**). The overall composition is shown in **Figure V.4**. Although the main bacterial species identified here were consistent with the previous analysis made by read profiling, some differences merit highlighting. Whereas MetaPhlAn attributed 35% of the reads of A4 to *S. parauberis*, the marker gene analysis detected only 6.9% of this species and further detected another streptococcal species, *S. parasuis,* which retained 55% of the reads. This species was also detected at low levels in four other samples, while *S. parauberis* was found in eight samples, generally at low levels. This analysis also confirmed the frequent presence at low levels of several species of Enterobacteriaceae such as *E. coli*, *K. pneumoniae* and *C. freundii*, and of Enterococcaceae such as *E. faecalis* and *E. faecium.* Lastly, it also established that *Lc. lactis* subsp. *lactis* was detected in all samples, whereas the *cremoris* subspecies was detected at concentrations ten times lower in S8 and S10-b. A specific analysis by read mapping confirmed the presence of *Lc. lactis* subsp. *cremoris* at 5-15% lower abundance than that of the *lactis* subspecies in most samples.

Finally, 14 species of yeasts whose genomes are absent from the MetaPhlan database were identified by marker gene analysis. The most frequent and abundant were *Pichia kudriavzevii* and, to a lesser extent, *S. cerevisiae* and *Kluyveromyces marxianus*. Nevertheless, yeast relative abundance was generally below 1%, except in Labneh El Darff where it reached 8%. A more sensitive analysis performed by read mapping (**Appendix IV.6)** confirmed the presence of these yeasts at low levels in more samples, as well as that of *Torulaspora delbrueckii.*

Food metagenomic samples may contain animal reads, which may significantly bias the analysis when they are in high abundance. Since the present products are manufactured from goat milk, we tested for the presence of *Capra hircus* reads that were detected at levels ranging from

2.3 to 72% (**Appendix IV.3**). They were notably abundant in Labneh El Darff samples where they accounted for more than 50%, as a consequence of the process mode where the product is drained in a goat skin.

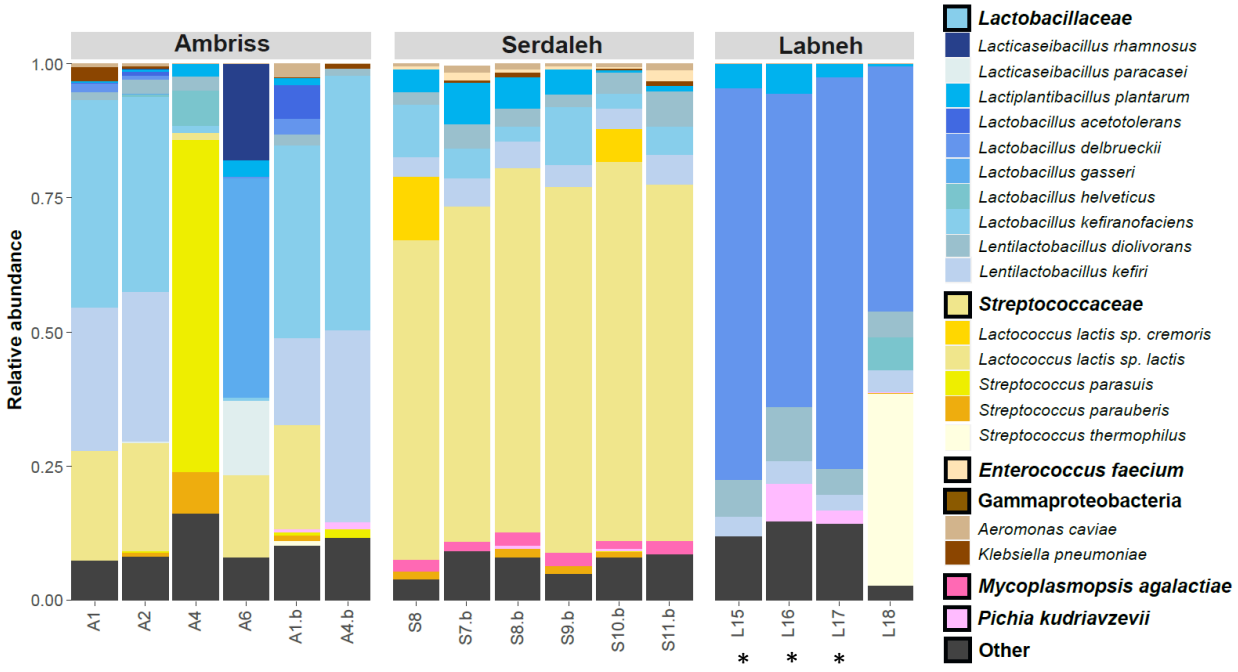

FIGURE V-5. RELATIVE ABUNDANCE OF MICROBIAL COMPOSITION OF AMBRISS, SERDALEH AND LABNEH EL DARF. VALUES WERE CALCULATED FROM THE COVERAGE OF TH YCHF MARKER GEN ASSEMBLED IN THE METAGENOMES. THE ASTERISK (*) INDICATES A LOW DEPTH OF THE SEQUENCES FOR L15, L16 AND L17 SAMPLES.

V.4.3.2. MAGs and strain-level analyses of LAB

A total of 62 prokaryotic Metagenome Assembled Genomes (MAGs) of high quality were reconstituted from the metagenome assemblies (**Appendix IV.7**). The majority of the MAGs correspond to Lactobacillaceae such as *Lb. diolivorans* (12), *Len. kefiri* (10), *Lb. kefiranofaciens* (9), *L. plantarum* (5) and *Lb. gasseri* (1). The two yogurt species *Lb. delbrueckii* subsp. *bulgaricus* (1) and *S. thermophilus* (1) were also recovered from Labneh El Darff and MAGs of *Lactococcus lactis* subsp. *lactis* (5) were reconstituted from Ambriss and Serdaleh products. In addition, the results included MAGs of the goat pathogen *M. agalactiae* (6) from Serdaleh samples, and of milk

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contaminants such as *S. parauberis* (3), *S. parasuis* (1), *Escherichia coli* (1), *Klebsiella pneumoniae* (1), *Aeromonas caviae* (2) and *Acetobacter spp.* (2).

Further detailed analysis was performed with the *lactococci* and *lactobacilli* MAGs and genome sequences of the most frequent and sometimes dominant species in the three products. Phylogenomic analysis of the *Lc. lactis* FME150 genome and the five *Lc. lactis* MAGs was performed with 215 reference genomes of this species, representing strains from its two main subspecies (*lactis* and *cremoris*) and isolated from different environments (dairy, vegetable, animal, etc.) (**Figure V.6**). In this analysis, five groups could be highlighted, one of which included all the representatives of the *cremoris* subspecies (brown branches), and three were formed by (i) industrial dairy strains (in red), (ii) diacetylactis biovariant strains (in blue), and (iii) dairy strains isolated from traditional products (in violet). The last group (green branches), which was the most diverse, contained environmental strains (plants, animals, etc.). The five *Lc. lactis* MAGs from Ambriss sand Serdaleh were included in this last group (highlighted by external red arrows).

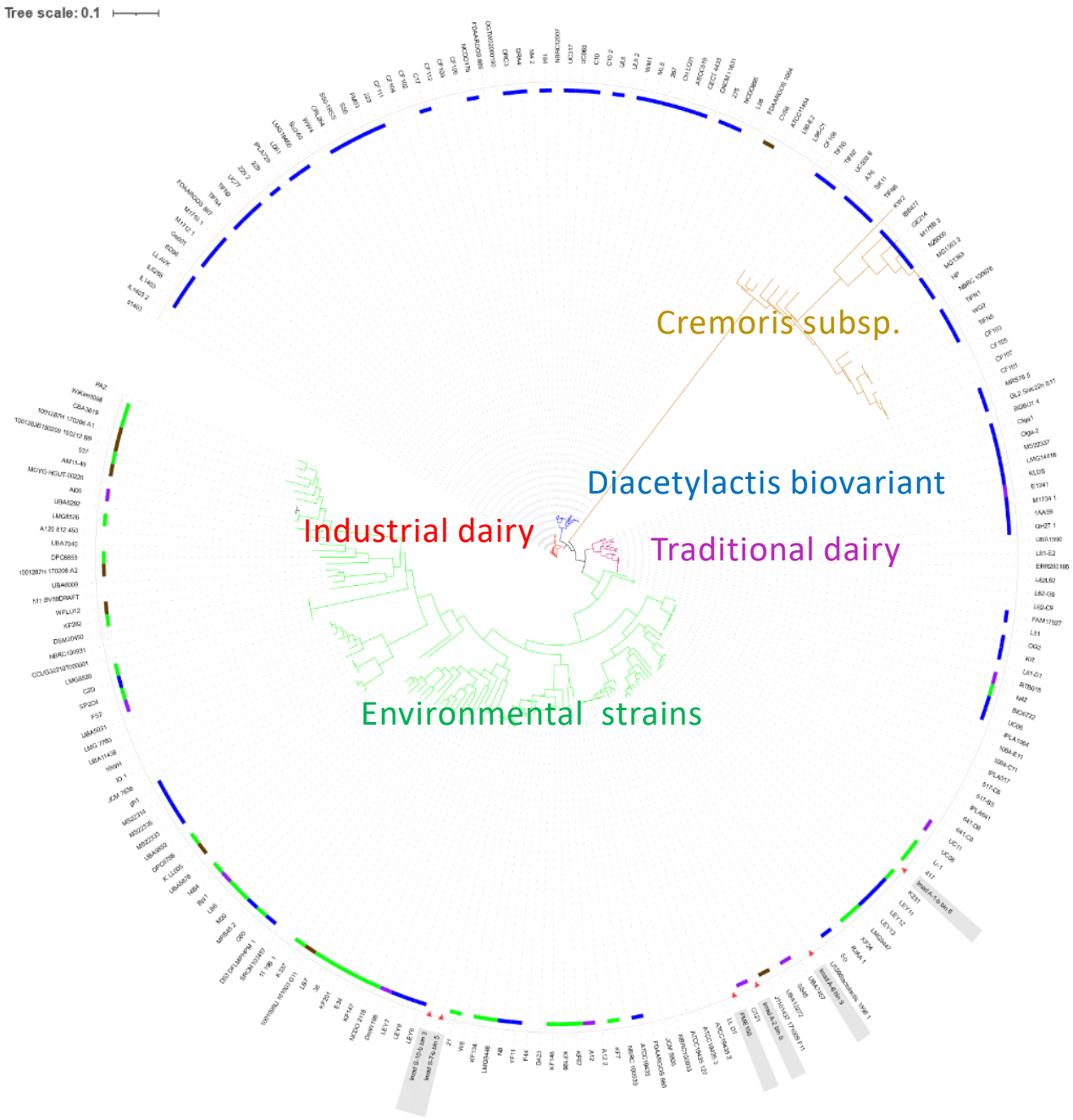

FIGURE V-6. PHLOGENOMIC TREE FOR *LACTOCOCCUS LACTIS*, HGHLIGHTING THE DIFFERENT GROUPS OF *L.LACTIS*. *THE COLORS OF THE BRACHES MAKE IT POSSIBLE TO VISUALIZE CREMORIS SUBSPECIES (BROWN), ENVIRONMENTAL SP.LACTIS STRAINS (GREEN), INDUSTRIAL DAIRY SP.(RED), DIACETYLACTIS* BIOVARIANT (BLUE) AND *LACTIS* SP FROM TRADITIONAL DAIRY PRODUCTS (VIOLET). THE INTERNAL CIRCLE PRESENTS THE ENVIRONMENT FROM WHICH THE

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STRAINS WERE ISOLATED (BLUE: MILK; GREEN: VEGETABLE; PURPLE: ANIMAL). AMBRISS AND SERDALEH MAGS AND THE ISOLATED STRAIN GENOME ARE HIGHLIGHTED BY EXTERNAL RED ARROWS AND NAMES ARE SHADED IN GRAY

A similar study was done with *Lb. kefiranofaciens*, *Len. kefiri Lb. diolivorans* and *Lpb. plantarum* **(Figure V.7)**. The *Lb. kefiranofaciens* FME151 genome and the eight *Lb. kefiranofaciens* MAGs assembled from Serdaleh (6) and Ambriss (3) metagenomes were compared to seven genomes present in the NCBI database, including those of the two type strains for the subspecies *kefiranofaciens* and *kefirgranum*. These strains were isolated from kefirs of different origins. ANI analysis showed that all strains shared at least 98.1% identity and could be divided into two main groups, one made by kefir strains and the second by Ambriss and Serdaleh strains, where ANI were ~99.2-99.4% within each group and ~98.2-98.4% between groups. Further phylogenomic analyses **(Figure V.7A)** showed that two groups could be differentiated within kefir strains, a first one with three strains sharing over 99.9% identity and including the *Lb. kefiranofaciens* subsp. *kefiranofaciens* type strain, and a second one with four strains that were more divergent between themselves, sharing 99.0 to 99.8% identity and including the *Lb. kefiranofaciens* subsp. *kefirgranum* type strain. Lastly, Ambriss and Serdaleh strains also formed two groups sharing 99.4 to 99.8% ANI within and 99.1 to 99.3% outside of their group. Analysis of gene composition confirmed and refined these results. First, it showed a very high relatedness of three *L. kefiranofaciens* subsp. *kefiranofaciens* strains from kefir that shared more than 95% of their genes (> 2,325 out of ~2,440 genes), while within each of the other groups, the strains were more divergent between themselves (87% of shared genes in the *kefirgranum* group, 88% in the Serdaleh group and 92% in the Ambriss group). Seventeen EPS-related genes involved in kefiran production present in a 14.4-kb region were present only in the three former strains, whereas only the first part of the operon containing the genes of enzymes involved in EPS regulation and priming was conserved in the other strains. Furthermore, in the latter strains, the genes involved in polymerization, chain length determination and export that could be characterized appeared to be different in each strain, indicating that they produce polysaccharides other than kefiran.

The *Len. kefiri* FME71 genome and the ten *Len. kefiri* MAGs assembled from Ambriss (3), Serdaleh (6) and Labneh El Darff (1) were compared to the ten reference genomes present in the

NCBI database, including seven strains isolated from kefir, two from human gut and one from yogurt. This analysis showed that all strains shared very high ANI values (> 99.55%), indicating that they were closely related **(Appendix IV.8**). Analysis of their phylogenomic tree did not indicate clustering as a function of the origin of the strains (**Figure V.7B**). Furthermore, the analysis of the core/pan genome showed that all strains shared ~2,148 genes out of ~2400. The analysis of the specific genes in the different strains indicated that they code mostly for "hypothetical proteins" (50%) and mobile element proteins (25%).

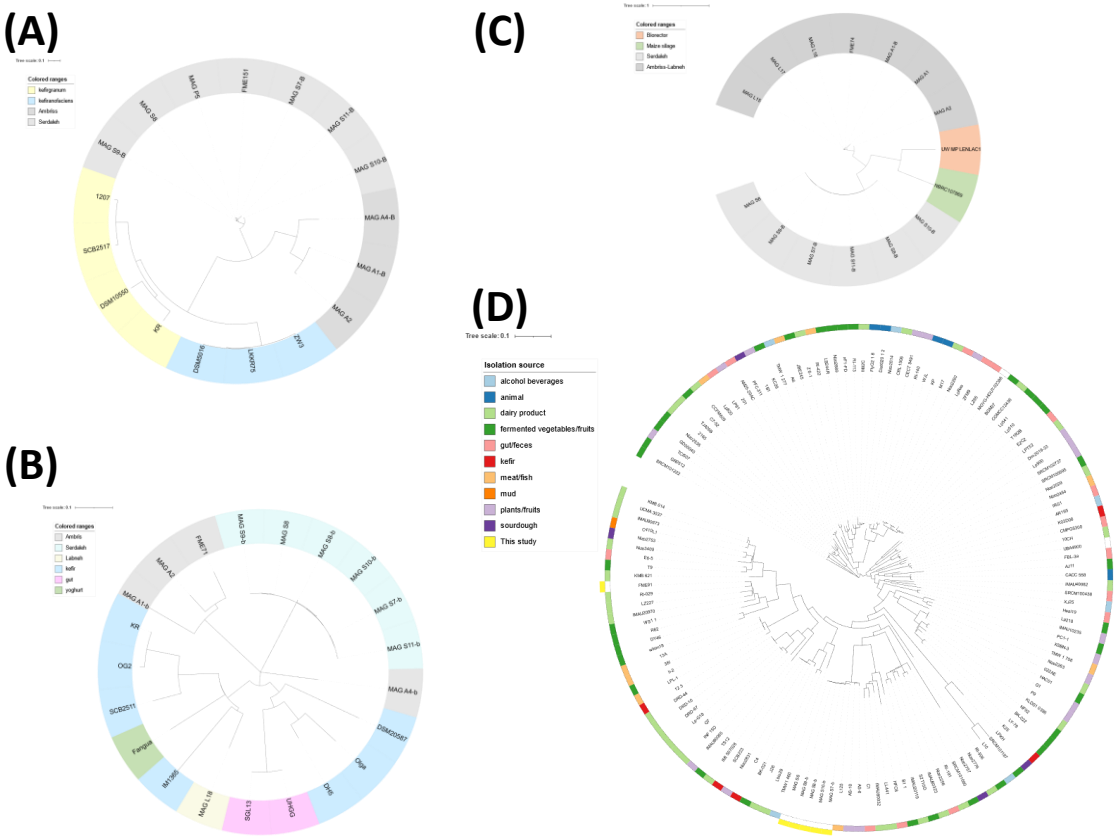

FIGURE V-7. PYLOGENOMIC TREE FOR LB.KEFIRANOFACIENS (A), LEN.KEFIRI (B), LB.DIOLIVORANS (C) AND LPB.PLANTARUM (D).

ANI analysis of *Lb. diolivorans* FME74 and of our 12 MAG strains with that of the type strain (isolated from maize silage) and one MAG from an ultra-filtered-milk-permeate fermenting bioreactor showed that these strains could be clustered into four groups (**Figure V.7C**), displaying at least ~97.7 to 98.4% ANI . Serdaleh MAGs formed a group of very closely related strains (ANI

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> 99.9%), Ambriss and Labneh MAGs formed a second homogenous group (ANI > 99.1%), whereas the bioreactor MAG and the type strain were isolated.

The sequences of *Lpb. plantarum* FME91 strain and of the five MAGs obtained from Serdaleh were analyzed with a set of 145 *Lpb. plantarum* strains isolated from different ecosystems, including several types of food, animals, gut, etc. The five MAGs from Serdaleh appeared to be almost identical (ANI > 99.97%), but unrelated to that of FME91 from Ambriss (ANI ~92.7%), and they ranged between 95.7 and 99.7-99.9, with an average of 98.7 with the strains of the set (**Appendix IV.8**). An overview of the phylogenic relationship between these strains is given by the tree obtained by kSNP analysis (**Figure V.7D**). Strains were dispersed irrespective of their environmental origin. The Ambriss strain FME91 was closely related to those isolated from milk or artisanal dairy products, whereas the Serdaleh strains were closely related to those isolated from meat, fruits and vegetables.

V.4.3.3. Detection of pathogens by genomic analysis

Marker gene analysis showed that undesirable species were present in several Ambriss and Serdaleh samples. All Serdaleh samples collected in 2019 contained low levels (1-4%) of the goat pathogen *M. agalactiae*. Moreover, zoonotic streptococcal species, namely *S. parauberis* and *S. parasuis*, were detected at 0.5-2% in several samples of Ambriss and Serdaleh and, remarkably, at 7 and 55%, respectively, in Ambriss A4. *S. aureus* was detected at low levels in Serdaleh S10-b. Finally, Enterobacteria such as *E. coli* and *K. pneumoniae* were detected in about half of the samples at levels < 1% (except for *K. pneumoniae* present at 2.7% in Ambriss A1). Deep profiling analysis in the metagenome samples was performed to detect the presence at low abundance of pathogen species potentially found in dairy products (**Appendix IV.6**). No *Brucella abortus, Listeria monocytogenes*, *Mycobacterium vaccae* or *Staphylococcus agalactiae* were detected. However, *S. aureus* was detected at low levels in five Serdaleh samples.

Further analyses were performed on these undesirable species, which were sufficiently covered to yield MAGs. An ANI analysis of these MAGs with representative genomes of these species confirmed the taxonomic classification previously obtained by the *ychF* marker. For example, *M. agalactiae, S. parauberis* and *K. pneumoniae* MAGs share 99.8, 98.5 and 98.4% ANI with the type strains of their species, *E. coli* MAG; 99.1% with *E. coli* K12 laboratory strain, *S.*

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parasuis MAG; and 97-98% with four genome references of this species present in the NCBI database.

Gene content analysis was performed with *S. parasuis* MAG_A4, which appears to be dominant in Ambriss A4. In particular, genes involved in lactose metabolism were searched to explain the fitness of this strain in milk fermentation. Among the two systems used for lactose utilization, MAG-A4 contains the Lac-PTS system, including ten genes encoding the Lac-PTS transporter, the phospho-beta-galactosidase, the tagatose pathway, glucokinase, a repressor and a gene of unknown function (LacX). A blast search with these genes provided a hit at 97-99% nucleotide identity on its entire length with an ICESsuJH1308-1 mobile element present in *S. suis* JH1308 and a lower identity with several other *S. suis* strains. However, none of the nine *S. parasuis* genomes available contained these genes. These data show that *S. parasuis* MAG_A4 acquired lactose utilization genes by horizontal transfer, possibly from *S. suis* species.

Interestingly, MAGs from *M. agalactiae* were of very good quality, probably due to particular features of their sequences, such as genomic guanine-cytosine content (~29.7%) and di-tri-nucleotide composition, allowing a better binning. In particular, MAGs from Serdaleh S8 and S9 consisted of only four and ten contigs, respectively. ANI analysis indicated that the strains from the Serdaleh samples were very closely related to each other (ANI > 99.9%), as well as to the type strain (ANI ~99.85%; **Appendix IV.8**). Phylogenomic analysis confirmed the close relationship of *M. agalactiae* Serdaleh MAGs together and with PG2^T (**Figure V.8**). Furthermore, gene content analysis indicates that these two MAGs differed from PG2^T by less than 20 genes encoding hypothetical proteins.

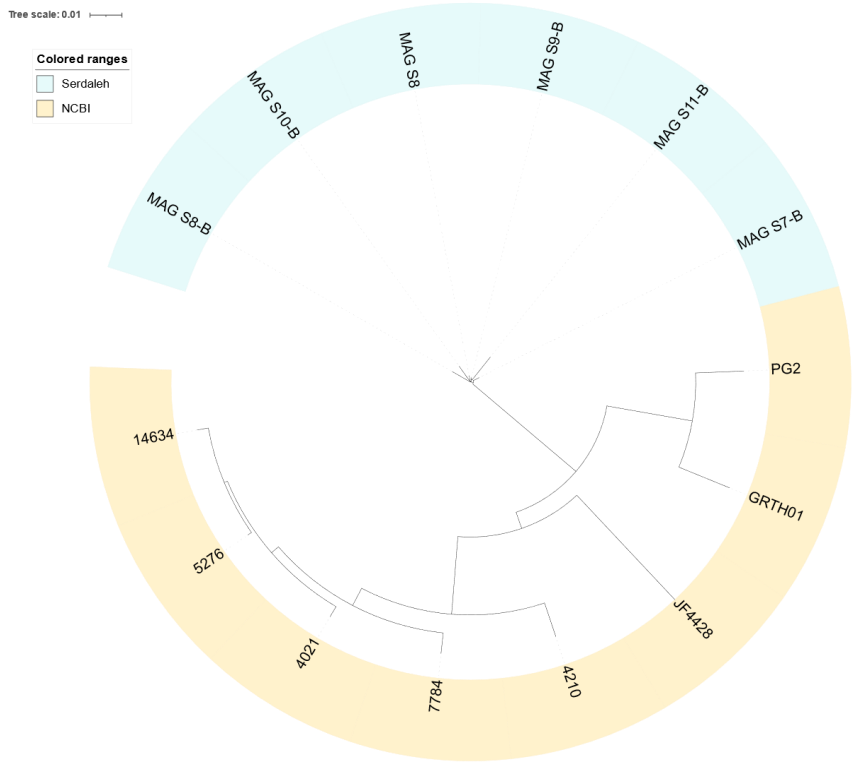

FIGURE V-8. PHYLOGENOMIC TREE FOR *M.AGALACTIAE*.

V.4.3.4. Detection of antibiotic resistance genes

A mapping-based approach against a comprehensive collection of antibiotic resistance genes was performed. A total of 18 ABR genes were identified, belonging to seven different classes of antibiotics (**Figure IV.9**). They were detected in almost all samples, and notably those from Serdaleh had higher levels and more diverse genes than those from Ambriss. Concerning the Labneh samples, only sample L18 presented detectable amounts of ABR. However, it cannot be concluded that L15, L16 and L17 samples did not contain ABR because there were not enough useful reads in these samples due to the presence of goat DNA.

Overall, the most abundantly detected antibiotic classes were aminoglycosides, MLS (Macrolide, Lincosamide, Streptogramin), nucleosides and tetracycline. In particular, Serdaleh samples contained high levels of aminoglycoside (*aad*(6) and APH(3'')-IIIa), MLS (*ermB*),

nucleosides (*sat-4*) and tetracycline (*tetS*). The coverage of these genes is similar to that of *Lc. lactis*, which was the highly dominant species in these samples. Since this level of coverage allows a reliable level of assembly, we searched for contigs carrying these determinants by BLAST analysis in Serdaleh samples.

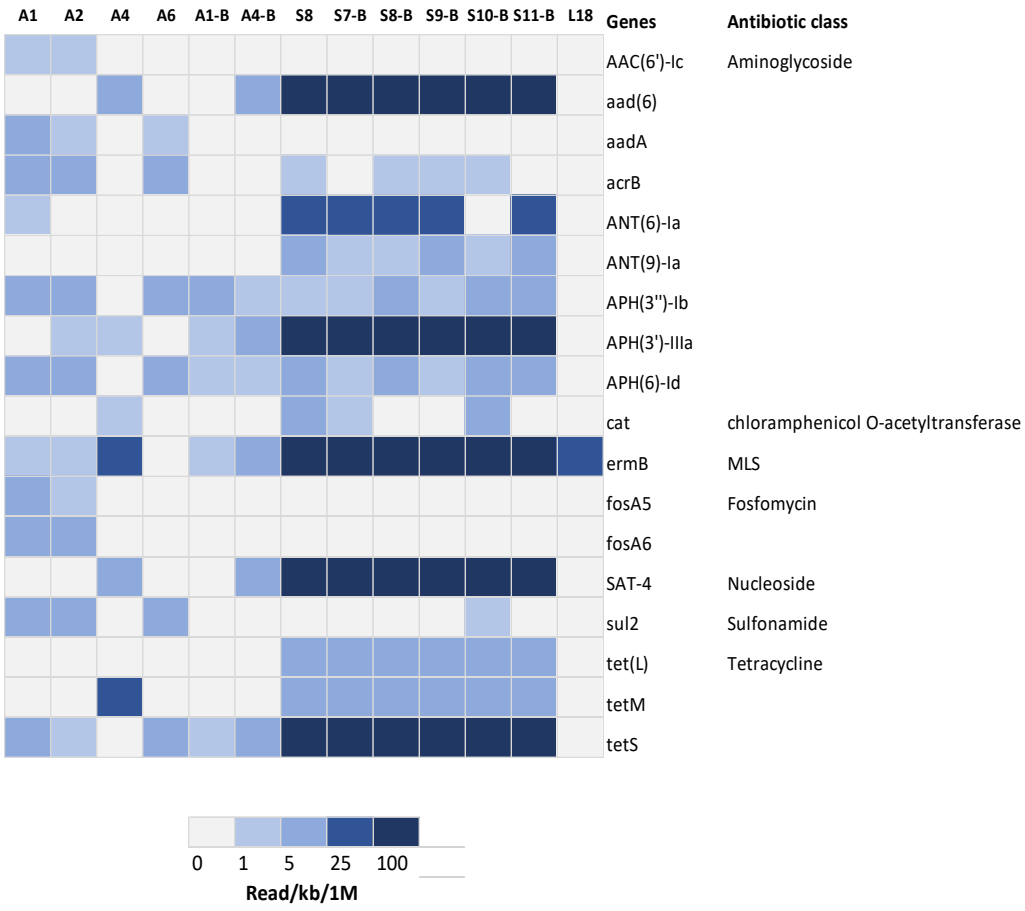

FIGURE V-9. HEATMAP SHOWING DISTRIBUTION OF THE 18 ANTIBIOTIC RESISTANCE GENES IN READS/KB WITHIN THE 13 METAGENOMIC SAMPLES.

MLS: Macrolide, Lincosamide and Streptogramin.

This search pointed out two contigs of 12,360 and 3,370 nucleotides in sample S10-b, with coverages ~2 times that of *Lc. lactis*, and carrying, respectively, the four first determinants and *tetS*. The first contig is ~98% identical on 76-85% of its length to large plasmids from *E. faecium*, *E. faecalis* and *Staphylococcus pseudointermedius*. The second contig is identical on its full length

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to regions of plasmids and genome drafts of various food bacteria such as *L. raffinolactis, Lc. lactis, S. thermophilus, Carnobacterium divergence* and *Listeria innocua*. The other Serdaleh metagenomes contained contigs of comparable sizes indicating similar events.

The metagenome of Serdaleh S10-b also contained a ~6.6 kb contig carrying the *tetL, tetM* and *cat* genes that was identical on its entire length to regions of plasmids from *E. faecalis*. The coverage of this contig is 40 times lower compared to the two former ones, which is a level corresponding to that of *Len. kefiri, Lb. kefiranofaciens* and *Lb. diolivorans*. None of the other metagenomes contained these three genes together on one contig. Nevertheless, samples S8, S7-b, S8-b, S9-b and S11-b contained contigs of ~5 and 0.9 kb carrying *tetLM* and *cat* genes, respectively. The coverages of these contigs were significantly different in the other assemblies where *tetLM* coverage was 2 to 10 times higher that of *cat* genes, indicating that their organization might be different from that found in S10-b.

Lastly, in Ambriss A4, *tetM* was found on a ~32 kb contig of S. *parasuis, ermB* on a 7 kb contig sharing 90% identity on 3 kb with plasmids of various Lactobacillaceae, *cat* on a ~3 kb contig sharing 93% identity to *L. garviae* plasmids, *aad* and *sat* on a ~23 kb contig sharing 93% identity on ~12 kb (including *aad* and *sat*) of the *S. agalactiae* B111 chromosome and 99% identity on 4-6 kb of *E. faecalis* plasmids and the *S. pseudointermedius* chromosome.

V.5. Discussion

Traditional Lebanese fermented goat milk products are perceived in Lebanon as having an important historical and cultural heritage, but their microbiota are still poorly characterized. A detailed characterization of 50 producers allowed us to select those who used genuine traditional processes for the production of Ambriss, Serdaleh and Labneh El Darff. The manufacturing processes were established and the physico-chemical characteristics of the products were determined. Our survey also made it possible to define the socio-economic profiles of the producers, which confirmed the danger of extinction of these products, thus making it possible to require a Protected Designation of Origin label. This study explored the microbial biodiversity of 34 samples of three fermented goat milk products from three regions by combining microbiological, genomic and metagenomic approaches.

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The samples analyzed here shared similar technical properties for pH, salinity, temperature and microbial counts as those of the same products previously studied representative of these products in Lebanon.

V.5.1. Microbial originality of traditional Lebanese fermented goat milk products

An overall view of the microbiota is provided by metagenetic analysis, which deciphers species or lower taxonomic levels. This study clearly separated Labneh samples from those of Ambriss and Serdaleh. Labneh contained a relatively simple microbiota, strongly dominated by *Lb. delbrueckii*, which was almost absent from the other products. Furthermore, it contained *Lb. diolivorans*, *Len. kefiri* and *S. thermophilus* to a lesser extent. The dominance of *Lb. delbrueckii* is due to the way Labneh is inoculated by Laban, whose composition is that of yogurt made of *Lb. delbrueckii* and *S. thermophilus* (Chammas *et al*., 2006b). The dominance of *Lb. bulgaricus* is probably due to its better resistance to acidity than *S. thermophilus* (Béal and Chammas, 2012). Similar observations were made with yogurt and Doogh (also known as Ayran), a traditional Iranian fermented ewe milk product (Davati and Hesami, 2021). The presence of *Lb. diolivorans* and *Len. kefiri*, which are also found in Ambriss and Serdaleh, might be due to the specificity of the local goat milk. Moreover, the conditioning of Labneh in a goatskin may also influence the microbial composition by releasing nutrients or other metabolic products, as indicated by the very high level of goat DNA obtained from the metagenomic data for Labneh. Interestingly, *Lb. diolivorans* is usually found in vegetable and fruit fermentation, whereas it is occasionally reported in dairy products (Tamang *et al*. 2005; C. Zhang *et al*., 2010; Zheng *et al*., 2020). To the best of our knowledge, it was isolated from one raw milk in Brazil (Agostini *et al*., 2018), traditional yogurts in Iran (RoushanZadeh *et alet al*., 2014), traditional fermented dairy products in Mongolia (Yu *et al*., 2011), and detected in metagenomic samples of Koumiss (You *et al*., 2022; Kozhakhmetov *et al*., 2014). The present study is thus the first one reporting the genome of *Lb. diolivorans* isolated from a dairy product.

Ambriss and Serdaleh samples contained, as dominant species, *Lc. lactis* and *L. kefiranofaciens*, which were almost absent from Labneh, followed by *Len. kefiri* and, to a lesser extent, by *Lb. diolivorans* and *Lpb. plantarum*. These results wree in coherent with a recent study as reported by Abi Khalil *et al.*, (2023) using a smilar metagenetic study. a Interestingly, this

composition was reminiscent of that of dairy kefir, a fermented milk with health properties, which is produced by the inoculation of a stable and complex microbial community made of lactic acid bacteria and yeasts entrapped in gelatinous grains (Blasche *et al*., 2021; Prado *et al*., 2015; Nalbantoglu *et al*., 2014). In this product, the dominance of *Lb. kefiranofaciens*, which otherwise grows poorly alone in milk, is attributed to the metabolic cooperation it develops with the community, and the synthesis of an exopolysaccharide.

In this respect, growth conditions in Ambriss and Serdaleh, where the microbiota remained essentially in a coagulated milk matrix, displayed strong similarities with the one encountered in the kefir grain, which may thus explain the dominance of this particular *Lactobacillus* and of several sub-dominant lactic acid bacteria. MAGs and genome comparisons of *Lb. kefiranofaciens* and *Len. kefiri* confirm the very close phylogenomic relationship between kefir, Ambriss and Serdaleh strains. Their gene content mainly differed by unknown functions and mobile elements. In *Lb. kefiranofaciens* strains isolated from Ambriss and Serdaleh and in *Lb. kefirgranum* subspecies strains, only the first six genes encoding the general functions of initiating and controlling EPS synthesis were conserved, whereas glycosyl transferase conferring the specific structure of EPS units differed. This fact explains why these strains did not produce kefiran, the specific polysaccharide for kefir grain formation. Lastly, metabolic cooperation in Ambriss and Serdaleh certainly differed from that of kefir, as suggested by the low level of *Leuconostoc* and *Acetobacter* species, which play an important role in milk fermentation and citrate metabolism (Laëtitia *et al*., 2014). Nevertheless, the low input of milk on a the coagulated milk matrix gradually offers an environment for this "slow growing species" that is *Lb. kefiranofaciens* (Blasche *et al*., 2021; Georgalaki *et al*., 2021). Since the microbiota of Ambriss and Serdaleh were not inoculated, the environmental origin of *Lb. kefiranofaciens* in these products still remains unknown. Interestingly, this species was also found to be dominant in several samples of Airag, a traditional Mongolian fermented mare milk made in horse-hide containers (Watanabe *et al*., 2008) and in Koumiss (M. Zhang *et al*., 2020), neither of which contain grain.

In the present set of samples, the balance between *Lc. lactis* and *Lb. kefiranofaciens* appeared to be the main factor differentiating Ambriss and Serdaleh, the former generally containing lower levels of *Lc. lactis* and higher levels of *Lb. kefiranofaciens*. Nevertheless, the

samples of these two similarly processed products, originating from two distinct regions, could not be clearly separated since some samples of Serdaleh were also dominated by *Lb. kefiranofaciens*. Although Serdaleh samples were obtained at lower temperatures, displayed higher salt content, and reached lower pH in comparison to Ambriss samples, these factors do not fully explain this population shift. Alternatively, there may be subtle differences in production methods, such as the quantity of milk added at once, or a potential mixing that modifies the speed of milk infiltration through the product, which may affect the metabolism of the microbial communities in different ways, as discussed earlier. For example, if a significant quantity of the milk poured into the jar is not mixed with the curd, it may favor a rapid planktonic development of *Lc. lactis*, compared to the slow solid-state development where *Lb. kefiranofaciens* and *Len. kefiri* compete more effectively.

A second important outcome of this study is the detection of uncultivated species as dominant microbiota in Ambriss and Serdaleh. While the initial failure to isolate *Lb. kefiranofaciens* from the samples can be explained by the fact that this species is fastidious to grow and requires specific media and growth conditions (Georgalaki *et al*., 2021), the lack of recovery of *Lc. lactis* is less understandable. Indeed, this bacterium is routinely cultured from different environments such as plant or dairy products (Soundharrajan *et al*., 2021; Li *et al*., 2020). In this work, we thus tested the hypothesis that *Lc. lactis* cells were viable but not-cultivable (VBNC) (Oliver, 2010) and would thus require specific conditions to recover growth throughout their transfer from the product to M17 medium. Actually, a transfer of several hours in media containing goat milk allowed a straight forward isolation of strains of this species. This result indicates that the production process of Ambriss and Serdaleh induced a particular physiological state in *L. lactis* cells that hampers its growth recovery, thus corresponding to the definition of VBNC. Indeed, during the Ambriss and Serdaleh process, various environmental factors (such as pH, salinity and temperature) and the physiological age of the culture could induce the entry of bacterial cells into a VBNC state. Interestingly, the present process is still reversible, which is rarely the case (Dong *et al.*, 2020), and hence offers a reproducible model for the study of the entry into the VBNC state. Further work would be required to understand why *Lc. lactis* growth recovery was deprived when directly plated on M17.

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V.5.2. Potential safety concerns

A wide metagenomic search for bacteria potentially involved in public health issues indicated the presence of DNA of several kinds of undesirable species. Among hygiene indicators, Enterobacteria were detected at low levels in about half of the samples, as well as *S. aureus*, which was detected at low levels, mostly in Serdaleh samples. These results are comparable to those of a recent study on raw cow milk where, from a total of 195 samples collected from different Lebanese farms, 65% and 17% of the samples were non-compliant with national standards for *Escherichia coli* and *S. aureus*, respectively (Joubrane *et al*., 2022). Enterobacteriaceae are often reported as a typical bacterial population in raw milk from many mammalian origins (Ramos and Nascimento, 2021). They can also be transmitted to milk and dairy products by fecal contamination during the milking process or because of poor hygiene conditions (Giraffa, 2003). *S. aureus* was highly prevalent in some products and was the most frequent contaminant of milk and goat milk products (Oliveira *et al*., 2020; Xing *et al*., 2016). The transmission of *S. aureus* to milk is due to its ability to form biofilms and to remain on surfaces and utensils, its presence being mainly attributed to poor handling during processing (Kümmel *et al*., 2016).

A striking finding was the presence of several zoonotic streptococcal species such as *S. parauberis* and *S. parasuis*, which were detected at 0.5-2% in several samples of Ambriss and Serdaleh, respectively, and even at 7 and 55% in Ambriss A4. *S. parasuis* is an important pathogen for humans and pigs (Nomoto *et al*., 2015) and was already isolated as a contaminant in raw camel milk. Intriguingly, in Ambriss A4, *S. parasuis* was strongly dominant over the traditional lactic acid bacteria, which accounted for only 10% of the population. The fact that the pH of this sample has a value similar to that of the other samples indicates that the strain of *S. parasuis* present in this sample is an acidifying strain. Indeed, our analysis revealed the presence of ten genes encoding a Lac-PTS system in its genome, similar to the one that allows efficient lactose utilization in several lactic acid bacteria such as *Lc. lactis* (Aleksandrzak-Piekarczyk, 2013). These genes were probably acquired by a horizontal gene transfer from *S. suis*. Adaptation to lactose utilization in milk has already been observed for other non-starter streptococci, in particular, *S. infantarius* in fermented dairy products from East and West Africa (Jans *et al*., 2013) and in artisanal cheese from Brazil (Kothe *et al*., 2021).

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Serdaleh samples contained low levels (1-4%) of the goat pathogen *M. agalactiae*. This bacterium is one of the causal agents of contagious agalactiae, a serious enzootic disease of small ruminants, which notably occurs in the Mediterranean Basin (Kumar *et al*., 2014). Once it has infected the animal, it disseminates though the blood to different mucosa, including the mammary gland, and can consequently be found in milk. *M. agalactiae* has already been detected in artisanal cheeses via non-cultural techniques (Martín-Platero *et al*., 2009). Its presence here is, therefore, a sign that the herd in the Chouf region was infected with this pathogen.

Finally, transmissible antibiotic resistance genes, which are considered to be a growing threat to public health (Ghimpețeanu *et al*., 2022; Yadav & Kapley, 2021), were detected. In particular, Serdaleh samples contained a multi-resistance cassette carrying aad(6) and APH(3'')-IIIa, ermB and sat-4, as well as tetracycline resistance genes. Since these antibiotics are used in prophylaxis for *M. agalactiae*, it is likely that such treatment contaminated the milk and favored the emergence of antibiotic resistant lactic acid bacteria strains.

V.6. Conclusion

In this study, we analyzed three artisanal Lebanese fermented products (Ambriss, Serdaleh and Labneh El Darff) that are processed from the raw milk of the Black Baladi goat, a local breed. The process consists of the daily introduction of salted milk or Laban into a terracotta jar or a goatskin over several months, together with the draining of the whey, until the container is full. This production remains highly artisanal and offers opportunities for the empowerment of women, but it is in danger of extinction due to the low volumes that are produced, the age of the producers and the absence of marketing. In industrial products that are inoculated with starters or by backslopping, the microbiota rapidly develops in large batches of milk, whereas in the current products, it develops slowly during percolation of the milk that is added periodically. During batch growth, fast-growing microorganisms are highly favored, whereas in a dense community, slow-growing bacteria can compete more effectively, as is the case with the well-described kefir grain where *Lb. kefiranofaciens* clearly predominates despite its poor growth performance in milk. Interestingly, kefir is promoted as a probiotic beverage and *Lb. kefiranofaciens* is presented as a key factor in this attribute (Georgalaki *et al*., 2021). Although no studies have yet proven this, traditional Lebanese goat milk products are believed to deliver health benefits, and it would thus

be interesting to further test this possibility. These products are also interesting as sources of biodiversity because they contain strains of *Lb. kefiranofaciens* and *Len. kefiri*, which are genetically quite different from those present in kefir that are highly conserved.

Furthermore, progress has to be made to ensure the safety of these products from herd prophylaxis to milk chain management via a global action in order to avoid contamination by potential pathogens and the spread of antibiotic resistance genes. Finally, this study also provides key elements related to the microbiology and microbiota functioning of these products that will facilitate the development of strategies to improve these processes and preserve their identity.

Acknowledgements

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VI. RESULTS AND DISCUSSION: Technological and functional properties of some lactic acid bacteria isolates

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The technological properties of the selected isolates of lactic acid bacteria from Ambriss, Serdaleh and Labneh El Darff have been characterized in order to point out some strains that could be selected for further applications, either for the manufacture of well controlled fermented milks Ambriss, Serdaleh or Labneh El Darff, or for the elaboration of other kinds of fermented dairy products. These technological properties concern acidification activity of the bacteria in goat milk, physical and rheological characteristics of goat milk fermented with single isolates, composition in volatile organic compounds of the fermented products, lipolytic and proteolytic activities of the strains as well as their resistance to gastrointestinal stress.

VI.1. Acidification activity

VI.1.1. Raw results of acidification activity

The acidification activity of each of the identified isolates was quantified by using the Cinac system as described by Chammas *et al*. (2006b). Thanks to on-line measurement of pH and calculation of acidification rate, the Cinac system allowed recording seven acidification kinetic descriptors:

- The maximum acidification rate in absolute values (Vmax, in upH/min),
- The time at which the maximum acidification rate was achieved (tmax, in min),
- The pH at which maximum acidification rate occurred (pHmax),
- The times to reach pH 5.5, pH 5.0 and pH 4.5 (tpH5.5, tpH5.0, tpH4.5 respectively, in min),
- The final pH attained at the end of the fermentation, when no more pH variation was observed (pHf).

These parameters were measured for 40 strains, and all measurements were triplicated. The 40 isolates were identified by 16S rRNA as *Lactococcus lactis* (n=3), *Pediococcus acidilactici* (n=2), *Lentilactobacillus kefiri* (n=5), *Lactiplantibacillus plantarum* (n=8), *Lacticaseibacillus rhamnosus* (n=3), *Lentilactobacillus kisonensis* (n=2), *Lactobacillus kefiranofaciens* (n=1), *Levilactobacillus brevis* (n=6), *Lentilactobacillus diolivorans* (n=5), *Enterococcus durans* (n=5). The mean values and their standard deviations of these parameters are reported in **Table V.1.**

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TABLE VI-1. MEAN VALUES OF ACIDIFICATION PARAMETERS OF 40 LAB STRAINS COLLECTED FROM AMBRISS, SERDALEH AND LABNEH EL DARFF PRODUCTS AND IDENTIFIED BY 16S RNA SEQUENCING.

Region	Producer	Product type	Species identified by 16S	Strain code	Vmax (upH/min)	tmax (min)	pHmax	tpH5.5 (min)	tpH5.0 (min)	tpH4.5 (min)	Final pH
Chouf	P3	Serdaleh Milk	*Lactococcus lactis*	LL01	-0.0067±0.0002	510±2	5.80±0.0	562±5	689±4	1009±8	4.29±0.02
Chouf	P3	Serdaleh Milk	*Lactococcus lactis*	LL03	-0.0061± 0.0003	280±8	5.98±0.05	422±5	774±31	1268±80	4.08±0.0
Bekaa	P1	Ambriss	*Pediococcus acidilactici*	PA04	-0.0018±0.0001	1642±72	5.70±0.01	1778±95	2444±132	--	4.80±0.06
Bekaa	P1	Ambriss	*Pediococcus acidilactici*	PA05	-0.0020±0.0002	1664±36	5.73±0.06	1800±71	2542±76	--	4.86±0.05
Chouf	P4	Serdaleh	*Lentilactobacillus kefiri*	LK06	-0.0015±0.0004	1887±89	5.48±0.01	1876±84	2643±17	3999±173	4.48±0.03
Chouf	P3	Serdaleh	*Lentilactobacillus kefiri*	LK07	-0.0029±0.0007	639±45	5.60±0.1	689±32	943±61	1351±128	4.01±0.08
Chouf	P3	Serdaleh	*Lentilactobacillus kefiri*	LK08	-0.0041±0.0003	630±193	5.68±0.37	711±27	970±55	1358±108	4.05±0.01
Chouf	P3	Serdaleh	*Lentilactobacillus kefiri*	LK09	-0.0031±0.0006	640±106	5.60±0.18	686±25	921±46	1291±67	4.01±0.05
Chouf	P4	Serdaleh	*Lentilactobacillus kefiri*	LK10	-0.0036±0.0006	564±29	5.71±0.08	661±16	880±6	1241±5	4.03±0.01
Bekaa	P2	Ambriss	*Lactiplantibacillus plantarum*	LPP11	-0.0039±0.001	711±265	5.47±0.5	693±0	929±0	1295±9	4.01±0.00
Bekaa	P1	Ambriss Milk	*Lactiplantibacillus plantarum*	LP12	-0.0031±0.0002	623±254	5.67±0.43	696±55	933±63	1394±216	4.06±0.00
Chouf	P3	Serdaleh Milk	*Lactiplantibacillus plantarum*	LP13	-0.0033±0.0001	761±82	5.55±0.21	797±22	1015±37	1427±47	4.13±0.03
Bekaa	P2	Ambriss	*Lactiplantibacillus plantarum*	LP14	-0.0013±0.000	2429±0	5.21±0.04	2102±0	2627±44	3564±78	4.37±0.00
South	P5	Labneh	*Lactiplantibacillus plantarum*	LP15	-0.0028±0.000	859±8	5.37±0.01	806±13	1043±4	1479±17	4.12±0.01
South	P6	Laban	*Lactiplantibacillus plantarum*	LP17	-0.0025±0.0009	1102±191	5.37±0.32	1016±8	1327±3	1691±38	3.80±0.06

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TABLE VI-I. MEAN VALUES OF ACIDIFICATION PARAMETERS OF 40 LAB STRAINS COLLECTED FROM AMBRISS, SERDALEH AND LABNEH EL DARFF PRODUCTS AND IDENTIFIED BY 16S RNA SEQUENCING.

South	P6	Laban	*Lactiplantibacillus plantarum*	LP18	-0.0031±0.000	672±8	5.63±0.04	730±10	969±27	1314±88	3.98±0.04
Bekaa	P1	Ambriss Milk	*Lactiplantibacillus plantarum*	LP19	-0.0033±0.0001	646±12	5.54±0.04	664±6	892±8	1229±5	3.98±0.00
Bekaa	P1	Ambriss	*Lacticaseibacillus rhamnosus*	LR20	-0.0025±0.000	1510±0	5.51±0.0	1513±0	1751±0	2224±0	4.05±0.07
Bekaa	P2	Ambriss	*Lacticaseibacillus rhamnosus*	LR21	-0.0063±0.0014	747±83	5.65±0.14	864±5	1150±3	1417±20	3.33±0.02
Bekaa	P1	Ambriss	*Lacticaseibacillus rhamnosus*	LR22	-0.0042± 0.0005	541±29	5.58±0.09	566±2	741±0	936±0	3.62±0.00
South	P6	Labneh	*Lactobacillus kisonensis /parakefiri/buchneri*	LKN24	-0.0038±0.002	599±23	5.73±0.16	697±52	929±55	1350±184	4.05±0.00
Bekaa	P2	Ambriss	*Lactobacillus kefiranofaciens*	LKF26	-0.0031±0.0006	630±82	5.73±0.20	739±21	995±35	1437±93	4.05±0.02
South	P5	Laban	*Lactobacillus kisonensis /parakefiri/buchneri*	LKN28	-0.0028±0.0007	1906±6	5.52±0.02	1916±19	2160±38	2632±33	3.85±0.10
South	P6	Labneh	*Levilactobacillus brevis*	LB31	-0.0022±0.000	1435±59	5.75±0.00	1571±57	2111±47	--±--	5.01±0.05
South	P6	Labneh	*Levilactobacillus brevis*	LB33	-0.0024±0.002	1635±238	5.51±0.29	1652±101	1988±83	2492±253	4.01±0.07
South	P5	Labneh	*Levilactobacillus brevis*	LB34	-0.0022±0.0001	831±32	5.56±0.09	861±19	1139±27	164±76	4.12±0.01
South	P5	Labneh	*Levilactobacillus brevis*	LB35	-0.0030±0.0003	828±57	5.63±0.13	895±15	1121±5	1506±2	4.04±0.04
South	P5	Laban	*Levilactobacillus brevis*	LB36	-0.0029±0.0009	616±68	5.79±0.15	763±22	1032±20	1496±33	4.12±0.03
South	P5	Laban	*Levilactobacillus brevis*	LB37	-0.0032±0.0003	763±8	5.45±0.19	744±79	955±89	1344±116	4.05±0.04
Chouf	P3	Serdaleh	*Lactobacillus diolivorans*	LD40	-0.0032±0.0004	548±14	5.80±0.03	729±50	973±56	1424±118	4.16±0.03
Chouf	P3	Serdaleh Milk	*Lactobacillus diolivorans*	LD43	-0.0032±0.0005	610±6	5.88±0.01	778±9	1004±3	1461±40	4.08±0.04
Chouf	P3	Serdaleh Milk	*Lactobacillus diolivorans*	LD44	-0.0029±0.0003	796±12	5.46±0.03	779±26	987±48	1292±37	3.99±0.03
Chouf	P5	Serdaleh	*Lactobacillus diolivorans*	LD45	-0.0032±0.000	636±0	5.65±0.00	752±71	991±70	1502±282	4.18±0.16

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TABLE VI-I. MEAN VALUES OF ACIDIFICATION PARAMETERS OF 40 LAB COLLECTED FROM AMBRISS, SERDALEH AND LABNEH EL DARFF PRODUCTS AND IDENTIFIED BY 16S RNA SEQUENCING.

South	P5	Labneh	*Lactobacillus diolivorans*	LD46	-0.0029±0.0001	810±12	5.44±0.07	786±17	997±24	1366±42	4.20±0.27
Chouf	P3	Serdaleh Milk	*Lactococcus lactis* ssp *cremoris*	LC50	-0.006±---	202±---	6.19±--	371±--	677±--	1326±--	3.96±--
Bekaa	P2	Ambriss	*Enterococcus durans*	ED52	-0.0048±0.0001	246±10	6.06±0.05	502±37	1067±172	3389±603	4.39±0.08
Bekaa	P1	Ambriss	*Enterococcus durans*	ED53	-0.0051±0.0003	228±2	6.09±0.01	452±23	955±35	2205±235	4.31±0.14
Bekaa	P2	Ambriss	*Enterococcus durans*	ED55	-0.0049±0.0001	228±14	6.11±0.03	486±41	1022±178	2470±372	4.34±0.09
Bekaa	P2	Ambriss	*Enterococcus durans*	ED57	-0.0047±0.0001	231±0	6.12±0.01	511±30	1109±94	2903±679	4.43±0.06
Bekaa	P1	Ambriss	*Enterococcus durans*	ED65	-0.0052±0.0006	210±10	6.08±0.05	443±21	970±26	2878±1220	4.44±0.02

Vmax: maximum acidification rate in absolute values (in upH/min), tmax: time at which the maximum acidification rate was achieved (in min), pHmax: pH at which maximum acidification rate occurred, tpH5.5, tpH5.0, and tpH4.5: times to reach pH 5.5, pH 5.0 and pH 4.5 respectively (in min), pHf: final pH attained at the end of the fermentation

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According to these descriptors, the acidification rates (Vmax, in absolute values) varied between 0.0067 and 0.0013 upH/min. The highest value was obtained with the strain *Lc. lactis* LL01, whereas the lowest one characterized the strain *Lpb. plantarum* LP14. The pHmax values were comprised between 6.19 and 5.21, thus showing a large range of values. The time tmax to reach Vmax varied between 202 min (for *Lc. cremoris* LC50) and 2429 min (for *Lpb. plantarum* LP14), corresponding to a range comprised between 3h12 and 40h29. The times to reach pH 4.5 also strongly differed between the strains, from 936 min (15h36) for *Lb. rhamnosus* LR22 to 3999 min (66h39) for *Lb. kefiri* LK06. These very large ranges of fermentation times indicate a great diversity between the strains when cultivated in goat milk.

VI.1.2. Statistical correlations between the acidification parameters

The statistical relationships between the acidification parameters were determined using the Pearson's correlation tool by considering all the tested strains. This approach makes it possible to reduce the number of parameters, if needed, to characterize the tested strains for further statistical analyses. The parameter Vmax was negatively correlated with pHmax (r = -0.72). The parameter tpH5.5 was positively correlated with tmax (r = 0.98) and tpH5.0 (r = 0.96). The parameter tpH4.5 was only slightly correlated to tpH5.0 (r = 0.75) and the parameter pHf was not correlated to the other parameters. Consequently, in order to reduce the number of descriptors for further analyses, the four parameters Vmax, tpH5.5, tpH4.5 and pHf were retained.

VI.1.3. Hierarchical cluster analysis of the strains by considering the acidification parameters

A Hierarchical Cluster Analysis (HCA) was conducted in order to separate the strains into 4 groups. The mean values of acidification parameters for each cluster are presented in **Table VI.2**.

TABLE VI-2. MEAN VALUES OF ACIDIFICATION PARAMETERS CHARACTERIZING THE 4 CLUSTERS AND THEIR REPRESENTATIVE STRIANS OBTAINED BY HCA FOR THE 40 LAB STRAINS

Cluster	Representative Strain	Vmax (upH/min)	tpH5.5 (min)	tpH4.5 (min)	pHf
A (n = 27)	LK08	0.0041±0.0012	710±171	1357±155	4.05±0.18
B (n = 5)	PA05	0.0020±0.0003	1800±340	3999±217	4.86±0.24
C (n = 3)	LB33	0.0024±0.0002	1651±165	2492±169	4.00±0.09
D (n = 5)	ED65	0.0052±0.0002	442±11	2878±405	4.43±0.05

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n = number of strains, Vmax: maximum acidification rate (in absolute value, in upH/min), tpH5.5 and tpH4.5: times to reach pH 5.5 and pH 4.5 (in min), respectively, pHf: final pH at the end of the fermentation

The classification of the strains into 4 clusters is given in **Table VI.3.** Cluster A included 67.5% of the strains and was represented by the strain *Len. kefiri* LK08. It included a large diversity of strains form different genera and species *(Lc. lactis, Lc. lactis ssp. cremoris, Len kefiri, Lb. kisonensis, Lb. kefiranofaciens, Lpb. plantarum, L. rhamnosus, Lev. brevis, Lb. diolivorans*). It exhibited a moderate value of Vmax, low values of tpH5.5 and tpH4.5 and a low final pH value. Cluster B included 12.5% of the strainss showing very low values of Vmax, high values of pH5.5 and tpH4.5 and high values of final pH; It included the two strains of *P. acidilactici* and was represented by the strain *P. acidilactici* PA05. Strains belonging to Cluster C (7.5% of the strains, represented by the strain *Lev. brevis* LB33) displayed similar characteristics to those of cluster B by considering Vmax and tpH5.5, but lower values of tpH4.5 and final pH. Finally, cluster D contained all strains of *E. durans* and was represented by the strain *E. durans* ED65. It enclosed 12.5% of the isolates and was characterized by the most acidifying strains as they displayed the highest Vmax and the lowest tpH5.5. However, the tpH4.5 value remained high and the final pH was intermediate.

TABLE VI-3. CLUSTER CLASSIFICATION OBTAINED BY HCA OF THE 40 LAB STRAINS ACCORDING TO THE DESCRIPTORS OF ACIDIFICATION ACTIVITY

Clusters	A	B	C	D
Strain code	LL01	PA04	LR20	ED52
	LL03	PA05	LKN28	ED53
	LK07	LK06	LB33	ED55
	LK08	LP14		ED57
	LK09	LB31		ED65
	LK10			
	LP11			
	LP12			
	LP13			
	LP15			
	LP17			
	LP18			
	LP19			
	LR21			
	LR22			
	LKN24			

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LKF26
LB34
LB35
LB36
LB37
LD40
LD43
LD44
LD45
LD46
LC50

Another HCA was conducted, by considering only the Vmax parameter, in order to differentiate the strains according to their acidification rate. Four groups were identified by HCA. The classification of the strains into these 4 clusters is presented into **Table VI.4**.

TABLE VI-4. CLUSTER CLASSIFICATION BY HCA OF 40 STRAINS ACCORDING TO THEIR ACIDIFICATION RATE VM BY CINAC SYSTEM.

Cluster	**A**	**B**	**C**	**D**
Strain code	LL01	PA04	LK07	LK08
	LL03	PA05	LK09	LK10
	LR21	LK06	LP12	LPP11
	LC50	LP14	LP13	LR22
		LP17	LP15	LKN24
		LR20	LP18	ED52
		LB31	LP19	ED53
		LB33	LKF26	ED55
		LB34	LKN28	ED57
			LB35	ED65
			LB36	
			LB37	
			LD40	
			LD43	
			LD44	
			LD45	
			LD46	

Cluster A (10% of the strains) contained all of the *Lactococcus strains* (LL01, LL03, LC50) and one *L. rhamnosus* (LR21). They were fast acidifying strains (Vm = 0.0063±0.0002 upH/min) when compared to Cluster B (22.5%), including the two *P. acidilactici* and Cluster C (42.5%), including all the *Lb. diolivorans* (Vm = 0.002± 0.0004 and 0.003± 0.0003 upH /min respectively).

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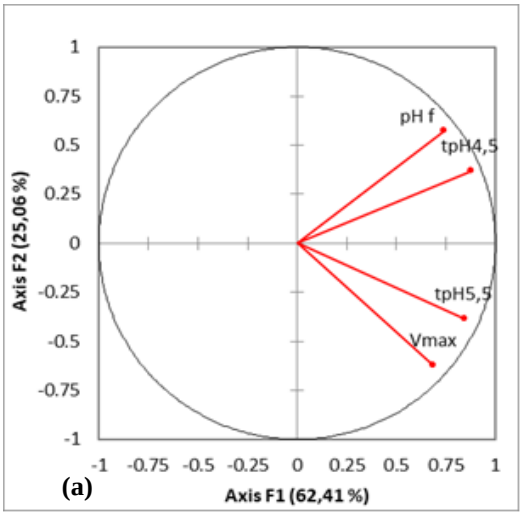
Cluster D (25%) included all of the *E. durans* and was characterized by a moderate acidification rate (Vm = 0.0044± 0.0005 u.pH/min). The other strains were divided among 2 or 3 of these clusters, thus indicating a diversity of their acidification rate within each species.

VI.1.4. Principal Component Analysis of the strainss according to their acidification parameters

To separate the strains according to their clusters, a Principal Component Analysis (PCA) allowed identifying the groups of strains according to their attributes. The two principal components PC1 and PC2 explained 87.5% of the variance. Following the Kaiser's rule, only the two first components were retained as their eigenvalues were respectively equal to 2.50 and 1.00, whereas that of the third component was equal to 0.40.



(a)

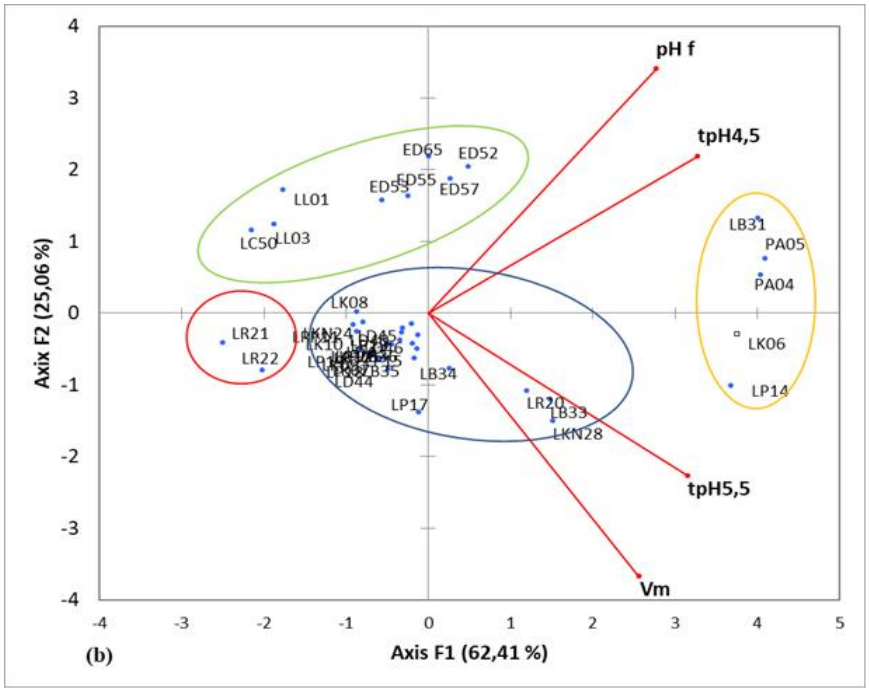


FIGURE VI-1. GRAPHICAL REPRESENTATION OF THE PRINCIPAL COMPONENT ANALYSIS OF THE ACIDIFICATION PROPERTIES FOR 40 LAB STRAINS SHOWING PC1 VS. PC2 AXIS

(a) factor loadings; (b) scores of the tested strains. Vm: maximum acidification rate (upH/min), tpH5.5: time to reach pH 5.5 (min), tpH4.5: time to reach pH 4.5 (min), pHf: Final pH attained at the end of the fermentation process

According to **Figure VI.1a**, all of the 4 attributes of acidification activity: Vm, tpH5.5, tpH4.5 and pHf positively contributed to the first axis PC1 which accounted for 62.41 % of the total variation. The second component PC2 represented 25.06 % of the variability and was able to differentiate the attributes tpH4.5 and pHf (on the positive side) from the attributes Vmax and tpH5.5 (on the positive side).

On the other hand, the plot of factors scores separated the isolates into 4 distinct groups **(Figure VI.1b).** All of the *Enterococcus* (n=5) and *Lactococcus* (n= 3) strains were grouped in the upper left part of the plot (green ellipse). They were characterized by a high acidification rate, a low tpH5.5, but high values of tpH4.5 and final pH. These isolates started the acidification rapidly, but the end of the process was slower and the products were not too acidic. The plot distinguished the two *L. rhamnosus* strains LR21 and LR22 (red ellipse) that showed a high rate of acidification,

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a short tpH4.5 and a very low pHf. In the right part of the graph (orange ellipse), five strains were identified by their low Vmax but high tpH5.5, tpH4.5 and pHf. They included the *Pediococcus* strains PA04 and PA05, and the isolates LK06, LP14 and LB31. The last group (blue ellipse) was composed of 25 isolates. They were characterized by medium values of Vm, tpH5.5, tpH4.5 and pHf.

VI.1.5. Results summary and analysis

From these results, a real diversity existed among the isolates by considering their acidification activity, and only a few strains displayed specific characteristics. More specifically, these acidification characteristics were first established in this study for the following species: *Lb. diolivorans*, *Lb. kisonensis*, *Len. kefiri*, *Pediococcus acidilactici*, *Lpb. plantarum* and *Lb. rhamnosus.*

In addition to this outcome, common characteristics were observed within the genus *Enterococcus and Lactococcus* and the species *L. rhamnosus* and *P. acidilactici*. Conversely, the acidification characteristics of the other species (*Lev. brevis, Lb. diolivorans Len. kefiri, Lb. kisonensis* and *Lpb. plantarum*) were strain–dependent and varied by considering their acidification rate, times to reach pH 5.5 and pH 4.5 and final pH.

By comparing the acidification descriptors of our isolates to native Lactic acid bacteria isolated from traditional Lebanese Laban, higher acidification rates and shorter times to reach pH 4.5 were presented for *Lb. bulgaricus* (0.0111 ± 0.0017 u.pH/min; 337 min) and for *Streptococcus thermophilus* (0.0085 ± 0.0009 u.pH/min; 297 min). Although all of the isolate were considered as slow acidifying strains (Vm < 0.0067 upH/min) with a long time to reach tpH4.5 (> 936 min), however some strains namely *Lc lactis* ssp *cremoris* LC50 and *L. rhamnosus* LR21 could reached a low pH at the end of the fermentation process of 3.96 and 3.33 respectively.

Acidifying properties are known to be ascribed to strain and species specificities (Spinnler and Corrieu, 1989; Xanthopoulos *et al*., 2001; Badis *et al*. 2004; Chammas *et al*. 2006b; Malek *et al*., 2012). These characteristics of the isolates affect the final acidity and the texture of the fermented milk products (McAuliffe, 2018). Badis *et al*. (2004) also explained the differences in acidification abilities between strains by their capacity to use the culture medium.

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The highest maximum acidification rate was attributed to the *Lc. lactis* and *Lc. lactis* ssp *cremoris* strains, which ranged from 0.0061 to 0.0067 upH/min in absolute values. They also displayed the shortest time to reach pH 5.5. As *Lc. lactis* species is considered as not so fast acidifier (Wouters *et al*., 2002), this indicates that all the *Lactococci* strains isolated in this work were globally poor acidifying. Strains of *cremoris* species are often favored as defined starters because they tend to cause less bitterness (Li *et al*., 2020).

The rapid pH decrease during milk fermentation may support protein hydrolysis, thus producing more free amino nitrogen that affect the final sensory quality of the product (Ayad *et al*., 2004). The *E. durans* strains presented low acidifying activity, as their maximum acidification rate Vm varied from 0.0047 to 0.0052 upH/min. This range is comparable to that obtained by Malek *et al*., (2012), varying from 0.0018–0.0041 upH/min for *E. faecium*. This is confirmed by the high value of tpH4.5 range (2205 -3389 min) for *E. durans*, which is linked to a long latency period. These values are higher than tpH4.5 of *L. rhamnosus* (969-2224 min) or lactococci strains (1009- 1329 min).

Finally, our results highlighted that the lactic acid bacteria collected from the Lebanese fermented goat milks globally displayed a low acidification activity, thus justifying the high duration of the fermentation processes realized by the artisanal producers.

VI.2. Physical and rheological characterization

VI.2.1. Raw results of physical and rheological properties

The physical and rheological parameters of the products inoculated with each of the 40 strains were quantified. Using Texture Profile Analysis (TPA), the firmness, the cohesiveness and the fracturability of the products were quantified (Chammas *et al*., 2006b). A rotary viscometer allowed quantifying the apparent viscosity according to Damian, (2013) and syneresis was measured after centrifugation of the product according to Gentès *et al*., (2011). The mean values of the physical and rheological parameters of the analyzed fermented milk products are presented in **Table VI.5**.

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TABLE VI-5. MEAN VALUES OF PHYSICAL AND RHEOLOGICAL CHARACTERISTICS OF 40 LAB STRAINS COLLECTED FROM AMBRISS, SERDALEH AND LABNEH EL DARFF PRODUCTS AND IDENTIFIED BY 16S RNA SEQUENCING.

Region	Producer	Product type	Strain code	Species identified by 16S	FRA (N)	FIR (N)	COH	AV (Pa.s)	Syneresis (%)
Chouf	P3	Serdaleh Milk	LL01	*Lactococcus lactis*	0.34±0.04	1.29±0.02	0.87±0.02	0.48±0.04	12.53±0.43
Chouf	P3	Serdaleh Milk	LL03	*Lactococcus lactis*	0.31±0.02	1.35±0.08	0.91±0.04	0.814±0.05	12,72±0.80
Bekaa	P1	Ambriss	PA04	*Pediococcus acidilactici*	0.44±0.02	2.34±0.10	0.80±0.01	0.92±0.03	39.47±1.47
Bekaa	P1	Ambriss	PA05	*Pediococcus acidilactici*	0.72±0.06	1.94±0.15	0.90±0.02	0.13±0.01	43.05±1.07
Chouf	P4	Serdaleh	LK06	*Lentilactobacillus kefiri*	0.46±0.02	2.07±0.17	0.87±0.01	0.62±0.04	4.94±0.37
Chouf	P3	Serdaleh	LK07	*Lentilactobacillus kefiri*	0.50±0.02	2.25±0.11	0.84±0.04	0.12±0.0	4.60±0.34
Chouf	P3	Serdaleh	LK08	*Lentilactobacillus kefiri*	0.41±0.0	2.16±0.13	0.85±0.08	0.11±0.0	4.36±0.17
Chouf	P3	Serdaleh	LK09	*Lentilactobacillus kefiri*	0.47±0.03	2.44±0.1	0.78±0.04	0.33±0.11	8.38±0.48
Chouf	P4	Serdaleh	LK10	*Lentilactobacillus kefiri*	0.58±0.03	3.49±0.21	0.76±0.03	0.68±0.0	6.23±0.31
Bekaa	P2	Ambriss	LPP11	*Lactiplantibacillus plantarum*	0.57±0.05	2.92±0.27	0.80±0.06	1.15±0.03	6.59±0.6
Bekaa	P1	Ambriss Milk	LP12	*Lactiplantibacillus plantarum*	0.47±0.03	3.99±0.27	0.92±0.04	0.37±0.01	28.71±0.77
Chouf	P3	Serdaleh Milk	LP13	*Lactiplantibacillus plantarum*	0.60±0.0	1.62±0.12	0.91±0.01	1.12±0.03	60.27±0.74
Bekaa	P2	Ambriss	LP14	*Lactiplantibacillus plantarum*	0.34±0.0	2.75±0.14	0.69±0.06	0.35±0.00	68.35±1.37
South	P5	Labneh	LP15	*Lactiplantibacillus plantarum*	0.77±0.08	2.21±0.6	0.91±0.02	0.42±0.01	73.07±1.78
South	P6	Laban	LP17	*Lactiplantibacillus plantarum*	0.59±0.02	3.10±0.13	0.77±0.06	0.20±0.01	8.11±0.44
South	P6	Laban	LP18	*Lactiplantibacillus plantarum*	0.48±0.02	1.99±0.12	0.82±0.02	0.28±0.00	9.98±0.07
Bekaa	P1	Ambriss Milk	LP19	*Lactiplantibacillus plantarum*	0.53±0.02	2.20±0.13	0.79±0.01	0.40±0.02	8.73±0.19
Bekaa	P1	Ambriss	LR20	*Lacticaseibacillus rhamnosus*	0.55±0.02	1.84±0.10	0.86±0.06	0.71±0.03	67.29±0.98
Bekaa	P2	Ambriss	LR21	*Lacticaseibacillus rhamnosus*	0.49±0.04	2.12±0.11	0.83±0.01	0.73±0.05	5.93±0.18
Bekaa	P1	Ambriss	LR22	*Lacticaseibacillus rhamnosus*	0.50±0.02	2.14±0.02	0.81±0.06	0.75±0.03	5.91±0.20
South	P6	Labneh	LKN24	*Lactobacillus kisonensis/parakefiri/buchneri*	0.52±0.04	2.36±0.08	0.84±0.04	0.88±0.04	7.96±0.06
Bekaa	P2	Ambriss	LKF26	*Lactobacillus kefiranofaciens*	0.46±0.02	2.56±0.23	0.87±0.03	0.82±0.03	7.56±0.17
South	P5	Laban	LKN28	*Lactobacillus kisonensis/parakefiri/buchneri*	0.22±0.0	1.30±0.19	0.90±0.07	0.39±0.02	16.00±0.42
South	P6	Labneh	LB31	*Levilactobacillus brevis*	0.77±0.04	2.62±0.16	0.81±0.04	0.86±0.01	70.87±2.54
South	P6	Labneh	LB33	*Levilactobacillus brevis*	0.33±0.02	1.91±0.06	0.83±0.08	0.65±0.04	9.49±1.36
South	P5	Labneh	LB34	*Levilactobacillus brevis*	0.59±0.02	1.96±0.15	0.81±0.02	0.81±0.01	53.34±1.67
South	P5	Labneh	LB35	*Levilactobacillus brevis*	1.11±0.06	3.52±0.28	0.73±0.05	0.79±0.01	58.43±1.87
South	P5	Laban	LB36	*Levilactobacillus brevis*	1.02±0.06	2.29±0.16	0.77±0.01	0.79±0.01	21.20±1.39
South	P5	Laban	LB37	*Levilactobacillus brevis*	0.35±0.02	1.66±0.11	0.85±0.02	0.84±0.01	46.15±2.85

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Region	Producer	Product type	Strain code	Species identified by 16S	FRA (N)	FIR (N)	COH	AV (Pa.s)	Syneresis (%)
Chouf	P3	Serdaleh	LD40	*Lactobacillus diolivorans*	0.53±0.02	2.69±0.22	0.79±0.04	1.73±0.03	4.08±0.23
Chouf	P3	Serdaleh Milk	LD43	*Lactobacillus diolivorans*	0.43±0.08	2.06±0.09	0.83±0.04	1.33±0.02	8.20±0.52
Chouf	P3	Serdaleh Milk	LD44	*Lactobacillus diolivorans*	0.79±0.04	4.36±0.24	0.78±0.03	1.28±0.02	1.12±0.07
Chouf	P5	Serdaleh	LD45	*Lactobacillus diolivorans*	0.86±0.06	3.37±0.26	0.75±0.05	1.57±0.03	1.28±0.06
South	P5	Labneh	LD46	*Lactobacillus diolivorans*	1.03±0.03	4.37±0.08	0.76±0.05	1.39±0.02	2.16±0.08
Chouf	P3	Serdaleh Milk	LC50	*Lactococcus lactis ssp cremoris*	0.45±0.04	2.38±0.08	0.83±0.01	1.01±0.09	11.15±0.63
Bekaa	P2	Ambriss	ED52	*Enterococcus durans*	0.35±0.02	1.94±0.17	0.75±0.03	0.71±0.05	8.42±0.14
Bekaa	P1	Ambriss	ED53	*Enterococcus durans*	0.44±0.02	2.12±0.09	0.83±0.04	0.71±0.02	4.46±0.14
Bekaa	P2	Ambriss	ED55	*Enterococcus durans*	0.51±0.02	2.41±0.13	0.82±0.03	0.93±0.02	8.49±0.50
Bekaa	P2	Ambriss	ED57	*Enterococcus durans*	0.41±0.0	2.18±0.05	0.83±0.02	0.80±0.08	2.22±0.04
Bekaa	P1	Ambriss	ED65	*Enterococcus durans*	0.26±0.0	1.14±0.12	0.88±0.04	0.83±0.04	16.00±0.42

FRA: Fracturability (in N); FIR: Firmness (in N); COH: Cohesiveness; AP: Apparent viscosity (Pa.s); SYN: syneresis (%)

The firmness parameter varied between 1.14 and 4.37 N. The highest values were observed with the *Lb. diolivorans* strains LD44 and LD46 (4.36 and 4.37 N) which are originated from « Serdaleh Milk » and Labneh El Darff respectively. Conversely, the fermented milks obtained with the genus *Lactococcus* and *Enterococcus* displayed a low firmness (1.14 to 1.94 N). The other strains generated a large diversity of firmness in the products.

By considering fracturability, the values ranged between 0.22 and 1.11 N. The highest values were obtained with three *Lb. diolivorans* and three *Lev. brevis* (0.77 to 1.11 N) whereas the lowest values were achieved in the products elaborated with the *Lactococcus* and *Enterococcus* strains. The other isolated generated a large diversity of fracturability of the products.

The cohesiveness of the products was comprised between 0.69 and 0.92. The *Lb. plantarum* isolates LP12, LP13 and LP15 showed the highest cohesiveness (ranging from 0.91 to 0.92) when compared to the other fermented milks. The other isolated displayed various levels of cohesiveness, without any variability within a given species.

The apparent viscosity varied considerably in the fermented products obtained with the different strains. It ranged between 0.11 and 1.73 Pa.s. Most of the *Lb. diolivorans* led to highly

viscous products (1.28 to 1.73Pa.s), whereas *Lb. kefiri* isolates induced low viscous products (0.11 to 0.68 Pa.s). The viscosity measured in the other fermented milks varied within the other species.

Finally, by considering syneresis, fermented milks displayed a wide range of values (1.1% to 73.1%). Most of the products obtained with *Lb. diolivorans*, and *Len. kefiri* and *E. durans* showed low syneresis, ranging from 1.1% to 4.9%. Conversely, the products obtained with the species *Lpb. plantarum, Lev. brevis* and *P. acidilactici* showed high syneresis (21.2% to 73.1%). The other species displayed a higher diversity by considering syneresis.

VI.2.2. Correlations between the physical and rheological parameters

The statistical relationship between the physical and rheological parameters was observed using the Pearson's correlation tool. The Firmness parameter was positively correlated with Fracturability (r = 0.63). The later was not significantly correlated with Apparent Viscosity (r = 0.29) and Syneresis (r = 0.22). Cohesiveness was negatively but not significantly correlated with Syneresis (r = -0.37), Firmness (r = -0.48) and Apparent Viscosity (r = -0.24).

VI.2.3. Hierarchical cluster analysis of the strains by considering physical and rheological parameters

Based on the statistical correlations between the technological and physical parameters, Cohesiveness, Firmness, Syneresis and Apparent Viscosity were retained for the cluster analysis, and results of Fracturability will be analyzed together with those of Firmness. Regarding the results of HCA, the LAB were separated into 4 distinct groups (**Table VI.6).**

TABLE VI-6. CLUSTER CLASSIFICATION OBTAINED BY HCA OF THE 40 STRAINS ACCORDING TO THEIR PHYSICAL AND RHEOLOGICAL PARAMETERS.

Cluster	A	B	C	D
Strains	LL01	PA04	LP12	LP13
	LL03	PA05	LKN28	LP14
	LK06	LB37	LB36	LP15
	LK07		ED65	LR20
	LK08			LB31
	LK09			LB34
	LK10			LB35
	LPP11			

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LP17
LP18
LP19
LR21
LR22
LKN24
LKF26
LB33
LD40
LD43
LD44
LD45
LD46
LC50
ED52
ED53
ED55
ED57

The first group was the largest and the most diversified one, with 26 strains belonging to 9 species. The second group included three strains of which two *P. acidilactici*. The third group comprised four strains from four species. The last group was constituted by seven strains, including three *Lpb. plantarum* and three *Lev. brevis*. The mean values of the parameters of the clusters are presented in **Table VI.7**.

TABLE VI-7. MEAN VALUES OF PHYSICAL AND RHEOLOGICAL PARAMETERS OF THE DIFFERENT CLUSTERS OF 40 LAB STRAINS OBTAINED BY HCA.

Cluster	Cohesiveness	Firmness (N)	Apparent viscosity (PA.s)	Syneresis (%)	Fracturability (N)
A (n=26)	0.81±0.04	2.59±0.74	0.81±0.42	6.32±3.14	0.53±0.16
B (n = 3)	0.85±0.04	1.98±0.28	0.63±0.35	42.89±2.73	0.50±0.16
C (n = 4)	0.87±0.06	2.18±1.13	0.60±0.22	20.48±5.20	0.49±0.32
D (n = 7)	0.82±0.08	2.36±0.60	0.72±0.25	64.52±6.72	0.67±0.22

n = number of strains

The fermented products elaborated with the isolates belonging in Cluster A (65% of the strains) showed the highest values of Firmness and Apparent Viscosity; however, they were characterized by low Syneresis and moderate Cohesiveness compared to the other clusters.

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Fermented milks obtained from Cluster B (7.5%) displayed the lowest Firmness (1.98±0.28 N). Samples of Cluster C (10%) were the highest in Cohesiveness (0.87±0.06), but showed the lowest values in Firmness, Fracturability, Apparent Viscosity and Syneresis. On the contrary, the products obtained with the strains of Cluster D (17.5%) displayed the highest values for Syneresis (64.52%) and Fracturability (0.67±0.22 N). The distribution of the species into the clusters indicated that the *lactococci*, *Len. kefiri*, *L. rhamnosus* and most of *E. durans* and *L. rhamnosus* isolates were grouped in cluster A. The two isolates of *P. acidilactici* belonged cluster B. The isolates corresponding to *Lev. brevis*, *Lpb. plantarum* and *Lb. kisonensis* were distributed into 2 or 3 clusters, thus indicating a larger range of physical and rheological properties for these strains.

VI.2.4. Principal Component Analysis of the technological parameters

In order to separate the isolates according to their physical and rheological properties, a Principal Component Analysis (PCA) of the attributes was carried out **(Figure VI.2)**.

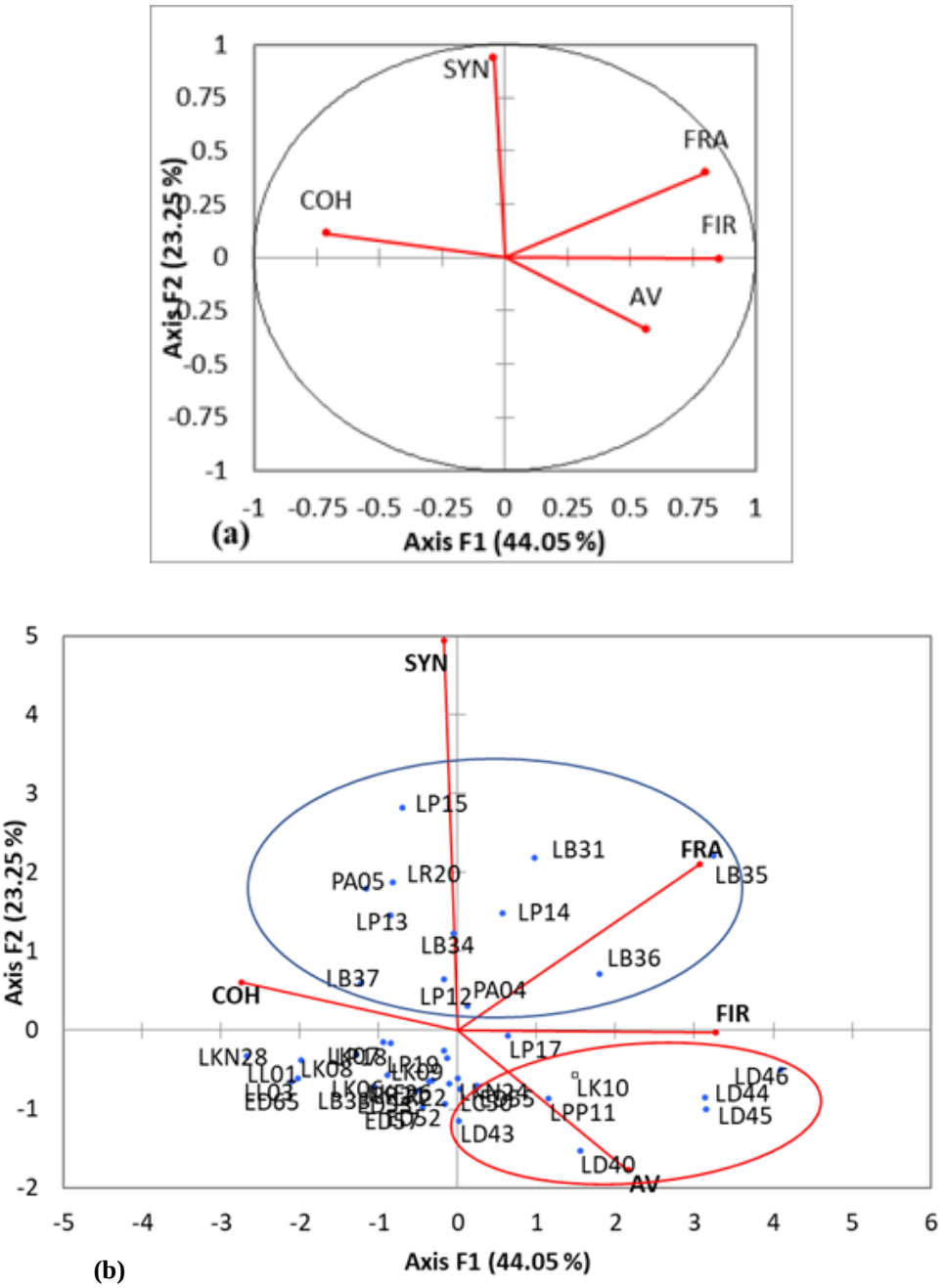

FIGURE VI-2. GRAPHICAL REPRESENTATION OF THE PRINCIPAL COMPONENT ANALYSIS OF THE PHYSICAL AND RHEOLOGICAL PROPERTIES OF FERMENTED MILKS OBTAINED WITH 40 LAB STRAINS SHOWING PC1 VS. PC2 AXIS

(a) Factor loadings; **(b)** scores of the tested isolates. COH: Cohesiveness, SYN: Syneresis (%), FRA: Fracturability (N), Firmness (N) and AV: Apparent Viscosity (Pa.s).

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The plot shows that two principal components PC1 and PC2 were able to explain 67.30% of the variance (Figure IV.2a). The third axis PC3 was dropped because its eigenvalue (0.77) was lower than one according to Kaiser's rule. The factor scores of the sample **(Figure VI.2b)** show that the first axis PC1 (44.05% of the total variation) distinguished the strains according to Firmness (FIR), Fracturability (FRA) as indicated from the previous correlations, Cohesiveness (COH), Apparent Viscosity (AV) variables. The second axis PC2 (23.25% of the variation) was explained by Syneresis (SYN).

The PCA discriminated five *Lb. diolivorans* strains, one *Lpb. plantarum* (LP11), one *Len. kefiri* (LK10) and one *E. durans* (ED55) that formed one group in the lower right part of the graph (red ellipse). This group was characterized by high Firmness and Apparent Viscosity but low Cohesiveness and low Syneresis. In the upper part of the plot were located srain which presented high values of Syneresis, but low values of Apparent Viscosity. This group included all of *P. acidilactici* strains, most of *Lev. brevis* and of three *Lpb. plantarum*. The remaining bacteria were separated because they displayed together low Syneresis and low Firmness and Fracturability.

VI.2.5. Results summary and analysis

Rheological and physical properties of fermented milk products play an essential role in sensory characteristics and consumer acceptability (Mende *et al*., 2016; Ozcan, 2013). These are mainly affected by milk composition, manufacturing process and culture type (Sodini *et al*., 2004), and are largely dependent on the structural arrangement and microstructure of the protein network in the fermented milk (Delikanli and Ozcan, 2014; Nguyen *et al*., 2018).

A high degree of firmness, which is positively correlated to fracturability, was displayed by all the fermented milks inoculated with the LAB isolates. This physical parameter ranged from 1.14 N for *E. durans* ED65 to 4.37 N for *Lb. diolivorans* LD46. These attributes were higher than those obtained in traditional Laban by (Chammas *et al*., 2006b), who obtained a firmness value of 0.529 N for *Lb. bulgaricus* and byMarshall and Rawson, (1999) in fermented milks (0.31 N and 0.64 N). In addition, our results were higher than those recorded by Mituniewicz-Małek *et al*.,(2017) for goat milk yogurt (0.2 N and 0.26 N).

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Cohesiveness is another indicator that characterizes the structural integrity of fermented milks (Nishinari and Fang, 2018, Prajapathi *et al*., 2020). Many authors suggested that a high cohesiveness is related to the water-binding ability of EPS which limits the whey separation of the fermented product and the loosening of the protein gel structure (Prajapathi *et al*., 2020; Patel and Prajapati, 2013**).** The values of cohesiveness obtained in this study varied between 0.69 and 0.92, thus showing a low variability. They were in agreement with the values indicated by Chammas *et al*., (2006) for fermented milks with strains which are isolated from Laban. In addition, these authors reported higher values of cohesiveness for *lactobacilli* (1.09) but lower values (0.676) for streptococci strains. By considering the species *Lpb. plantarum*, Zhao and Liang (2022) demonstrated that the supplementation of yogurt with the strain *Lpb. plantarum* MC5 strain improved significantly the cohesiveness of the product. In our study, the strains *Lpb. plantarum* LP12, LP13 and LP15 led to highly cohesive gels, in accordance with this work. Our values were also higher than those recently recorded by Szopa and Pawlos, (2023), whose values reached 0.58, 0.67 and 0.76 when inoculated with *L. paracasei*, *L. rhamnosus* or *Lb. acidophilus*, respectively.

Results obtained from the tested fermented products showed a low apparent viscosity (<1.73 Pa.s) for all LAB srains, thus characterizing low viscous gels. These results were in agreement with those reported by Chammas *et al*., (2006b) who displayed apparent viscosity values comprised between 0.01 Pa.s and 1.28 Pa.s for *S. thermophilus* and between 0.29 Pa.s and 0.82 Pa.s for *Lb. bulgaricus*. The mean value of viscosity for *E. durans* strains (0.80 Pa.s) was slightly higher than those observed by Malek *et al*., (2012) for 35 strains of *E. faecium* isolated from traditional Egyptian cheeses (0.05 to 0.41 Pa.s). The viscosity of a fermented milk is generally ascribed to the production of exopolysaccharides (EPS) by polymer-producing strains (Marshall and Rawson, 1999; Béal *et al*., 1999; Li *et al*., 2012). These EPS strongly influence the physicochemical and rheological properties of fermented milk products, thus improving the viscosity of the products and reducing the syneresis (Mende *et al*., 2022). Recently, Zhao and Liang, (2022) demonstrated that the addition of *Lpb. plantarum* strain culture to goat milk fermented with thermophilic yogurt culture significantly increased the apparent viscosity of the fermented product from 1.90 Pa.s to 12.24 Pa.s. These values of viscosity are however, considerably higher than those observed in our study.

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The syneresis measured in the fermented milks obtained with the 40 strains largely varied from 1.1 % to 73.1 %. The lowest values were recorded for the species *Lb. diolivorans*, *Len. kefiri* and *E. durans*. In contrast, most of the *Lpb. plantarum* strains displayed high syneresis (73.07 % for LP15). When comparing these results with previous ones, one can observe that the strains isolated in our study led to an important syneresis compared to those obtained by Szopa and Pawlos, (2023). In their work, syneresis was strain dependent and attained 45.6 % for *L. paracasei*, 47.0 % for *L. casei*, 56.6 % for *Lb. acidophilus* and 52.36 % for *L. rhamnosus* in fermented goat milk products. According to Verruck *et al*., (2019) and Gomes *et al*., (2013), the microstructure of fermented goat milks is more delicate, less resistant to deformation and more susceptible to syneresis in comparison with the microstructure of cow and sheep fermented milks. In addition, since cohesiveness and syneresis are interesting properties within the context of manufacturing traditional Lebanese fermented goat milk products such as Ambriss or Serdaleh, some *Lpb. plantarum* strains isolated in this study could be retained as starter cultures for their ability to reduce the water holding capacity and to increase productivity per production season.

VI.3. Quantification of produced volatile organic compounds

VI.3.1. Raw results of aroma compounds composition

The gas chromatography coupled to mass spectrometry (GC-MS) technique was used to analyse the volatile organic compounds (VOC) composition of milks fermented upon completion of fermentation with lactic acid bacteria isolates. The analyses were done on a selection of 15 strains among 40, including four *Lentilactobacillus diolivorans,* two *Lentilactobacillus kefiri,* two *Lactococcus lactis,* two *Lactiplantibacillus plantarum,* two *Levilactobacillus brevis,* one *Lactobacillus kisonensis, one Lacticaseibacillus rhamnosus* and one *Enterococcus durans.* These isolates were selected according to their different characteristics, by considering acidification activity and physical and rheological properties. They were individually inoculated in goat milk and one commercial Laban (LL) was used as a control sample. In total 17 aroma compounds were quantified, belonging to six groups: acids (6), alcohols (4), ketones (3), furans (2), aldehydes (1) and esters (1). The mean values of these compounds are presented in **Table VI.8.**

The results of VOC concentrations were first verified against the method limit of quantification (LQ = 0.1 ppm). Considerable amount of acetic acid (AA) were recorded in all

samples, ranging from 135.5 mg.kg^{-1} with *E. durans* ED55 to 2437.6 mg.kg^{-1}with *Lb. diolivorans* LD45. Butyric acid (BA) showed high relative abundance in all samples, ranging from 105.5 to 5009.8 mg.kg^{-1}, the highest concentration being reached with *Lev. brevis* LB31. Alcohol compounds are another major group of aroma founds in the tested fermented milks. Among the investigated samples, there were considerable differences in ethanol compound content (2.4 to 917.3 mg.kg^{-1}), the highest levels being found in samples fermented by *Lb. diolivorans* strains LD40 and LD45, reaching 508.9 and 917.3 mg.kg^{-1} respectively. *Lc. lactis* LL03 and *Len. kefiri* LK07 samples presented similarities in 2-furanmethanol (FM) and 2-furancarboxaldehyde (FCA). These two compounds have only been detected in these two samples, at concentrations comparable to those detected in the control Laban sample (LL) (67.4 and 81.3 mg.kg^{-1} for FCA and FM respectively). The aroma compound 2,3-butanedione (BTD) was only detected in milks fermented with LD44, LD46 and LP14, at concentrations varying between 21.8 and 43.8 mg/kg^{-1}.

TABLE VI-8. MEAN CONCENTRATIONS VALUES OF 17 VOLATILE ORGANIC COMPOUNDS MEASURED IN FERMENTED MILK SAMPLES BY 15 STRAINS

Strain number	Product type	Region	Producer	Species identified by 16S	Strain code	Acetaldehyde	Acetic acid	Benzoic acid	Butyric acid (butanoic)	n-Decanoic acid (capric)	Hexanoic acid (caproic)	Octanoic acid (caprylic)	2,3-Butanediol	Butanol	Ethanol	n-Propanol	Acetone	Acetoin	2,3-Butanedione (diacetyl)	Furfural	Furfuryl alcohol	Ethylacetate
						ACH	AA	BA	BUTA	CAP	CAPO	CAPY	BTA	BUT	ETH	PRO	ACT	ACO	BTD	FCA	FM	ETA
S03	Serdaleh milk	Chouf	P3	*Lactococcus lactis*	LL03	7.5	350.2	0.1	268.2	0.8	18.0	2.5	0.0	0.0	6.5	0.0	2.8	14.4	0.2	67.5	3.0	0.0
S07	Serdaleh	Chouf	P3	*Lentilactobacillus kefiri*	LK07	0.0	644.3	0.1	642.1	0.9	20.7	2.6	0.0	0.0	103.4	0.0	1.2	0.0	0.2	33.9	2.8	0.0
S10	Serdaleh	Chouf	P4	*Lentilactobacillus kefiri*	LK10	0.0	201.9	0.1	187.5	0.2	12.6	1.9	0.0	0.0	5.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0
S14	Ambriss	Bekaa	P2	*Lactiplantibacillus plantarum*	LP14	0.0	272.9	0.1	105.5	0.0	5.7	1.2	0.0	0.0	2.4	0.0	0.0	20.2	43.8	0.0	0.0	0.0
S15	Labneh	South	P5	*Lactiplantibacillus plantarum*	LP15	0.0	392.5	0.1	1137.1	0.0	11.6	1.8	55.0	0.0	61.6	36.5	0.0	1.8	0.0	0.0	0.0	0.0
S21	Ambriss	Bekaa	P2	*Lacticaseibacillus rhamnosus*	LR21	0.0	1214.4	0.1	2003.4	1.8	25.2	3.3	0.0	0.0	21.0	0.0	3.2	31.4	0.2	0.0	0.0	0.0
S28	Laban	South	P5	*Lactobacillus kisonensis/parakefiri /buchneri*	LKN28	0.0	728.5	0.1	232.4	0.0	7.2	1.5	30.1	0.0	21.9	67.6	0.0	8.5	0.0	0.0	0.0	0.0
S31	Labneh	South	P6	*Levilactobacillus brevis*	LB31	0.0	489.1	0.0	5009.9	0.4	77.4	1.4	0.0	10.2	35.9	34.1	0.0	0.0	0.0	0.0	0.0	0.0
S35	Labneh	South	P5	*Levilactobacillus brevis*	LB35	0.0	686.4	0.1	216.9	0.1	18.6	2.4	0.0	0.0	43.0	0.0	0.0	10.1	0.0	0.0	0.0	0.0
S11	Serdaleh	Chouf	P3	*Lentilactobacillus diolivorans*	LD40	0.0	582.3	0.1	2668.0	0.8	82.8	2.2	0.0	18.2	508.9	89.2	0.0	0.0	0.0	0.0	0.0	0.0

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S40	Serdaleh milk	Chouf	P3	*Lentilactobacillus diolivorans*	LD44	43.6	594.1	0.0	118.4	0.0	70.4	2.7	128.3	0.0	5.3	67.3	65.0	33.8	21.8	0.0	0.0	0.0
S44	Serdaleh	Chouf	P5	*Lentilactobacillus diolivorans*	LD45	0.0	2437.7	0.5	1178.0	0.3	99.2	1.8	182.9	0.0	917.3	227.6	64.5	0.0	0.0	0.0	0.0	6.0
S45	Labneh	South	P5	*Lentilactobacillus diolivorans*	LD46	0.0	587.0	0.2	268.0	1.4	174.6	2.9	238.0	17.4	87.0	74.9	0.0	3.1	43.7	0.0	0.0	0.0
S46	Serdaleh milk	Chouf	P3	*Lactococcus lactis ssp cremoris*	LC50	43.4	187.3	0.0	182.3	0.6	22.9	1.7	0.0	0.0	4.1	0.0	1.1	15.0	0.0	0.0	0.0	0.0
S46	Ambriss	Bekaa	P2	*Enterococcus durans*	ED55	0.0	135.5	0.0	667.7	0.6	13.7	1.9	0.0	0.0	11.2	0.0	1.2	12.1	0.0	0.0	0.0	0.0
-	LL	-	-	-	-	57.2	477.3	0.4	123.0	0.2	10.6	1.0	22.9	0.0	7.8	0.0	2.5	22.8	43.7	67.4	8.1	0.0

All concentrations are given in $mg.kg^{-1}$; LL: control Laban sample.

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VI.3.2. Correlations between the volatile organic compounds

Some volatile compounds were well correlated as shown by the correlation matrix according to Pearson's correlations. Acetaldehyde was significantly negatively correlated to 2-furancarboxaldehyde (r =-0.78). Benzoic acid was weakly correlated to 2,3-butanediol (r = 0.54) and acetone (r = 0.52) but strongly correlated to ethanol (r = 0.74), n-propanol (r = 0.84) and acetic acid (r = 0.85). Correlations were also found by considering alcohols like butanol and 2,3-butanediol (r = 0.58) and n-propanol and ethanol (r = 0.83). Acetic acid was positively correlated to ethanol (r = 0.79), acetone (r = 0.54), benzoic acid (r = 0.85) and n-propanol (r = 0.82). Aroma compounds that characterized the goat milk odor such as hexanoic acid was correlated to butanol (r = 0.53) and 2,3-butanediol (r = 0.77), while octanoic acid was significantly correlated to n-decanoic acid (r = 0.70).

VI.3.3. Hierarchical cluster analysis of the stains by considering the production of aroma compounds

An HCA helped classifying the aroma compounds into 3 main clusters. The mean values for each cluster are presented in **Table VI.9,** and the distribution of the strains within the clusters is given in **Table VI.10**.

TABLE VI-9. MEAN VALUES OF FLAVOR COMPOUNDS CONCENTRATIONS (IN MG.KG-1) OF THE DIFFERENT CLUSTERS SEPARATING THE 15 LAB STRAINS.

Cluster	ACH	AA	BA	BUTA	CAP	CAPO	CAPY	BTA	BUT
A (n = 11)	8,6	434,6	0,1	366,0	0,4	34,2	2,1	41,0	1,6
B (n = 3)	0,0	1411,5	0,2	1949,8	1,0	69,1	2,4	61,0	6,1
C (n = 1)	0,0	489,1	0,0	5009,9	0,4	77,4	1,4	0,0	10,2

Cluster	ETH	PRO	ACT	ACO	BTD	FCA	FM	ETA
A (n = 11)	32,0	22,4	6,5	10,8	10,0	9,2	0,5	0,0
B (n = 3)	482,4	105,6	22,6	10,5	0,1	0,0	0,0	2,0
C (n = 1)	35,9	34,1	0,0	0,0	0,0	0,0	0,0	0,0

n: number of strains; ACH: acetaldehyde; AA: acetic acid; BA: benzoic acid; BUTA: butyric acid; CAP: capric (n-decanoic) acid; CAPO: caproic (hexanoic) acid; CAPY: caprylic (octanoic) acid; BTA: butanediol; BUT: butanol: ETH: ethanol; PRO: n-propanol: ACT: acetone; ACO: acetoin; BTD: 2,3-butanedione; FCA: 2-furancarboxaldehyde (furfural); FM: furnamethanol; ETA: ethylacetate.

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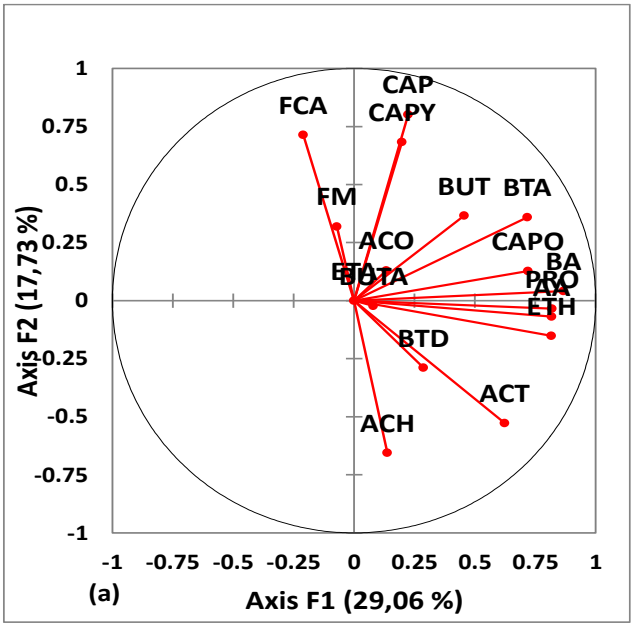
TABLE VI-10. CLUSTER CLASSIFICATION OBTAINED BY HCA OF THE 15 STRAINS ACCORDING TO THEIR ABILITY TO SYNTHESIZE FLAVOR COMPOUNDS

Cluster	A	B	C
Strain code	LL03	LR21	LB31
	LK07	LD40	
	LK10	LD45	
	LP14		
	LP15		
	LKN28		
	LB35		
	LD46		
	LD44		
	LC50		
	ED55		

Cluster A (73% of the strains) was characterized by the highest concentrations in acetaldehyde, butanediol and 2-furancarboxaldehyde. It included 11 strains belonging to the species *Lc. lactis*, *Len. kefiri*, *Lpb. plantarum*, *Lb. kisonensis*, *Lev. brevis*, *Lb. diolivorans* and *E. durans*. Cluster B (20 %) grouped only three isolates, notably two strains of *Lb. diolivorans* LD40, LD45 and *L. rhamnosus* LR21 and was characterized by high levels of acetic acid and butanediol. The last cluster C (7 %) included only one isolate of *Lev. brevis* (LB31) which produced significant amounts of butyric and hexanoic acid but didn't yield acetone, acetoin none 2,3-butanedione.

VI.3.4. Principal Component Analysis of the different aromas

To classify the 15 strains according to the volatile organic compounds they produced in sheep milk by fermentation, a Principal Component Analysis (PCA) was carried out **(Figure VI.3)**. The first three principal components (PC) explained 61.72% of the relationship between the attributes and the samples. The first component PC1 accounted for 29.06% of the total variation, and was characterized by eight aroma compounds: acetone, 2,3 butanediol, ethanol, n-propanol, acetic acid, benzoic acid, hexanoic acid and ethylacetate. PC2 was responsible for 17.73% of the variation and was characterized by acetaldehyde, n-decanoic acid, octanoic acid and 2-Furancarboxaldehyde. The third component PC3 represented 14.93% of the total variation and was able to describe butanol and 2,3-butanedione (diacetyl) contents.



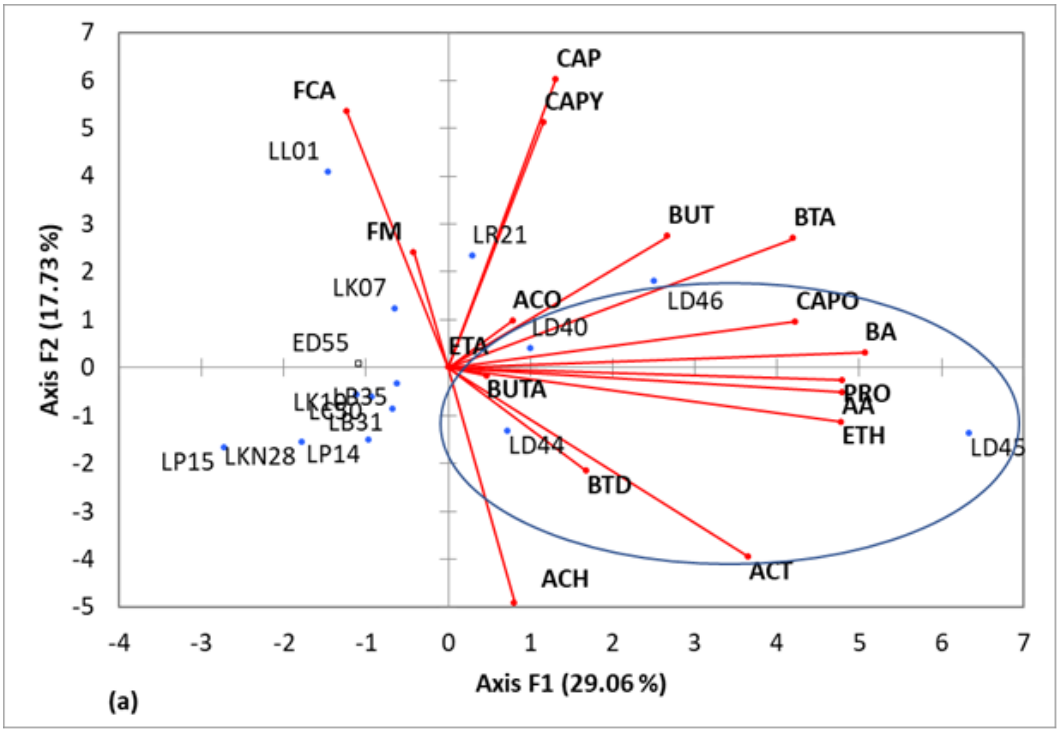


FIGURE VI-3. GRAPHICAL REPRESENTATION OF THE PRINCIPAL COMPONENT ANALYSIS OF THE VOLATILE ORGANIC COMPOUNDS PRODUCED BY THE MILK FERMENTED SAMPLES INOCULATED INDIVIDUALLY BY THE 15 LAB STRAINS SHOWING PC1 VS. PC2 AXIS

(a) Factor loading (b) scores of the tested isolates. ACH: Acetaldehyde; AA: acetic acid; BA: benzoic acid; BUTA: butyric acid; CAP: capric (n-decanoic) acid; CAPO: caproic (hexanoic) acid; CAPY: caprylic (octanoic) acid; BTA: butanediol; BUT: butanol: ETH: ethanol; PRO: n-propanol: ACT: acetone; ACO: acetoin: BTD: 2,3-butanedione: FCA: 2-furancarboxaldehyde; ETA: ethylacetate.

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From **Figure VI.3**, it could be observed that the four *Lb. diolivorans* strain (LD40, LD44, LD45 and LD46) were discriminated in the right part of the plot. They displayed high concentration values of acetone, hexanoic acid and n-propanol, but low levels of n-decanoic acid which is characteristic of goat milk flavor. However, a high variability was observed within this species, as only LD44 produced acetaldehyde (44.3 $mg.kg^{-1}$) as compared to the three other strains. Of note, the concentration values of hexanoic acid and n- propanol ranged from 22.9 $mg.kg^{-1}$ to 174.6 $mg.kg^{-1}$ and from 67.3 $mg.kg^{-1}$ to 227.6 $mg.kg^{-1}$ respectively.

VI.3.5. Results summary and analysis

Flavor is one of the sensory properties of food or any product, which is determined by the senses of taste and smell. It plays an important role in determining the consumers acceptability and preference of milk and fermented milks (Bezerra *et al*., 2017; Erkaya and Şengül, 2011). Taste and smell are complex phenomena in themselves, and their interaction with other sensory properties (acidity, texture, viscosity) increases the complexity of human perception (Ott *et al*. 2000; Queiroga *et al*., 2019). In fermented milks, other factors, such as the kind of milk and the fat content affect and interact with the flavor properties of the products (Ott *et al*, 2000; Chen *et al*., 2017a). The main volatile compounds that are involved in the flavor of fermented milks are aldehydes, alcohols, acids, ketones, esters, and hydrocarbons. A lot of works addressed the composition of volatile compounds of fermented milks. Most of them considered yogurt, which may contain about 50 different compounds (Ott *et al*., 2000; Dan *et al*., 2017a). In our study, the total number of volatile organic compounds varied from 7 to 13, depending on the strain used for the preparation of the fermented goat milk. The most abundant aroma were volatile fatty acids (acetic, benzoic, butyric, n-decanoic, hexanoic and octanoic acids), followed by alcohols (ethanol, butanol, 2,3-butanediol, n-propanol), ketones (acetone, acetoin, diacetyl), carbonyl compounds (acetaldehyde), furans (furfural and 2-furanmethanol) and esters (ethyl acetate). These results are similar to those reported by Huang *et al*., (2020a), who detected 19 compounds in goat yogurt prepared from a culture mix of 1:1 of *S. thermophilus Lb. bulgaricus* strains.

Not all strains belonging to the same species behave similarly regarding aroma production. This indicates that aroma production of these strains is more strain dependent than species dependent. More specifically, an intra-species variability was detected in *Len. kefiri* (LK07 and LK10) by considering the furfural content, and in *Lb. diolivorans* (LD40, LD44, LD45 and LD46) by considering ethanol and 2,3-butanedione (diacetyl). .

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Acetic acid is one of the important acid compound produced by heterofermentative lactic acid bacteria. It contributes to a sharp vinegar-like flavor in milk fermented products (R Muelas et al., 2018). In this study, various amounts of acetic acid were recorded in the fermented samples, ranging from 7.5 to 2437.7 $mg.kg^{-1}$. By considering that the favorable concentration of acetic acid in yogurt is comprised between 0.5 and 18.8 mg/L (Alonso and Fraga, 2001), and that the mean acetic acid content of laban is around 178 mg/L (Chammas *et al*., 2006b), most of the fermented products obtained in this study displayed an overall vinegar flavor.

Most of the volatile acids, which are produced during the fermentation process, derived mainly from fat decomposition of the C4 - C20 chains of milk, and partly from fermentation by lactic acid bacteria (Cheng, 2010b). Butyric acid is responsible for the cheese flavor. Octanoic acid contributes positively to the perception of wax, soap, goaty, musty, rancid and fruity aroma. The aroma of hexanoic and octanoic acids characterized a "goaty" smell effect in fermented products (De Santis *et al* 2019; Salles *et al*., 2002) and cheesy notes in soft cheeses (Shu *et al*., 2016; Bao *et al*., 2016). Butanoic and hexanoic acids are known to confer sweaty, cheesy and lipolyzed notes to Parmigiano (Qian and Reineccius, 2003). In this study, hexanoic acid was prominent over decanoic (capric) and octanoic (caprylic) acids in all fermented milks. It reached high levels of concentration (up to 174 $mg.kg^{-1}$), which is significantly higher than the concentrations obtained by Muelas *et al*. (2018) (18.1 and 26.1 ng/L).

Acetaldehyde, acetoin and diacetyl are important volatile compounds that significantly contribute to the flavor of fermented milks (Ott *et al*. 2000). Acetaldehyde (ACH) is considered as a main flavor compound in fermented milk products and exhibits a green apple or nutty flavor (Cheng, 2010b). Ketones have a herbal fruity or even floral aroma, they are intermediate compounds and can be reduced to secondary alcohols (Bezerra *et al*., 2017b). Methyl ketones are the result of lipolysis and produced from the free fatty acids through oxidation (Hayaloglu *et al*., 2013). According to Tian *et al*. (2019), acetaldehyde and diacetyl are the main factors involved in the aroma quality of yogurht when fermented with 2 starains of *Lpb plantarum*. From Settachaimongkon *et al*., (2014b), acetaldehyde is predominantly generated by the metabolism of threonine. The optimal concentrations of acetaldehyde in yogurt ranged from 14 to 20 mg/kg, while less than 8.0 mg/kg resulted in weak flavor, and high quantities of acetaldehyde (>200 mg/kg) led to an "astringent" off-flavor yogurt (Chen et al., 2017b). In their study, Buran *et al*., (2021) measured acetaldehyde concentration of 19.02 mg/L^{-1} and 2,3-

butanedione (diacetyl) concentration of 3.18 mg/L^{-1} ppm in a kefir-like fermented goat milk product. These values are lower than those obtained in our study, with the strain *Lb. diolivorans* LD44 (43.6 mg.kg^{-1} for acetaldehyde) and with the strain *Lb. diolivorans* LD40 (43.6 mg.kg^{-1} for diacetyl). Acetoin (ACO) is known for its creamy, vanilla and buttery flavor, and has an effect on the odor quality of fermented milks. As it corresponds to a reduced form of diacetyl, it displays has a weaker flavor than diacetyl. In our study, acetoin concentration varied between 1.8 mg.kg^{-1} (*Lpb. plantarum* LP15) and 33.8 mg.kg^{-1} (*Lb. diolivorans* LD 44). The latter value was higher than those obtained by Erkaya and Şengül (2011) in traditional goat milk yogurt which showed a peak area of only 1.25 % compared to all other detected components. The studies of Muelas *et al*., (2022) also reported same low peak area of 1.7 % for acetoin resulting from fermented milk produced from goat fed with Broccoli and Artichoke plant by products and inoculated with the *Lb. delbrueckii* MA400 strain.

Ethanol is one of the main alcohol compounds in yogurt as the final product of glucose metabolism or amino acid degradation (Dan *et al*., 2019). It contributes positively to the perception of mild and ether aroma, but only at low concentrations (Tian *et al*., 2019). It is the final product of glucose metabolism or amino acid degradation in milk by heterofermentative bacteria (Dan *et al*., 2017; Tunick, 2014). In this study, considerable differences were observed among the samples regarding this compound. High levels of ethanol (295.2 to 917.3 mg.kg^{-1}) were found in samples fermented by *Lb. diolivorans* (LD40 and LD45). These values are higher than those observed by Erkaya and Şengül, (2011) in fermented goat milk. When compared to kefir grains, the levels of ethanol were detected at percentages and ranged from 1% to 3.75 % in 4 traditional fermented products (Dertli and Çon, 2017). In fact, it is confirmed that ethanol, ethyl acetate, ethyl butyrate, 2-butanone, acetone, acetoin, diacetyl and acetaldehyde are representative of alcohol, ketone, ester and aldehyde compounds in kefir grains as reported by (Aghlara *et al*., 2009).

From this analysis, the aroma compounds synthesized by the lactic acid bacteria collected from fermented goat milk products in Lebanon are not so different from those found in other fermented milks. However, some differences were observed between the species, which may help for the selection of strains showing specific sensory characteristics.

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VI.4. Lipolytic activity

The degree of lipolysis by lactic acid bacteria helps determining the selection of the strains used as starter cultures (Toe *et al*., 2019), as this property is relevant for flavor development in fermented milk products (Tanasupawat *et al*., 2015; Garcia-Cano *et al*.,2019). In this study, the lipolytic activity of 35 strains of lactic acid bacteria has been measured qualitatively, using the Agar spot test described by(Katz *et al*., 2002).

None of these 35 strains demonstrated any lipolytic properties. This result was not somewhat astonishing, as lactic acid bacteria are generally considered to be weakly lipolytic , relatively to the genera *Penicillium, Pseudomonas* (Katz *et al*., 2002; González *et al*., 2007), *Acinetobacter* and *Flavobacterium* (Serhan *et al*., 2009). According to Matthews et al., (2004), lactic acid bacteria are generally acknowledged as weakly lipolytic. However, some authors reported that lipolytic activity has been found in different *Lactobacilli* species: *Lb. helveticus*, *Lb. delbrueckii*, *Lb. bulgaricus, Lb. casei, Lpb. plantarum,* and *L. acidophilus* (Fernández *et al*., 2000). The study of Karam *et al*., (2012) showed that lipolytic activity of *Lactobacillus, Lactococcus, Enterococcus* and *Leuconostoc* is significantly expressed on MRS supplemented with natural substrates like butter and olive oil. More recently, García-Cano *et al*., (2019) screened 137 LAB strains from various dairy products for their lipolytic abilities. Strains which exhibited this characteristic represented only 50.3% of the tested strains. In addition, this activity also depends on the nature of the substrates used for the bacterial growth. By considering goat milk products, only few data were previously reported by Moulay et al., (2006) who presented *Lb. lactis* subsp *lactis* and *Enterococcus* spp as weak lipolytic strains. Finally, our results of weak lipolytic activity were also in line with those reported by Ebadi Nezhad *et al*., (2020) who worked on technological characteristics of LAB from Motal, a traditional Iranian raw goat milk cheese, noting no lipolytic activity by *Lactobacillus* strains when cultured on cream fat agar plates.

Consequently, from our results and this analysis, a great diversity exists between species and strains of lactic acid bacteria, by considering their lipolytic activity.

VI.5. Proteolytic activity

The ability of lactic acid bacteria to produce exocellular protease is a desired characteristic (Donkor *et al*., 2007), since proteolysis of caseins has an essential impact to improve texture and enhance flavor and aroma of dairy fermented products (El-Ghaish *et al*.,

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2011). This trait is mostly important in milk that contains low concentration of free amino acids. In this study, the proteolytic activity of the bacteria has been determined qualitatively according to the method of Guillemard, (1986).

None of the 35 strains showed a capability of proteolysis on milk plate count agar medium. This result is in disagreement with the study of Atanasova *et al*., (2014), which showed that most *Lpb. plantarum* and *Lc. lactis* ssp. *lactis* strains can hydrolyze casein. They also disagree with the work García-Cano *et al*., (2019) who demonstrated that 61.3% of 137 LAB strains isolated from various dairy products displayed a significant proteolytic activity. However, our results are more comparable to those reported by Franciosi *et al*., (2009), who observed that only 12% of the isolated stains (eight out of 63) presented this characteristic when cultured on skim cow milk agar medium. In the studies of Gobbetti *et al*., (1999), it was demonstrated that proteinase activity is strain dependent and is affected by the substrate. This is confirmed by Kieliszek *et al*., (2021) who indicated that proteolytic behavior of lactic acid bacteria varies from one medium to another and depends on its chemical composition

A high intra- and inter-specific variability is commonly observed for autochthonous LAB strains isolated from raw milk (Franciosi *et al.,* 2009) or from raw milk cheese (Ebadi Nezhad et al., 2020). By considering raw goat milk, similar results to our study showing low proteolytic activity were observed by Yelnety et al., (2014). The authors recorded that only two *Lpb. plantarum* and one *Lb. pentosus* exhibited significant proteolytic activity from all of the 26 LAB isolated strain. De Almeida Júnior *et al.*, (2015) also reported that most of the LAB (34%) collected from 42 goat milk samples revealed poor proteolytic activity and only 2 were characterized for their proteolysis capabilities.

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Marroki, (2014) reported a desirable proteolytic activity in 11 strains belonging to *Lpb. plantarum, L. rhamnosus* and *Lb. fermentum* species isolated from Algerian raw goat milk.

From this analysis, it can be concluded that lactic acid bacteria are generally weakly proteolytic, even if a diversity is observed according to the species, the strains and the authors.

VI.6. Resistance to gastro-intestinal stress

Except for the *Enterococcus* genus strains, which are isolated from Ambriss product, all other strains can be considered as part of the indigenous microbiota and can be safely used as they gained the GRAS status (Hanchi *et al*., 2018), which limits the safety risks associated to their use as probiotics (Wang *et al*., 2020). For this reason, the *Enterococcus* strains were excluded from the gastrointestinal stress analysis. This analysis was conducted by applying two successive stress conditions, to simulate the gastric compartment (gastric juice for 3h at pH 3.0 and 37°C) and the duodenal compartment (bile salts and pancreatin for 3h at 37°C). The ability of bacterial cells to tolerate these two successive stresses was calculated as survival rate (in %).

VI.6.1. Raw results of resistance to gastrointestinal stress

The resistance of 35 strains of LAB to this double gastrointestinal stress is shown in **Table IV.11** below. Two strains showed the lower resistance (survival rates < 60%); five strains showed survival rates comprised between 64% and 72%; 13 isolates displayed survival rates varying between 80 and 90%; 15 strains were highly resistant to gastrointestinal stress as their survival rates were higher than 90%. Two strains were completely resistant to the simulated gastrointestinal environment: *Lb. brevis* LB37 and *P. acidilactici* PA05, when compared to *Lb. diolivorans* LD46 and *Lc. lactis* LC50 presenting the lowest survival rate of about 59%

VI.6.2. Hierarchical cluster analysis of the strains by considering their resistance to gastrointestinal stress

According to the results of HCA, the resistance of the 35 LAB strains to gastro-intestinal stress was distributed into 4 groups **(Table VI.11).**

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TABLE VI-11. CLUSTERS OF SURVIVAL RATES (%) OF 35 LACTIC ACID BACTERIA COLLECTED FROM AMBRISS, SERDALEH AND LABNEH EL DARFF PRODUCTS AND SUBMITTED TO GASTRO-INTESTINAL STRESS.

Cluster	Strain code	Viable count in Buffer Solution (Log CFU/mL)	Viable count after treatment (Log CFU/mL)	Survival rate (%) and standard deviation
A	LD46	9.7	5.7 (±1.6)	58.5 (±16.1)
	LC50	8.5	5.0 (±0.4)	59.0 (±5.2)
	LK08	9.3	6.0 (±0.0)	64.5 (±0.0)
B	LK06	6.3	4.2 (±0.1)	67.1 (±1.2)
	LL03	8.7	6.0 (±0.0)	69.6 (±0.3)
	LL01	8.8	6.3 (±0.0)	70.9 (±0.6)
	LD43	7.3	5.3 (±0.5)	71.9 (±6.8)
C	PA04	9.9	8.0 (±1.0)	80.9 (±9.9)
	LR20	9.1	7.5 (±0.0)	82.4 (±0.5)
	LR21	9.9	8.2 (±0.2)	82.5 (±1.7)
	LK09	9.9	8.6 (±1.8)	87.0 (±18.1)
	LKN28	10.2	8.6 (±0.1)	84.1 (±0.9)
	LP13	6.3	5.5 (±0.0)	87.0 (±0.4)
	LP14	10.1	8.6 (±0.1)	85.5 (±1.1)
	LP15	8.3	7.3 (±0.3)	87.8 (±3.2)
	LP17	10.1	8.6 (±0.2)	85.2 (±2.1)
	LD40	10.4	8.9 (±0.2)	85.2 (±2.4)
	LB33	10.3	8.8 (±0.3)	85.3 (±2.7)
	LB35	10.1	8.9 (±0.3)	88.4 (±2.9)
	LB36	10.1	8.7 (±1.2)	86.2 (±11.7)
D	LD44	6.0	5.4 (±0.2)	90.8 (±4.1)
	LD45	8.9	8.1 (±0.2)	90.3 (±2.1)
	LP12	10.2	9.4 (±0.4)	92.1 (±3.6)
	LP19	10.1	9.7 (±0.1)	96.0 (±0.8)
	LP18	10.2	9.3 (±0.2)	91.4 (±2.1)
	LK07	10.1	9.9 (±0.1)	97.5 (±1.0)
	LK22	8.2	7.5 (±0.3)	91.7 (±3.4)
	LKN24	9.2	9.0 (±0.0)	97.1 (±0.2)
	LKF26	8.5	8.2 (±0.1)	97.4 (±1.0)
	LB31	9.7	9.1 (±0.2)	93.4 (±2.1)
	LB34	9.7	9.2 (±0.4)	94.9 (±3.8)
	LPP11	8.9	8.8 (±0.0)	99.3 (±0.1)
	LK10	10.2	10.1 (±0.0)	99.5 (±0.3)
	LB37	8.7	9.1 (±0.4)	100.0 (±4.8)
	PA05	10.1	10.2 (±0.5)	100.0 (±4.7)

*Numbers in parentheses represent the standard deviation of the repeatability (rSD).

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Cluster A (8.6 % of the strains) encompassed the weakest strains with low survival rates ranging from 58.5 to 64.5%. Cluster B (11.4 %) included strains with a better resistance to gastrointestinal conditions, with death rates around 30%. More resistant strains were grouped in Cluster C (37.1 %) with death rates comprised between 13% and 20%. *Lev. brevis* LB35 was the most resistant in this group. Cluster D (42.9 %) was characterized by strains demonstrating the higher resistance capacity. The death rates in this cluster ranged between 0 and 10%. Isolates from this group were heterogonous, as they belong to all types of species except for *Lc. lactis*. The most resistant were *Lev. brevis* LB37, *P. acidilactici* PA05, *Len. kefiri* LK10 and *Lpb. paraplantarum* LPP11 strains

As these results indicated that the lactic acid bacteria strains showed heterogeneity by considering their tolerance to simulated gastrointestinal conditions, the species were classified from the least resistant to the most resistant (**Table VI.12**).

TABLE VI-12. MEAN VALUES OF SURVIVAL RATES (%) OF THE LAB SPECIES ISOLATED FROM AMBRISS, SERDALEH AND LABNEH EL DARFF PRODUCTS AND SUBMITTED TO GASTRO-INTESTINAL STRESS.

Species	**Survival rate (%)**
Lactococcus lactis (n = 3)	66.3 (9.7)*
Lentilactobacillus kefiri (n = 5)	83.1 (19.9)
Lacticaseibacillus rhamnosus (n = 3)	85.4 (6.0)
Lactobacillus diolivorans (n = 5)	86.1 (16.7)
Lactobacillus kisonensis/parakefiri (n = 2)	86.4 (11.4)
Lactiplantibacillus plantarum (n= 8)	90.0 (4.3)
Pediococcus acidilactici (n = 2)	91.0 (15.7)
Levilactobacillus brevis (n = 6)	92.4 (6.7)
Lactobacillus kefiranofaciens (n = 1)	97.5

*Numbers in parentheses represent the % of variability among strains of the same species, expressed as coefficient of variation.

The *Lb. kefiranofaciens* strain was the most resistant with a survival rate of 97.5% followed by *Lev. brevis* at a mean survival rate of 92.4%. On the contrary, *Lc. lactis* spp *lactis* showed the least resistance as expressed by the lowest survival rates (66.3 %). The species *Len. kefiri* and *Lb. diolivorans* presented the highest interspecies variability by considering resistance to gastro-intestinal stress. No significant difference ($P>0.05$) was observed between species for survival rates due to high heterogeneity between the strains belonging to the same species.

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VI.6.3. Results summary and analysis

Beside their positive effect of health, one of the most important selection criterion for potential probiotic cultures is their resistance to the low pH of the gastric juice in the stomach and to the bile salts in the duodenum (Ruiz *et al*., 2013; Tambekar and Bhutada, 2010).

Our results indicated that some of the lactic acid bacteria, which were isolated from the traditional Lebanese fermented goat milk products, had the prerequisites to survive in the gastrointestinal tract. More specifically, *Lb. kefiranofaciens, Lev. brevis, P. acidilactici* and *Lpb. plantarum* strains showed a high survival rate in adverse gastrointestinal conditions, even if this resistance is strain-specific due to the high diversity within the same species. By comparing our results with to those of Shehata *et al*., (2016) obtained with two traditional Egyptian fermented milks (Boza and Rayeb), the surviving percentage of *Lc. lactis* (66.3 %) was close (even slightly lower) to that observed in their study (68 – 88.3 %). In addition, the survival rate of *L. rhamnosus* (85.4%) was slightly higher than that of the same species isolated from Egyptian traditional Boza (76.2%). The survival rate of *Lpb. plantarum* to gastrointestinal stress (90 %) was higher than those reported by Zhang *et al*., (2016) with strains isolated from Tibetan Qula, a fermented yak milk product (71% - 82%).

From this analysis, the resistance of some lactic acid bacteria isolated in this study was either lower or higher to that reported in previous studies, with different behaviors depending on the species. In addition, the resistance level of some lactic acid bacteria species isolated from traditional goat milk products was demonstrated for the first time in this study for the following species: *Len. kefiri, Lb. kefiranofaciens, Lev. brevis, Lb. diolivorans, Lb. kisonensis* and *P. acidilactici*. Finally, the type of milk may also affect this resistance, since goat milk influences the gastrointestinal environment and its microbial community differently to cow milk (Prosser, 2021; de Assis *et al*., 2016).

VI.7. Combined analysis of all technological and functional properties

In order classify the 15 previous LAB strains isolated from Ambriss, Serdaleh and Labneh El Darff according to their technological properties, a global principal component analysis based on representative parameters of acidification, texture, viscosity and aroma production has been performed (**Figure VI.4)**. The results concerning lipolytic and proteolytic activities were not included as they have not sufficiently discriminated the strains. Similarly,

the results regarding the resistance to gastrointestinal stress were not included due the variability observed.

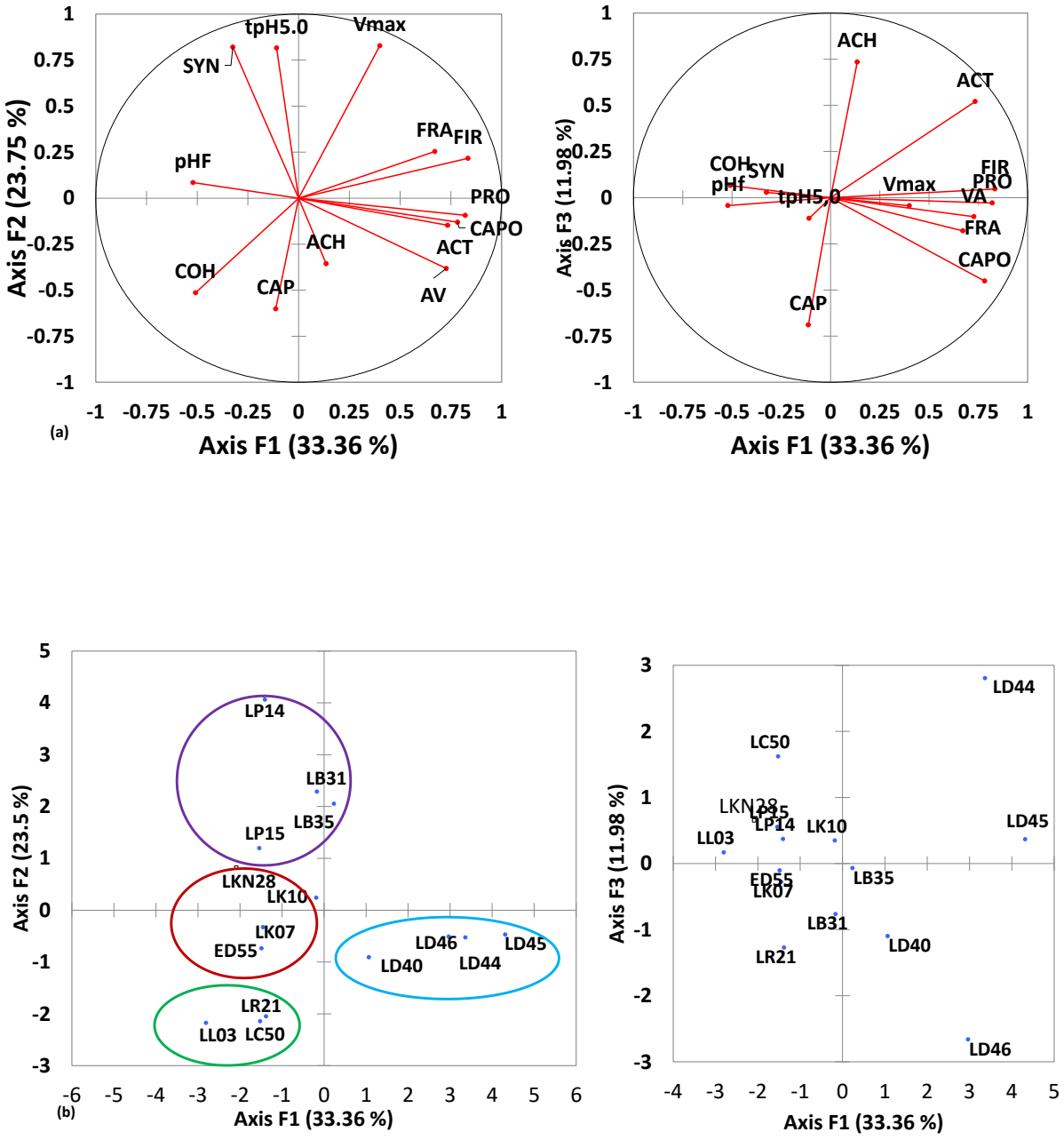

FIGURE VI-4. GRAPHICAL REPRESENTATION OF THE PRINCIPAL COMPONENT ANALYSIS OF ALL TECHNOLOGICAL PROPERTIES DEMONSTRATED BY THE 15 LAB COLLECTED TO PRODUCE FERMENTED MILKS, SHOWING PC1 VS. PC2 AXIS.

(a) Factor loadings; **(b)** scores of the strains. Vmax: Maximum acidification rate; tpH5.0: times to reach pH5.0; pHf: Final pH; COH: Cohesiveness; FRA: Fracturability; AV: Apparent viscosity; SYN:

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Syneresis; ACH: acetaldehyde; ACT: Acetone; CAP: Capric (n-decanoic) acid; CAPO: Caproic (hexanoic) acid; PRO: n-Propanol.

The 15 strains were taxonomically classified into *Lentilactobacillus* (4), *Lactobacillus* (3), *Lactococcus* (2), *Lactiplantibacillus* (2), *Levilactobacillus* (2), *Lacticaseibacillus* (1) and *Enterococcus* (1). To accomplish this approach, a reduced number of descriptors was retained from each type of the previous analyses. In total 13 descriptors were kept from the three functional analyses: three from acidification measurements: maximum acidification rate (Vm), time to reach pH 5.0 (tpH5.0) and final pH of the fermented product (pHf); four of the five physical and rheological parameters: Cohesiveness (COH), Fracturability (FRA), Apparent Viscosity (AP) and syneresis (SYN); five out of the 17 quantified aroma compounds: acetaldehyde (ACH), acetone (ACT), capric (n-decanoic) acid (CAP), caproic (hexanoic) acid (CAPO) and n-propanol (PRO). These descriptors were chosen with the aim to efficiently discriminate the isolates as shown in the previous chapters.

From **Figure VI.4a,** the first three principal components explained 69.09% of the variability between the samples. The first component PC1 accounted for 33.36% of the total variance. It was characterized by three attributes of aroma: acetone, caproic acid and n-propanol and three attributes of physical parameters: Fracturability, Firmness and Apparent Viscosity. These attributes were opposed to pHf, Cohesiveness and Syneresis. The PC2 explained 23.75% of the variation and was characterized by the parameter Syneresis that was opposed to Cohesiveness, by two attributes of acidification: Vm and tpH5.0 and by the attribute capric acid. The third axis PC3 explained 11.98% of the variability and was characterized by the attribute acetaldehyde that was opposed to the attribute capric acid, and to a less extent by the attribute acetone.

The score plot of the first 2 components made it possible to separate the 13 strains into four distinct groups **(Figure VI.4b).** The first group (blue ellipse) is located in the right lower part of the plot. It assembled all the *Lb. diolivorans* (LD40, LD44, LD45 and LD46) strains. This group presented high values of acidification rate, but low values of tpH5.0 and final pH, high values of apparent viscosity, fracturability, firmness but low values of syneresis, and significant amounts of all aroma compounds. The two *Lc.* strains (LL03, LC50) and one *L. rhamnosus* (LR21) formed a second group (green ellipse) in the lower left part of the graph. These strains presented high values of acidification rate, final pH, cohesiveness and capric acid

content. However, they displayed low values of firmness and fracturability, as well as other aroma compounds. The third group (red ellipse) included two *Len. kefiri* (LK07, LK10), one *Lb. kisonensis* (LK28) and one *E. durans* (LD50). It showed high values of final pH and cohesiveness but medium values for all other parameters. The *Lev. brevis* (LB31 and LB35) and *Lpb. plantarum* strains (LP14 and LP15) formed the last group that was presented at the upper part of the graph (violet ellipse). These strains generated fermented milks showing high tpH5.0 and syneresis but low acidification rate, cohesiveness and capric acid content. The other volatile organic compounds were less present in these samples. By considering the first and third components of the PCA, a strong discrimination of the four *Lb. diolivorans* strains appeared. These bacteria differed according to their ability to produce the three aroma compounds, acetaldehyde, acetone and capric acid.

VI.8. Selection of strains with specific technological properties

From the data obtained by considering all technological and functional parameters of the analyzed lactic acid strains, some could be highlighted, in order to be selected as culture strains for further production of Lebanese local fermented dairy products.

The *Lc. lactis* LD03 and *Lc. lactis* spp. *cremoris* LC50 strains, which were obtained from raw goat milk used in the production of Serdaleh, showed high rate of acidification, short time to reach pH5.0, very low syneresis, high apparent viscosity and cohesiveness, in addition to a high level of the goat flavor capric acid. These characteristics make them suitable to be used as culture strains for the production of Lebanese Labneh product. In fact, Labneh made from drainage of goat Laban is appreciated by the consumers because of its acidic and "goaty" taste that may be improved by these bacteria. In addition, the reduced whey loss during draining may improve the yield production rate of this product.

In contrast, the *Lb. diolivorans* group and in particular the LD44 and LD45 which were obtained from Serdaleh products, led to fermented products showing high firmness and fracturability, an important production of hexanoic acid, acetone and n-propanol, and a moderate final pH. These characteristics make them good candidates to be used in the aging stages of the traditional cheese production, like Akkawi cheese which lack firm consistency and aroma at the end of production process.

On the other hand, the *Lpb. plantarum* strains LP14 and LP15 which were obtained from Ambriss and Labneh El Darff respectively, exhibited slow rate of acidification, moderate

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firmness and final pH, together an aromatic profile rich in butyric and acetic acid. Nonetheless, they showed a remarkably high rate of syneresis which makes them candidate for the production of traditional Serdaleh or Ambriss, mainly by accelerating the process of whey drainage from the jars, and hence increasing the final productivity rate per production season.

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VII. GENERAL DISCUSSION

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The production process of the three Lebanese products Ambriss, Serdaleh and Labneh El Darff is regionally defined, entirely artisanal and seasonal as it strictly depends on the availability of fresh raw goat milk, which confirms previous reports by Abiad *et al*., (2022) and Dimassi *et al*.,(2020). The combination of the culture dependent approach and the in-depth, fine-tuned metagenomic approach corroborates the specificity and particularity of Ambriss and Serdaleh when compared to traditional fermented raw goat milk products like Algerian "Bouhezza" (Boudalia *et al*., 2020), Greek goat milk Kefir (Kazou *et al*., 2021), Moroccan or Egyptian "Raib" (Ayad *et al*., 2004; Hamama, 1992) or Iranian tribes "Yogurt" (S. RoushanZadeh *et al*., 2014). The microbial composition of Labneh El Darff is similar, to a certain extent, to the Lebanese Laban which shows a remarkable dominance of *L. delbrueckii* spp *bulgaricus* as observed by (Béal and Chammas, 2012), but also a noteworthy resemblance to Ambriss and Serdaleh due to the presence of *Lb. diolivorans, Len. kefiri* and *Lev. brevis*.

In spite of the simple manufacturing process, the artisanal Ambriss and Serdaleh showed a high microbial richness with the contribution of marker gene analysis, a total of 104 bacterial species and 15 fungal species were distributed among 4 families: *Lactobacillaceae, Streptococcaceae, Gammaproteobacteria, and Mycoplasmopsis agalactiae*. In addition, the identification of the microbial species by the phylogenomic analysis of the MAG highlighted the presence of *Lc. lactis, Lb. kefiranofaciens, Len. kefiri, Lb. diolivorans* and *Lpb. plantarum.* Given the hypothesis that MAG corresponded to the genome of the dominant strains of the corresponding species in the sample, it is possible to compare these strains to those isolated from other environments and which sequences are available in databases. It is shown that the strains from our products are clearly not originating from starters strain cultures, and likely specific from them. This finding is well illustrated with *Lb. kefiranofaciens,* which are from different lineages from those dominant in kefir.

This scheme is also applicable to the isolated *Lb. diolivorans* and to *Lpb. plantarum* strains. The former species occasionally reported in dairy products, is typically isolated from corn silage (Krooneman *et al*., 2002) or from fermented fruits and vegetables (Zhang *et al*., 2010; Zheng *et al*., 2020). And it is generally selected for its technological properties such as the production of 1,3-propanediol from glycerol as spoilage organisms from cider (Garai-Ibabe *et al*., 2008) and recommended for its ability to regulate acidity and extend the stability of silage which is prepared from difficult to ensile raw materials (EFSA FEEDAP Panel,

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2016).The native *Lb. diolivorans* strains of our study present however much more interesting functional characteristics such as high firmness and fracturability, important typical goat aromas and a moderate final pH. Many authors observed that indigenous *Lactiplantibacillus plantarum* strains, which were isolated from kefir grains or fermented milk products, increased acetaldehyde, diacetyl, and acetoin concentration and improved yogurt flavor and organoleptic characteristics (Tian *et al*., 2019; Gallegos *et al*., 2017) as well as water holding capacity, apparent viscosity and cohesiveness (Zhao and Liang, 2022). Additional attributes could be added to *Lactiplantibacillus plantarum* LP14 and LP15 strains which were isolated from Ambriss and Labneh El Darff, since they exhibited particular properties like low acidification rate and a high rate of syneresis in the formed gels. Interestingly all these gels displayed a high degree of firmness compared to other Lebanese or Mediterranean fermented milk products (Chammas *et al*., 2006b).

As a matter of fact, most typical and artisanal fermented milk products (Abiad *et al*., 2022; Boudalia *et al*., 2020; Muelas *et al*., 2018) and artisanal cheeses (Tilocca *et al*., 2022) are made from raw, unprocessed milk carrying a high rate of microbial diversity, importance of which is still largely debated. The presently studied traditional Ambriss and Serdaleh products showed a richness of 20 identified species belonging to *Lactobacillaceae, Streptococcaceae*, Gammaproteobacteria groups and *Mycoplasmopsis agalactiae* compared to about 6 identified species in "Xueo", raw Yak fermented milk (Ao *et al*., 2012) or 7 species in “Tarag” raw goat fermented milk product (J. Yu *et al*., 2011).

Interestingly, *Lactococcus lactis* species which is found to be dominant in Serdaleh and subdominant in Ambriss could not be isolated by culture dependent methods. This led us to suggest that *Lc. lactis* cells were viable but not-cultivable (VBNC) according to Oliver, (2010) and would require specific conditions to recover its growth from the product to M17 medium. Actually, a transfer of several hours in media supplemented with goat milk and enriched with salt allowed a straightforward isolation of this species. These results clearly indicate that the production process of Ambriss and Serdaleh induced a particular metabolic state that hampered *Lactococcus* species recovery, and as such corresponds to the definition of VBNC. Indeed, during the manufacturing process of Ambriss and Serdaleh, various environmental factors such as pH, salinity and temperature, together with the physiological age of the culture could induce the entry of bacterial cells into a VBNC state. In addition, this

process is still reversible, which is rarely the case (Dong *et al*., 2020), and hence offers a reproducible model for the study of the entry into the VBNC state.

Understanding the origin and the original niche of the present strains in these spontaneously formed ecosystems appears to be crucial in order to preserve the products, either for its controlled reproduction or to design a controlled starter culture mix. The initial step of fermentation at the beginning of the season is likely crucial to determine the microbiota of the product, as it produces an initial inoculum to which milk is constantly added. The microorganisms that are developing therein are originating from the goat milk, which might be contaminated by the animal, the environment of milking and production area, the used vessels and tools and finally, by the terracotta jar itself in the case of Ambriss and Serdaleh. In the case of Labneh El Darff, the microorganisms from Laban (often made initially by yogurt inoculation) are setting the ground of the ecosystem made of *Lb. delbrueckii*, completed then by the development of milk microbiota (contaminated as previously described) and likely the inner goat skin microbiota, even though the skin has been heavily processed before its use. Thus, the reconstruction and assembly of these ecosystems should take into consideration all these inputs.

The natural microbial biodiversity of these fermented goat milk products may offer new possibilities to develop industrial dairy products. The wild LAB strains which were isolated from this microbiota were studied for their growth characteristics and functionalities since these strains could possess interesting phenotypic and genotypic traits. The *Lactobacillus kefiranofaciens* strains, which were isolated from Ambriss, could probably produce polysaccharide due to their EPS related genes.

The artisanal Ambriss and Serdaleh are similarly processed, however, their microbial balance is different. The main dominant strains found in Ambriss were *Lb*. *kefiranofaciens* followed by *Len*. *kefiri* and *Lc lactis*. In contrast, Serdaleh product, which is manufactured following a diversified process of Ambriss, was dominated by *Lactococcus lactis,* followed by *Lb*. *kefiranofaciens*. In fact, *Lactococcus lactis* is a mesophilic bacterium with an optimum growth around 30 °C (Chen *et al*., 2015), tolerating mild to high concentrations of salt, for up to 4% according to Gonzalez *et al*., (2017) and Kim *et al*., (2002). These characteristics might partially explain its dominance in the salty Serdaleh product. On the other hand, and despite its high resistance to acidic environment, the *Lb. kefiranofaciens* might be outgrowth during

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fermentation by *Lc. lactis* species despite being initially dominant at early stages (Georgalaki *et al*., 2021). This dynamic might be probably related to the high acidification rate of *Lactococcus* spp as observed in LL01 and LC50 strains.

The metagenomics assemblies, as well as functional analyses based on deep profiling, allowed not only a refined identification of pathogenic or native isolates in their microbial habitat, but also the genomic characterization for each strain like polysaccharide production and presence of ABR genes. The detection of aminoglycosides, MLS, nucleosides and tetracycline genes in most of Ambriss and all of Serdaleh samples suggest an irresponsible use of antibiotics by Lebanese farmers. This practice might be linked to the easy access to antibiotics, interrupted or absent communication with the local veterinary authorities and lack of self-assessment and regular inspection (Dankar *et al*., 2022). These findings propose a necessary intervention, rectification measures and fast corrective actions to be considered in the current animal health and goat husbandry sector.

As a consequence of these bad practices, the detection of pathogenic bacteria by MAG analysis like *Streptococcus parasuis, parauberis* and *Mycoplasmopsis agalactiae* in the collected samples was expected at least in one of the two production regions. The first two pathogens cause diseases similar to *S. suis*, including meningitis, pneumonia, septicemia and arthritis (Guo *et al*., 2022). In small ruminants, *Mycoplasma agalactiae* is the classic agent responsible for Contagious Agalactiae (CA) syndrome producing clinical signs in the mammary glands, joints and eyes (Amores *et al*., 2012). In acute cases, variations at individual and herd level were reported whether goats are affected on herd size, structure and husbandry (Jaÿ and Tardy, 2019). This may partially explain the detection of this pathogen in Chouf region and not in Bekaa.

In addition the detection of C*apra hircus* reads in metagenomic samples of Labneh El Darff might bias the final analysis (Zhang *et al*., 2021). However, a high percentage of reads are a clear indicator of sample contamination with milk somatic cells either from an infected udder (Petzer *et al*., 2017) or by cross contamination from EL Darff “goat skin sack” used to drain Laban. This outcome points out the need to implement effective and modern herd management measures

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VIII. General Conclusion and Perspectives

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The Lebanese traditional fermented goat milk products were characterized by culture-dependent and -independent analyses, using metagenetic and metagenomic approaches. The results obtained by these two methods were substantially different, in particular for Ambriss and Serdaleh. Although no studies have yet proven this, traditional Lebanese goat milk products are believed to deliver health benefits, and it would thus be interesting to further test this possibility. These products are also interesting as sources of biodiversity because they contain strains of *Lb. kefiranofaciens* and *Len. kefiri*, which are genetically quite different from those present in kefir that are highly conserved.

In this study the technological characteristics, and in particular the acidification characteristics were established for the first time for the following species: *Lb. diolivorans, Lb kisonensis, Len kefiri, Pediococcus acidilactici, Lpb. plantarum and L. rhamnosus*. Since these lactic acid bacteria displayed a low acidification activity, they justify the high duration of the fermentation processes realized by the artisanal producers. The *Lpb. plantarum* LP14 and LP15 strains, which exhibited a low acidification rate and a high rate of syneresis make them useful for the production of traditional Serdaleh or Ambriss. In contrast, the strains *Lb. diolivorans* LD44 and LD45 make them good candidates to be used in the ripening stages of traditional cheese production, to achieve high firmness and appreciated flavor. Moreover, most of lactic acid bacteria are resistant to gastrointestinal stress. In this regard *Lb. kefiranofaciens* and *Lev. brevis* were the most resistant, and thus could be considered as candidate probiotic cultures. The tested fermented products showed a low apparent viscosity for all of the strains, thus characterizing low viscous gels which might be ascribed to low EPS production strains. Furthermore, progress has to be made to ensure the safety of these products from herd prophylaxis to milk chain management in order to avoid contamination by potential pathogens and the spread of antibiotic resistance genes. Finally, this study also provides key elements related to the microbiology and microbiota functioning of these products which will facilitate the development of strategies to improve these processes and preserve their identity. In this regard several perspectives can be proposed for future investigations

First, when analyzing the results which are related to genomic characterization and functional properties of the LAB isolates, two strategy approaches could be proposed to preserve these products. The first one depends on the conservation, sustainability and development of the traditional production system of these products, while the second is driven towards the local dairy industry through the preparation of specific starter cultures.

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To implement the requirements of the first approach, the strategy is assembled towards the implementation of simultaneous and multiple axes activities. Some research studies need to be conducted by LARI (Lebanese Agricultural Research Institute) to establish a DNA gene and bacterial stain bank. This activity will concern all of the native strains implicated in the manufacturing process from collection, identification, preservation, storage till the dissemination of the prepared starter cultures. Following this basic phase, a development and surveillance plan will track the different chain production stages of these artisanal products. In this regard, technical assistance will be provided for the producers concerning the availability of necessary tools such as the restoration of terracotta jars, or other suitable aluminum container, in addition to the provision of guaranteed bacterial cultures. The socio-economic aspect of this approach imposes the training of producers or producers/farmers specially to sustain women empowerment. New young age cluster is targeted in this process by the creation of marketing policies and the establishment of specialized cooperatives. Complementary extension services and training could include good management practices, safe handling techniques, goat management husbandry and veterinary services. In parallel, the enacting of regulatory decrees for the regulation of these productions such as standards and norms (Lebanese Standards, quality control programs) and a law project are implemented on the long term.

Concerning the second approach, it depends on the assembly single strain starter or culture mix starter from the selected strains to be used in industrial dairy production. This strategy also implies the development of different technical aspects such as “tailor cut” culture preparation according to the previously determined microbial growth dynamics. In addition, a complete knowledge must be available regarding strain growth specifications such as protocooperation, development of probable adverse conditions, viability related to microbial environment such as phage infestation during preparation. Storage, post storage conditions and revival conditions will be also of special importance.

The second aspect will include economical outcomes of such innovation related to marketing impacts, consumer’s preference and the development of regulations. Finally, the adoption of different aspects for the dissemination plans of these starter cultures on selected traditional producers groups or on the dairy industrial associations should be considered.

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APPENDICES

APPENDIX 1 PRODUCERS'S SURVEY QUESTIONNAIRE

Institut de Recherches Agronomiques Libanais

AgroParisTech INRAE

Université Libanaise
Faculté d"Agronomie

Producers' sruvey

Traditional dairy Lebanese products

NUM_ENQ	**Nb of Survey**	
DATE	**Date of interview**	

1. Socio Demographic Data

	CODE	Question	Response
1.0	NOM	Full Name of the interviewee (respondent)	
1.1	SX	Sex	0- Male 1- Female
1.2	AGE	Age (years)	
1.3	GOV	Governorat (mohafazat)	2- Beirut 3- Mount-Lebanon

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			4- South Lebanon 5- Nabatyeh 6- North Lebanon 7- Akkar 8- Bekaa 9- Baalbek-Hermel
1.4	CAZ	Caza	
1.5	REG	Region/village	
1.6	ALT	Altitude (meters)	
1.7	TOT_FAM	Number of persons in charge	
1.8	SIT_FAM	Social Status	10- Single 11- Married 12- Divorced 13- Separated 14- Widow(er)
1.9	EDU	Education	15- Illettrate 16- Primary 17- Complementary 18- Secondary 19- Vocational and technical level / speciality 20- Academic university
1.10	PRO	Profession	21- Farmer 22- Artisan/merchant/ enterprise manager 23- Employer 24- Worker 25- Retired 26- Non-nactive/ housekeeper
1.11	REV	Monthly income in Lebanese pounds (LL)	27- < 600.000 28- 600.000 – 1.000.000

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			29- 1.000.000– .500.000 30- 1.500.000– .000.000 31- > 2.000.000
1.12	ELV	Are you producer/breeder or only dairy producer ?? ***If response is 32 go to section 2*** ***If resposne is 33 go to section 3***	32- Producer/animal breeder /Farmer 33- Producer

1.A Additional Information	
Address of the interviewee	
Tel nb	
Number of visit (s) and date (s)	

2. Rearing activities

	CODE	**Question**	**Response**
2.0	NB FE	Number of producing (lactating) females	
2.1	RACE	Type of goat race	34- Black 35- Chami 36- Saanen 37- Alpine
2.2	SYS_PROD	Production system	38- Extensif 39- Semi-intensive 40- Semi-nomade

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			41- Vertical-Transhumance 42- Horizontal Transhumance
2.3	PAT	Mean number of grazing hours per day during summer season	
2.4	PAT	Mean number of grazing hours per day during fall-summer season.	
2.5	CONC	Quantity of Feed concentrate fed (g) /head / day if applicable	
2.6	PROD_LAIT	Mean Quantity of milk produced (L/j)	
2.7	PROD_TEMP	Milk production period (availability) or lactaion period (months)	
2.8	PROD_SAIS	Milk production season	43- Winter 44- Summer 45- Spring 46- Fall
2.9	DEST_LAIT	Destination of milk	47- Auto-consommation 48- Commercialisation 49- Mix uof both

3. Cheese/ fermented product production

	CODE	Question	Réponse

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3.0	TYPE_PRDT	Type of cheese / fermented milk product produced	50- Ambriss 51- Serdaleh 52- Labneh El Darff 53- Other types (Akkawi – Baladi, Halloumi….) 94- 50+53 95 : 51 + 53 96 : 52+53
3.1	ANNEE_PRDT	Producers' experience in the production of traditional cheese? (years)	54- < one 55- 1- 3 56- 3 - 5 57- 5 - 10 58- > 10
3.2	DEST_PRDT	Destination of the final product	59- Self - consumption 60- Individual marketing 61- Cooperative marketing 62- Third party (middle man) marketing 63- National/ international fair exhibitions/ Souks
3.3	M1_LAIT	Type of dairy primary materials used in the manufacturing process	64- Goat Milk 65- Goat Laban 66- Arisheh 67- Mixed Milks 90- 64+65
3.4	M1_SOURCE	Source of primary dairy materials used in the	68- Self sufficiency/autonomy 69- Local breeder

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		manufacturing process of cheese	70- Hallab/middle man 71- Milk collection center 72- Experimetanl research center 91- Mix
3.5	M1_NON_LAIT	Type of non- dairy primary materials used in the manufacturing process of cheese	73- Commercial starter 74- In-house (backslopping starter) 75- Goat skin 76- Terracota jarr 77- Cloth bag 78- Plastic barrel 79- Wooden spoon 92 - 77+78+ 79 93- 77+76+79 97- 74+75 98- 73+75
3.6	DUREE_PRDT	Lenght period of the Manufacturing process (in months)	80- 1 - 3 81- 4 - 6 82- 7 - 9 83- 9 - 12 84- > 12 months
3.7	QT_PRDT	Mean Quantity of product produced (Kg/ lot)	
3.8	QT_ LAIT	Quantity of milk used in each lot (L/lot)	
3.9	NB_LOT	Number of cheese lot (batches) yearly produced	

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3.10	PRIX	Cheese/fermented product consumer's price per kg in lebanese pounds (Kg/LL)	
3.11	CDNT	Cheese conditioning and presentation	85- Balls form 86- Bloc form 87- Packed in small glass jars 88- Packed in plastic jars 89- In Bulk

4. Description of the manufacturing process (Flow chart)

APPENDIX 2 LIST OF TRADITIONAL GOAT MILK PRODUCERS AND THEIR LOCATION

LP: Labneh el Darff producers, S: Serdaleh producers, P: Ambriss producers

Survey Nb	Producer code	Gender	Age (Yrs)	Governorate	Caza	Region	Altitude (m)
1	P1	F	75	Baalbeck-Hermel	Baalbeck	Douris	900
2	P2	F	60	Bekaa	Zahleh	Messa	1000
3	P3	F	45	Bekaa	Baalbeck	Fahouur	900
4	P4	M	55	Bekaa	Zahleh	Ali nahri	950
5	S3	M	67	Mount-Lebanon	Chouf	Mriste	1350
6	S4	M	71	Mount-Lebanon	Chouf	Niha	1050
7	S5	F	62	Mount-Lebanon	Chouf	Mriste	1350
8	LP1	M	58	South-Lebanon	Bint Jbeil	Rmeych	550
10	P5	M	52	Bekaa	Bekaa West	Ammiq	850
11	P6	M	40	Bekaa	Bekaa West	kherbit Anafar	1900

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12	P7	M	57	Bekaa	Bekaa West	Sagbin	1100
13	P8	F	64	Bekaa	Bekaa West	Manara	1160
14	P9	F	47	Baalbeck-Hermel	Baalbeck	Hermel	1100
15	P10	F	45	Bekaa	Bekaa West	Sagbin	1100
16	P11	F	62	Baalbeck-Hermel	Baalbeck	Younin	1200
17	P12	F	55	Baalbeck-Hermel	Baalbeck	Hour taala	1050
18	S6	F	54	Mount-Lebanon	Chouf	Maasir el chouf	1300
19	S7	M	43	Mount-Lebanon	Chouf	Maasir el chouf	1300
20	P13	F	49	Baalbeck-Hermel	Baalbeck	Arsal	1500
21	P14	M	55	Bekaa	Baalbeck	Baalbak	1150
22	P15	F	54	Baalbeck-Hermel	Baalbeck	Younin	1200
23	P16	M	60	Bekaa	Bekaa West	Aana	970
24	S8	F	55	Baalbeck-Hermel	Chouf	Bater	1200
25	S9	F	29	Mount-Lebanon	Chouf	Bater	800
26	S10	F	45	Mount-Lebanon	Chouf	Khraybeh	1050
27	S11	M	57	Mount-Lebanon	Chouf	Botmeh	950
28	S12	F	47	Mount-Lebanon	Chouf	Kfarnabrakh	1000
29	S13	M	55	Mount-Lebanon	Chouf	Bsonnyy	600
30	S14	M	56	Mount-Lebanon	Chouf	Kfarnabrakh	1050
31	LP17	F	63	South-Lebanon	Bint Jbeil	Rmeych	550
32	LP18	M	63	South-Lebanon	Bint Jbeil	Rmeych	430
33	P19	M	63	South-Lebanon	Bint Jbeil	Ramiah	570
34	P20	M	40	Bekaa	Bekaa West	Kherbit anafar	1900
35	P21	M	57	Bekaa	Bekaa West	Kherbit anafar	1100
36	P22	F	64	Bekaa	Bekaa West	Manara	1160
37	P23	F	47	Baalbeck-Hermel	Baalbeck	Hermel	1100
38	P24	F	45	Bekaa	Bekaa West	Hermel	1100
39	P25	F	62	Baalbeck-Hermel	Baalbeck	Younin	1200
40	P26	F	55	Baalbeck-Hermel	Baalbeck	Hour taala	1050
41	S15	F	29	Mount-Lebanon	Chouf	Bater	800
42	S16	F	45	Mount-Lebanon	Chouf	Bater	1050
43	S17	M	57	Mount-Lebanon	Chouf	Botmeh	950

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44	S18	F	47	Mount-Lebanon	Chouf	Kfarnabrakh	1000
45	S19	M	55	Mount-Lebanon	Chouf	Bsonnyy	600
46	S20	M	56	Mount-Lebanon	Chouf	Botmeh	1050
47	S21	F	62	Mount-Lebanon	Chouf	Mriste	1350
48	S22	F	62	Mount-Lebanon	Chouf	Mriste	1300
49	S23	F	62	Mount-Lebanon	Chouf	Botmeh	1400
50	P27	F	78	Bekaa	Zahleh	Ali nahri	950

APPENDIX 3 PHYSICO-CHEMICAL PARAMETERS AND CULTURE COUNTS OF AMBRISS, SERDALEH AND LABNEH EL DARFF SAMPLES AS A FUNCTION OF THEEI PRODUCERS AND PRODUCTION DATES.

	Ambriss	P1	2019	Surface	May	4.24	1.94	29	1.2E+07	1.1E+06	3.0E+05	*
A1-b	Ambriss	P1	2019	Surface	July	4.73	2.12	33	2.0E+06	2.1E+05	1.3E+05	*
A2	Ambriss	P2	2019	Surface	May	4.43	2.08	28	1.9E+07	7.9E+07	6.4E+05	*
A2-b	Ambriss	P2	2019	Surface	July	4.60	2.24	29	1.0E+06	4.5E+04	3.8E+05	
A3	Ambriss	P3	2019	Surface	May	4.62	1.87	32	1.2E+05	8.0E+05	1.1E+04	
A3-b	Ambriss	P3	2019	Surface	July	4.70	2.20	30	1.1E+06	2.2E+05	1.5E+05	
A4	Ambriss	P4	2019	Surface	May	4.78	0.87	30	1.1E+02	9.0E+01	2.6E+05	*
A4-b	Ambriss	P4	2019	Surface	July	4.80	1.32	32	7.2E+06	1.1E+04	1.8E+05	*
A5	Ambriss	P5	2019	Surface	May	4.08	1.19	32	< 10	3.6E+03	1.1E+04	
A5-b	Ambriss	P5	2019	Surface	July	4.42	1.05	33	7.3E+05	1.7E+04	8.0E+05	
A6	Ambriss	P6	2019	Surface	May	4.65	1.07	31	4.9E+05	1.6E+04	1.5E+06	*
S7	Serdaleh	P7	2019	Surface	May	3.58	3.59	-	1.8E+05	4.0E+05	3.0E+05	
S7-b	Serdaleh	P7	2019	Surface	July	4.11	4.01	22	1.3E+06	1.7E+06	8.7E+04	*
S8	Serdaleh	P8	2019	Surface	May	3.70	4.29	22	2.9E+04	2.9E+05	3.0E+05	*
S8-b	Serdaleh	P8	2019	Surface	July	3.67	4.12	23	4.5E+05	2.1E+05	5.7E+05	*
S9	Serdaleh	P9	2019	Surface	May	3.74	3.14	25	8.2E+04	2.6E+05	3.0E+05	
S9-b	Serdaleh	P9	2019	Surface	July	3.93	3.18	24	7.3E+04	1.7E+06	1.4E+06	*
S10	Serdaleh	P10	2019	Surface	May	3.57	4.50	23	4.3E+06	3.9E+05	3.0E+05	
S10-b	Serdaleh	P10	2019	Surface	July	3.73	5.04	22	6.4E+05	2.8E+04	4.1E+04	*
S11	Serdaleh	P11	2019	Surface	May	3.85	3.19	24	2.3E+05	7.0E+03	3.0E+05	
S11-b	Serdaleh	P11	2019	Surface	July	3.77	2.92	22	4.7E+06	2.0E+04	4.9E+05	*
S12	Serdaleh	P12	2019	Surface	May	3.94	3.08	20	2.9E+05	2.8E+04	3.0E+05	
S13	Serdaleh	P13	2019	Surface	May	4.12	2.69	24	1.3E+07	2.0E+05	3.0E+05	
S14	Serdaleh	P14	2019	Surface	May	3.94	3.69	25	3.6E+04	< 10	1.0E+04	
S19-C	Serdaleh	P3	2018	Middle	May	3.31	3.64	24	3.5E+07	9.5E+04	2.3E+06	
S19-C-b	Serdaleh	P3	2018	Middle	July	3.01	2.70	25	2.7E+07	6.5E+04	1.4E+05	
S19-b	Serdaleh	P3	2018	Surface	July	3.33	3.80	-	8.8E+08	< 10	< 10	
S20	Serdaleh	P4	2018	Surface	May	3.46	4.18	22	3.5E+08	< 10	3.5E+08	
S20-C	Serdaleh	P4	2018	Middle	May	3.28	4.60	-	2.8E+06	< 10	2.8E+06	
S20-C-b	Serdaleh	P4	2018	Middle	July	3.60	4.18	-	2.8E+06	1.1E+04	2.9E+04	
L15	Labneh	P15	2019	Surface	May	3.87	1.49	32	1.1E+05	9.0E+02	< 10	*
L16	Labneh	P16	2019	Surface	May	3.62	1.28	31	3.9E+07	5.0E+04	< 10	*
L17	Labneh	P17	2019	Surface	May	3.87	1.10	34	2.7E+06	2.4E+05	< 10	*
L18	Labneh	P18	2019	Surface	May	4.26	1.75	34	9.4E+06	< 10	< 10	*

The asterix (*) indicates samples selected for shotgun metagenomic analysis.

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APPENDIX 4 THE ANALYSIS OF THE TAXONOMICAL COMPOSITION BASED ON (OUT) DATA

			Producer	P1	P1	P2	P2	P3	P3	P4	P4	P5	P5	P6	P7	P7	P8	P8	P9	P9	P10	P10	P11	P11	P12	P13	P14	P3	P3	P3	P4	P4	P4	P15	P16	P17	P18
			Year	2019	2019	2019	2019	2019	2019	2019	2019	2019	2019	2019	2019	2019	2019	2019	2019	2019	2019	2019	2019	2019	2019	2019	2019	2018	2018	2018	2018	2018	2018	2019	2019	2019	2019
			Month	May	July	May	July	May	July	May	July	May	July	May	May	July	May	July	May	July	May	July	May	July	May	May	May	May	July	July	May	May	July	May	May	May	May
			Jar																																		
			Middle/Surface	Surface	Surface	Surface	Surface	Surface	Surface	Surface	Surface	Surface	Surface	Surface	Surface	Surface	Surface	Surface	Surface	Surface	Surface	Surface	Surface	Surface	Surface	Surface	Surface	Middle	Middle	Surface	Surface	Middle	Middle	Surface	Surface	Surface	Surface
Species	seed_sequ	observatio	observation_sun	A1	A1-b	A2	A2-b	A3	A3-b	A4	A4-b	A5	A5-b	A6	S7	S7-b	S8	S8-b	S9	S9-b	S10	S10-b	S11	S11-b	S12	S13	S14	S19-C	S19-C-b	S19-b	S20	S20-C	S20-C-b	L15	L16	L17	L18
Lactococcus lactis	AGGGAATC	Cluster_1	182342	19	2.99983	15.306	0.4416	4.4516	10.421	0.1712	0.5573	6.5077	0.1582	16.699	59.801	60.035	58.184	60.208	59.822	58.223	59.793	63.431	60.596	58.315	25.13	24.046	5.6064	9.5828	9.2646	11.55	82.873	79.644	40.135	0	0.0087	0	0
Lactobacillus kefiranofaciens	AGGGAATC	Cluster_3	105543	24.171	34.2132	22.04	78.59	48.81	0	0.9008	45.628	41.542	38.474	0.0907	13.962	9.6445	18.492	5.6835	12.352	8.8615	10.806	6.5907	9.5295	7.8505	36.116	1.1924	0.2311	10.928	52.45	57.174	0.0608	0.1867	17.073	0.0369	0.0087	0	0
Lentilactobacillus kefiri	AGGGAATC	Cluster_4	64740	28.998	29.3363	34.546	12.239	22.38	2.1238	0.4988	40.041	21.82	52.353	0.0453	5.4332	6.791	3.6622	10.43	6.0893	4.3805	5.4819	5.9871	6.081	7.0994	5.4455	34.668	34.666	12.324	5.7416	6.2181	3.3111	4.6929	23.771	1.1669	7.5537	3.0973	3.1882
Lactobacillus delbrueckii	AGGGAATC	Cluster_5	29926	0.1414	4.21485	0.1304	0	0.0287	0	0	0	0.0117	0	0.0151	0	0	0	0.039	0.0247	0	0.0069	0	0.006	0	0.0146	0.0438	0.0514	0.0128	0.1107	0	0.3807	0.0151	0.0182	82.226	67.446	80.855	55.454
Lentilactobacillus hilgardii-dioliv	AGGGAATC	Cluster_7	15413	2.5457	1.3826	2.1278	1.4116	2.3943	0.6014	0.0596	1.348	1.4801	1.0808	0.0151	2.6165	6.9743	2.6799	7.6665	2.4637	13.265	1.6762	9.7259	2.0071	11.615	0.2412	4.6275	0.1455	0.8089	0.1588	0.2483	0.2957	0.2195	1.1103	7.5421	17.325	9.5212	4.3429
Lactobacillus acetotolerans	AGGGAATC	Cluster_6	12457	0.3935	4.67572	0.5285	0	0	0	0.0149	0	0.0059	0	0	0	0	0	0	0	0	0	0	0	0	0	2.3739	0.0171	25.93	7.2625	9.0766	0	0.005	0.4004	0	0	0	0
Lactiplantibacillus plantarum-pe	AGGGAATC	Cluster_9	8806	0.0307	0.22624	0.0137	0	0	0.9972	0.7295	0	0	0	0.7859	1.3083	1.642	1.0763	2.8886	1.6233	1.9658	2.1914	2.3037	1.8761	2.2175	8.9979	0.1313	4.0486	0.9408	1.1599	1.6497	3.8458	2.0235	5.0419	0.0123	0	0.0632	0
Streptococcus parasuis-sanguini	AGGGAATC	Cluster_8	8174	0	0.00838	0.1991	0.0079	0.3656	0	53.51	1.2501	1.4096	0.3691	0	0	0	0	0	0	0	0	0	0	0	0	0.0109	0	1.8135	0.0385	0.0046	0.002	0.0025	0.0546	0	0	0	0
Aeromonas caviae > sp.	GGGGAATA	Cluster_10	6539	0.5657	1.45802	0.1167	0.2129	2.0072	0.0076	0.0596	0.5347	3.6474	0.0659	0.0756	1.5942	2.9571	1.8564	1.9283	3.3125	2.732	2.4456	2.1312	3.4663	1.7883	6.1618	6.3779	0.137	0.1831	0.1636	0.0278	0.245	0.2296	2.7485	0	0.0173	0	0
Lentilactobacillus buchneri	AGGGAATC	Cluster_12	6042	0.2706	0.25138	0.4187	0.071	0.1219	36.051	0	0.241	0.0705	0.1779	0.9521	0.4146	0.4304	0.2745	0.3747	0.3543	0.3328	0.316	0.3265	0.2918	0.5007	0.0439	0.9627	0.0171	0.0043	0	0	0.0041	0.0151	0	1.6706	0.4591	1.7462	0.1004
Escherichia-Shigella	GGGGAATA	Cluster_11	5278	0.5288	0.01676	0.6795	0.0789	0.1864	4.3465	0.0074	0	0.7342	0	17.123	0.7864	0.9087	0.6934	0.9134	0.5686	0.7817	2.0334	0.8993	0.9827	0.8405	0.2851	0.2407	2.8075	1.2899	0.1925	0.0928	1.0612	1.5113	0.3094	0	0.0347	0.0079	0
Klebsiella pneumoniae	GGGGAATA	Cluster_13	5096	4.9499	0	4.0154	0.1183	1.6774	0	0	0	1.1336	0	0	0.0286	0.0797	0	0.039	0	0.0077	0.0137	0.0185	0.006	0.0358	0.0585	1.3893	10.836	0.6641	4.2497	1.7773	0.0324	0.0177	0.0728	0.0123	0	0	0
Lactobacillus helveticus > grou	AGGGAATC	Cluster_14	4795	0	0.01676	0.0069	0.3549	10.344	0	1.1688	0	14.261	0	0.0151	0	0	0	0	0	0	0	0	0	0	0	0	4.8446	0.0085	0.0096	0	0.002	0	0.0182	0	0	0	1.8326
Limosilactobacillus sp.	AGGGAATC	Cluster_29	4194	1.9861	2.69817	3.4114	1.7901	1.1541	0	0.0298	2.9068	1.4977	3.5521	0	1.0866	0.4543	0.8524	0.3825	0.8652	0.2554	0.7213	0.1294	0.8696	0.304	1.3449	0.3282	0.9843	0.0724	0.0337	0.0673	0	0.0151	0.1638	0.2948	0.9615	0.5452	0.7406
Citrobacter sp.	GGGGAATA	Cluster_17	3859	0	0.00838	0.0206	0	0	0	0	0.0602	0.0059	0	0	0.0643	0.0558	0.0433	0.1249	0.0494	0.0464	0.0962	0.0801	0.0834	0.0805	0.0292	1.7175	0.0086	6.7433	0.9048	0.594	1.0187	2.5836	0.4914	0	0.0087	0	0.0126
Citrobacter sp._2	GGGGAATA	Cluster_15	3423	0.0123	0.00838	0.0069	0	0	0.0152	0	0.0075	0	0	0	0.0357	0.0399	0.0578	0.0078	0.0165	0.0387	0.0893	0.0308	0.0179	0.0268	0.0073	0.6017	0.0086	4.9808	3.1476	1.6984	0.8789	0.7771	0.2002	0	0	0	0
Enterobacter-Klebsiella	GGGGAATA	Cluster_26	2982	0.7317	0.03352	0.5903	0.0079	0.0143	0.0761	0	0.0301	0.0411	0	0	0	0.0159	0.0072	0.0078	0.0494	0.0155	0.0412	0.0123	0.0238	0.0268	0.0219	1.1377	0.3766	1.7795	2.7	1.4362	1.207	0.8276	0.8009	0.0369	0	0	0.0251
Lactobacillus helveticus	AGGGAATC	Cluster_16	2804	0.0307	0.00838	0.0892	0	0	0	11.39	0	0	0	0	0	0	0	0	0.0082	0	0	0	0	0	0	0.0109	9.073	0	0	0	0	0	0	0	0	0	2.4225
Citrobacter sp._3	GGGGAATA	Cluster_18	2635	0.1783	0.00838	0.048	0	0.0215	0.0228	0	0	0	0	0	0.1573	0.1993	0.1878	0.1405	0.1236	0.1006	0.3229	0.2772	0.1132	0.1073	0.095	0.1422	0.0257	4.6318	2.1176	1.7401	0.0365	0.0303	0.2366	0	0	0	0
Lactobacillus gasseri	AGGGAATC	Cluster_19	2632	0	0	0.1716	0	0	2.7632	0.0149	0	0.0529	0	33.716	0	0	0.0072	0	0	0	0	0	0	0	0	0	0	0	0.0048	0	0	0	0	0	0	0	0
Klebsiella pneumoniae varicola	GGGGAATA	Cluster_24	2622	0.7871	0	0.707	0.1735	0.2724	0	0.0149	0	0.6226	0	0	0.0286	0.0877	0.0217	0.0546	0.0247	0.0464	0.0275	0.0123	0.0179	0.1967	0.212	0.8752	10.725	0.447	1.7374	0.7401	0.0162	0.005	0.0182	0	0	0	0
Enterobacter sp.	GGGGAATA	Cluster_20	2619	1.5741	0.01676	1.6336	0.0552	0	0.609	0.0074	0.0377	0.0235	0.0132	0.2418	0.2145	0.7492	0.1734	0.4138	0.2307	0.1238	0.5908	0.3449	0.2561	0.1431	1.4765	2.899	0.0342	2.218	1.1406	0.5104	0.0547	0.1135	0.6553	0.0614	0	0	0
Mycoplasmopsis agalactiae	AGGGAATA	Cluster_25	2556	0	0	0	0	0	0	0	0	0	0	0	2.0089	1.0601	1.5964	1.2101	1.9034	1.4163	2.1776	1.1518	2.0488	1.4217	1.1987	1.1159	0.6762	0	0	0	0	0	0	0	0	0	0
Streptococcus thermophilus	AGGGAATC	Cluster_21	2445	0	0.84632	0	0.0079	0	0.0304	0	1.6643	0.0176	0.0923	0.0907	0	0.008	0.0072	0	0.0165	0	0	0	0	0	0	0.0438	0.0086	0.0213	0	0.0023	0.0122	0.0025	0.0182	0	1.7758	0.5294	22.593
Klebsiella pneumoniae_2	GGGGAATA	Cluster_22	2245	1.4819	0	0.8237	0.0158	0.3011	0	0	0	0.1292	0	0.0302	0	0.0558	0	0	0.0165	0.0155	0	0.0308	0	0.0626	0.0439	0.5579	7.3526	0.3831	1.9203	0.8747	0.0101	0.0126	0	0	0	0	0
Lacticaseibacillus rhamnosus	AGGGAATC	Cluster_38	2110	0	0.00838	0.1373	0.0079	0	5.3056	0.0074	0.0075	0	0	20.78	0	0	0	0	0	0	0.0069	0	0	0	0	0	0.0599	0.0085	0	0.0093	0	0	0	0	0	0	0
Lactococcus sp.	AGGGAATC	Cluster_36	1924	0.4489	0.14245	0.7962	0.0079	0.0215	0.2284	0	0.0377	0.1175	0	1.1939	1.3512	0.542	1.358	0.5465	1.0959	0.5882	0.8793	0.3881	1.2269	0.5544	0.7383	0.9955	0.1198	0.0426	0.0241	0.0302	0.0446	0.3482	0.0546	0	0	0	0
Chryseobacterium sp.	GAGGAATA	Cluster_49	1645	0	0	0.0412	0	0	0	12.194	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0126
Lactobacillus helveticus_2	AGGGAATC	Cluster_85	1638	0.8117	1.02229	1.3934	0.623	0.4731	0	0.0149	1.3103	0.4992	1.4103	0	0.5219	0.2949	0.5345	0.1171	0.4285	0.2167	0.316	0.1663	0.3097	0.1878	0.709	0.0328	0.0428	0.017	0.0241	0.0441	0	0	0.0546	0	0	0	0
Limosilactobacillus sp._2	AGGGAATC	Cluster_92	1536	0.1845	0.12569	0.2265	0.1341	0.129	0	0.0074	0.1205	0.047	0.0791	0	0.4075	0.4703	0.4912	0.2342	0.3955	0.5031	0.2473	0.2279	0.2263	0.3308	0.0804	0.0656	0.0086	0	0	0	0	0	0.0182	4.1887	1.8798	1.8568	1.2426
Enterobacter-Klebsiella_2	GGGGAATA	Cluster_57	1477	0.5719	0.02514	0.254	0	0	0	0	0.0753	0	0	0.0302	0.0143	0.0399	0	0.0078	0.0082	0	0.055	0.0493	0.006	0.0358	0.0073	0.4048	0.0257	0.8642	1.9829	0.9443	0.2329	0.2725	0.2912	0	0	0	0
Aeromonas sp.	GGGGAATA	Cluster_41	1443	0.0061	0.64522	0.1167	0.0079	1.9498	2.5653	0.9157	0.256	0.9397	0.0461	0.0151	0.0858	0.1674	0.0795	0.1796	0.1978	0.1006	0.1786	0.1786	0.2799	0.0984	0.2047	0.6236	0.0514	0.0724	0.0481	0.007	0.0648	0.0858	0.1638	0	0	0	0
Acetobacter sp.	GGGGAATA	Cluster_30	1255	0.3382	9.51064	0.2402	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.2954	0	0.0128	0	0	0	0	0	0	0	0	0
Lactobacillus sp.	AGGGAATC	Cluster_48	1131	0.6702	0.63684	0.9678	0.6151	0.4086	0	0.0074	0.9715	0.5462	1.1006	0	0.2431	0.1196	0.2023	0.0781	0.2472	0.0774	0.1992	0.0554	0.1846	0.0984	0.3289	0.1094	0	0	0	0.0302	0	0.0076	0.0364	0	0	0	0
Streptococcus parauberis	AGGGAATC	Cluster_54	1039	0.2152	0.05866	0.2471	0	0	0	4.0348	0	0.0117	0	0	0.1144	0.1355	0.2673	0.5153	0.0659	0.2322	0.3366	0.2156	0.2859	0.1431	0.6359	0.0109	0	0	0	0	0.0081	0.0025	0.0364	0	0	0	0
Aeromonas sp._2	GGGGAATA	Cluster_58	1000	0	0.10055	0.0549	0	0	5.3665	1.4144	0	0	0	0.0756	0.0143	0	0	0	0	0.0077	0.0756	0.0062	0.0357	0	0	0.0109	0.2996	0.0255	0.0433	0.007	0.0101	0	0	0	0	0	0
Lactococcus lactis_2	AGGGAATC	Cluster_44	990	0.1783	0.01676	0.2608	0.0079	0.0287	0.0685	0	0.0075	0.047	0	0.3476	0.6792	0.2949	0.4695	0.2186	0.6592	0.2941	0.5015	0.2833	0.6015	0.2682	0.4166	0.4595	0.0856	0.0213	0	0.0603	0.077	0.2498	0.091	0	0	0	0
Lactococcus sp._2	AGGGAATC	Cluster_43	970	0	0.03352	0	0.0315	0.2366	0	0.0447	0.2636	0.6343	0.033	0	0.0786	0.0717	0.0578	0.0468	0.0082	0.0929	0.0687	0.0678	0.0655	0.0536	0.2047	0.0766	0	0.6599	0.385	0.8051	0.0041	0.005	1.2559	0	0	0	0
Escherichia-Shigella_2	GGGGAATA	Cluster_78	913	0.2029	0.00838	0.0824	0	0	0	0	0	0	0	0.0605	0	0	0.0144	0	0	0.0077	0.0206	0	0	0	0	3.26	0.642	0.1575	0.2503	0.2158	0.32	0.2069	1.1103	0.0123	0	0	0
Clostridium tyrobutyricum	GGGGAATA	Cluster_60	882	0	0	0.0137	0	0	4.9783	1.6526	0	0	0	0.0605	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lentilactobacillus kefiri_2	AGGGAATC	Cluster_47	880	0.2767	0.20111	0.4942	0.0552	0.086	0	0	0.128	0.0529	0.1186	0	0.286	0.3746	0.1878	0.4294	0.4038	0.3483	0.1786	0.3511	0.2859	0.3487	0.0658	1.3784	0.0086	0.0128	0	0	0.002	0.0606	0	0.0491	0.4591	0.1106	0.113
Citrobacter sp._4	GGGGAATA	Cluster_56	804	0	0.00838	0.0137	0	0	0	0	0	0	0	0.0151	0.0572	0.0478	0.065	0.0625	0.0412	0.0387	0.0824	0.0739	0.0596	0.0536	0.0365	0.9955	0.0257	0.0937	0	0.0046	0.4091	0.984	0.0728	0	0	0	0
Lentilactobacillus sp.	AGGGAATC	Cluster_52	804	0	0	0	0	0	5.5797	0	0	0.0059	0	0.136	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.3194	0	0.2765	0
Aeromonas sp._3	GGGGAATA	Cluster_53	791	0	0	0	0	0	5.9679	0	0	0	0	0.0605	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0128	0	0	0	0	0	0	0	0	0
Citrobacter sp._5	GGGGAATA	Cluster_63	789	0.0615	0.06704	0.0412	0.0079	0	0.1218	0	0.0377	0.0529	0	0	0.2359	0.3268	0.1878	0.242	0.1071	0.1703	0.4465	0.1725	0.1489	0.1162	0.0439	0.0766	0	0.3023	0.1877	0.0766	0.2774	0.2321	0.9465	0	0	0	0
Acinetobacter sp.	GGGGAATA	Cluster_11	705	0.7317	0.05028	0.5354	0.0079	0	0.0457	0.3871	0	0	0	0.0453	0.1144	0.0638	0.0361	0.0937	0.1071	0.2012	0.0824	0.0986	0.1489	0.0715	0.0658	0.0547	0.0428	0.6897	0.1588	0.0882	0.0547	0.0404	0.0546	0.0123	0	0	0
Raoultella ornithinolytica	GGGGAATA	Cluster_59	654	0	0	0.0069	0	0	0.0457	0	0	0	0	0	0.0214	0.008	0.0072	0.0468	0.0247	0.0077	0	0.0123	0.0298	0.0089	0.0219	0	0.0599	0.6684	0.6594	0.5429	0.1175	0.0555	0.1092	0	0	0	0
Enterococcus sp.	AGGGAATC	Cluster_65	633	0.2767	0.01676	0.1922	0	0.2509	0	0.1936	0.0075	0.1057	0	0.0302	0.0786	0.0877	0.1228	0.1249	0.0742	0.1161	0.3023	0.1355	0.0298	0.2325	0.1096	0.0328	0.1284	0.0085	0.0963	0.123	0.1944	0.2195	0.1638	0	0	0	0
Lentilactobacillus kefiri_3	AGGGAATC	Cluster_11	618	0.2214	0.05028	0.2814	0.0079	0	0	0.0447	0.0151	0.0411	0.0198	0	0.3003	0.2391	0.0939	0.1874	0.239	0.0774	0.2404	0.1478	0.3752	0.2057	0.1389	0.5251	0.077	0.017	0.0096	0.007	0.0284	0.2952	0.1274	0	0	0	0
Lactiplantibacillus	AGGGAATC	Cluster_89	602	0.0061	0.01676	0	0	0	0.137	0.067	0	0	0	0.1965	0.1716	0.1594	0.2023	0.242	0.1813	0.1625	0.316	0.2094	0.2263	0.1788	0.9648	0.0438	0.4451	0.017	0	0.0302	0.0243	0.1312	0.0728	0	0	0.0158	0
Lactobacillus sp. (new)	AGGGAATC	Cluster_76	600	0.0123	0.01676	0	0	0	0	0.0744	0	0	0	0	0.1716	0.1435	0.3034	0.0781	0.3131	0.0929	0.1923	0.037	0.2323	0.0447	2.1636	0	0.2568	0	0.0241	0.065	0	0.0025	0	0.0491	0	0	0
Acinetobacter sp._2	GGGGAATA	Cluster_12	546	1.0761	0.00838	0.6589	0	0	0	0.1117	0.0151	0	0	0	0.0071	0.0159	0.0289	0.0234	0.0082	0.0155	0.0206	0.0123	0.0476	0	0.0219	0.0109	0.0599	0.6471	0.0818	0.051	0.0385	0.0126	0.091	0	0	0	0
Lentilactobacillus buchneri_2	AGGGAATC	Cluster_69	546	0	0	0	0	0	4.1562	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lactobacillus kefiranofaciens_2	AGGGAATC	Cluster_98	535	0.123	0.0419	0.1236	0.0237	0.0215	0	0.0521	0.0301	0.0529	0.0066	0	0.3932	0.1913	0.6284	0.0625	0.4202	0.178	0.2954	0.1109	0.3454	0.0715	0.4678	0	0	0.0085	0.0096	0.0394	0	0.0101	0.0182	0	0	0	0
Streptococcus equinus-infantari	AGGGAATC	Cluster_93	457	0	0	0	0	0.0502	0	0	0.0753	0	0.0066	0	0	0.0638	0	0	0	0.0077	0.0069	0	0	0	0	0	0.1541	1.5198	0.0048	0.0023	0.083	0.0252	0.0182	0	0	0	0
Raoultella ornithinolytica_2	GGGGAATA	Cluster_10	438	0	0	0.0137	0	0	0.0304	0	0	0	0	0	0.0071	0.008	0.0072	0.0156	0	0.0155	0.0137	0	0.006	0.0179	0	0.0109	0	0.6428	0.3561	0.2575	0.1256	0.0454	0.0546	0	0	0	0
Lentilactobacillus buchneri_3	AGGGAATC	Cluster_91	433	0.0061	0.00838	0.0069	0	0	0.236	0	0	0	0	0	0.1501	0.1196	0.1156	0.0937	0.1236	0.0697	0.1443	0.117	0.2204	0.0984	0.6725	0.0328	0.4451	0.0043	0.0048	0.0046	0.0284	0.1388	0.0364	0	0	0	0.0126
Lactococcus sp._3	AGGGAATC	Cluster_80	432	0.1168	0.02514	0.1236	0.0158	0.0072	0	0.0074	0.0151	0.094	0	0.2267	0.336	0.1435	0.5129	0.0546	0.3461	0.1084	0.2267	0.0616	0.2204	0.0805	0.3947	0.0109	0.0342	0	0.0048	0.0093	0.002	0.005	0	0	0	0	0
Enterobacter sp._2	GGGGAATA	Cluster_94	417	0	0	0	0	0	0	0	0	0.0411	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.281	0	0.0116	0.3605	0.3936	0.091	0	0	0	0
Lactococcus lactis_3	AGGGAATC	Cluster_95	390	0.0061	0	0.048	0	0	0	0	0	0.0059	0	0.0756	0.4504	0.0638	0.3756	0.0312	0.4038	0.0619	0.3091	0.037	0.4407	0.0179	0.1096	0.0438	0	0	0	0	0.002	0.1135	0	0	0	0	0
Serratia sp.	GGGGAATA	Cluster_11	373	1.2421	0	0.9953	0	0	0.0457	0	0.0678	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0085	0.0337	0	0.002	0	0.0182	0	0	0	0
Staphylococcus simulans	AGGGAATC	Cluster_11	370	0	0	0	0	0	0	0	0	0	0	0	0.0071	0.0239	0.0144	0	0.0082	0.0077	0.0137	0.0308	0.0298	0.0358	0	0	0	0.0085	0	0	0.3929	0.3785	0	0	0	0	0
Lentilactobacillus kefiri_4	AGGGAATC	Cluster_96	363	0.0061	0.03352	0	0	0	0	0	0	0	0	0	0.1144	0.0877	0.0722	0.1561	0.1566	0.031	0.1305	0.0431	0.1727	0.0805	0.5336	0.0438	0.2996	0.0085	0.0048	0.0093	0.0263	0.1867	0.1456	0	0	0	0
Aeromonas sp._4	GGGGAATA	Cluster_13	349	0.0246	0.00838	0.0137	0	0.0287	0	0	0	0.047	0	0	0.0786	0.0638	0.1011	0.0078	0.1236	0.0464	0.2404	0.0431	0.2323	0.0447	0.2851	1.1706	0.0086	0.0766	0.0096	0.007	0.0081	0.0227	0.1092	0	0	0	0
Citrobacter sp._6	GGGGAATA	Cluster_97	345	0	0.03352	0	0	0	2.5424	0	0	0	0	0	0	0	0	0	0	0	0	0	0.006	0	0	0	0.0428	0.0043	0	0	0	0	0	0	0	0	0
Pediococcus parvulus	AGGGAATC	Cluster_10	339	0	0.00838	0	0	0	0	0	0	0	0	0	0.1287	0.2232	0.1517	0.2186	0.2802	0.2477	0.0756	0.2464	0.1429	0.2414	0.0146	0.1532	0.0086	0	0	0	0.0061	0.005	0	0.2702	0.0173	0.2291	0
Pediococcus pentosaceus	AGGGAATC	Cluster_86	339	0	0	0.0206	0	0	0	2.5013	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Raoultella terrigena	GGGGAATA	Cluster_14	337	0.0369	0	0.0412	0	0	0.0304	0	0	0	0	0	0	0	0	0.0078	0.0082	0	0.0069	0	0	0	0	0.0109	0	0.728	0.0289	0.0302	0.1499	0.1287	0.0364	0	0	0	0
Enterobacter sp._3	GGGGAATA	Cluster_14	333	0	0	0.0137	0	0	0.0381	0	0.6928	0	0.0395	0.0151	0	0	0	0	0	0.0077	0	0.0062	0	0	0	0.1203	0	0.5619	0.0433	0.0023	0.0729	0.0606	0.182	0.0123	0.0087	0	0
Lactobacillus delbrueckii_2	AGGGAATC	Cluster_12	332	0	0.15083	0.0137	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0109	0	0	0	0	0.0041	0	0	1.2284	0.9182	0.6005	0.3389
Acetobacter orientalis	GGGGAATA	Cluster_90	329	1.3528	0	0.7413	0	0	0	0.0074	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lactococcus sp._4	AGGGAATC	Cluster_11	329	0	0	0	0	0	0	0	0	0	0	0.0605	0.1716	0.0956	0.1228	0.0781	0.0906	0.0542	0.1992	0.0616	0.2382	0.0894	0.2631	0.0547	0.0342	0	0	0	0.0587	0.2044	0	0	0	0	0
Klebsiella pneumoniae_3	GGGGAATA	Cluster_14	320	0.0246	0	0.1373	0	0.0072	0.0457	0	0	0	0	0.1814	0.0786	0.0159	0.0289	0.0156	0.0165	0	0.2061	0.0308	0.0715	0	0.0146	0.0328	0.2996	0.3491	0	0	0.0203	0.1918	0.0182	0	0	0	0
Lentilactobacillus kefiri_5	AGGGAATC	Cluster_13	314	0.0799	0.02514	0.103	0	0	0	0	0.0075	0.0059	0	0	0.143	0.0956	0.1156	0.1249	0.1566	0.0851	0.1305	0.0862	0.1965	0.0715	0.0731	0.3063	0.0086	0.0128	0	0.0046	0.0263	0.1312	0.0728	0	0	0	0
Staphylococcus aureus	AGGGAATC	Cluster_11	294	0	0	0	0	0	0	0	0	0	0	0	0.0143	0.0319	0.0072	0.0078	0.0165	0.0155	0.0343	0.0062	0.0238	0.0626	0.0219	0	0	0	0.0048	0	0.399	0.1615	0	0	0	0	0
Lentilactobacillus kefiri_6	AGGGAATC	Cluster_10	264	0.0861	0	0.1647	0	0.0072	0	0	0	0.0235	0	0	0.1716	0.0797	0.0361	0.1093	0.1566	0.0464	0.103	0.0554	0.137	0.0984	0.0365	0.2188	0.0257	0.0128	0.0048	0.0023	0.0162	0.1009	0.0728	0	0	0	0
Enterococcus faecalis	AGGGAATC	Cluster_10	262	0	0	0	0.0079	0	0.0609	0.0596	0.0904	0.0646	0	0.0605	0.0143	0.0478	0.0795	0.0312	0.0659	0.0929	0.1786	0.2217	0.0536	0.1341	0.0731	0	0.2311	0.0298	0.0241	0.0394	0.0142	0.0378	0.0182	0	0	0	0
Enhydrobacter sp.	GGGGAATA	Cluster_10	258	0	0.01676	0.0412	0	0	0	0.7891	0.0377	0	0.033	0	0.0786	0.1196	0.0722	0.1015	0.033	0.1238	0.1168	0.0801	0.0596	0.0805	0.0731	0	0	0.017	0	0	0.0041	0	0	0	0	0	0
Lactococcus sp._5	AGGGAATC	Cluster_13	245	0.0307	0	0.048	0	0	0	0.0223	0	0	0	0	0.2002	0.0558	0.2167	0.1015	0.0824	0.0774	0.3572	0.0554	0.2859	0.0089	0.1535	0	0	0	0	0	0	0.0025	0	0	0	0	0
Lactobacillus helveticus_3	AGGGAATC	Cluster_14	243	0.0246	0	0	0	0	0	0	0	0	0	0	0.1001	0.0478	0.1228	0.0234	0.0906	0.0619	0.103	0.0123	0.1965	0.0358	0.8333	0.0109	0	0	0.0193	0.0116	0	0	0.0364	0	0	0	0
Aeromonas sp._5	GGGGAATA	Cluster_10	242	0.0061	1.74292	0	0	0	0.0761	0.0074	0	0	0	0.136	0	0	0	0	0.0082	0	0	0	0.006	0	0	0	0	0.0085	0	0.0186	0	0.0025	0	0	0	0	0
Raoultella ornithinolytica_3	GGGGAATA	Cluster_12	230	0	0	0.0137	0	0	0.0076	0	0	0	0	0	0.0143	0	0	0.0078	0.0165	0.031	0.0069	0.0123	0.0119	0.0268	0.0073	0	0.1198	0.5917	0.0337	0.0673	0.0182	0.0227	0.0182	0	0	0	0.0126
Serratia sp._2	GGGGAATA	Cluster_17	230	0	0	0.048	0	0	1.2712	0	0	0	0	0.0907	0.0357	0.0159	0.0072	0	0	0.0155	0.0275	0	0.006	0	0.0146	0	0.0599	0.0894	0.0048	0.0046	0.002	0.0025	0	0	0	0	0
Rothia endophytica	GGGGAATA	Cluster_12	221	0	0	0.0069	0	0	0	0.2829	0	0.1527	0	0	0	0.008	0	0	0	0.0077	0	0	0	0	0	0	0	0.6428	0	0.0023	0	0	0.0364	0	0	0	0
Lentilactobacillus rapi	AGGGAATC	Cluster_22	218	0.0307	0.02514	0.0137	0.481	0.6093	0	0	0.1581	0.0881	0.0527	0.0151	0	0	0	0.0078	0	0.0155	0	0	0	0	0	0.0656	0	0	0.0048	0.0162	0	0	0	0	0	0	0
Lactiplantibacillus plantarum-pe	AGGGAATC	Cluster_19	208	0	0	0	0	0	0	0.0521	0	0	0	0.0151	0.0572	0.0638	0.0289	0.1405	0.0824	0.0464	0.1237	0.0431	0.1013	0.0268	0.1608	0	0.0086	0.0043	0.0048	0.0023	0.0405	0.1388	0	0	0	0	0
Streptococcus agalactiae	AGGGAATC	Cluster_12	205	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.8387	0	0.0139	0.002	0	0.0182	0	0	0	0
Lactobacillus gasseri_2	AGGGAATC	Cluster_16	202	0	0	0.0206	0	0	0.2893	0	0	0	0	2.4331	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Streptococcus pluranimalium	AGGGAATC	Cluster_11	200	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0082	0	0	0	0	0	0	0	0	0.2086	0.0096	0.0116	0.2045	0.1034	0.0182	0	0	0	0
Leuconostoc mesenteroides	AGGGAATC	Cluster_12	198	0.0246	0.23462	0.0275	0.0315	0	0	0.0149	0	0	0	0	0.0143	0.0558	0.0072	0	0.0082	0.0387	0.055	0.0554	0.0298	0.0268	0	0	0.0257	0.1575	0.0241	0.0603	0.0628	0.0303	0.0182	0	0	0	0
Lactococcus raffinolactis	AGGGAATC	Cluster_13	195	0.0061	0.02514	0.0206	0	0	0	0	0	0	0	0	0.0929	0.1514	0.1156	0.1093	0.1071	0.1393	0.0412	0.154	0.1132	0.1073	0.0146	0.2297	0.0342	0.017	0	0	0	0	0.0364	0	0	0	0
Enterobacter sp._4	GGGGAATA	Cluster_17	193	0	0	0	0	0	0	0	0	0	0	0	0.1215	0.1833	0.0578	0.1171	0.1318	0.1548	0.0756	0.1047	0.2323	0.2057	0.0073	0	0	0.0128	0	0	0	0	0	0	0	0	0
Macrococcus caseolyticus	AGGGAATC	Cluster_12	193	0.1045	0	0.1647	0	0.0573	0	0.4615	0	0.1057	0	0	0.0143	0.0319	0.0506	0.0937	0	0.0077	0.0206	0.0616	0.0417	0.0179	0.0365	0.0656	0.0257	0.0085	0	0	0	0	0	0	0	0	0
Streptococcus thermophilus_2	AGGGAATC	Cluster_17	191	0	0.07541	0	0	0	0.0152	0	0.0904	0	0.0066	0.0302	0	0	0	0	0.0082	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1299	0.0158	1.8451
Streptococcus salivarius	AGGGAATC	Cluster_25	189	0	0.05028	0	0	0	0	0	0.0377	0	0.0066	0.0151	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.104	0.0316	2.0083
Enterococcus sp._2	AGGGAATC	Cluster_14	188	0	0.01676	0	0	0	0	0.6625	0	0	0	0	0	0	0.0072	0.0078	0.0082	0.0155	0.0137	0.0246	0.0119	0.0179	0	0.4266	0.0171	0.0043	0	0.0162	0.0567	0.005	0.0546	0	0	0	0
Klebsiella pneumoniae_4	GGGGAATA	Cluster_13	184	0.4919	0	0.3912	0	0.1505	0	0	0	0.1057	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0043	0.0289	0.0023	0	0	0	0	0	0	0
Serratia sp._3	GGGGAATA	Cluster_14	173	0	0	0.0069	0	0	1.119	0	0	0	0	0.1511	0	0	0	0	0	0	0	0	0	0.0089	0.0073	0	0.0599	0.0128	0.0048	0.0046	0	0	0	0	0	0	0
Acinetobacter sp._3	GGGGAATA	Cluster_16	171	0	0.00838	0	0	0	0	0	0	0	0	0	0.0286	0.0478	0.065	0.0312	0.0247	0.0464	0.0206	0.0678	0.0596	0.0358	0	0	0	0.2341	0.077	0.0487	0.0142	0.0202	0.0364	0	0	0.0079	0
Pseudomonas sp.	GGGGAATA	Cluster_16	162	0	0.00838	0	0	0	0	0	0	0	0	0	0.05	0.2471	0.0072	0.1874	0.1154	0.0697	0.0962	0.0986	0.1429	0.1609	0.0146	0	0	0.0043	0	0	0	0	0	0	0	0	0
Lentilactobacillus kefiri_7	GGGGAATA	Cluster_24	161	0	0.12569	0.0618	0.071	0.0143	0	0.0074	0.0602	0.0352	0.112	0	0.0071	0.008	0.0144	0.0625	0.033	0.0077	0	0.0185	0.0179	0.0626	0	0.0766	0.1455	0.0851	0	0.0116	0.0122	0.005	0	0	0.0606	0	0
Lentilactobacillus sp._2	AGGGAATC	Cluster_21	160	0.0061	0	0.0206	0.0079	0	0	0	0.0075	0.0059	0.0066	0	0.1001	0.1036	0.0722	0.1171	0.0494	0.2244	0.0481	0.1417	0.0298	0.1609	0.0073	0.0547	0	0	0	0.0046	0.002	0.0076	0	0	0	0	0
Enterobacter sp._5	GGGGAATA	Cluster_17	154	0	0	0.0069	0	0	0.1903	0	0	0.0352	0	0.1814	0.0643	0.0558	0.0578	0.0078	0.1236	0.0155	0.1717	0.0062	0.1251	0.0358	0.0439	0	0	0.0213	0	0	0	0.0126	0.0182	0	0	0	0
Lactobacillus	AGGGAATC	Cluster_15	154	0.0123	0.3268	0.0137	0.0079	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.3807
Lactobacillus kefiranofaciens_3	AGGGAATC	Cluster_26	151	0.0061	0.07541	0.0549	0.3943	0.043	0	0.0074	0.113	0.0352	0.0461	0	0.0286	0.0399	0.0289	0.0156	0.0165	0.0232	0.0137	0.0123	0.0298	0.0447	0.0292	0.0109	0	0.0085	0.0048	0.0139	0	0	0	0	0	0	0
Clostridium perfringens	GGGGAATA	Cluster_16	150	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0073	0	1.2326	0	0	0	0.0081	0.0025	0	0	0	0	0
Klebsiella sp.	GGGGAATA	Cluster_19	150	0.1968	0	0.103	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.175	0.1969	0.0596	0.0385	0.007	0.0425	0.0404	0.0364	0	0	0	0
Lacticaseibacillus casei	AGGGAATC	Cluster_30	145	0	0	0.0206	0	0	0.0609	0	0	0	0	2.0251	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pseudomonas putida	GGGGAATA	Cluster_14	145	0	0.01676	0	0	0	0	0	0	0.0066	0	0	0.0429	0.0319	0	0.1718	0.0494	0.0697	0.316	0.0924	0.1608	0.0358	0.0073	0	0	0	0	0	0.002	0	0	0	0.0087	0	0
Leuconostoc mesenteroides_2	AGGGAATC	Cluster_13	141	0	0.15921	0	0	0	0.2816	0.0074	0	0	0	0.0302	0	0.008	0	0.0234	0	0	0	0	0.006	0.0268	0.0073	0.0656	0	0.2682	0.0048	0	0.002	0.0025	0.0182	0	0	0	0
Enterobacter-Klebsiella_3	GGGGAATA	Cluster_14	140	0	0	0	0	0	0	0	0.0075	0	0	0	0.05	0.0877	0.065	0.0937	0.0494	0.0697	0.055	0.0862	0.0774	0.0805	0.0073	0	0.0171	0.0639	0.0481	0.0093	0.0122	0.005	0	0.0123	0	0	0
Lactobacillus kefiranofaciens_4	AGGGAATT	Cluster_17	140	0	0	0	0.7886	0.2509	0	0	0	0.0235	0.0066	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lactococcus sp._6	AGGGAATC	Cluster_23	139	0.0061	0.00838	0.0343	0	0	0	0	0	0	0	0	0.1144	0.0399	0.1011	0.1327	0.0494	0.0619	0.158	0.0185	0.1608	0.0268	0.0731	0	0	0	0	0	0	0	0	0	0	0	0
Lentilactobacillus kefiri_8	AGGGAATC	Cluster_14	137	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0109	0.0086	0.0468	0.284	0.1508	0	0	0	0	0	0	0
Lentilactobacillus kefiri_9	AGGGAATC	Cluster_24	135	0.0246	0.02514	0.048	0	0.0143	0	0	0.0075	0	0	0	0.0929	0.0478	0.0722	0.0859	0.0659	0.0155	0.0481	0.0308	0.0536	0.0358	0.0146	0.1422	0	0.0085	0	0	0.0061	0.058	0	0	0	0	0
Vagococcus carniphilus	AGGGAATC	Cluster_15	135	0	0	0	0	0	0	0.8561	0	0	0	0.0756	0	0	0	0	0	0	0	0	0	0	0	0	0	0.017	0	0	0.0162	0.005	0.0182	0	0	0	0
Streptococcus equinus	AGGGAATC	Cluster_18	132	0	0	0	0	0.0072	0	0.938	0.0226	0.0117	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Staphylococcus simulans_2	AGGGAATC	Cluster_15	130	0	0	0	0	0	0	0	0	0	0	0	0.0214	0.0239	0.0072	0.0156	0	0.031	0.0069	0.0185	0.0238	0.0179	0	0	0	0	0	0	0.1458	0.0883	0	0	0	0	0
Acinetobacter sp._4	GGGGAATA	Cluster_18	126	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.5364	0	0	0	0	0	0	0	0	0
Levilactobacillus brevis	AGGGAATC	Cluster_19	125	0	0	0.0137	0.0237	0	0.1903	0.0819	0	0	0	0.3174	0	0.0159	0	0	0.0082	0.0387	0.0137	0	0	0	0.0877	0	0	0	0	0	0.0122	0.0101	0	0.1106	0.0347	0.1422	0
Lentilactobacillus sp._3	AGGGAATC	Cluster_22	123	0	0	0	0	0	0	0	0	0	0	0	0.0572	0.1036	0.0722	0.0859	0.0906	0.1161	0.0343	0.0801	0.0476	0.1431	0.0146	0.0219	0	0.0128	0	0	0.002	0.0126	0	0	0	0	0
Lactobacillus sp._2	AGGGAATC	Cluster_22	121	0.043	0.36869	0.0755	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0328	0	0.0979	0.0241	0.065	0	0	0	0	0	0	0
Lactobacillus helveticus_4	AGGGAATC	Cluster_23	119	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.4937
Acinetobacter sp._5	GGGGAATA	Cluster_38	118	0.0123	0.02514	0.0618	0	0	0.1446	0.0893	0	0	0	0	0	0	0	0.0234	0.0082	0.0387	0.0137	0.0246	0.0119	0.0179	0.0365	0.0328	0	0.0724	0	0.0046	0.0344	0.0252	0	0	0	0	0
Lactococcus sp._7	AGGGAATC	Cluster_20	116	0.0061	0	0.0275	0	0	0	0	0	0	0	0	0.0357	0.008	0.0217	0.0078	0.0247	0.0232	0.1305	0.037	0.0357	0.0358	0.0292	0.0328	0.0086	0	0	0.0023	0.0041	0.1236	0	0	0	0	0
Lentilactobacillus sp._4	AGGGAATC	Cluster_18	116	0.0123	0	0	0	0	0	0	0	0	0	0	0.0357	0.0797	0.0578	0.039	0.0577	0.1548	0.0206	0.1294	0.0417	0.1878	0	0.0438	0	0.0043	0	0	0	0.005	0	0	0	0	0
Lentilactobacillus sp._5	AGGGAATC	Cluster_21	115	0.0184	0.00838	0	0	0	0	0	0	0	0	0	0.0429	0.0956	0.0506	0.1093	0.0412	0.1703	0.0412	0.0616	0.0357	0.1431	0.0219	0.0109	0	0	0	0.0023	0	0.0025	0.0364	0	0	0	0
Lactococcus sp._8	AGGGAATC	Cluster_23	114	0	0	0.0137	0	0.0072	0	0.0149	0	0	0	0.0302	0.0715	0.0399	0.0217	0.0234	0.033	0.0232	0.103	0.0062	0.1013	0.0089	0.0585	0.0109	0	0.0596	0	0.0116	0.0122	0.0278	0	0	0	0	0
Weissella sp.	AGGGAATC	Cluster_18	114	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.4513	0.0193	0.0093	0	0	0	0	0	0	0
Aeromonas sp._6	GGGGAATA	Cluster_31	112	0	0	0.0069	0	0	0	0	0	0.0176	0	0	0.0786	0.0319	0.065	0.0078	0.033	0.0077	0.055	0.0246	0.137	0	0.0658	0.3282	0	0.0085	0	0	0	0.005	0	0	0	0	0
Chryseobacterium hominis	GAGGAATA	Cluster_20	112	0.0307	0	0.0069	0.0237	0	0	0.6923	0	0	0.0132	0	0	0	0	0	0	0	0.0069	0	0.0119	0	0	0.0109	0	0.0128	0	0.0023	0	0	0	0	0	0	0
Streptococcus pseudoporcinus	AGGGAATC	Cluster_28	108	0	0	0	0	0	0	0.7965	0.0075	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acinetobacter sp._6	GGGGAATA	Cluster_24	104	0.0246	0.03352	0	0	0	0.5785	0	0.1054	0.0235	0.0066	0	0	0	0	0	0	0	0	0	0	0	0	0.0109	0	0	0	0	0	0	0	0	0	0	0
Escherichia-Shigella_3	GGGGAATA	Cluster_21	104	0.3812	0	0.2883	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Limosilactobacillus sp._3	AGGGAATC	Cluster_31	103	0.0492	0.07541	0.0824	0.0394	0.0287	0	0.0074	0.0753	0.0411	0.0725	0	0.0429	0	0.0289	0.0078	0.033	0.0077	0	0	0.0119	0.0089	0.0512	0	0.0257	0.0085	0	0.0023	0	0	0	0.0123	0.0173	0	0.0126
Klebsiella pneumoniae_5	GGGGAATA	Cluster_21	102	0.0061	0	0.0275	0.0394	0	0	0	0	0	0	0	0.0071	0	0.0072	0	0	0	0	0	0.006	0	0	0.1094	0.0257	0.0809	0.1829	0.0394	0.002	0	0	0	0.0087	0	0
Mycoplasmopsis agalactiae_2	AGGGAATC	Cluster_29	102	0	0	0	0	0	0	0	0	0	0	0	0.1001	0.0319	0.0939	0	0.0824	0.0232	0.1374	0.0123	0.1429	0.0536	0.0219	0.0219	0	0	0	0	0	0	0	0	0	0	0.0126
Lactobacillus helveticus_5	AGGGAATC	Cluster_20	97	0	0	0	0.0473	0.1505	0	0.0298	0	0.3876	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lactococcus sp._9	AGGGAATC	Cluster_20	96	0	0	0	0	0	0	0	0	0	0	0	0.0786	0.008	0.0939	0.0468	0.0989	0.0542	0.0343	0.0554	0.0893	0.0626	0	0.0547	0	0	0.0048	0.007	0	0.0025	0	0	0	0	0
Lactococcus sp._10	AGGGAATC	Cluster_34	95	0.0123	0	0.0206	0	0	0	0	0	0	0	0.0151	0.0858	0.0478	0.0217	0.0156	0.0659	0.0077	0.0343	0	0.1132	0.0358	0.0219	0.0109	0	0	0	0	0	0.0631	0	0	0	0	0
Lactobacillus kefiranofaciens_5	GGGGAATA	Cluster_30	94	0.0061	0.02514	0.0206	0.1025	0.0287	0	0	0.0075	0.0117	0	0	0	0.008	0.0217	0	0.0165	0.0077	0.0069	0	0.006	0.0358	0.0146	0	0	0.0128	0.0578	0.0835	0	0	0.0182	0	0	0	0
Lactobacillus fornicalis	AGGGAATC	Cluster_29	93	0	0	0	0	0	0	0	0	0	0	0	0.0786	0.0239	0.0506	0.0078	0.0577	0.0077	0.0687	0.0185	0.0536	0.0089	0.2631	0	0.0257	0	0	0	0	0	0	0.0123	0	0	0
Streptococcus parauberis_2	AGGGAATC	Cluster_20	92	0.0061	0	0.0206	0	0	0	0	0	0	0	0	0.0357	0.0399	0.065	0.1093	0.0247	0.0387	0.0756	0.0308	0.0893	0.0268	0.0877	0	0	0	0	0	0	0.0025	0	0	0	0	0
Streptococcus equinus_2	AGGGAATC	Cluster_25	91	0	0	0	0	0.0072	0	0.5509	0.0452	0.0529	0.0066	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Enterobacter sp._6	GGGGAATA	Cluster_35	90	0.0123	0	0.0549	0	0	0.0152	0	0	0.0059	0	0.0605	0	0.008	0.0072	0.0078	0.0082	0.0155	0.0412	0.0062	0.0417	0	0.0073	0.0219	0.0685	0.0468	0	0	0.0041	0.0732	0	0	0	0	0
Lactiplantibacillus plantarum	AGGGAATC	Cluster_23	88	0	0	0	0	0	0	0	0	0	0	0	0.0429	0.008	0.0361	0.039	0.033	0.0155	0.0618	0.0246	0.0298	0.0536	0.0439	0	0	0	0	0.007	0.0263	0.0454	0.0182	0	0	0	0
Lactobacillus helveticus_6	AGGGAATC	Cluster_29	87	0	0	0	0	0	0	0.5955	0	0.0176	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0171	0	0	0	0	0	0	0	0	0	0.0251
Lactococcus sp._11	AGGGAATC	Cluster_26	86	0	0	0	0	0	0	0	0	0	0	0	0.0214	0.0319	0.0578	0.0312	0.0412	0.0387	0.0206	0.0986	0.0715	0.0715	0	0.1313	0	0.0043	0.0193	0.0023	0	0	0	0	0	0	0
Lentilactobacillus sp._6	AGGGAATC	Cluster_27	85	0.0123	0	0	0	0	0	0	0	0	0	0	0.0286	0.0558	0.0361	0.0546	0.0494	0.1006	0.0206	0.0678	0.0476	0.1341	0	0.0219	0	0	0	0	0.002	0.0025	0	0	0	0	0
Aeromonas sp._7	GGGGAATA	Cluster_21	84	0.0061	0.05866	0.0755	0.0079	0	0	0	0.1054	0.0294	0.0066	0	0	0	0	0.0156	0	0	0	0	0	0.0089	0	0.0328	0	0.017	0	0	0.0304	0.0378	0.0728	0	0	0	0
Lactiplantibacillus plantarum_2	AGGGAATC	Cluster_25	84	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.719	0	0	0	0	0	0	0	0	0	0
Lactococcus sp._12	AGGGAATC	Cluster_29	84	0	0	0.0206	0	0	0	0	0	0.0059	0	0.0453	0.0143	0.0638	0.0506	0.0781	0.0659	0.0619	0.0206	0.0185	0.0536	0.0626	0	0.0109	0.0257	0	0	0	0.0041	0.0151	0	0	0	0	0
Enterococcus sp._3	AGGGAATC	Cluster_28	82	0.0184	0	0.0275	0	0	0	0	0	0	0	0.0151	0.0071	0.008	0.0361	0.0234	0.033	0.0155	0.0756	0.0185	0.0179	0.0447	0.0146	0	0	0	0	0	0.0122	0.0681	0.0182	0	0	0	0
Lentilactobacillus kefiri_10	AGGGAATC	Cluster_26	82	0.0061	0.0419	0.0549	0.0237	0.0143	0	0	0.0828	0.0117	0.0659	0	0	0.0159	0.0072	0.0156	0.0165	0.0155	0.0137	0.0062	0.0119	0.0447	0	0.0328	0.0942	0.0043	0	0	0	0.005	0	0	0.0347	0	0
Lactobacillus kefiranofaciens_6	AGGGAATC	Cluster_43	80	0.0246	0.0419	0.0618	0.0315	0	0	0	0.0151	0	0.0395	0	0.0643	0.0159	0.065	0	0.0494	0.0077	0.0069	0.0185	0.0238	0.0089	0.1023	0	0	0	0	0	0	0	0	0	0	0	0
Enterococcus sp._4	AGGGAATC	Cluster_28	77	0.0369	0	0.1098	0	0.0072	0	0	0.0151	0.0117	0.0066	0.0151	0	0.008	0.0072	0.0078	0.0165	0	0.0412	0.0062	0.0179	0	0.0219	0.1532	0.0428	0	0	0	0	0.0202	0.0546	0	0	0	0
Lentilactobacillus sp._7	AGGGAATC	Cluster_54	77	0	0	0.0137	0.0079	0.0072	0	0	0.0075	0	0.0066	0	0.0214	0.0239	0.0361	0	0	0	0.0069	0.0246	0.006	0.0089	0	0	0	0	0	0	0	0	0	0.172	0.104	0.1738	0.0628
Lactococcus sp._13	AGGGAATC	Cluster_44	75	0.0061	0	0	0	0	0	0	0	0.0059	0	0	0.0643	0.0319	0.0289	0.0156	0.033	0.0387	0.0481	0.0123	0.0834	0.0179	0.1023	0.0219	0	0.0085	0	0	0	0	0.0364	0	0	0	0
Streptococcus uberis	AGGGAATC	Cluster_29	75	0	0	0	0	0	0	0.2485	0	0.2307	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0109	0.0086	0.0085	0	0.007	0	0	0	0	0	0	0
Pasteurella multocida	GGGGAATA	Cluster_24	73	0	0	0	0	0	0	0	0	0	0	0	0.0643	0.0159	0.0289	0.0312	0.0659	0.0697	0.055	0.0554	0.0476	0.0984	0.0073	0	0	0	0	0	0	0	0	0	0	0	0
Lactococcus sp._14	AGGGAATC	Cluster_28	72	0	0.00838	0	0	0	0	0	0	0	0	0	0.0715	0.0558	0.0506	0.039	0.0494	0.0232	0.0481	0.0185	0.0536	0.0358	0.0073	0.0438	0	0	0	0.007	0	0.005	0	0	0	0	0
Lactococcus sp._15	AGGGAATC	Cluster_39	72	0	0	0	0	0	0	0	0	0.0529	0	0	0.0429	0.0399	0.0361	0.0078	0.033	0.0077	0.0687	0.0062	0.0715	0.0179	0.0731	0.0219	0	0	0	0.0023	0	0	0.0546	0	0	0	0
Lactobacillus delbrueckii_3	AGGGAATC	Cluster_27	71	0	0.01676	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.2088	0.3465	0.0869	0.0126
Escherichia sp.	GGGGAATA	Cluster_30	69	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0383	0.0337	0.0464	0.0284	0.0479	0	0	0	0	0
Lentilactobacillus kefiri_11	AGGGAATC	Cluster_25	68	0.0922	0	0.0275	0	0	0	0	0	0.0176	0	0	0.0286	0.0159	0.0217	0.0312	0.0247	0	0.0206	0.0062	0.0298	0.0179	0	0.0547	0	0.0043	0	0	0.0061	0.0202	0.0364	0	0	0	0
Acinetobacter sp._7	GGGGAATA	Cluster_23	66	0	0.0419	0	0	0	0	0	0	0	0	0.0151	0	0	0	0	0	0	0	0	0	0	0	0	0	0.2001	0.0193	0.0139	0	0	0.0546	0	0	0	0
Lactobacillus kefiranofaciens_7	AGGGAATC	Cluster_37	64	0.0061	0	0.0137	0	0	0	0	0	0	0.0066	0	0.1072	0	0.1156	0	0.0659	0.0077	0.0206	0	0.0238	0.0358	0.0658	0	0	0	0	0	0	0	0	0	0	0	0
Lactobacillus sp._3	AGGGAATC	Cluster_32	63	0	0.14245	0	0.3627	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lentilactobacillus sp._8	GGGGAATA	Cluster_58	63	0.0061	0.01676	0.0069	0.0079	0.0072	0	0	0	0	0.0066	0	0	0.0319	0	0.039	0	0.0542	0.0069	0.0246	0.0119	0.0536	0	0.0219	0	0	0	0	0	0	0	0.0614	0.1473	0.0237	0
Acinetobacter sp._8	GGGGAATA	Cluster_34	62	0	0	0.0069	0	0.0072	0	0.0298	0.0075	0	0	0	0.0071	0.0319	0.0361	0.0312	0.0165	0.0155	0.0069	0.0246	0.0119	0.0179	0.0219	0	0	0	0	0	0.002	0.005	0.1456	0	0.1126	0	0.0126
Staphylococcus sp.	AGGGAATC	Cluster_37	61	0	0	0.0069	0	0	0	0.0223	0	0	0	0	0.0143	0.0239	0	0.0468	0.033	0.0155	0.0069	0.0062	0.0179	0.0447	0.0073	0.0219	0.0086	0.0085	0	0.0325	0.0101	0.005	0	0.0246	0	0.0079	0
Lentilactobacillus sp._9	AGGGAATC	Cluster_44	60	0.0307	0	0	0.0473	0	0.0076	0.0893	0.0452	0.0411	0.0198	0	0	0	0	0.0546	0.0082	0.0155	0	0.0123	0.0298	0	0	0	0	0	0	0	0	0	0	0.0123	0.0087	0	0.0126
Enterococcus sp._5	AGGGAATC	Cluster_31	59	0.0553	0	0.0206	0.0473	0	0	0	0.0452	0.0587	0.0066	0.0151	0.0071	0.0239	0.0072	0.0234	0	0.0077	0.0069	0.0246	0.006	0.0089	0.0219	0.0109	0	0	0	0.0023	0.002	0	0.0182	0	0	0	0
Lactococcus lactis_4	AGGGAATC	Cluster_44	59	0.0123	0	0.0069	0	0	0	0	0	0	0	0.0151	0.0286	0	0.065	0	0.0247	0.0077	0.0756	0	0.0834	0.0089	0.0365	0.0109	0	0	0	0	0	0.0151	0	0	0	0	0
Lentilactobacillus sp._10	GGGGAATA	Cluster_37	59	0.0184	0.00838	0.0275	0.0315	0	0.0381	0.0521	0	0.0587	0	0	0	0.008	0.0144	0	0.0247	0.0077	0	0.0123	0	0.0179	0.0146	0.0109	0.0257	0	0	0	0	0.0101	0	0.0246	0	0.0079	0.0126
Pseudomonas sp._2	GGGGAATA	Cluster_25	59	0	0.49439	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Streptococcus dysgalactiae	AGGGAATC	Cluster_37	59	0	0	0	0	0	0	0	0	0	0	0	0.0143	0.0399	0.0144	0.039	0.033	0.0387	0.0824	0.0739	0.0357	0.0268	0.0219	0	0	0	0	0	0	0	0	0	0	0	0
Enterobacteriaceae	GGGGAATA	Cluster_36	58	0	0	0	0	0	0	0	0	0	0	0	0.0071	0	0	0	0	0.0387	0	0.0123	0	0.0268	0	0	0.0428	0	0	0.0882	0.0081	0	0	0	0	0	0
Lactobacillus sp._4	GGGGAATA	Cluster_33	57	0	0.01676	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1958	0.0096	0.0162	0	0	0	0	0	0	0
Corynebacterium sp.	GGGGAATA	Cluster_27	56	0.1906	0	0	0	0	0	0.0223	0	0	0	0	0.0071	0.008	0.0144	0.0078	0	0.0155	0.0069	0.0123	0	0	0.0146	0	0.0086	0.0085	0	0.0023	0.0101	0.0025	0	0	0	0	0
Latilactobacillus sakei	AGGGAATC	Cluster_33	56	0	0	0	0	0	0	0	0	0	0	0	0.0357	0.0319	0.0506	0.0078	0.1401	0.0387	0.0275	0.0246	0.0298	0.0268	0	0	0.0086	0	0	0	0	0	0	0	0	0	0
Lactobacillus delbrueckii_4	AGGGAATC	Cluster_48	53	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.6652
Lentilactobacillus buchneri_4	AGGGAATC	Cluster_38	53	0	0.00838	0	0	0	0.0152	0	0	0	0	0	0.0214	0	0.0144	0.0156	0.0577	0.0077	0.0343	0	0.0715	0.0089	0.0877	0.0109	0.0171	0	0	0	0	0.005	0	0	0	0	0
Lactococcus sp._16	AGGGAATC	Cluster_43	50	0	0	0.0069	0	0	0	0	0	0	0	0	0.05	0	0.0578	0.0156	0.0247	0.0077	0.055	0.0062	0.0893	0.0089	0.0219	0	0	0	0	0	0	0	0	0	0	0	0
Serratia sp._4	GGGGAATA	Cluster_56	49	0.0738	0	0.2334	0	0	0	0	0.0075	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0048	0.0023	0	0	0	0	0	0	0
Escherichia-Shigella_4	GGGGAATA	Cluster_35	47	0.0123	0	0.0069	0	0	0	0	0	0	0	0.0453	0	0	0	0	0	0	0.0069	0	0.006	0	0	0	0	0.0979	0	0	0.002	0.0378	0	0	0	0	0
Limosilactobacillus sp._4	AGGGAATC	Cluster_80	47	0	0	0	0	0	0	0	0	0	0	0	0.0715	0.008	0.0361	0.0156	0.0165	0	0.0069	0	0.0298	0.0089	0.0146	0	0	0	0	0	0	0	0	0.1474	0	0.0474	0
Escherichia-Shigella_5	GGGGAATA	Cluster_29	46	0.0123	0	0.0137	0	0	0.0076	0	0	0	0	0.136	0	0.008	0.0289	0	0.0082	0	0.0687	0	0.0238	0	0.0073	0.0219	0	0.0298	0	0.0023	0.002	0	0	0	0	0	0
Lactiplantibacillus plantarum-pe	AGGGAATC	Cluster_34	46	0	0	0	0	0	0	0	0	0	0	0	0.0429	0.0159	0	0.0078	0.0412	0.0077	0.0137	0	0.0119	0.0358	0.0073	0	0.0086	0	0	0	0.0081	0.0429	0	0	0	0	0
Hafnia alvei	GGGGAATA	Cluster_56	45	0	0.01676	0	0	0	0	0	0	0	0	0	0.0143	0.0239	0	0	0.0494	0	0	0.0246	0.0298	0.0089	0	0.0547	0.1027	0.017	0	0	0.002	0	0	0	0	0	0
Lactiplantibacillus plajomi	AGGGAATC	Cluster_41	44	0	0	0	0	0	0	0	0	0	0	0	0.0357	0.008	0.0144	0.0156	0.0494	0.0077	0.0343	0.0062	0.0476	0.0089	0.0292	0	0.0086	0	0	0	0	0.0177	0	0	0	0	0
Lentilactobacillus buchneri_5	AGGGAATC	Cluster_42	44	0	0	0	0	0	0	0	0	0	0	0	0.05	0	0.0361	0.0078	0.0082	0	0.0618	0	0.0715	0	0.0219	0	0	0	0	0	0	0.0126	0.0182	0	0	0	0
Psychrobacter celer	GGGGAATA	Cluster_70	44	0	0	0	0	0	0	0	0	0	0	0	0.0357	0.0399	0.0217	0.0234	0.0082	0.031	0.0069	0.0616	0.0357	0.0536	0	0	0	0	0	0	0	0	0	0	0	0	0
Undibacterium sp.	GGGGAATT	Cluster_35	44	0	0	0	0	0.3154	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lactiplantibacillus sp.	AGGGAATC	Cluster_35	43	0	0	0	0	0	0	0	0	0	0	0	0.0214	0	0.0144	0.0156	0	0	0.0412	0.0062	0.0119	0.0089	0.0512	0	0.0342	0.0085	0	0	0.0081	0.0227	0	0	0	0	0
Lactobacillus kefiranofaciens_8	GGGGAATA	Cluster_56	43	0	0.01676	0.0343	0.1262	0.0072	0	0	0.0075	0.0059	0.0198	0	0	0.0159	0.0072	0.0078	0	0.0155	0	0	0.006	0.0179	0	0	0	0	0	0.0116	0	0	0	0	0	0	0
Lactococcus sp._17	AGGGAATC	Cluster_32	42	0	0	0	0	0	0	0	0	0	0	0	0	0.008	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0304	0.0656	0	0	0	0	0
Lactococcus sp._18	GGGGAATA	Cluster_42	41	0.0061	0	0.0137	0	0	0.0076	0	0	0	0	0	0	0.0558	0.0144	0.0156	0.0247	0.0387	0.0206	0.0123	0.006	0.0268	0	0.0109	0.0086	0.0085	0	0.0023	0.002	0.0076	0	0	0	0	0
Oceanobacillus sp.	AGGGAATC	Cluster_33	41	0	0	0.0069	0	0.0143	0	0.1936	0	0.0705	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chryseobacterium bovis	GAGGAATA	Cluster_35	40	0	0.02514	0	0	0	0	0.2233	0.0301	0	0	0	0.0071	0	0	0	0.0082	0	0	0	0	0.0089	0	0	0	0	0	0	0	0	0	0	0	0	0
Lactococcus sp._19	AGGGAATC	Cluster_34	40	0	0.00838	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.006	0	0	0	0	0.0043	0	0	0.0203	0.0681	0	0	0	0	0
Wautersiella falsenii	GAGGAATA	Cluster_53	40	0	0	0.0137	0	0	0	0	0	0	0	0	0.0071	0.0159	0.0217	0.0234	0.0165	0.0155	0.0069	0.0246	0.0536	0	0	0.0219	0	0	0.0096	0	0.0041	0.0025	0.0728	0	0	0	0
Lactococcus sp._20	AGGGAATC	Cluster_42	39	0	0	0	0	0	0	0	0	0	0	0	0	0.0159	0.0361	0.0078	0	0.031	0.1237	0.0123	0.0238	0	0.0219	0	0	0	0	0	0	0	0	0	0	0	0
Streptococcus equinus_3	AGGGAATC	Cluster_35	39	0	0	0.0275	0	0.0072	0	0.0223	0.0226	0.0059	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1107	0	0.0023	0	0	0	0	0	0	0
Escherichia-Shigella_6	GGGGAATA	Cluster_93	38	0	0	0.0137	0.0079	0	0.0152	0	0	0	0	0.4534	0.0143	0	0.0072	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lactobacillus kefiranofaciens_9	AGGGAATC	Cluster_79	37	0.0061	0.02514	0.0069	0.1025	0.0072	0	0	0.0377	0.0176	0.0132	0	0.0071	0	0	0.0156	0	0.0077	0	0	0.006	0.0089	0.0073	0	0	0	0.0048	0	0	0	0	0	0	0	0
Escherichia-Shigella_7	GGGGAATA	Cluster_83	36	0	0	0.0069	0	0	0.0076	0	0	0	0	0.0453	0.0286	0	0.0217	0	0.0247	0.0155	0.0481	0.0062	0.0476	0.0089	0	0	0	0.0085	0	0	0	0	0	0	0	0	0
Lactococcus lactis_5	AGGGAATC	Cluster_28	36	0	0	0	0	0	0	0	0	0	0	0.0151	0.0357	0.0159	0.0361	0	0.0412	0	0.0343	0.0062	0.006	0.0089	0.0073	0.0109	0	0	0	0	0.0202	0	0	0	0	0	0
Lactococcus sp._21	AGGGAATC	Cluster_48	36	0	0	0	0	0	0	0	0	0	0	0	0.0214	0.0159	0.0072	0.0234	0.0247	0.0155	0.0962	0.0123	0.0238	0.0179	0	0	0	0	0	0	0	0	0	0	0	0	0

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APPENDIX 5 PERCENTAGE (%) OF *CAPRA HIRCUS* READS ALIGNED IN EACH FERMENTED FOOD METAGENOME

	Samples	% Reads aligned with *Capra hircus*
AMBRISS	**A1**	7.33
	A2	9.08
	A4	49.22
	A6	13.4
	A1-b	8.26
	A4-b	0.34
LABNEH	**L15**	71.85
	L16	58.48
	L17	61.89
	L18	11.58
SERDALEH	**S8**	2.35
	S7-b	7.44
	S8-b	8.49
	S9-b	7.44
	S10-b	7.69
	S11-b	9.29

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Appendix 6 Relative abundance of microbial composition of 16 traditional fermented goat milk metagenomes from Lebanon using the MetaPhlAn taxonomic assignment method.

A- By occurence

	Kingd	Phylu	Class	Order	Family	Genus	Species	A1	A2	A4	A6	A1-b	A4-b	S8	S7-b	S8-b	S9-b	S10-b	S11-b	L15	L16	L17	L18	Occurrence
k__Bacteria|p__Firmicutes|c__Bacilli|o__Lactobacillales|f__Lactobacillaceae|g__Lactobacillus|s__Lactobacillus_kefiri	k__B	p__F	c__Bacilli	o__Lactobacillales	f__Lactobacillaceae	g__Lactobacillus	s__Lactobacillus_kefiri	25.8	37.1	1.13	0.22	24.4	49.1	6.88	8.33	7.61	7.59	5.24	8.11	1.52	7.95	3.01	5.88	16
k__Bacteria|p__Firmicutes|c__Bacilli|o__Lactobacillales|f__Lactobacillaceae|g__Lactobacillus|s__Lactobacillus_diolivorans	k__B	p__F	c__Bacilli	o__Lactobacillales	f__Lactobacillaceae	g__Lactobacillus	s__Lactobacillus_diolivorans	1.41	2.46	0	0	2.25	1.45	2.03	4.17	2.82	7.51	3.3	5.76	7.99	14	7.52	4.45	14
k__Bacteria|p__Firmicutes|c__Bacilli|o__Lactobacillales|f__Streptococcaceae|g__Lactococcus|s__Lactococcus_lactis	k__B	p__F	c__Bacilli	o__Lactobacillales	f__Streptococcaceae	g__Lactococcus	s__Lactococcus_lactis	17.7	18.9	0.34	12	15.1	0.82	67.2	62.5	68.1	59.3	72.7	61.7	0	0	0	0.01	13
k__Bacteria|p__Firmicutes|c__Bacilli|o__Lactobacillales|f__Lactobacillaceae|g__Lactobacillus|s__Lactobacillus_kefiranofaciens	k__B	p__F	c__Bacilli	o__Lactobacillales	f__Lactobacillaceae	g__Lactobacillus	s__Lactobacillus_kefiranofaciens	40.6	27.8	2	0.24	37.4	43.8	8.19	3.93	2.2	4.94	2.35	3.87	0	0	0	0	12
k__Bacteria|p__Firmicutes|c__Bacilli|o__Lactobacillales|f__Lactobacillaceae|g__Lactobacillus|s__Lactobacillus_plantarum	k__B	p__F	c__Bacilli	o__Lactobacillales	f__Lactobacillaceae	g__Lactobacillus	s__Lactobacillus_plantarum	0	0.05	4.37	1.53	1.43	0.03	5.29	9.73	7.88	9.86	6.29	8.9	0	0	0	0.16	12
k__Bacteria|p__Firmicutes|c__Bacilli|o__Lactobacillales|f__Streptococcaceae|g__Streptococcus|s__Streptococcus_parauberis	k__B	p__F	c__Bacilli	o__Lactobacillales	f__Streptococcaceae	g__Streptococcus	s__Streptococcus_parauberis	0.07	0.36	35.6	0	1.43	0	1.71	0.37	1.7	0.52	0.54	0.49	0	0	0	0.13	12
k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Aeromonadales|f__Aeromonadaceae|g__Aeromonas|s__Aeromonas_media	k__B	p__P	c__Gammaproteobacteria	o__Aeromonadales	f__Aeromonadaceae	g__Aeromonas	s__Aeromonas_media	0.15	0.11	0.97	0.27	0.91	0.14	0.15	0.54	0.27	0.5	0.34	0.55	0	0	0	0	12
k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Aeromonadales|f__Aeromonadaceae|g__Aeromonas|s__Aeromonas_caviae	k__B	p__P	c__Gammaproteobacteria	o__Aeromonadales	f__Aeromonadaceae	g__Aeromonas	s__Aeromonas_caviae	0.36	0.13	0	0.27	2.44	0.16	0.26	1.44	0.56	0.82	0.86	0.68	0	0	0	0	11
k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Pseudomonadales|f__Moraxellaceae|g__Acinetobacter|s__Acinetobacter_johnsonii	k__B	p__P	c__Gammaproteobacteria	o__Pseudomonadales	f__Moraxellaceae	g__Acinetobacter	s__Acinetobacter_johnsonii	0.42	1.22	0.33	0.03	0.08	0	0.05	0.09	0.05	0.11	0.06	0.1	0	0	0	0	11
k__Bacteria|p__Firmicutes|c__Bacilli|o__Bacillales|f__Staphylococcaceae|g__Macrococcus|s__Macrococcus_caseolyticus	k__B	p__F	c__Bacilli	o__Bacillales	f__Staphylococcaceae	g__Macrococcus	s__Macrococcus_caseolyticus	0.02	0.27	2.25	0	0.01	0	0.2	0.08	0.22	0.13	0.09	0.12	0	0	0	0	10
k__Bacteria|p__Firmicutes|c__Bacilli|o__Lactobacillales|f__Enterococcaceae|g__Enterococcus|s__Enterococcus_faecium	k__B	p__F	c__Bacilli	o__Lactobacillales	f__Enterococcaceae	g__Enterococcus	s__Enterococcus_faecium	0	0	0.39	0.01	0.03	0	0.81	0.79	1.23	1.11	0.77	1.77	0	0	0	0.01	10
k__Bacteria|p__Firmicutes|c__Bacilli|o__Lactobacillales|f__Leuconostocaceae|g__Leuconostoc|s__Leuconostoc_mesenteroides	k__B	p__F	c__Bacilli	o__Lactobacillales	f__Leuconostocaceae	g__Leuconostoc	s__Leuconostoc_mesenteroides	0.06	0.1	0	0	0.46	0	0.09	0.3	0.18	0.11	0.19	0.23	0	0	0	0	9
k__Bacteria|p__Firmicutes|c__Bacilli|o__Lactobacillales|f__Streptococcaceae|g__Lactococcus|s__Lactococcus_raffinolactis	k__B	p__F	c__Bacilli	o__Lactobacillales	f__Streptococcaceae	g__Lactococcus	s__Lactococcus_raffinolactis	0.01	0.03	0	0	0.04	0	0.7	0.88	0.4	0.83	1.16	0.87	0	0	0	0	9
k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Enterobacterales|f__Enterobacteriaceae|g__Citrobacter|s__Citrobacter_freundii	k__B	p__P	c__Gammaproteobacteria	o__Enterobacterales	f__Enterobacteriaceae	g__Citrobacter	s__Citrobacter_freundii	0.03	0.03	0	0	0.04	0	0.13	0.35	0.13	0.09	0.14	0.04	0	0	0	0	9
k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Enterobacterales|f__Enterobacteriaceae|g__Enterobacter|s__Enterobacter_cloacae_complex	k__B	p__P	c__Gammaproteobacteria	o__Enterobacterales	f__Enterobacteriaceae	g__Enterobacter	s__Enterobacter_cloacae_complex	0.65	0.93	0	0.16	0	0	0.06	0.3	0.16	0.1	0.13	0.11	0	0	0	0	9
k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Enterobacterales|f__Enterobacteriaceae|g__Escherichia|s__Escherichia_coli	k__B	p__P	c__Gammaproteobacteria	o__Enterobacterales	f__Enterobacteriaceae	g__Escherichia	s__Escherichia_coli	0.35	0.52	0	7.12	0	0	0.48	0.66	1.24	0.55	0.74	0.52	0	0	0	0	9
k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Pseudomonadales|f__Moraxellaceae|g__Moraxella|s__Moraxella_osloensis	k__B	p__P	c__Gammaproteobacteria	o__Pseudomonadales	f__Moraxellaceae	g__Moraxella	s__Moraxella_osloensis	0	0.01	1.67	0	0.01	0	0.06	0.06	0.11	0.03	0.07	0.1	0	0	0	0	9
k__Bacteria|p__Firmicutes|c__Bacilli|o__Lactobacillales|f__Enterococcaceae|g__Enterococcus|s__Enterococcus_faecalis	k__B	p__F	c__Bacilli	o__Lactobacillales	f__Enterococcaceae	g__Enterococcus	s__Enterococcus_faecalis	0	0	0	0.2	0	0.13	0.45	0.77	0.9	0.49	1.36	0.87	0	0	0	0	8
k__Bacteria|p__Firmicutes|c__Bacilli|o__Lactobacillales|f__Lactobacillaceae|g__Pediococcus|s__Pediococcus_parvulus	k__B	p__F	c__Bacilli	o__Lactobacillales	f__Lactobacillaceae	g__Pediococcus	s__Pediococcus_parvulus	0	0	0	0	0	0	0.18	0.19	0.13	0.22	0.1	0.18	0.12	0	0.12	0	8
k__Bacteria|p__Firmicutes|c__Bacilli|o__Lactobacillales|f__Lactobacillaceae|g__Pediococcus|s__Pediococcus_sp	k__B	p__F	c__Bacilli	o__Lactobacillales	f__Lactobacillaceae	g__Pediococcus	s__Pediococcus_sp	0	0	0	0	0	0	0.4	0.36	0.21	0.44	0.24	0.31	0.18	0	0.26	0	8
k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhodospirillales|f__Rhodospirillales_unclassified|g__Enhydrobacter|s__Enhydrobacter_aerosaccus	k__B	p__P	c__Alphaproteobacteria	o__Rhodospirillales	f__Rhodospirillales_unclassified	g__Enhydrobacter	s__Enhydrobacter_aerosaccus	0	0.01	1.06	0	0	0	0.04	0.05	0.05	0.06	0.02	0.09	0	0	0	0	8
k__Bacteria|p__Firmicutes|c__Bacilli|o__Lactobacillales|f__Lactobacillaceae|g__Lactobacillus|s__Lactobacillus_brevis	k__B	p__F	c__Bacilli	o__Lactobacillales	f__Lactobacillaceae	g__Lactobacillus	s__Lactobacillus_brevis	0.01	0.01	0	1.08	0.04	0	0	0	0	0.07	0	0	0.11	0	0.07	0	7
k__Bacteria|p__Firmicutes|c__Bacilli|o__Lactobacillales|f__Lactobacillaceae|g__Lactobacillus|s__Lactobacillus_delbrueckii	k__B	p__F	c__Bacilli	o__Lactobacillales	f__Lactobacillaceae	g__Lactobacillus	s__Lactobacillus_delbrueckii	1.3	1.22	0	0	3.81	0	0	0	0	0	0	0	86.1	77.2	86.7	49.2	7
k__Bacteria|p__Firmicutes|c__Bacilli|o__Lactobacillales|f__Lactobacillaceae|g__Lactobacillus|s__Lactobacillus_paraplantarum	k__B	p__F	c__Bacilli	o__Lactobacillales	f__Lactobacillaceae	g__Lactobacillus	s__Lactobacillus_paraplantarum	0	0	0	0	0.01	0	0.09	0.31	0.1	0.28	0.1	0.22	0	0	0	0	7
k__Bacteria|p__Firmicutes|c__Bacilli|o__Lactobacillales|f__Streptococcaceae|g__Lactococcus|s__Lactococcus_garvieae	k__B	p__F	c__Bacilli	o__Lactobacillales	f__Streptococcaceae	g__Lactococcus	s__Lactococcus_garvieae	0	0	0	0	0	0.13	0.03	0.02	0.02	0.02	0.02	0	0	0	0	0	7
k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Enterobacterales|f__Enterobacteriaceae|g__Citrobacter|s__Citrobacter_youngae	k__B	p__P	c__Gammaproteobacteria	o__Enterobacterales	f__Enterobacteriaceae	g__Citrobacter	s__Citrobacter_youngae	0.02	0.02	0	0	0.02	0	0	0.09	0	0.02	0.03	0.05	0	0	0	0	7
k__Bacteria|p__Tenericutes|c__Mollicutes|o__Mycoplasmatales|f__Mycoplasmataceae|g__Mycoplasma|s__Mycoplasma_agalactiae	k__B	p__T	c__Mollicutes	o__Mycoplasmatales	f__Mycoplasmataceae	g__Mycoplasma	s__Mycoplasma_agalactiae	0	0	0	0	0.06	0	4.02	3.16	3.54	3.77	2.63	3.58	0	0	0	0	7
k__Bacteria|p__Firmicutes|c__Bacilli|o__Bacillales|f__Staphylococcaceae|g__Staphylococcus|s__Staphylococcus_aureus	k__B	p__F	c__Bacilli	o__Bacillales	f__Staphylococcaceae	g__Staphylococcus	s__Staphylococcus_aureus	0	0	0	0	0	0	0.02	0.01	0.01	0.02	0.01	0	0	0	0	0	6
k__Bacteria|p__Firmicutes|c__Bacilli|o__Bacillales|f__Staphylococcaceae|g__Staphylococcus|s__Staphylococcus_simulans	k__B	p__F	c__Bacilli	o__Bacillales	f__Staphylococcaceae	g__Staphylococcus	s__Staphylococcus_simulans	0	0	0	0	0	0	0.03	0.01	0	0.05	0.02	0.05	0	0	0	0	6
k__Bacteria|p__Firmicutes|c__Bacilli|o__Lactobacillales|f__Lactobacillaceae|g__Lactobacillus|s__Lactobacillus_helveticus	k__B	p__F	c__Bacilli	o__Lactobacillales	f__Lactobacillaceae	g__Lactobacillus	s__Lactobacillus_helveticus	0	0.01	26.6	0.07	0.12	0.02	0	0	0	0	0	0	0	0	0	6.19	6
k__Bacteria|p__Firmicutes|c__Bacilli|o__Lactobacillales|f__Lactobacillaceae|g__Lactobacillus|s__Lactobacillus_sakei	k__B	p__F	c__Bacilli	o__Lactobacillales	f__Lactobacillaceae	g__Lactobacillus	s__Lactobacillus_sakei	0	0	0	0	0	0	0.12	0.17	0.08	0.18	0.11	0.32	0	0	0	0	6
k__Bacteria|p__Firmicutes|c__Bacilli|o__Lactobacillales|f__Streptococcaceae|g__Lactococcus|s__Lactococcus_petauri	k__B	p__F	c__Bacilli	o__Lactobacillales	f__Streptococcaceae	g__Lactococcus	s__Lactococcus_petauri	0	0	0	0	0.01	1.47	0.1	0	0	0.01	0	0	0	0	0	0	6
k__Bacteria|p__Firmicutes|c__Bacilli|o__Lactobacillales|f__Streptococcaceae|g__Streptococcus|s__Streptococcus_thermophilus	k__B	p__F	c__Bacilli	o__Lactobacillales	f__Streptococcaceae	g__Streptococcus	s__Streptococcus_thermophilus	0	0	0	0.08	0.76	1.47	0	0	0	0	0	0	0	0.79	0.29	33.9	6
k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Enterobacterales|f__Enterobacteriaceae|g__Raoultella|s__Raoultella_terrigena	k__B	p__P	c__Gammaproteobacteria	o__Enterobacterales	f__Enterobacteriaceae	g__Raoultella	s__Raoultella_terrigena	0.1	0.06	0	0.02	0	0	0	0.02	0	0	0.06	0.01	0	0	0	0	6
k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Pasteurellales|f__Pasteurellaceae|g__Pasteurella|s__Pasteurella_multocida	k__B	p__P	c__Gammaproteobacteria	o__Pasteurellales	f__Pasteurellaceae	g__Pasteurella	s__Pasteurella_multocida	0	0	0	0	0	0	0.1	0.06	0.02	0.16	0.12	0.13	0	0	0	0	6
k__Bacteria|p__Firmicutes|c__Bacilli|o__Lactobacillales|f__Leuconostocaceae|g__Leuconostoc|s__Leuconostoc_pseudomesenteroides	k__B	p__F	c__Bacilli	o__Lactobacillales	f__Leuconostocaceae	g__Leuconostoc	s__Leuconostoc_pseudomesenteroides	0	0	0	0.13	0.01	0	0	0	0	0	0	0	0	0	0	0	5
k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Enterobacterales|f__Enterobacteriaceae|g__Citrobacter|s__Citrobacter_braakii	k__B	p__P	c__Gammaproteobacteria	o__Enterobacterales	f__Enterobacteriaceae	g__Citrobacter	s__Citrobacter_braakii	0	0	0	0	0	0	0.03	0.05	0.03	0	0.06	0.03	0	0	0	0	5
k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Enterobacterales|f__Yersiniaceae|g__Serratia|s__Serratia_liquefaciens	k__B	p__P	c__Gammaproteobacteria	o__Enterobacterales	f__Yersiniaceae	g__Serratia	s__Serratia_liquefaciens	0.01	0	0	0.36	0	0	0	0	0	0	0.01	0	0	0	0	0	5
k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Pseudomonadales|f__Pseudomonadaceae|g__Pseudomonas|s__Pseudomonas_putida_group	k__B	p__P	c__Gammaproteobacteria	o__Pseudomonadales	f__Pseudomonadaceae	g__Pseudomonas	s__Pseudomonas_putida_group	0	0	0	0	0	0	0	0.13	0.04	0.02	0.02	0.06	0	0	0	0	5
k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Micrococcales|f__Micrococcaceae|g__Kocuria|s__Kocuria_rhizophila	k__B	p__A	c__Actinobacteria	o__Micrococcales	f__Micrococcaceae	g__Kocuria	s__Kocuria_rhizophila	0	0	0	0	0	0	0	0	0	0.01	0	0.01	0	0	0	0	4
k__Bacteria|p__Firmicutes|c__Bacilli|o__Lactobacillales|f__Lactobacillaceae|g__Lactobacillus|s__Lactobacillus_buchneri	k__B	p__F	c__Bacilli	o__Lactobacillales	f__Lactobacillaceae	g__Lactobacillus	s__Lactobacillus_buchneri	0	0.01	0	2.23	0	0	0	0	0	0	0	0	3.81	0	2	0	4
k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Enterobacterales|f__Enterobacteriaceae|g__Klebsiella|s__Klebsiella_pneumoniae	k__B	p__P	c__Gammaproteobacteria	o__Enterobacterales	f__Enterobacteriaceae	g__Klebsiella	s__Klebsiella_pneumoniae	6	3.88	0	0.03	0	0	0	0	0	0	0	0.11	0	0	0	0	4
k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Enterobacterales|f__Enterobacteriaceae|g__Klebsiella|s__Klebsiella_variicola	k__B	p__P	c__Gammaproteobacteria	o__Enterobacterales	f__Enterobacteriaceae	g__Klebsiella	s__Klebsiella_variicola	1.56	1.13	0	0.01	0	0	0	0	0	0	0	0.03	0	0	0	0	4
k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Enterobacterales|f__Enterobacteriaceae|g__Kluyvera|s__Kluyvera_cryocrescens	k__B	p__P	c__Gammaproteobacteria	o__Enterobacterales	f__Enterobacteriaceae	g__Kluyvera	s__Kluyvera_cryocrescens	0	0	0	0	0	0	0	0.01	0	0.01	0	0	0	0	0	0	4
k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Pseudomonadales|f__Moraxellaceae|g__Acinetobacter|s__Acinetobacter_ursingii	k__B	p__P	c__Gammaproteobacteria	o__Pseudomonadales	f__Moraxellaceae	g__Acinetobacter	s__Acinetobacter_ursingii	0	0	0	0	0	0	0.02	0	0	0.01	0	0	0	0	0	0	4
k__Bacteria|p__Firmicutes|c__Bacilli|o__Bacillales|f__Staphylococcaceae|g__Staphylococcus|s__Staphylococcus_petrasii	k__B	p__F	c__Bacilli	o__Bacillales	f__Staphylococcaceae	g__Staphylococcus	s__Staphylococcus_petrasii	0	0	0	0	0	0	0	0.01	0	0	0.01	0	0	0	0	0	3
k__Bacteria|p__Firmicutes|c__Bacilli|o__Lactobacillales|f__Enterococcaceae|g__Enterococcus|s__Enterococcus_durans	k__B	p__F	c__Bacilli	o__Lactobacillales	f__Enterococcaceae	g__Enterococcus	s__Enterococcus_durans	0.03	0.46	0	0	0.03	0	0	0	0	0	0	0	0	0	0	0	3
k__Bacteria|p__Firmicutes|c__Bacilli|o__Lactobacillales|f__Lactobacillaceae|g__Lactobacillus|s__Lactobacillus_acetotolerans	k__B	p__F	c__Bacilli	o__Lactobacillales	f__Lactobacillaceae	g__Lactobacillus	s__Lactobacillus_acetotolerans	0.05	0.41	0	0	6.09	0	0	0	0	0	0	0	0	0	0	0	3
k__Bacteria|p__Firmicutes|c__Bacilli|o__Lactobacillales|f__Lactobacillaceae|g__Lactobacillus|s__Lactobacillus_coryniformis	k__B	p__F	c__Bacilli	o__Lactobacillales	f__Lactobacillaceae	g__Lactobacillus	s__Lactobacillus_coryniformis	0	0	0	0	0	0	0	0.03	0	0.02	0.02	0	0	0	0	0	3
k__Bacteria|p__Firmicutes|c__Bacilli|o__Lactobacillales|f__Lactobacillaceae|g__Pediococcus|s__Pediococcus_pentosaceus	k__B	p__F	c__Bacilli	o__Lactobacillales	f__Lactobacillaceae	g__Pediococcus	s__Pediococcus_pentosaceus	0	0.01	9.33	0	0	0	0	0	0	0	0	0	0	0	0	0.04	3
k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhodospirillales|f__Acetobacteraceae|g__Acetobacter|s__Acetobacter_fabarum	k__B	p__P	c__Alphaproteobacteria	o__Rhodospirillales	f__Acetobacteraceae	g__Acetobacter	s__Acetobacter_fabarum	0.11	0.08	0	0	2.08	0	0	0	0	0	0	0	0	0	0	0	3
k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhodospirillales|f__Acetobacteraceae|g__Acetobacter|s__Acetobacter_orientalis	k__B	p__P	c__Alphaproteobacteria	o__Rhodospirillales	f__Acetobacteraceae	g__Acetobacter	s__Acetobacter_orientalis	1.18	0.87	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Aeromonadales|f__Aeromonadaceae|g__Aeromonas|s__Aeromonas_veronii	k__B	p__P	c__Gammaproteobacteria	o__Aeromonadales	f__Aeromonadaceae	g__Aeromonas	s__Aeromonas_veronii	0	0	0	0	0.38	0.13	0	0	0	0	0	0	0	0	0	0	3
k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Enterobacterales|f__Enterobacteriaceae|g__Klebsiella|s__Klebsiella_michiganensis	k__B	p__P	c__Gammaproteobacteria	o__Enterobacterales	f__Enterobacteriaceae	g__Klebsiella	s__Klebsiella_michiganensis	0.77	0.64	0	0	0	0.01	0	0	0	0	0	0	0	0	0	0	3
k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Enterobacterales|f__Enterobacteriaceae|g__Klebsiella|s__Klebsiella_quasipneumoniae	k__B	p__P	c__Gammaproteobacteria	o__Enterobacterales	f__Enterobacteriaceae	g__Klebsiella	s__Klebsiella_quasipneumoniae	0.36	0.22	0	0	0	0	0	0	0	0	0	0.03	0	0	0	0	3
k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Enterobacterales|f__Hafniaceae|g__Hafnia|s__Hafnia_alvei	k__B	p__P	c__Gammaproteobacteria	o__Enterobacterales	f__Hafniaceae	g__Hafnia	s__Hafnia_alvei	0	0	0	0	0.01	0	0.02	0	0	0	0	0	0	0	0	0	3
k__Eukaryota|p__Ascomycota|c__Saccharomycetes|o__Saccharomycetales|f__Saccharomycetaceae|g__Saccharomyces|s__Saccharomyces_cerevisiae	k__E	p__A	c__Saccharomycetes	o__Saccharomycetales	f__Saccharomycetaceae	g__Saccharomyces	s__Saccharomyces_cerevisiae	0	0	0	0	0	0	0	0	0	0	0	0	0.13	0	0	0.03	3
k__Bacteria|p__Firmicutes|c__Bacilli|o__Bacillales|f__Staphylococcaceae|g__Staphylococcus|s__Staphylococcus_haemolyticus	k__B	p__F	c__Bacilli	o__Bacillales	f__Staphylococcaceae	g__Staphylococcus	s__Staphylococcus_haemolyticus	0	0	0	0	0	0	0.01	0	0	0	0	0	0	0	0	0	2
k__Bacteria|p__Firmicutes|c__Bacilli|o__Lactobacillales|f__Aerococcaceae|g__Aerococcus|s__Aerococcus_urinaeequi	k__B	p__F	c__Bacilli	o__Lactobacillales	f__Aerococcaceae	g__Aerococcus	s__Aerococcus_urinaeequi	0	0	0	0	0	0	0	0	0	0	0	0.02	0	0	0	0	2
k__Bacteria|p__Firmicutes|c__Bacilli|o__Lactobacillales|f__Enterococcaceae|g__Enterococcus|s__Enterococcus_casseliflavus	k__B	p__F	c__Bacilli	o__Lactobacillales	f__Enterococcaceae	g__Enterococcus	s__Enterococcus_casseliflavus	0	0	3.84	0	0	0	0	0	0	0	0	0	0	0	0	0	2
k__Bacteria|p__Firmicutes|c__Bacilli|o__Lactobacillales|f__Enterococcaceae|g__Enterococcus|s__Enterococcus_italicus	k__B	p__F	c__Bacilli	o__Lactobacillales	f__Enterococcaceae	g__Enterococcus	s__Enterococcus_italicus	0	0.07	0	0	0.02	0	0	0	0	0	0	0	0	0	0	0	2
k__Bacteria|p__Firmicutes|c__Bacilli|o__Lactobacillales|f__Lactobacillaceae|g__Lactobacillus|s__Lactobacillus_gasseri	k__B	p__F	c__Bacilli	o__Lactobacillales	f__Lactobacillaceae	g__Lactobacillus	s__Lactobacillus_gasseri	0	0.01	0	11.8	0	0	0	0	0	0	0	0	0	0	0	0	2
k__Bacteria|p__Firmicutes|c__Bacilli|o__Lactobacillales|f__Lactobacillaceae|g__Lactobacillus|s__Lactobacillus_kisonensis	k__B	p__F	c__Bacilli	o__Lactobacillales	f__Lactobacillaceae	g__Lactobacillus	s__Lactobacillus_kisonensis	0.04	0.07	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
k__Bacteria|p__Firmicutes|c__Bacilli|o__Lactobacillales|f__Lactobacillaceae|g__Lactobacillus|s__Lactobacillus_paragasseri	k__B	p__F	c__Bacilli	o__Lactobacillales	f__Lactobacillaceae	g__Lactobacillus	s__Lactobacillus_paragasseri	0	0.07	0	43.6	0	0	0	0	0	0	0	0	0	0	0	0	2
k__Bacteria|p__Firmicutes|c__Bacilli|o__Lactobacillales|f__Lactobacillaceae|g__Lactobacillus|s__Lactobacillus_rhamnosus	k__B	p__F	c__Bacilli	o__Lactobacillales	f__Lactobacillaceae	g__Lactobacillus	s__Lactobacillus_rhamnosus	0	0.06	0	18.5	0	0	0	0	0	0	0	0	0	0	0	0	2
k__Bacteria|p__Firmicutes|c__Bacilli|o__Lactobacillales|f__Streptococcaceae|g__Lactococcus|s__Lactococcus_chungangensis	k__B	p__F	c__Bacilli	o__Lactobacillales	f__Streptococcaceae	g__Lactococcus	s__Lactococcus_chungangensis	0	0	0	0	0	0	0.01	0	0	0	0.05	0	0	0	0	0	2
k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Clostridiaceae|g__Clostridium|s__Clostridium_tyrobutyricum	k__B	p__F	c__Clostridia	o__Clostridiales	f__Clostridiaceae	g__Clostridium	s__Clostridium_tyrobutyricum	0	0	1.98	0.04	0	0	0	0	0	0	0	0	0	0	0	0	2
k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhodospirillales|f__Acetobacteraceae|g__Acetobacter|s__Acetobacter_syzygii	k__B	p__P	c__Alphaproteobacteria	o__Rhodospirillales	f__Acetobacteraceae	g__Acetobacter	s__Acetobacter_syzygii	0.05	0.05	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Aeromonadales|f__Aeromonadaceae|g__Aeromonas|s__Aeromonas_allosaccharophila	k__B	p__P	c__Gammaproteobacteria	o__Aeromonadales	f__Aeromonadaceae	g__Aeromonas	s__Aeromonas_allosaccharophila	0	0	0	0.02	0.05	0	0	0	0	0	0	0	0	0	0	0	2
k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Aeromonadales|f__Aeromonadaceae|g__Aeromonas|s__Aeromonas_hydrophila	k__B	p__P	c__Gammaproteobacteria	o__Aeromonadales	f__Aeromonadaceae	g__Aeromonas	s__Aeromonas_hydrophila	0	0	0	0.03	0	0	0	0	0	0	0	0	0	0	0	0	2
k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Enterobacterales|f__Enterobacteriaceae|g__Klebsiella|s__Klebsiella_oxytoca	k__B	p__P	c__Gammaproteobacteria	o__Enterobacterales	f__Enterobacteriaceae	g__Klebsiella	s__Klebsiella_oxytoca	0.31	0.36	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Enterobacterales|f__Enterobacteriaceae|g__Raoultella|s__Raoultella_ornithinolytica	k__B	p__P	c__Gammaproteobacteria	o__Enterobacterales	f__Enterobacteriaceae	g__Raoultella	s__Raoultella_ornithinolytica	0	0.01	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Enterobacterales|f__Yersiniaceae|g__Serratia|s__Serratia_marcescens	k__B	p__P	c__Gammaproteobacteria	o__Enterobacterales	f__Yersiniaceae	g__Serratia	s__Serratia_marcescens	0.39	0.28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Pseudomonadales|f__Moraxellaceae|g__Acinetobacter|s__Acinetobacter_junii	k__B	p__P	c__Gammaproteobacteria	o__Pseudomonadales	f__Moraxellaceae	g__Acinetobacter	s__Acinetobacter_junii	0	0	0	0	0	0	0.01	0	0	0	0	0	0	0	0	0	2
k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Micrococcales|f__Dermabacteraceae|g__Brachybacterium|s__Brachybacterium_nesterenkovii	k__B	p__A	c__Actinobacteria	o__Micrococcales	f__Dermabacteraceae	g__Brachybacterium	s__Brachybacterium_nesterenkovii	0	0	0.35	0	0	0	0	0	0	0	0	0	0	0	0	0	1
k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Micrococcales|f__Micrococcaceae|g__Kocuria|s__Kocuria_varians	k__B	p__A	c__Actinobacteria	o__Micrococcales	f__Micrococcaceae	g__Kocuria	s__Kocuria_varians	0	0	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Micrococcales|f__Micrococcaceae|g__Rothia|s__Rothia_sp	k__B	p__A	c__Actinobacteria	o__Micrococcales	f__Micrococcaceae	g__Rothia	s__Rothia_sp	0	0	0.08	0	0	0	0	0	0	0	0	0	0	0	0	0	1
k__Bacteria|p__Bacteroidetes|c__Flavobacteriia|o__Flavobacteriales|f__Flavobacteriaceae|g__Chryseobacterium|s__Chryseobacterium_haifense	k__B	p__B	c__Flavobacteriia	o__Flavobacteriales	f__Flavobacteriaceae	g__Chryseobacterium	s__Chryseobacterium_haifense	0	0	4.24	0	0	0	0	0	0	0	0	0	0	0	0	0	1
k__Bacteria|p__Firmicutes|c__Bacilli|o__Bacillales|f__Bacillaceae|g__Bacillus|s__Bacillus_cereus_group	k__B	p__F	c__Bacilli	o__Bacillales	f__Bacillaceae	g__Bacillus	s__Bacillus_cereus_group	0	0	0	0.01	0	0	0	0	0	0	0	0	0	0	0	0	1
k__Bacteria|p__Firmicutes|c__Bacilli|o__Lactobacillales|f__Aerococcaceae|g__Aerococcus|s__Aerococcus_viridans	k__B	p__F	c__Bacilli	o__Lactobacillales	f__Aerococcaceae	g__Aerococcus	s__Aerococcus_viridans	0	0	0	0	0	0	0	0	0	0	0	0.01	0	0	0	0	1
k__Bacteria|p__Firmicutes|c__Bacilli|o__Lactobacillales|f__Enterococcaceae|g__Melissococcus|s__Melissococcus_sp_OM08_11BH	k__B	p__F	c__Bacilli	o__Lactobacillales	f__Enterococcaceae	g__Melissococcus	s__Melissococcus_sp_OM08_11BH	0	0	3.36	0	0	0	0	0	0	0	0	0	0	0	0	0	1
k__Bacteria|p__Firmicutes|c__Bacilli|o__Lactobacillales|f__Enterococcaceae|g__Vagococcus|s__Vagococcus_teuberi	k__B	p__F	c__Bacilli	o__Lactobacillales	f__Enterococcaceae	g__Vagococcus	s__Vagococcus_teuberi	0	0	0	0.01	0	0	0	0	0	0	0	0	0	0	0	0	1
k__Bacteria|p__Firmicutes|c__Bacilli|o__Lactobacillales|f__Lactobacillaceae|g__Lactobacillus|s__Lactobacillus_parafarraginis	k__B	p__F	c__Bacilli	o__Lactobacillales	f__Lactobacillaceae	g__Lactobacillus	s__Lactobacillus_parafarraginis	0	0	0	0	0.05	0	0	0	0	0	0	0	0	0	0	0	1
k__Bacteria|p__Firmicutes|c__Bacilli|o__Lactobacillales|f__Lactobacillaceae|g__Lactobacillus|s__Lactobacillus_rapi	k__B	p__F	c__Bacilli	o__Lactobacillales	f__Lactobacillaceae	g__Lactobacillus	s__Lactobacillus_rapi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
k__Bacteria|p__Firmicutes|c__Bacilli|o__Lactobacillales|f__Leuconostocaceae|g__Leuconostoc|s__Leuconostoc_citreum	k__B	p__F	c__Bacilli	o__Lactobacillales	f__Leuconostocaceae	g__Leuconostoc	s__Leuconostoc_citreum	0	0	0	0	0.17	0	0	0	0	0	0	0	0	0	0	0	1
k__Bacteria|p__Firmicutes|c__Bacilli|o__Lactobacillales|f__Leuconostocaceae|g__Leuconostoc|s__Leuconostoc_lactis	k__B	p__F	c__Bacilli	o__Lactobacillales	f__Leuconostocaceae	g__Leuconostoc	s__Leuconostoc_lactis	0	0.02	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
k__Bacteria|p__Firmicutes|c__Bacilli|o__Lactobacillales|f__Streptococcaceae|g__Lactococcus|s__Lactococcus_piscium	k__B	p__F	c__Bacilli	o__Lactobacillales	f__Streptococcaceae	g__Lactococcus	s__Lactococcus_piscium	0	0	0	0	0.11	0	0	0	0	0	0	0	0	0	0	0	1
k__Bacteria|p__Firmicutes|c__Bacilli|o__Lactobacillales|f__Streptococcaceae|g__Streptococcus|s__Streptococcus_macedonicus	k__B	p__F	c__Bacilli	o__Lactobacillales	f__Streptococcaceae	g__Streptococcus	s__Streptococcus_macedonicus	0	0	0	0	0	0.13	0	0	0	0	0	0	0	0	0	0	1
k__Bacteria|p__Firmicutes|c__Bacilli|o__Lactobacillales|f__Streptococcaceae|g__Streptococcus|s__Streptococcus_uberis	k__B	p__F	c__Bacilli	o__Lactobacillales	f__Streptococcaceae	g__Streptococcus	s__Streptococcus_uberis	0	0	0	0	0	0.92	0	0	0	0	0	0	0	0	0	0	1
k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Aeromonadales|f__Aeromonadaceae|g__Aeromonas|s__Aeromonas_bestiarum	k__B	p__P	c__Gammaproteobacteria	o__Aeromonadales	f__Aeromonadaceae	g__Aeromonas	s__Aeromonas_bestiarum	0	0	0	0.02	0	0	0	0	0	0	0	0	0	0	0	0	1
k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Enterobacterales|f__Enterobacteriaceae|g__Citrobacter|s__Citrobacter_pasteurii	k__B	p__P	c__Gammaproteobacteria	o__Enterobacterales	f__Enterobacteriaceae	g__Citrobacter	s__Citrobacter_pasteurii	0	0	0	0	0.01	0	0	0	0	0	0	0	0	0	0	0	1
k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Enterobacterales|f__Enterobacteriaceae|g__Citrobacter|s__Citrobacter_portucalensis	k__B	p__P	c__Gammaproteobacteria	o__Enterobacterales	f__Enterobacteriaceae	g__Citrobacter	s__Citrobacter_portucalensis	0	0.02	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Enterobacterales|f__Enterobacteriaceae|g__Raoultella|s__Raoultella_planticola	k__B	p__P	c__Gammaproteobacteria	o__Enterobacterales	f__Enterobacteriaceae	g__Raoultella	s__Raoultella_planticola	0.02	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Pseudomonadales|f__Moraxellaceae|g__Acinetobacter|s__Acinetobacter_bereziniae	k__B	p__P	c__Gammaproteobacteria	o__Pseudomonadales	f__Moraxellaceae	g__Acinetobacter	s__Acinetobacter_bereziniae	0	0	0	0	0	0.01	0	0	0	0	0	0	0	0	0	0	1
k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Pseudomonadales|f__Moraxellaceae|g__Acinetobacter|s__Acinetobacter_guillouiae	k__B	p__P	c__Gammaproteobacteria	o__Pseudomonadales	f__Moraxellaceae	g__Acinetobacter	s__Acinetobacter_guillouiae	0	0	0	0	0.01	0	0	0	0	0	0	0	0	0	0	0	1
k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Pseudomonadales|f__Moraxellaceae|g__Acinetobacter|s__Acinetobacter_idrijaensis	k__B	p__P	c__Gammaproteobacteria	o__Pseudomonadales	f__Moraxellaceae	g__Acinetobacter	s__Acinetobacter_idrijaensis	0	0	0	0	0.02	0	0	0	0	0	0	0	0	0	0	0	1
k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Pseudomonadales|f__Pseudomonadaceae|g__Pseudomonas|s__Pseudomonas_fluorescens_group	k__B	p__P	c__Gammaproteobacteria	o__Pseudomonadales	f__Pseudomonadaceae	g__Pseudomonas	s__Pseudomonas_fluorescens_group	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Pseudomonadales|f__Pseudomonadaceae|g__Pseudomonas|s__Pseudomonas_helleri	k__B	p__P	c__Gammaproteobacteria	o__Pseudomonadales	f__Pseudomonadaceae	g__Pseudomonas	s__Pseudomonas_helleri	0	0	0	0	0.04	0	0	0	0	0	0	0	0	0	0	0	1
k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Pseudomonadales|f__Pseudomonadaceae|g__Pseudomonas|s__Pseudomonas_lurida	k__B	p__P	c__Gammaproteobacteria	o__Pseudomonadales	f__Pseudomonadaceae	g__Pseudomonas	s__Pseudomonas_lurida	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1

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B- By Taxonomy

Kingdom	Phylum	Class	Order	Family	Genus	Species	A1	A2	A4	A6	A1-b	A4-b	S8	S7-b	S8-b	S9-b	S10-b	S11-b	L15	L16	L17	L18
k_Bacteria	p_Actinobacteria	c_Actinobacteria	o_Micrococcales	f_Dermabacteraceae	g_Brachybacterium	s_Brachybacterium_nesterenkovii	0	0	0.35	0	0	0	0	0	0	0	0	0	0	0	0	0
k_Bacteria	p_Actinobacteria	c_Actinobacteria	o_Micrococcales	f_Micrococcaceae	g_Kocuria	s_Kocuria_rhizophila	0	0	0	0	0	0	0	0	0	0.01	0	0.01	0	0	0	0
k_Bacteria	p_Actinobacteria	c_Actinobacteria	o_Micrococcales	f_Micrococcaceae	g_Kocuria	s_Kocuria_varians	0	0	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0
k_Bacteria	p_Actinobacteria	c_Actinobacteria	o_Micrococcales	f_Micrococcaceae	g_Rothia	s_Rothia_sp	0	0	0.08	0	0	0	0	0	0	0	0	0	0	0	0	0
k_Bacteria	p_Bacteroidetes	c_Flavobacteriia	o_Flavobacteriales	f_Flavobacteriaceae	g_Chryseobacterium	s_Chryseobacterium_haifense	0	0	4.24	0	0	0	0	0	0	0	0	0	0	0	0	0
k_Bacteria	p_Firmicutes	c_Bacilli	o_Bacillales	f_Bacillaceae	g_Bacillus	s_Bacillus_cereus_group	0	0	0	0.01	0	0	0	0	0	0	0	0	0	0	0	0
k_Bacteria	p_Firmicutes	c_Bacilli	o_Bacillales	f_Staphylococcaceae	g_Macrococcus	s_Macrococcus_caseolyticus	0.02	0.27	2.25	0	0.01	0	0.2	0.08	0.22	0.13	0.09	0.12	0	0	0	0
k_Bacteria	p_Firmicutes	c_Bacilli	o_Bacillales	f_Staphylococcaceae	g_Staphylococcus	s_Staphylococcus_aureus	0	0	0	0	0	0	0.02	0.01	0.01	0.02	0.01	0	0	0	0	0
k_Bacteria	p_Firmicutes	c_Bacilli	o_Bacillales	f_Staphylococcaceae	g_Staphylococcus	s_Staphylococcus_haemolyticus	0	0	0	0	0	0	0.01	0	0	0	0	0	0	0	0	0
k_Bacteria	p_Firmicutes	c_Bacilli	o_Bacillales	f_Staphylococcaceae	g_Staphylococcus	s_Staphylococcus_petrasii	0	0	0	0	0	0	0	0.01	0	0	0.01	0	0	0	0	0
k_Bacteria	p_Firmicutes	c_Bacilli	o_Bacillales	f_Staphylococcaceae	g_Staphylococcus	s_Staphylococcus_simulans	0	0	0	0	0	0	0.03	0.01	0	0.05	0.02	0.05	0	0	0	0
k_Bacteria	p_Firmicutes	c_Bacilli	o_Lactobacillales	f_Aerococcaceae	g_Aerococcus	s_Aerococcus_urinaeequi	0	0	0	0	0	0	0	0	0	0	0	0.02	0	0	0	0
k_Bacteria	p_Firmicutes	c_Bacilli	o_Lactobacillales	f_Aerococcaceae	g_Aerococcus	s_Aerococcus_viridans	0	0	0	0	0	0	0	0	0	0	0	0.01	0	0	0	0
k_Bacteria	p_Firmicutes	c_Bacilli	o_Lactobacillales	f_Enterococcaceae	g_Enterococcus	s_Enterococcus_casseliflavus	0	0	3.84	0	0	0	0	0	0	0	0	0	0	0	0	0
k_Bacteria	p_Firmicutes	c_Bacilli	o_Lactobacillales	f_Enterococcaceae	g_Enterococcus	s_Enterococcus_durans	0.03	0.46	0	0	0.03	0	0	0	0	0	0	0	0	0	0	0
k_Bacteria	p_Firmicutes	c_Bacilli	o_Lactobacillales	f_Enterococcaceae	g_Enterococcus	s_Enterococcus_faecalis	0	0	0	0.2	0	0.13	0.45	0.77	0.9	0.49	1.36	0.87	0	0	0	0
k_Bacteria	p_Firmicutes	c_Bacilli	o_Lactobacillales	f_Enterococcaceae	g_Enterococcus	s_Enterococcus_faecium	0	0	0.39	0.01	0.03	0	0.81	0.79	1.23	1.11	0.77	1.77	0	0	0	0.01
k_Bacteria	p_Firmicutes	c_Bacilli	o_Lactobacillales	f_Enterococcaceae	g_Enterococcus	s_Enterococcus_italicus	0	0.07	0	0	0.02	0	0	0	0	0	0	0	0	0	0	0
k_Bacteria	p_Firmicutes	c_Bacilli	o_Lactobacillales	f_Enterococcaceae	g_Melissococcus	s_Melissococcus_sp_OMO8_1	0	0	3.36	0	0	0	0	0	0	0	0	0	0	0	0	0
k_Bacteria	p_Firmicutes	c_Bacilli	o_Lactobacillales	f_Enterococcaceae	g_Vagococcus	s_Vagococcus_teuberi	0	0	0	0.01	0	0	0	0	0	0	0	0	0	0	0	0
k_Bacteria	p_Firmicutes	c_Bacilli	o_Lactobacillales	f_Lactobacillaceae	g_Lactobacillus	s_Lactobacillus_acetotolerans	0.05	0.41	0	0	6.09	0	0	0	0	0	0	0	0	0	0	0
k_Bacteria	p_Firmicutes	c_Bacilli	o_Lactobacillales	f_Lactobacillaceae	g_Lactobacillus	s_Lactobacillus_brevis	0.01	0.01	0	1.08	0.04	0	0	0	0	0.07	0	0	0.11	0	0.07	0
k_Bacteria	p_Firmicutes	c_Bacilli	o_Lactobacillales	f_Lactobacillaceae	g_Lactobacillus	s_Lactobacillus_buchneri	0	0.01	0	2.23	0	0	0	0	0	0	0	0	3.81	0	2	0
k_Bacteria	p_Firmicutes	c_Bacilli	o_Lactobacillales	f_Lactobacillaceae	g_Lactobacillus	s_Lactobacillus_coryniformis	0	0	0	0	0	0	0	0.03	0	0.02	0.02	0	0	0	0	0
k_Bacteria	p_Firmicutes	c_Bacilli	o_Lactobacillales	f_Lactobacillaceae	g_Lactobacillus	s_Lactobacillus_delbrueckii	1.3	1.22	0	0	3.81	0	0	0	0	0	0	0	86.1	77.2	86.7	49.2
k_Bacteria	p_Firmicutes	c_Bacilli	o_Lactobacillales	f_Lactobacillaceae	g_Lactobacillus	s_Lactobacillus_diolivorans	1.41	2.46	0	0	2.25	1.45	2.03	4.17	2.82	7.51	3.3	5.76	7.99	14	7.52	4.43
k_Bacteria	p_Firmicutes	c_Bacilli	o_Lactobacillales	f_Lactobacillaceae	g_Lactobacillus	s_Lactobacillus_gasseri	0	0.01	0	11.8	0	0	0	0	0	0	0	0	0	0	0	0
k_Bacteria	p_Firmicutes	c_Bacilli	o_Lactobacillales	f_Lactobacillaceae	g_Lactobacillus	s_Lactobacillus_helveticus	0	0.01	26.6	0.07	0.12	0.02	0	0	0	0	0	0	0	0	0	6.19
k_Bacteria	p_Firmicutes	c_Bacilli	o_Lactobacillales	f_Lactobacillaceae	g_Lactobacillus	s_Lactobacillus_kefiranofaciens	40.6	27.8	2	0.24	37.4	43.8	8.19	3.93	2.2	4.94	2.35	3.87	0	0	0	0
k_Bacteria	p_Firmicutes	c_Bacilli	o_Lactobacillales	f_Lactobacillaceae	g_Lactobacillus	s_Lactobacillus_kefiri	25.8	37.1	1.13	0.22	24.4	49.1	6.88	8.33	7.61	7.59	5.24	8.11	1.52	7.95	3.01	5.88
k_Bacteria	p_Firmicutes	c_Bacilli	o_Lactobacillales	f_Lactobacillaceae	g_Lactobacillus	s_Lactobacillus_kisonensis	0.04	0.07	0	0	0	0	0	0	0	0	0	0	0	0	0	0
k_Bacteria	p_Firmicutes	c_Bacilli	o_Lactobacillales	f_Lactobacillaceae	g_Lactobacillus	s_Lactobacillus_parafarraginis	0	0	0	0	0.05	0	0	0	0	0	0	0	0	0	0	0
k_Bacteria	p_Firmicutes	c_Bacilli	o_Lactobacillales	f_Lactobacillaceae	g_Lactobacillus	s_Lactobacillus_paragasseri	0	0.07	0	43.6	0	0	0	0	0	0	0	0	0	0	0	0
k_Bacteria	p_Firmicutes	c_Bacilli	o_Lactobacillales	f_Lactobacillaceae	g_Lactobacillus	s_Lactobacillus_paraplantarum	0	0	0	0	0.01	0	0.09	0.31	0.1	0.28	0.1	0.22	0	0	0	0
k_Bacteria	p_Firmicutes	c_Bacilli	o_Lactobacillales	f_Lactobacillaceae	g_Lactobacillus	s_Lactobacillus_plantarum	0	0.05	4.37	1.53	1.43	0.03	5.29	9.73	7.88	9.86	6.29	8.9	0	0	0	0.16
k_Bacteria	p_Firmicutes	c_Bacilli	o_Lactobacillales	f_Lactobacillaceae	g_Lactobacillus	s_Lactobacillus_rapi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
k_Bacteria	p_Firmicutes	c_Bacilli	o_Lactobacillales	f_Lactobacillaceae	g_Lactobacillus	s_Lactobacillus_rhamnosus	0	0.06	0	18.5	0	0	0	0	0	0	0	0	0	0	0	0
k_Bacteria	p_Firmicutes	c_Bacilli	o_Lactobacillales	f_Lactobacillaceae	g_Lactobacillus	s_Lactobacillus_sakei	0	0	0	0	0	0	0.12	0.17	0.08	0.18	0.11	0.32	0	0	0	0
k_Bacteria	p_Firmicutes	c_Bacilli	o_Lactobacillales	f_Lactobacillaceae	g_Pediococcus	s_Pediococcus_parvulus	0	0	0	0	0	0	0.18	0.19	0.13	0.22	0.1	0.18	0.12	0	0.12	0
k_Bacteria	p_Firmicutes	c_Bacilli	o_Lactobacillales	f_Lactobacillaceae	g_Pediococcus	s_Pediococcus_pentosaceus	0	0.01	9.33	0	0	0	0	0	0	0	0	0	0	0	0	0.04
k_Bacteria	p_Firmicutes	c_Bacilli	o_Lactobacillales	f_Lactobacillaceae	g_Pediococcus	s_Pediococcus_sp	0	0	0	0	0	0	0.4	0.36	0.21	0.44	0.24	0.31	0.18	0	0.26	0
k_Bacteria	p_Firmicutes	c_Bacilli	o_Lactobacillales	f_Leuconostocaceae	g_Leuconostoc	s_Leuconostoc_citreum	0	0	0	0	0.17	0	0	0	0	0	0	0	0	0	0	0
k_Bacteria	p_Firmicutes	c_Bacilli	o_Lactobacillales	f_Leuconostocaceae	g_Leuconostoc	s_Leuconostoc_lactis	0	0.02	0	0	0	0	0	0	0	0	0	0	0	0	0	0
k_Bacteria	p_Firmicutes	c_Bacilli	o_Lactobacillales	f_Leuconostocaceae	g_Leuconostoc	s_Leuconostoc_mesenteroides	0.06	0.1	0	0	0.46	0	0.09	0.3	0.18	0.11	0.19	0.23	0	0	0	0
k_Bacteria	p_Firmicutes	c_Bacilli	o_Lactobacillales	f_Leuconostocaceae	g_Leuconostoc	s_Leuconostoc_pseudomesenteroides	0	0	0	0.13	0.01	0	0	0	0	0	0	0	0	0	0	0
k_Bacteria	p_Firmicutes	c_Bacilli	o_Lactobacillales	f_Streptococcaceae	g_Lactococcus	s_Lactococcus_chungangensis	0	0	0	0	0	0	0.01	0	0	0	0.05	0	0	0	0	0
k_Bacteria	p_Firmicutes	c_Bacilli	o_Lactobacillales	f_Streptococcaceae	g_Lactococcus	s_Lactococcus_garvieae	0	0	0	0	0	0.13	0.03	0.02	0.02	0.02	0.02	0	0	0	0	0
k_Bacteria	p_Firmicutes	c_Bacilli	o_Lactobacillales	f_Streptococcaceae	g_Lactococcus	s_Lactococcus_lactis	17.7	18.9	0.34	12	15.1	0.82	67.2	62.5	68.1	59.3	72.7	61.7	0	0	0	0.01
k_Bacteria	p_Firmicutes	c_Bacilli	o_Lactobacillales	f_Streptococcaceae	g_Lactococcus	s_Lactococcus_petauri	0	0	0	0	0.01	1.47	0.1	0	0	0.01	0	0	0	0	0	0
k_Bacteria	p_Firmicutes	c_Bacilli	o_Lactobacillales	f_Streptococcaceae	g_Lactococcus	s_Lactococcus_piscium	0	0	0	0	0.11	0	0	0	0	0	0	0	0	0	0	0
k_Bacteria	p_Firmicutes	c_Bacilli	o_Lactobacillales	f_Streptococcaceae	g_Lactococcus	s_Lactococcus_raffinolactis	0.01	0.03	0	0	0.04	0	0.7	0.88	0.4	0.83	1.16	0.87	0	0	0	0
k_Bacteria	p_Firmicutes	c_Bacilli	o_Lactobacillales	f_Streptococcaceae	g_Streptococcus	s_Streptococcus_macedonicus	0	0	0	0	0	0.13	0	0	0	0	0	0	0	0	0	0
k_Bacteria	p_Firmicutes	c_Bacilli	o_Lactobacillales	f_Streptococcaceae	g_Streptococcus	s_Streptococcus_parauberis	0.07	0.36	35.6	0	1.43	0	1.71	0.37	1.7	0.52	0.54	0.49	0	0	0	0.13
k_Bacteria	p_Firmicutes	c_Bacilli	o_Lactobacillales	f_Streptococcaceae	g_Streptococcus	s_Streptococcus_thermophilus	0	0	0	0.08	0.76	1.47	0	0	0	0	0	0	0	0.79	0.29	33.9
k_Bacteria	p_Firmicutes	c_Bacilli	o_Lactobacillales	f_Streptococcaceae	g_Streptococcus	s_Streptococcus_uberis	0	0	0	0	0	0.92	0	0	0	0	0	0	0	0	0	0
k_Bacteria	p_Firmicutes	c_Clostridia	o_Clostridiales	f_Clostridiaceae	g_Clostridium	s_Clostridium_tyrobutyricum	0	0	1.98	0.04	0	0	0	0	0	0	0	0	0	0	0	0
k_Bacteria	p_Proteobacteria	c_Alphaproteobacteria	o_Rhodospirillales	f_Acetobacteraceae	g_Acetobacter	s_Acetobacter_fabarum	0.11	0.08	0	0	2.08	0	0	0	0	0	0	0	0	0	0	0
k_Bacteria	p_Proteobacteria	c_Alphaproteobacteria	o_Rhodospirillales	f_Acetobacteraceae	g_Acetobacter	s_Acetobacter_orientalis	1.18	0.87	0	0	0	0	0	0	0	0	0	0	0	0	0	0
k_Bacteria	p_Proteobacteria	c_Alphaproteobacteria	o_Rhodospirillales	f_Acetobacteraceae	g_Acetobacter	s_Acetobacter_syzygii	0.05	0.05	0	0	0	0	0	0	0	0	0	0	0	0	0	0
k_Bacteria	p_Proteobacteria	c_Alphaproteobacteria	o_Rhodospirillales	f_Rhodospirillales_unclassified	g_Enhydrobacter	s_Enhydrobacter_aerosaccus	0	0.01	1.06	0	0	0	0.04	0.05	0.05	0.06	0.02	0.09	0	0	0	0
k_Bacteria	p_Proteobacteria	c_Gammaproteobacteria	o_Aeromonadales	f_Aeromonadaceae	g_Aeromonas	s_Aeromonas_allosaccharophila	0	0	0	0.02	0.05	0	0	0	0	0	0	0	0	0	0	0
k_Bacteria	p_Proteobacteria	c_Gammaproteobacteria	o_Aeromonadales	f_Aeromonadaceae	g_Aeromonas	s_Aeromonas_bestiarum	0	0	0	0.02	0	0	0	0	0	0	0	0	0	0	0	0
k_Bacteria	p_Proteobacteria	c_Gammaproteobacteria	o_Aeromonadales	f_Aeromonadaceae	g_Aeromonas	s_Aeromonas_caviae	0.36	0.13	0	0.27	2.44	0.16	0.26	1.44	0.56	0.82	0.86	0.68	0	0	0	0
k_Bacteria	p_Proteobacteria	c_Gammaproteobacteria	o_Aeromonadales	f_Aeromonadaceae	g_Aeromonas	s_Aeromonas_hydrophila	0	0	0	0.03	0	0	0	0	0	0	0	0	0	0	0	0
k_Bacteria	p_Proteobacteria	c_Gammaproteobacteria	o_Aeromonadales	f_Aeromonadaceae	g_Aeromonas	s_Aeromonas_media	0.15	0.11	0.97	0.27	0.91	0.14	0.15	0.54	0.27	0.5	0.34	0.55	0	0	0	0
k_Bacteria	p_Proteobacteria	c_Gammaproteobacteria	o_Aeromonadales	f_Aeromonadaceae	g_Aeromonas	s_Aeromonas_veronii	0	0	0	0	0.38	0.13	0	0	0	0	0	0	0	0	0	0
k_Bacteria	p_Proteobacteria	c_Gammaproteobacteria	o_Enterobacterales	f_Enterobacteriaceae	g_Citrobacter	s_Citrobacter_braakii	0	0	0	0	0	0	0.03	0.05	0.03	0	0.06	0.03	0	0	0	0
k_Bacteria	p_Proteobacteria	c_Gammaproteobacteria	o_Enterobacterales	f_Enterobacteriaceae	g_Citrobacter	s_Citrobacter_freundii	0.03	0.03	0	0	0.04	0	0.13	0.35	0.13	0.09	0.14	0.04	0	0	0	0
k_Bacteria	p_Proteobacteria	c_Gammaproteobacteria	o_Enterobacterales	f_Enterobacteriaceae	g_Citrobacter	s_Citrobacter_pasteurii	0	0	0	0	0.01	0	0	0	0	0	0	0	0	0	0	0
k_Bacteria	p_Proteobacteria	c_Gammaproteobacteria	o_Enterobacterales	f_Enterobacteriaceae	g_Citrobacter	s_Citrobacter_portucalensis	0	0.02	0	0	0	0	0	0	0	0	0	0	0	0	0	0
k_Bacteria	p_Proteobacteria	c_Gammaproteobacteria	o_Enterobacterales	f_Enterobacteriaceae	g_Citrobacter	s_Citrobacter_youngae	0.02	0.02	0	0	0.02	0	0	0.09	0	0.02	0.03	0.05	0	0	0	0
k_Bacteria	p_Proteobacteria	c_Gammaproteobacteria	o_Enterobacterales	f_Enterobacteriaceae	g_Enterobacter	s_Enterobacter_cloacae_complex	0.65	0.93	0	0.16	0	0	0.06	0.3	0.16	0.1	0.13	0.11	0	0	0	0
k_Bacteria	p_Proteobacteria	c_Gammaproteobacteria	o_Enterobacterales	f_Enterobacteriaceae	g_Escherichia	s_Escherichia_coli	0.35	0.52	0	7.12	0	0	0.48	0.66	1.24	0.55	0.74	0.52	0	0	0	0
k_Bacteria	p_Proteobacteria	c_Gammaproteobacteria	o_Enterobacterales	f_Enterobacteriaceae	g_Klebsiella	s_Klebsiella_michiganensis	0.77	0.64	0	0	0	0.01	0	0	0	0	0	0	0	0	0	0
k_Bacteria	p_Proteobacteria	c_Gammaproteobacteria	o_Enterobacterales	f_Enterobacteriaceae	g_Klebsiella	s_Klebsiella_oxytoca	0.31	0.36	0	0	0	0	0	0	0	0	0	0	0	0	0	0
k_Bacteria	p_Proteobacteria	c_Gammaproteobacteria	o_Enterobacterales	f_Enterobacteriaceae	g_Klebsiella	s_Klebsiella_pneumoniae	6	3.88	0	0.03	0	0	0	0	0	0	0	0.11	0	0	0	0
k_Bacteria	p_Proteobacteria	c_Gammaproteobacteria	o_Enterobacterales	f_Enterobacteriaceae	g_Klebsiella	s_Klebsiella_quasipneumoniae	0.36	0.22	0	0	0	0	0	0	0	0	0	0.03	0	0	0	0
k_Bacteria	p_Proteobacteria	c_Gammaproteobacteria	o_Enterobacterales	f_Enterobacteriaceae	g_Klebsiella	s_Klebsiella_variicola	1.56	1.13	0	0.01	0	0	0	0	0	0	0	0.03	0	0	0	0
k_Bacteria	p_Proteobacteria	c_Gammaproteobacteria	o_Enterobacterales	f_Enterobacteriaceae	g_Kluyvera	s_Kluyvera_cryocrescens	0	0	0	0	0	0	0	0.01	0	0.01	0	0	0	0	0	0
k_Bacteria	p_Proteobacteria	c_Gammaproteobacteria	o_Enterobacterales	f_Enterobacteriaceae	g_Raoultella	s_Raoultella_ornithinolytica	0	0.01	0	0	0	0	0	0	0	0	0	0	0	0	0	0
k_Bacteria	p_Proteobacteria	c_Gammaproteobacteria	o_Enterobacterales	f_Enterobacteriaceae	g_Raoultella	s_Raoultella_planticola	0.02	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
k_Bacteria	p_Proteobacteria	c_Gammaproteobacteria	o_Enterobacterales	f_Enterobacteriaceae	g_Raoultella	s_Raoultella_terrigena	0.1	0.06	0	0.02	0	0	0	0.02	0	0	0.06	0.01	0	0	0	0
k_Bacteria	p_Proteobacteria	c_Gammaproteobacteria	o_Enterobacterales	f_Hafniaceae	g_Hafnia	s_Hafnia_alvei	0	0	0	0	0.01	0	0.02	0	0	0	0	0	0	0	0	0
k_Bacteria	p_Proteobacteria	c_Gammaproteobacteria	o_Enterobacterales	f_Yersiniaceae	g_Serratia	s_Serratia_liquefaciens	0.01	0	0	0.36	0	0	0	0	0	0	0.01	0	0	0	0	0
k_Bacteria	p_Proteobacteria	c_Gammaproteobacteria	o_Enterobacterales	f_Yersiniaceae	g_Serratia	s_Serratia_marcescens	0.39	0.28	0	0	0	0	0	0	0	0	0	0	0	0	0	0
k_Bacteria	p_Proteobacteria	c_Gammaproteobacteria	o_Pasteurellales	f_Pasteurellaceae	g_Pasteurella	s_Pasteurella_multocida	0	0	0	0	0	0	0.1	0.06	0.02	0.16	0.12	0.13	0	0	0	0
k_Bacteria	p_Proteobacteria	c_Gammaproteobacteria	o_Pseudomonadales	f_Moraxellaceae	g_Acinetobacter	s_Acinetobacter_bereziniae	0	0	0	0	0	0.01	0	0	0	0	0	0	0	0	0	0
k_Bacteria	p_Proteobacteria	c_Gammaproteobacteria	o_Pseudomonadales	f_Moraxellaceae	g_Acinetobacter	s_Acinetobacter_guillouiae	0	0	0	0	0.01	0	0	0	0	0	0	0	0	0	0	0
k_Bacteria	p_Proteobacteria	c_Gammaproteobacteria	o_Pseudomonadales	f_Moraxellaceae	g_Acinetobacter	s_Acinetobacter_idrijaensis	0	0	0	0	0.02	0	0	0	0	0	0	0	0	0	0	0
k_Bacteria	p_Proteobacteria	c_Gammaproteobacteria	o_Pseudomonadales	f_Moraxellaceae	g_Acinetobacter	s_Acinetobacter_johnsonii	0.42	1.22	0.33	0.03	0.08	0	0.05	0.09	0.05	0.11	0.06	0.1	0	0	0	0
k_Bacteria	p_Proteobacteria	c_Gammaproteobacteria	o_Pseudomonadales	f_Moraxellaceae	g_Acinetobacter	s_Acinetobacter_junii	0	0	0	0	0	0	0.01	0	0	0	0	0	0	0	0	0
k_Bacteria	p_Proteobacteria	c_Gammaproteobacteria	o_Pseudomonadales	f_Moraxellaceae	g_Acinetobacter	s_Acinetobacter_ursingii	0	0	0	0	0	0	0.02	0	0	0.01	0	0	0	0	0	0
k_Bacteria	p_Proteobacteria	c_Gammaproteobacteria	o_Pseudomonadales	f_Moraxellaceae	g_Moraxella	s_Moraxella_osloensis	0	0.01	1.67	0	0.01	0	0.06	0.06	0.11	0.03	0.07	0.1	0	0	0	0
k_Bacteria	p_Proteobacteria	c_Gammaproteobacteria	o_Pseudomonadales	f_Pseudomonadaceae	g_Pseudomonas	s_Pseudomonas_fluorescens	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
k_Bacteria	p_Proteobacteria	c_Gammaproteobacteria	o_Pseudomonadales	f_Pseudomonadaceae	g_Pseudomonas	s_Pseudomonas_helleri	0	0	0	0	0.04	0	0	0	0	0	0	0	0	0	0	0
k_Bacteria	p_Proteobacteria	c_Gammaproteobacteria	o_Pseudomonadales	f_Pseudomonadaceae	g_Pseudomonas	s_Pseudomonas_lutida	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
k_Bacteria	p_Proteobacteria	c_Gammaproteobacteria	o_Pseudomonadales	f_Pseudomonadaceae	g_Pseudomonas	s_Pseudomonas_putida_group	0	0	0	0	0	0	0	0.13	0.04	0.02	0.02	0.06	0	0	0	0
k_Bacteria	p_Tenericutes	c_Mollicutes	o_Mycoplasmatales	f_Mycoplasmataceae	g_Mycoplasma	s_Mycoplasma_agalactiae	0	0	0	0	0.06	0	4.02	3.16	3.54	3.77	2.63	3.58	0	0	0	0
k_Eukaryota	p_Ascomycota	c_Saccharomycetes	o_Saccharomycetales	f_Saccharomycetaceae	g_Saccharomyces	s_Saccharomyces_cerevisiae	0	0	0	0	0	0	0	0	0	0	0	0	0.13	0	0	0.03

APPENDIX 7 RELATIVE ABUNDANCE OF MICROBIAL COMPOSITION OF 16 TRADITIONAL FERMENTED GOAT MILK METAGENOMES FROM LEBANON USING YCHF MARKER GENE ASSIGNATION

Species	A1	A2	A4	A6	A1-b	A4-b	S8	S7-b	S8-b	S9-b	S10-b	S11-b	L15	L16	L17	L18	Freq	Max
Lactobacillus diolivorans	1.49	2.45	2.38	0	2.03	1.35	2.1	4.38	3.11	1.67	3.29	5.96	6.69	6.06	4.59	4.5	15	6.69
Lactiplantibacillus plantaru	0.23	0.24	1.95	2.94	1.34	0	4.29	7.71	5.76	3.33	0.33	0.95	4.37	3.27	2.23	0.3	15	7.71
Lactobacillus kefiri	26.7	26.3	0	0	16.3	33.8	3.76	5.28	4.59	2.92	3.2	4.99	3.62	2.66	2.68	4.04	14	33.80
Lactobacillus kefiranofacie	38.2	34.2	1.23	0.61	36.2	45	9.7	5.31	2.74	8.03	2.57	4.83	0	0	0	0	12	45.01
Lactococcus lactis sp. lactis	20.2	18.9	1.09	14.4	19.4	0	58.9	60.9	64.6	50	61.8	60.7	0	0	0	0	11	64.62
Escherichia coli	0.79	0.54	0	0.27	0	0.93	0.56	0.79	0.33	0.17	0.3	0.5	0	0	0	0	10	0.93
Aeromonas caviae	0.49	0.29	0	0	2.37	0	0.41	1.31	0.85	0.3	0.44	1.14	0	0	0	0	9	2.37
Streptococcus parauberis	0	0.44	6.9	0	1.08	0	1.37	0	1.51	1.08	0.8	0	0	0	0	0.18	8	6.90
Klebsiella pneumoniae	2.76	0.48	0	0	0.24	0.7	0	0.34	0.67	0	0.38	0.72	0	0	0	0	8	2.76
Lactobacillus delbrueckii	1.57	0.57	0	0.2	3	0	0	0	0	0	0	0	70.2	35.5	67.4	45.1	8	70.22
Enterococcus faecalis	0	0	0	0.35	0	0.57	0.66	0.71	0	0.51	0.88	1.28	0	0	0	0	7	1.28
Lactococcus raffinolactis	0.21	0.22	0	0	0	0	0.68	1.23	0	0.53	0.73	0.8	0	0	0	0	7	1.23
Pichia kudriavzevii	0	0	0	0	0.56	1.11	0	0	0.76	0	0.29	0	0	4.18	2.3	0.3	7	4.18
Enterococcus faecium	0	0	0	0	0	0	0.59	1.3	0.72	0.45	0.27	1.85	0	0	0	0	6	1.85
Mycoplasma agalactiae	0	0	0	0	0	0	2.33	1.69	2.31	1.84	1.3	2.2	0	0	0	0	6	2.33
Streptococcus parasuis	0	0.49	55.2	0	0.58	1.55	0	0	0	0	0.23	0	0	0	0	0	5	55.22
Citrobacter freundii	0	0.66	0	0.55	0	0.65	0	0	0.39	0	0	0.72	0	0	0	0	5	0.72
Pediococcus parvulus	0	0	0	0	0	0	0.53	0	0.4	0.4	0.48	0.69	0	0	0	0	5	0.69
Enterococcus sp.	0	0	0	0	0.19	0	0.31	0	1.4	0.4	0	0	0	0	0	0	4	1.40
Macrococcus caseolyticus	0	0.52	0	0	0.24	0	0.27	0	0	0.35	0	0	0	0	0	0	4	0.52
Lactobacillus helveticus	0	0.38	5.86	0	0	0	0	0	0	0	0	0	0	0	0	6.03	3	6.03
Acetobacter fabarum	0.18	0.78	0	0	2.62	0	0	0	0	0	0	0	0	0	0	0	3	2.62
Acetobacter orientalis	1.02	0.62	0	0	0	0.5	0	0	0	0	0	0	0	0	0	0	3	1.02
Acinetobacter johnsonii	0.45	0.73	0	0	0.22	0	0	0	0	0	0	0	0	0	0	0	3	0.73
Klebsiella michiganensis	0	0.41	0	0	0	0	0.19	0	0.39	0	0	0	0	0	0	0	3	0.41
Kluyveromyces marxianus	0	0	0	0.57	0	0	0	0	0	0	0	0	0	1.23	0	0.57	3	1.23
Lactobacillus acetotolerans	0.17	0.82	0	0	6.2	0	0	0	0	0	0	0	0	0	0	0	3	6.20
Lactobacillus brevis	0	0	0	0.95	0.37	0	0	0	0	0	0	0	2.66	0	0	0	3	2.66
Leuconostoc pseudomeser	0	0	0	0.64	0.27	0	0	0	0	0	0	0	0	0	0	0.19	3	0.64
Saccharomyces cerevisiae	0	0	0	0	0.29	0	0	0	0	0	0	0	0	0	1.48	0.71	3	1.48
Shigella sonnei	0	0	0	0	0	0	0	0	0.36	0.19	0.52	0	0	0	0	0	3	0.52
Streptococcus pyogenes	0	0	0	0	0	0	0	0	0	0	0.48	0.7	0	0	0	0.3	3	0.70
Streptococcus salivarius	0	0	0	0.24	0	1.09	0	0	0	0	0	0	0	1.77	0	0	3	1.77
Flavobacteriaceae bacteriu	0	0.22	2.99	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2.99
Aeromonas cavernicola	0	0	2.28	0	0	0	0	0	0	0	0	1.81	0	0	0	0	2	2.28
Pediococcus pentosaceus	0	0	1.51	0	0	0	0	0	0	0	0	0	0	0	0	0.14	2	1.51
Acetobacter lambici	0.59	0.48	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0.59
Aeromonas hydrophila	0	0	0	0.6	0	0	0	1.39	0	0	0	0	0	0	0	0	2	1.39
Debaryomyces hansenii CB	0	0	0	0	0	0	0	0.36	0	0	0.25	0	0	0	0	0	2	0.36
Enterococcus durans	0	0.51	0	0	0	0	0	0	0	0	0.56	0	0	0	0	0	2	0.56
Enterococcus hirae	0	0	0	0	0	0	0	0.72	0.74	0	0	0	0	0	0	0	2	0.74
Kluyveromyces marxianus	0	0	0	0.25	0	0	0	0	0	0	0	0	0	0	0	0.59	2	0.59
Lactobacillus buchneri	0	0	0	1.36	0	0	0	0	0	0	0	0	2.83	0	0	0	2	2.83
Lactobacillus crispatus	0.23	0	0	0.21	0	0	0	0	0	0	0	0	0	0	0	0	2	0.23
Lactobacillus curvatus	0	0	0	0	0	0	0	0	0	0	0.91	0.73	0	0	0	0	2	0.91
Lactobacillus gasseri	0	0.27	0	38.1	0	0	0	0	0	0	0	0	0	0	0	0	2	38.11
Lactobacillus paracasei	0	0.33	0	13	0	0	0	0	0	0	0	0	0	0	0	0	2	12.97
Lactobacillus reuteri	0	0	0	0	0	1.22	0	0	0	0	0	0	0	1.44	0	0	2	1.44
Lactococcus lactis sp. crem	0	0	0	0	0	0	11.6	0	0	0	5.4	0	0	0	0	0	2	11.56
Lentilactobacillus rhamnos	0	0.23	0	16.8	0	0	0	0	0	0	0	0	0	0	0	0	2	16.77
Leuconostoc mesenteroide	0	0.38	0	0	0.42	0	0	0	0	0	0	0	0	0	0	0	2	0.42
Moraxellaceae bacterium	0	0	0	0	0	0	0.24	0	0	0.18	0	0	0	0	0	0	2	0.24
Pasteurella multocida	0	0	0	0	0	0	0	0	0	0	0.24	0.62	0	0	0	0	2	0.62
Pediococcus acidilactici	0	0	0	0	0	1.85	0	0	0.37	0	0	0	0	0	0	0	2	1.85
Pediococcus ethanolidurar	0	0	0	0	0	0	0	0.67	0	0	0	0	1.44	0	0	0	2	1.44
Pseudomonas syringae	0	0	0	0	0.28	0	0	0	0.35	0	0	0	0	0	0	0	2	0.35
Psychrobacter sp.	0	0	0	0	0	0	0	0.75	0	0	0.31	0	0	0	0	0	2	0.75
Salmonella sp.	0	0	0	0.54	0	0	0	0.35	0	0	0	0	0	0	0	0	2	0.54
Streptococcus dysgalactiae	0	0	0	0	0	0	0.41	0	0	0.32	0	0	0	0	0	0	2	0.41
Streptococcus thermophilu	0	0	0	0	0.9	0	0	0	0	0	0	0	0	0	0	35.1	2	35.15
Melissococcus sp. OM08-11	0	0	2.47	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2.47
Moraxella boevrei	0	0	2.13	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2.13
Callithrix jacchus	0	0	1.75	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1.75
Clostridium tyrobutyricum	0	0	1.11	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1.11
Acetobacter lovaniensis	0.55	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.55
Actinobacillus	0	0	0	0	0	0	0	0	0	0	0.33	0	0	0	0	0	1	0.33
Aeromonas piscilcola	0	0	0	0.43	0	0	0	0	0	0	0	0	0	0	0	0	1	0.43
Aeromonas salmonicida	0	0.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.20
Aeromonas veronii	0	0	0	0	0.66	0	0	0	0	0	0	0	0	0	0	0	1	0.66
Bacillus	0	0.24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.24
Bacillus sp. 2A33	0	0	0	0	0	0	0	0	0.4	0	0	0	0	0	0	0	1	0.40
Candidatus Thioglobus	0	0	0	0	0.28	0	0	0	0	0	0	0	0	0	0	0	1	0.28
Deinococcus ficus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.16	1	0.16
Enterobacter asburiae	0	0.81	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.81
Enterobacter ludwigii	1.15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1.15
Enterobacter sp. 198	0	0	0	0	0	0	0	0	0	0.31	0	0	0	0	0	0	1	0.31
Enterobacter sp. 638	0	0	0	0.25	0	0	0	0	0	0	0	0	0	0	0	0	1	0.25
Enterococcus mundtii	0	0	0	0	0	0	0	0	0	0.26	0	0	0	0	0	0	1	0.26
Ewingia sp. OLMTSP16	0	0	0	0	0	0	0	0.57	0	0	0	0	0	0	0	0	1	0.57
Geotrichum candidum	0	0	0	0	0.42	0	0	0	0	0	0	0	0	0	0	0	1	0.42
Haemophilus parainfluenz	0.73	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.73
Kazachstania sp1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2.2	0	1	2.20
Kazachstania sp2	0	0	0	0	0	0	0	0	0	0	0	0	0	1.75	0	0	1	1.75
Klebsiella grimontii	0	0	0	0	0	0.52	0	0	0	0	0	0	0	0	0	0	1	0.52
Kluyveromyces lactis	0	0	0	0	0	0	0	0	0	0	0.5	0	0	0	0	0	1	0.50
Lactobacillus amylolyticus	0	0	0	0	0.6	0	0	0	0	0	0	0	0	0	0	0	1	0.60
Lactobacillus coryniformis	0	0	0	0	0	0	0	0.36	0	0	0	0	0	0	0	0	1	0.36
Lactobacillus otakiensis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.65	0	1	1.65
Lactobacillus yonginensis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.2	0	1	1.20
Lactococcus garvieae	0	0	0	0	0	2.2	0	0	0	0	0	0	0	0	0	0	1	2.20
Lactococcus piscium	0	0	0	0	0.37	0	0	0	0	0	0	0	0	0	0	0	1	0.37
Leminorella	0	0.23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.23
Leuconostoc suionicum	0	0	0	0	0	0	0	0	0.72	0	0	0	0	0	0	0	1	0.72
Leucothrix sp. C3212	0	0	0	0	0	0	0	0.32	0	0	0	0	0	0	0	0	1	0.32
Micavibrio aeruginosavoru	0.23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.23
Naumovozyma sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.26	0	1	1.26
Oenococcus oeni	0	0.26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.26
Pichia membranifaciens	0	0	0	0	0	0	0	0	0.61	0	0	0	0	0	0	0	1	0.61
Pichia membranifaciens	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.12	0	1	1.12
Pseudomonadales bacteriu	0	0	0	0	0.25	0	0	0	0	0	0	0	0	0	0	0	1	0.25
Pseudomonas	0	0	0	0	0	0	0	0	0	0	0	0	1.31	0	0	0	1	1.31
Pseudomonas juntendi	0	0	0	0	0	0	0	0	0.89	0	0	0	0	0	0	0	1	0.89
Pseudomonas putida	0	0	0	0	0.27	0	0	0	0	0	0	0	0	0	0	0	1	0.27
Pseudomonas savastanoi	0	0	0	0	0	0	0	0.71	0	0	0	0	0	0	0	0	1	0.71
Raoultella terrigena	0	0	0	0	0	0	0	0	0	0	0.35	0	0	0	0	0	1	0.35
Rhodobacteraceae bacteriu	0	0	0	0.21	0	0	0	0	0	0	0	0	0	0	0	0	1	0.21
Saccharomyces paradoxus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.99	1	0.99
Serratia marcescens	0.69	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.69
Shigella flexneri	0.58	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.58
Sphingobium yanoikuyae	0	0	0	0	0	0	0	0	0	0	0	0	1.61	0	0	0	1	1.61
Staphylococcus aureus	0	0	0	0	0	0	0	0	0	0	0.24	0	0	0	0	0	1	0.24
Staphylococcus sp.	0	0	0	0	0	0	0	0.3	0	0	0	0	0	0	0	0	1	0.30
Streptococcus salivarius	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.9	0	1	0.90
Streptococcus sp.	0	0	0	0	2.52	0.29	0	0	0.23	0	0	0	0	0	0	0	1	2.52
Streptococcus sp.	0	0	0	0	0	0.58	0	0	0	0	0	0	0	0	0	0	1	0.58
Streptococcus uberis	0	0	0	0	0	0.68	0	0	0	0	0	0	0	0	0	0	1	0.68
Tetrapisispora phaffii CBS	0	0	0	0	0	0	0	0	0	0	0	0	0	2.8	0	0	1	2.80
Torulaspora delbrueckii	0	0	0	0	0	0	0	0	0	0	0	0	1.57	0	0	0	1	1.57
Zygosaccharomyces sp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2.43	0	1	2.43

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APPENDIX 8 RELATIVE ABUNDANCE OF MICROBIAL COMPOSITION OF 16 TRADITIONAL FERMENTED GOAT MILK METAGENOMES FROM LEBANON USING THE FOOD MICROBIOME TOOL.

Species	A1	A2	A4	A6	A1-b	A4-b	S8	S7-b	S8-b	S9-b	S10-b	S11-b	L15	L16	L17	L18	freq	max
Pichia kudriavzevii	0.01	0.01	0.04	0.04	0.45	0.15	0.24	0.13	0.24	0.17	0.14	0.08	1.10	8.16	2.28	0.13	12	0.45
Lactococcus lactis IL1403	17.84	18.36	1.89	18.00	15.22	1.11	57.53	65.31	53.85	68.35	56.38	58.03	0.00	0.00	0.00	0.00	12	68.35
Lactobacillus kefiranofaciens ZW3	45.17	39.72	3.87	0.57	42.73	51.84	4.75	2.64	5.94	2.74	4.54	9.51	0.00	0.00	0.00	0.00	12	51.84
Lactobacillus kefiri DSM 20587	27.88	33.17	1.81	0.34	20.68	43.37	6.71	6.18	6.11	4.33	6.46	5.31	1.79	7.26	3.27	5.98	12	43.37
Aeromonas caviae FDAARGOS_72	0.39	0.24	0.00	0.51	2.41	0.26	1.61	0.78	1.18	1.02	0.95	0.39	0.00	0.00	0.00	0.00	11	2.41
Pichia membranifaciens	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.00	0.01	0.01	0.00	0.00	0.04	0.23	0.07	0.01	10	0.01
Streptococcus parauberis KCTC 11537	0.07	0.36	47.02	0.00	1.09	0.00	0.24	1.29	0.37	0.42	0.37	1.21	0.00	0.00	0.00	0.12	10	47.02
Lactococcus lactis SK11	1.06	0.38	0.00	0.89	1.98	0.00	10.01	7.14	9.33	8.74	9.71	12.88	0.00	0.00	0.00	0.00	10	12.88
Lactobacillus diolivorans DSM 14421	1.68	2.60	0.00	0.00	2.45	1.62	5.44	3.62	9.02	4.24	7.36	2.74	10.37	15.45	9.45	5.93	10	9.02
Lactobacillus plantarum WCFS1	0.00	0.03	6.30	2.92	1.08	0.00	8.06	6.57	8.11	5.18	7.72	4.50	0.00	0.00	0.00	0.18	10	8.11
Escherichia coli FAM21845	0.43	0.41	0.00	9.19	0.00	0.00	0.53	0.91	0.45	0.66	0.53	0.38	0.00	0.00	0.00	0.00	9	9.19
Torulaspora delbrueckii	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.91	0.54	0.69	0.00	8	0.01
Enterococcus faecalis JH2-2	0.00	0.00	0.00	0.23	0.00	0.10	0.54	1.01	0.52	1.14	0.67	0.43	0.00	0.00	0.00	0.00	8	1.14
Lactobacillus paraplantarum L-ZS9	0.00	0.00	0.00	0.14	0.07	0.00	0.63	0.51	0.63	0.47	0.64	0.35	0.00	0.00	0.00	0.00	8	0.64
Mycoplasma agalactiae PG2	0.00	0.00	0.00	0.00	0.05	0.00	2.70	3.16	3.16	1.34	3.02	3.40	0.00	0.00	0.00	0.00	7	3.40
Lactococcus raffinolactis NBRC 100932	0.00	0.03	0.00	0.00	0.00	0.00	0.72	0.32	0.65	0.90	0.69	0.52	0.00	0.00	0.00	0.00	7	0.90
Saccharomyces cerevisiae	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.31	0.22	0.15	0.05	6	0.01
Enterococcus faecium AT1E22	0.00	0.00	0.00	0.00	0.00	0.00	0.28	0.41	0.41	0.28	0.64	0.26	0.00	0.00	0.00	0.00	6	0.64
Staphylococcus aureus subsp. aureus NC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.05	0.04	0.04	0.04	0.00	0.00	0.00	0.00	5	0.05
Lactobacillus helveticus FAM8627	0.00	0.09	39.08	0.23	0.33	0.29	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	6.15	5	39.08
Kluyveromyces marxianus FIM-b	0.00	0.00	0.00	0.21	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.54	0.14	0.87	4	0.21
Klebsiella pneumoniae HS11286	3.89	2.85	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.00	0.00	0.00	0.00	4	3.89
Lactobacillus acetotolerans NBRC 13120	0.09	0.42	0.00	0.00	4.84	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3	4.84
Lactobacillus delbrueckii ATCC BAA-365	1.24	1.03	0.00	0.00	2.89	0.00	0.00	0.00	0.00	0.00	0.00	0.00	82.46	66.37	81.97	45.43	3	2.89
Acetobacter fabarum KR	0.24	0.13	0.00	0.00	2.88	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3	2.88
Streptococcus thermophilus LMD-9	0.00	0.00	0.00	0.25	0.76	1.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.24	0.34	35.15	3	1.25
Lactobacillus rhamnosus GG (ATCC 53103	0.00	0.04	0.00	21.51	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2	21.51
Lactobacillus paracasei ATCC 334	0.00	0.12	0.00	17.77	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2	17.77
Lactobacillus brevis ATCC 367	0.00	0.00	0.00	1.35	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2	1.35
Lactobacillus gasseri ATCC 33323	0.00	0.00	0.00	23.71	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1	23.71
Lactobacillus buchneri CD034	0.00	0.00	0.00	2.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.00	0.00	1.64	0.00	1	2.11
Listeria monocytogenes EGD-e	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0	0.00
Streptococcus agalactiae 2603V/R	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0	0.00
Streptococcus suis S735	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0	0.00
Brucella abortus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0	0.00
Mycobacterium vaccae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0	0.00

APPENDIX 9 QUALITY OF PROKARYOTIC MAGS

MAGid	lineage (UID)	NBGenoi	NBmarke	NBmarkerse	0	1	2	3	4	5+	Completene	Contaminati	StrainHeterc	Probable Species	Closely related species (by Auto MLST)	ANI
MAG_A-1.bin.4	o__Rhodospirillales (U	63	336	201	93	238	4	1	0	0	80.33	1.65	14.29	Acetobacter orientalis	Acetobacter orientalis 21F-2	96.40%
MAG_A-1-b.bin.15	o__Rhodospirillales (U	63	336	201	0	331	5	0	0	0	100	1.44	20	Acetobacter unclassified	Acetobacter sp. DsW 54	98.00%
MAG_A-2.bin.9	f__Moraxellaceae (UID	86	689	365	59	612	18	0	0	0	89.5	2.18	27.78	Acinetobacter johnsonii	Acinetobacter johnsonii CIP 64.6Acinet	96.30%
MAG_S-7-b.bin.11	c__Gammaproteobacte	899	312	185	75	235	2	0	0	0	85.15	0.72	0	Aeromonas caviae	Aeromonas caviae Ae398	97.60%
MAG_A-1-b.bin.2	c__Gammaproteobacte	899	312	185	54	254	4	0	0	0	81.6	1.53	75	Aeromonas caviae	Aeromonas caviae Ae398	97.90%
MAG_A-6.bin.11	f__Enterobacteriaceae	157	1005	324	4	994	7	0	0	0	99.76	0.75	14.29	Escherichia coli	Escherichia coli str. K-12 substr. MG165	99.10%
MAG_A-1.bin.1	f__Enterobacteriaceae	157	1005	324	145	808	52	0	0	0	91.19	5.66	48.08	Klebsiella pneumoniae	Klebsiella pneumoniae subsp. pneumo	98.90%
MAG_S-7-b.bin.9	o__Lactobacillales (UIC	85	367	162	1	361	5	0	0	0	99.38	2.78	0	Lactiplantibacillus plantarum	Lactobacillus plantarum subsp. plantaru	99.00%
MAG_S-8.bin.8	o__Lactobacillales (UIC	85	367	162	1	361	5	0	0	0	99.38	2.78	0	Lactiplantibacillus plantarum	Lactobacillus plantarum subsp. plantaru	98.90%
MAG_S-8-b.bin.3	o__Lactobacillales (UIC	85	367	162	1	361	5	0	0	0	99.38	2.78	0	Lactiplantibacillus plantarum	Lactobacillus plantarum subsp. plantaru	99.00%
MAG_S-9-b.bin.8	o__Lactobacillales (UIC	85	367	162	1	361	5	0	0	0	99.38	2.78	0	Lactiplantibacillus plantarum	Lactobacillus plantarum ZJ316	98.90%
MAG_S-10-b.bin.11	o__Lactobacillales (UIC	85	367	162	1	360	6	0	0	0	99.38	3.09	16.67	Lactiplantibacillus plantarum	Lactobacillus plantarum subsp. plantaru	98.90%
MAG_A-1-b.bin.13	g__Lactobacillus (UID3	91	430	154	48	381	1	0	0	0	95.96	0.65	0	Lactobacillus acetotolerans	Lactobacillus acetotolerans DSM 20749	97.40%
MAG_L-18.bin.8	g__Lactobacillus (UID3	91	430	154	29	401	0	0	0	0	91.79	0	0	Lactobacillus delbrueckii subsp. bu	Lactobacillus delbrueckii subsp. bulgari	98.70%
MAG_A-1-b.bin.1	g__Lactobacillus (UID3	91	430	154	77	352	1	0	0	0	80.12	0.09	100	Lactobacillus delbrueckii subsp. bu	Lactobacillus delbrueckii subsp. bulgari	98.30%
MAG_S-10-b.bin.8	o__Lactobacillales (UIC	75	376	160	3	372	1	0	0	0	98.94	0.62	0	Lentilactobacillus diolivorans	Lactobacillus diolivorans DSM 14421	98.20%
MAG_S-7-b.bin.12	o__Lactobacillales (UIC	75	376	160	3	372	1	0	0	0	98.94	0.62	0	Lentilactobacillus diolivorans	Lactobacillus diolivorans DSM 14421	98.20%
MAG_S-11-b.bin.10	o__Lactobacillales (UIC	75	376	160	4	369	3	0	0	0	98.31	1.25	66.67	Lentilactobacillus diolivorans	Lactobacillus diolivorans DSM 14421	98.20%
MAG_S-8-b.bin.9	o__Lactobacillales (UIC	75	376	160	12	362	2	0	0	0	97.45	0.78	0	Lentilactobacillus diolivorans	Lactobacillus diolivorans DSM 14421	98.10%
MAG_S-9-b.bin.9	o__Lactobacillales (UIC	75	376	160	12	363	1	0	0	0	96.53	0.62	0	Lentilactobacillus diolivorans	Lactobacillus diolivorans DSM 14421	98.10%
MAG_S-8.bin.9	o__Lactobacillales (UIC	75	376	160	12	363	1	0	0	0	96.53	0.62	0	Lentilactobacillus diolivorans	Lactobacillus diolivorans DSM 14421	98.10%
MAG_A-1-b.bin.10	o__Lactobacillales (UIC	75	376	160	28	342	6	0	0	0	94.87	2.75	16.67	Lentilactobacillus diolivorans	Lactobacillus diolivorans DSM 14421	98.10%
MAG_L-17.bin.3	o__Lactobacillales (UIC	75	376	160	42	331	3	0	0	0	94.59	0.96	66.67	Lentilactobacillus diolivorans	Lactobacillus diolivorans DSM 14421	98.10%
MAG_L-16.bin.5	o__Lactobacillales (UIC	75	376	160	19	350	7	0	0	0	94.52	1.36	57.14	Lentilactobacillus diolivorans	Lactobacillus diolivorans DSM 14421	98.10%
MAG_A-1.bin.3	o__Lactobacillales (UIC	75	376	160	74	300	2	0	0	0	88.43	0.78	0	Lentilactobacillus diolivorans	Lactobacillus diolivorans DSM 14421	97.80%
MAG_L-15.bin.2	o__Lactobacillales (UIC	75	376	160	50	321	5	0	0	0	86.39	0.48	60	Lentilactobacillus diolivorans	Lactobacillus diolivorans DSM 14421	97.80%
MAG_A-2.bin.13	o__Lactobacillales (UIC	75	376	160	64	311	1	0	0	0	84.43	0.62	0	Lentilactobacillus diolivorans	Lactobacillus diolivorans DSM 14421	98.00%
MAG_A-6.bin.4	g__Lactobacillus (UID3	58	449	129	1	443	4	1	0	0	99.22	2.93	14.29	Lactobacillus gasseri	Lactobacillus gasseri AL5	99.40%
MAG_A1-b	g__Lactobacillus	91	430	154	3	413	14	0	0	0	98.38	1.74	0	Lactobacillus kefiranofaciens	Lactobacillus kefiranofaciens subsp. kef	98.70%
MAG_A2	g__Lactobacillus	91	430	154	8	416	6	0	0	0	99.32	0.79	0	Lactobacillus kefiranofaciens	Lactobacillus kefiranofaciens subsp. kef	98.70%
MAG_A4-b	g__Lactobacillus	91	430	154	3	424	3	0	0	0	98.38	0.28	0	Lactobacillus kefiranofaciens	Lactobacillus kefiranofaciens subsp. kef	98.70%
MAG_S7-b	g__Lactobacillus	91	430	154	4	422	4	0	0	0	98.65	0.67	0	Lactobacillus kefiranofaciens	Lactobacillus kefiranofaciens subsp. kef	98.70%
MAG_S8	g__Lactobacillus	91	430	154	2	428	0	0	0	0	99.03	0	0	Lactobacillus kefiranofaciens	Lactobacillus kefiranofaciens subsp. kef	98.70%
MAG_S9-b	g__Lactobacillus	91	430	154	2	428	0	0	0	0	99.03	0	0	Lactobacillus kefiranofaciens	Lactobacillus kefiranofaciens subsp. kef	98.70%
MAG_S10-b	g__Lactobacillus	91	430	154	3	425	2	0	0	0	98.81	0.71	0	Lactobacillus kefiranofaciens	Lactobacillus kefiranofaciens subsp. kef	98.70%
MAG_S11-b	g__Lactobacillus	91	430	154	3	427	0	0	0	0	98.81	0	0	Lactobacillus kefiranofaciens	Lactobacillus kefiranofaciens subsp. kef	98.70%
MAG_A-6.bin.9	o__Lactobacillales (UIC	294	472	265	0	470	2	0	0	0	100	0.75	0	Lactococcus lactis subsp. lactis	Lactococcus lactis subsp lactis KF147	98.60%
MAG_A-2.bin.8	o__Lactobacillales (UIC	294	472	265	9	456	7	0	0	0	99.53	1.48	28.57	Lactococcus lactis subsp. lactis	Lactococcus lactis subsp lactis KF147	98.60%
MAG_A-1-b.bin.6	o__Lactobacillales (UIC	294	472	265	7	449	16	0	0	0	97.74	2.77	100	Lactococcus lactis subsp. lactis	Lactococcus lactis subsp lactis KF147	98.60%
MAG_S-10-b.bin.3	o__Lactobacillales (UIC	294	472	265	13	423	28	8	0	0	96.6	9.3	96.15	Lactococcus lactis subsp. lactis	Lactococcus lactis subsp lactis KF147	98.60%
MAG_S-7-b.bin.5	o__Lactobacillales (UIC	294	472	265	39	423	10	0	0	0	89.62	2.45	70	Lactococcus lactis subsp. lactis	Lactococcus lactis subsp lactis KF147	98.60%
MAG_S-9-b.bin.3	o__Lactobacillales (UIC	75	376	160	2	370	4	0	0	0	99.22	1.25	50	Lentilactobacillus kefiri	Lactobacillus kefiri DSM 20587	99.50%
MAG_S-8.bin.3	o__Lactobacillales (UIC	75	376	160	2	370	4	0	0	0	99.22	1.25	50	Lentilactobacillus kefiri	Lactobacillus kefiri DSM 20587	99.50%
MAG_S-8-b.bin.6	o__Lactobacillales (UIC	75	376	160	2	370	4	0	0	0	99.22	1.25	50	Lentilactobacillus kefiri	Lactobacillus kefiri DSM 20587	99.50%
MAG_S-11-b.bin.12	o__Lactobacillales (UIC	75	376	160	2	374	0	0	0	0	99.22	0	0	Lentilactobacillus kefiri	Lactobacillus kefiri DSM 20587	99.50%
MAG_A-1-b.bin.7	o__Lactobacillales (UIC	75	376	160	4	371	1	0	0	0	98.73	0.06	100	Lentilactobacillus kefiri	Lactobacillus kefiri DSM 20587	99.50%
MAG_S-10-b.bin.12	o__Lactobacillales (UIC	75	376	160	14	362	0	0	0	0	98.28	0	0	Lentilactobacillus kefiri	Lactobacillus kefiri DSM 20587	99.50%
MAG_A-2.bin.1	o__Lactobacillales (UIC	75	376	160	15	359	2	0	0	0	98.1	0.33	100	Lentilactobacillus kefiri	Lactobacillus kefiri DSM 20587	99.50%
MAG_L-18.bin.9	o__Lactobacillales (UIC	75	376	160	1	375	0	0	0	0	99.38	0	0	Lentilactobacillus kefiri	Lactobacillus kefiri DSM 20587	99.50%
MAG_A-4-b.bin.2	o__Lactobacillales (UIC	75	376	160	1	375	0	0	0	0	99.38	0	0	Lentilactobacillus kefiri	Lactobacillus kefiri DSM 20587	99.50%
MAG_S-7-b.bin.7	o__Lactobacillales (UIC	75	376	160	2	374	0	0	0	0	99.22	0	0	Lentilactobacillus kefiri	Lactobacillus kefiri DSM 20587	99.50%
MAG_S-8.bin.4	g__Mycoplasma (UID2	29	247	130	0	247	0	0	0	0	100	0	0	Mycoplasma agalactiae	Mycoplasma agalactiae PG2	99.90%
MAG_S-9-b.bin.4	g__Mycoplasma (UID2	29	247	130	0	247	0	0	0	0	100	0	0	Mycoplasma agalactiae	Mycoplasma agalactiae PG2	99.90%
MAG_S-7-b.bin.3	g__Mycoplasma (UID2	29	247	130	1	245	1	0	0	0	99.23	0.38	0	Mycoplasma agalactiae	Mycoplasma agalactiae PG2	99.90%
MAG_S-11-b.bin.11	g__Mycoplasma (UID2	29	247	130	2	243	2	0	0	0	99.04	1.15	0	Mycoplasma agalactiae	Mycoplasma agalactiae PG2	99.90%
MAG_S-10-b.bin.7	g__Mycoplasma (UID2	29	247	130	5	239	3	0	0	0	98.31	1.92	0	Mycoplasma agalactiae	Mycoplasma agalactiae PG2	99.90%
MAG_S-8-b.bin.11	g__Mycoplasma (UID2	29	247	130	19	224	4	0	0	0	93.3	1.67	50	Mycoplasma agalactiae	Mycoplasma agalactiae PG2	99.90%
MAG_S-8.bin.11	o__Lactobacillales (UIC	294	472	265	69	396	7	0	0	0	88.82	1.57	0	Streptococcus parauberis	Streptococcus parauberis SP-IIh	98.80%
MAG_S-9-b.bin.11	o__Lactobacillales (UIC	294	472	265	69	396	7	0	0	0	88.82	1.57	0	Streptococcus parauberis	Streptococcus parauberis SP-IIh	98.80%
MAG_A-4.bin.4	o__Lactobacillales (UIC	294	472	265	84	385	3	0	0	0	82.84	0.44	0	Streptococcus parauberis	Streptococcus parauberis SP-IIh	98.80%
MAG_A-4.bin.3	o__Lactobacillales (UIC	293	475	267	9	460	6	0	0	0	98.46	1.13	16.67	Streptococcus suis	Streptococcus suis BM407	88.60%
MAG_L-18.bin.7	o__Lactobacillales (UIC	293	475	267	6	466	3	0	0	0	99.13	0.75	0	Streptococcus thermophilus	Streptococcus thermophilus LMG 18311	99.20%

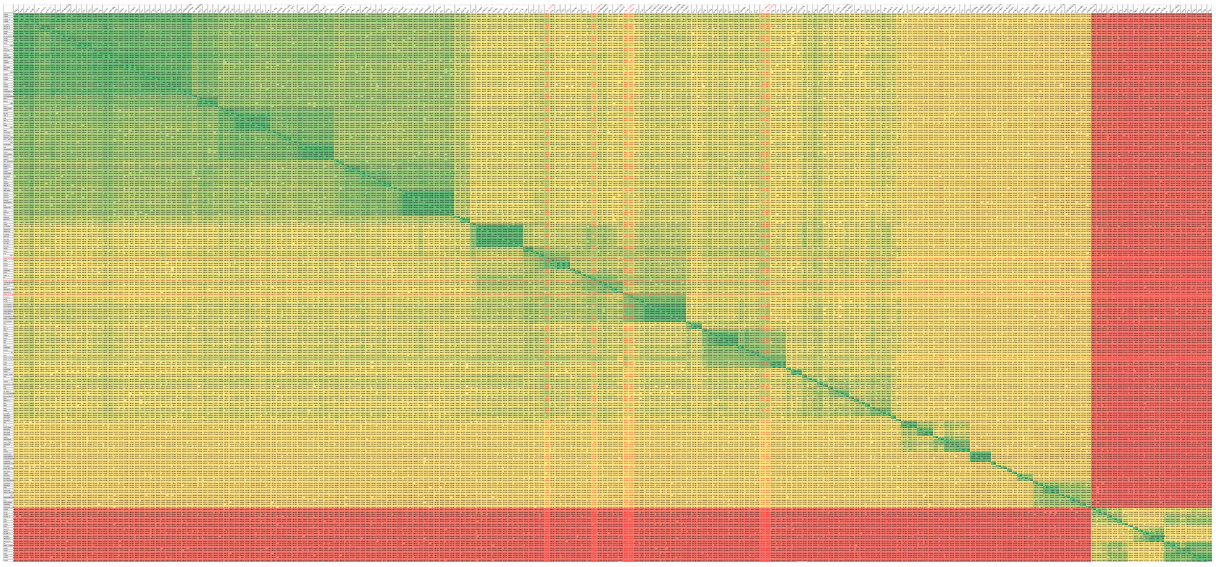
Ambriss

Serdaleh

Labneh El-Darff

APPENDIX 10 FASTANI VALUES FOR MAGS, ISOLATED STRAIN GENOMES AND NCBI REFERENCE GENOMES FOR L. LACTIS, L. KEFIRANOFACIENS, L. KEFIRI, L. DIOLIVORANS, L. PLANTARUM, M. AGALACTIAE AND S. PARASUIS

Lc. lactis



L. kefiranofacien

	MAG_S	MAG_S	FME15	MAG_P	MAG_S	MAG_S	MAG_S	MAG_A	MAG_A	MAG_A	ZW3	DSM50	LKKR75	1207	SCB251	DSM10	KR
MAG_S8	100	100	99.94	99.95	99.94	99.85	99.82	99.3	99.27	99.33	98.83	99.07	98.9	98.76	98.57	98.81	98.84
MAG_S9-B	100	100	99.95	99.95	99.88	99.89	99.79	99.3	99.28	99.34	98.87	99.09	98.93	98.85	98.58	98.94	98.92
FME151	99.94	99.95	100	99.99	99.85	99.91	99.83	99.22	99.28	99.22	98.77	98.73	98.83	98.49	98.43	98.71	98.63
MAG_P5	99.95	99.95	99.99	100	99.86	99.91	99.85	99.26	99.3	99.34	98.74	99.05	98.77	98.72	98.52	98.87	98.72
MAG_S7-B	99.94	99.88	99.85	99.86	100	99.83	99.8	99.39	99.48	99.45	98.86	99.01	99	98.79	98.57	98.97	98.83
MAG_S11-B	99.85	99.89	99.91	99.91	99.83	100	99.8	99.39	99.37	99.37	98.79	99.07	98.95	98.85	98.81	98.98	99.05
MAG_S10-B	99.82	99.79	99.83	99.85	99.8	99.8	100	99.46	99.4	99.52	98.84	98.98	98.94	98.89	98.78	98.86	98.93
MAG_A4-B	99.3	99.3	99.22	99.26	99.39	99.39	99.46	100	99.66	99.66	98.67	98.97	98.64	98.71	98.6	98.69	98.82
MAG_A2	99.27	99.28	99.28	99.3	99.48	99.37	99.4	99.66	100	99.86	98.79	98.88	98.78	98.69	98.69	98.7	98.72
MAG_A1-B	99.33	99.34	99.22	99.34	99.45	99.37	99.52	99.66	99.86	100	98.72	98.81	98.63	98.63	98.73	98.78	98.75
ZW3	98.83	98.87	98.77	98.74	98.86	98.79	98.84	98.67	98.79	98.72	100	99.84	99.96	99.1	99.22	99.09	99.23
DSM5016	99.07	99.09	98.73	99.05	99.01	99.07	98.98	98.97	98.88	98.81	99.84	100	99.92	99.38	99.41	99.38	99.3
LKKR75	98.9	98.93	98.83	98.77	99	98.95	98.94	98.64	98.78	98.63	99.96	99.92	100	99.18	99.26	99.25	99.22
1207	98.76	98.85	98.49	98.72	98.79	98.85	98.89	98.71	98.69	98.63	99.1	99.38	99.18	100	99.85	99.4	99.48
SCB2517	98.57	98.58	98.43	98.52	98.57	98.81	98.78	98.6	98.69	98.73	99.22	99.41	99.26	99.85	100	99.48	99.55
DSM10550	98.81	98.94	98.71	98.87	98.97	98.98	98.86	98.69	98.7	98.78	99.09	99.38	99.25	99.4	99.48	100	99.63
KR	98.84	98.92	98.63	98.72	98.83	99.05	98.93	98.82	98.72	98.75	99.23	99.3	99.22	99.48	99.55	99.63	100

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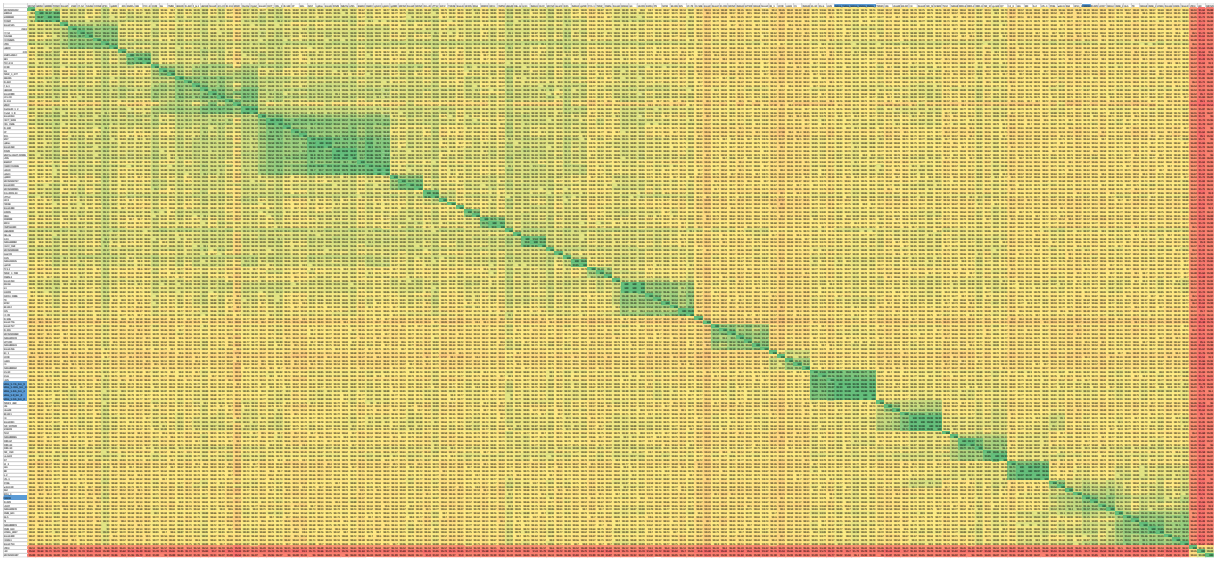
Len. kefiri

	MAG_A	FME71	MAG_A	MAG_S-	MAG_S-	MAG_S-	MAG_S-	MAG_S-	MAG_S-	MAG_A	DSM205	SGL13	UHGG_I	DH5	Olga	IM1365	MAG_L-	Fangua	SCB251	KR	OG2
MAG_A-1-b_bi	100	99.94	99.78	99.74	99.74	99.73	99.76	99.72	99.72	99.76	99.9	99.61	99.61	99.86	99.68	99.9	99.75	99.87	99.72	99.72	99.73
FME71	99.94	100	99.9	99.88	99.88	99.87	99.82	99.8	99.88	99.93	99.72	99.49	99.49	99.74	99.64	99.59	99.86	99.66	99.81	99.72	99.73
MAG_A-2_bin_	99.78	99.9	100	99.86	99.86	99.84	99.81	99.86	99.86	99.88	99.9	99.74	99.74	99.85	99.81	99.88	99.88	99.9	99.84	99.8	99.83
MAG_S-8_bin_	99.74	99.88	99.86	100	100	100	100	99.98	99.99	99.93	99.94	99.83	99.83	99.9	99.92	99.93	99.9	99.89	99.93	99.93	99.93
MAG_S-9-b_bi	99.74	99.88	99.86	100	100	100	100	99.98	99.99	99.93	99.94	99.83	99.83	99.9	99.92	99.93	99.9	99.89	99.93	99.93	99.93
MAG_S-11-b_b	99.73	99.87	99.84	100	100	100	100	99.98	99.99	99.91	99.87	99.79	99.79	99.87	99.87	99.91	99.92	99.85	99.84	99.85	99.81
MAG_S-7-b_bi	99.76	99.82	99.81	100	100	100	100	99.97	100	99.91	99.87	99.7	99.7	99.8	99.81	99.87	99.92	99.8	99.84	99.81	99.76
MAG_S-10-b_b	99.72	99.8	99.86	99.98	99.98	99.98	99.97	100	99.99	99.91	99.84	99.71	99.71	99.84	99.8	99.88	99.91	99.82	99.83	99.83	99.79
MAG_S-8-b_bi	99.72	99.88	99.86	99.99	99.99	99.99	100	99.99	100	99.91	99.93	99.81	99.81	99.9	99.9	99.95	99.91	99.89	99.88	99.92	99.88
MAG_A-4-b_bi	99.76	99.93	99.88	99.93	99.93	99.91	99.91	99.91	99.91	100	99.91	99.81	99.81	99.9	99.9	99.93	99.93	99.92	99.9	99.9	99.9
DSM20587	99.9	99.72	99.9	99.94	99.94	99.87	99.87	99.84	99.93	99.91	100	99.68	99.68	99.66	99.74	99.59	99.91	99.68	99.79	99.71	99.74
SGL13	99.61	99.49	99.74	99.83	99.83	99.79	99.7	99.71	99.81	99.81	99.68	100	100	99.84	99.55	99.57	99.81	99.75	99.69	99.63	99.64
UHGG_MGYG-H	99.61	99.49	99.74	99.83	99.83	99.79	99.7	99.71	99.81	99.81	99.68	100	100	99.84	99.55	99.57	99.81	99.75	99.69	99.63	99.64
DH5	99.86	99.74	99.85	99.9	99.9	99.87	99.8	99.84	99.9	99.9	99.66	99.84	99.84	100	99.78	99.72	99.92	99.74	99.77	99.77	99.77
Olga	99.68	99.64	99.81	99.92	99.92	99.87	99.81	99.8	99.9	99.9	99.74	99.55	99.55	99.78	100	99.64	99.89	99.52	99.79	99.76	99.76
IM1365	99.9	99.59	99.88	99.93	99.93	99.91	99.87	99.88	99.95	99.93	99.59	99.57	99.57	99.72	99.64	100	99.96	99.56	99.79	99.75	99.75
MAG_L-18_bin	99.75	99.86	99.88	99.9	99.9	99.92	99.92	99.91	99.91	99.93	99.91	99.81	99.81	99.92	99.89	99.96	100	99.95	99.81	99.9	99.89
Fangua	99.87	99.66	99.9	99.89	99.89	99.85	99.8	99.82	99.89	99.92	99.68	99.75	99.75	99.74	99.52	99.56	99.95	100	99.69	99.66	99.67
SCB2511	99.72	99.81	99.84	99.93	99.93	99.84	99.84	99.83	99.88	99.9	99.79	99.69	99.69	99.77	99.79	99.79	99.81	99.69	100	99.88	99.87
KR	99.72	99.72	99.8	99.93	99.93	99.85	99.81	99.83	99.92	99.9	99.71	99.63	99.63	99.77	99.76	99.75	99.9	99.66	99.88	100	100
OG2	99.73	99.73	99.83	99.93	99.93	99.81	99.76	99.79	99.88	99.9	99.74	99.64	99.64	99.77	99.76	99.75	99.89	99.67	99.87	100	100

L. diolivorans

	MAG_L-15_bin_2	FME74	MAG_A-1-b_bin_10	MAG_A-1_bin_3	MAG_A-2_bin_13	MAG_L-16_bin_5	MAG_L-17_bin_3	MAG_S-10-b_bin_8	MAG_S-11-b_bin_10	MAG_S-7-b_bin_12	MAG_S-8-b_bin_9	MAG_S-8_bin_9	MAG_S-9-b_bin_9	NBRC107869	UW_MP
MAG_L-15_b	100	99.71	99.11	98.4	99.25	99.15	99.25	97.87	97.79	97.86	97.77	97.84	97.84	97.43	97.28
FME74	99.71	100	99.78	99.55	99.5	99.75	99.83	98.45	98.47	98.43	98.45	98.42	98.42	97.91	97.9
MAG_A-1-b_	99.11	99.78	100	99.25	99.36	99.34	99.39	98.5	98.44	98.53	98.45	98.51	98.51	97.99	97.84
MAG_A-1_bi	98.4	99.55	99.25	100	98.92	98.72	98.85	98.29	98.32	98.26	98.3	98.27	98.27	97.65	97.59
MAG_A-2_bi	99.25	99.5	99.36	98.92	100	99.33	99.43	98.8	98.72	98.77	98.8	98.79	98.79	97.94	97.91
MAG_L-16_b	99.15	99.75	99.34	98.72	99.33	100	99.71	98.23	98.28	98.25	98.25	98.24	98.24	97.87	97.61
MAG_L-17_b	99.25	99.83	99.39	98.85	99.43	99.71	100	98.27	98.38	98.32	98.29	98.25	98.25	97.93	97.82
MAG_S-10-b_	97.87	98.45	98.5	98.29	98.8	98.23	98.27	100	99.95	99.95	99.93	99.97	99.97	98.14	97.99
MAG_S-11-b_	97.79	98.47	98.44	98.32	98.72	98.28	98.38	99.95	100	99.95	99.94	99.95	99.95	98.03	98.04
MAG_S-7-b_	97.86	98.43	98.53	98.26	98.77	98.25	98.32	99.95	99.95	100	99.94	99.95	99.95	98.01	97.97
MAG_S-8-b_	97.77	98.45	98.45	98.3	98.8	98.25	98.29	99.93	99.94	99.94	100	99.92	99.92	97.99	98.03
MAG_S-8_bi	97.84	98.42	98.51	98.27	98.79	98.24	98.25	99.97	99.95	99.95	99.92	100	100	98	98.02
MAG_S-9-b_	97.84	98.42	98.51	98.27	98.79	98.24	98.25	99.97	99.95	99.95	99.92	100	100	98	98.02
NBRC107869	97.43	97.91	97.99	97.65	97.94	97.87	97.93	98.14	98.03	98.01	97.99	98	98	100	97.84
UW_MP_LEN	97.28	97.9	97.84	97.59	97.91	97.61	97.82	97.99	98.04	97.97	98.03	98.02	98.02	97.84	100

L. plantarum



M. agalactiae

	MAG_S	MAG_S	MAG_S	MAG_S	MAG_S	MAG_S	GRTH0	PG2	JF4428	4210	7784	4021	14634	5276	4055
MAG_S-8-b_	100	99.67	99.67	99.68	99.43	99.71	99.63	99.88	99.85	99.79	99.84	99.87	99.84	99.84	96.14
MAG_S8	99.67	100	99.99	99.81	99.67	99.82	99.6	99.88	99.85	99.83	99.85	99.85	99.87	99.88	96.21
MAG_S-9-b_	99.67	99.99	100	99.76	99.65	99.82	99.65	99.88	99.86	99.79	99.86	99.87	99.87	99.87	96.14
MAG_S-10-b	99.68	99.81	99.76	100	99.63	99.72	99.56	99.86	99.85	99.83	99.83	99.85	99.85	99.85	96.09
MAG_S-11-b	99.43	99.67	99.65	99.63	100	99.57	99.62	99.86	99.85	99.7	99.86	99.85	99.79	99.86	96.26
MAG_S-7-b_	99.71	99.82	99.82	99.72	99.57	100	99.63	99.88	99.84	99.72	99.83	99.87	99.85	99.86	96.12
GRTH01	99.63	99.6	99.65	99.56	99.62	99.63	100	99.8	99.62	99.67	99.76	99.57	99.63	99.65	96.05
PG2	99.88	99.88	99.88	99.86	99.86	99.88	99.8	100	99.85	99.84	99.88	99.84	99.84	99.87	96.12
JF4428	99.85	99.85	99.86	99.85	99.85	99.84	99.62	99.85	100	99.78	99.86	99.88	99.86	99.87	96.1
4210	99.79	99.83	99.79	99.83	99.7	99.72	99.67	99.84	99.78	100	99.91	99.9	99.93	99.93	96.07
7784	99.84	99.85	99.86	99.83	99.86	99.83	99.76	99.88	99.86	99.91	100	99.94	99.93	99.89	96.22
4021	99.87	99.85	99.87	99.85	99.85	99.87	99.57	99.84	99.88	99.9	99.94	100	99.94	99.96	96.12
14634	99.84	99.87	99.87	99.85	99.79	99.85	99.63	99.84	99.86	99.93	99.93	99.94	100	99.99	96.17
5276	99.84	99.88	99.87	99.85	99.86	99.86	99.65	99.87	99.87	99.93	99.89	99.96	99.99	100	96.08
4055	96.14	96.21	96.14	96.09	96.26	96.12	96.05	96.12	96.1	96.07	96.22	96.12	96.17	96.08	100

S. parasu

#ANIb results	BS26	BS27	MAG-A4	H35	4253	SUT-503	SUT-286	SUT-328	SUT-380	SUT-7
BS26	*	99.98	98.29	97.35	97.28	94.89	94.46	94.66	94.63	93.35
BS27	100	*	98.33	97.38	97.31	95	94.6	94.73	94.68	93.51
MAG-A4	98.34	98.36	*	96.8	96.94	94.5	93.9	94.01	93.94	93.4
H35	97.4	97.4	97.01	*	96.73	95.13	94.62	94.86	94.86	93.45
4253	97.49	97.48	97.07	96.79	*	94.45	94.39	94.65	94.65	93.47

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Lebanese fermented goat milk products: From tradition to meta-omics

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Highlights

- Detailed analysis of the production chain of three emblematic Lebanese fermented goat milk products
- Ambriss and Serdaleh processes yield products with a composition similar to that of kefir
- Adaptation of the zoonotic agent *Streptococcus parasuis* to milk fermentation through the acquisition of lactose utilization genes
- Evidence of herd contamination by *Mycoplasmopsis agalactiae* through MAG analysis

1 Abstract

Ambriss, Serdaleh and Labneh El Darff are traditional Lebanese fermented goat milk products from the Bekaa Valley, the Chouf Mountains and Southern Lebanon, respectively. A questionnaire completed by 50 producers of these products showed that they are prepared by periodic percolation either by milk or Laban in amphora or goatskin during the lactation season. Production is carried out on a small scale and in a limited number of production units, often by elderly people, entailing a real risk that these products may disappear and that their corresponding microbial resources will be lost. In this study, 34 samples from 18 producers were characterized by culture-dependent and -independent analyses. The results obtained by these two methods were radically different, in particular for Ambriss and Serdaleh, the latter revealing the co-dominance of *Lactobacillus kefiranofaciens*, a fastidious-growing species, and of *Lactococcus lactis* in a viable but not culturable state. Overall, their composition is reminiscent of that of kefir grains. However, phylogenomic and functional analyses of the genomes of the keystone species *L. kefiranofaciens* show that they differed from those of kefir, for example, in terms of their polysaccharide genes, explaining the absence of grains. Labneh El Darff displays dominance of *Lactobacillus delbrueckii*, probably due to the addition of Laban. In addition, several zoonotic pathogens were found, including *Streptococcus parasuis*, which was dominant in one sample and which MAG analysis revealed has acquired lactose utilization genes by horizontal gene transfer. The contamination of the herd with *Mycoplasmopsis agalactiae* in the Chouf region was also revealed by MAG analysis of the Serdaleh samples. Antibiotic resistance genes were detected in most of the samples, particularly in the Serdaleh samples, where the dominant *L. lactis* strains possessed a plasmid with a multi-resistance island. Finally, this study opens the way for further analyses to shed light on the resilience of these ecosystems established in amphora or in goatskins and to improve hygiene practices for milk production.

Keywords: Microbial diversity, kefir, metagenomics, genomics, ecology, VBNC, metagenome-assembled genomes, LAB, *Lactobacillus kefiranofaciens*, *Streptococcus parasuis*, *Mycoplasmopsis agalactiae*

2 Introduction

The Mediterranean region is well known for its goat farming, providing about 21% of the world's goat milk supply (Miller & Lu, 2019; Dubeuf et al., 2016). Due to the nature of its composition, organoleptic characteristics and healthy attributes, goat milk has been the focus of interest in recent years (Prosser, 2021; Clark and Mora García, 2017). This sector has received increasing attention, particularly in Lebanon, and continues to grow (Kayouli, 2015). Goat milk products are highly appreciated by local consumers (Balaa & Marie, 2008), especially for their specific "goaty taste" (Semaan et al., 2011). Compared to cow milk, its casein proteins are more easily digested and it is better tolerated by people with mild lactose intolerance. It has a higher content of short-chain fatty acids in milk fat (Shu et al., 2016; Zenebe et al., 2014) and contains minerals such as magnesium, calcium and phosphorus (Abbas et al., 2014).

These goat milk products are made either from standardized milk at the industrial scale, such as Laban (Chammas et al., 2006a), Labneh (Serhan et al., 2016), Shankleesh (Serhan & Mattar, 2013: Toufeili et al., 1995), Kishk (Salameh et al., 2016), or artisanally from raw milk, such as Darfiyeh (Hosri & El Khoury, 2004), Aricheh (Serhan & Mattar, 2013), Serdaleh (Tabet et al., 2019), Ambriss (Dimassi et al., 2020) and Labneh El Darff (Semaan et al., 2011). The diversity of these dairy products not only enriches the Lebanese culinary culture and pantry (Massaad, 2017), but also represents an important source of empowerment for women in poor rural areas. Female-headed households effectively contribute to the development of the goat rearing sector and the manufacturing of goat milk products (Kayouli, 2015). The market share of this sector is not well characterized; however, 70% of all small ruminant industries are located in rural areas with limited market access (Saadeh, 2016). Among these fermented goat milk products, Ambriss, Serdaleh and Labneh El Darff, made with milk from the Black Baladi race that grazes in the Bekaa Valley, the Chouf Mountains and Southern Lebanon, respectively (**Figure 1A**), are real candidates to be awarded the PGI (Protected Geographical Origin) label according to the report of the SRVA (Service Romand de Vulgarisation Agricole; IDEAS Centre, 2005; Ministère de l'Economie et du Commerce de la République Libanaise, 2006). However, it appears that these products are in danger of disappearing, mainly due to commercial, regulatory and food safety concerns (Serhan & Mattar, 2018; Semaan et al., 2011). In fact, these products are made on a seasonal basis, at small or medium production scales and according to traditional methods (**Figure 1B**), by successive fermentation of raw goat milk in amphorae for Ambriss (Dimassi et al. 2020) and Serdaleh (Serhan and Mattar, 2013) and Laban in goatskins

for Labneh El Darff (Serhan et al. 2016; Nasbimana et al. 2005).

In the Mediterranean region, traditional fermented milk products are closely related to the area in which they are produced and the cultural history and heritage (Boyazoglu & Morand-Fehr, 2001). This fact has drawn the attention of many studies due to the unique microbial diversity of these products (Rhaiem et al., 2016; Abdalla & Hussain, 2010; Ayad et al., 2004; El Soda et al., 2003). Numerous genera of lactic acid bacteria (LAB) such as *Lactobacillus, Lactococcus, Enterococcus, Leuconostoc* and *Streptococcus* are generally involved in these transformations. However, only a few studies have described the native microbiota in the Lebanese fermented milk products, and the characterization of the isolates has been restricted to phenotypic and biochemical analyses. The first attempts were recorded on Laban, where 96 strains of *S. thermophilus* and *L. bulgaricus* were characterized (Chammas et al., 2006b). Serhan et al. (2009) then revealed the presence of the LAB *Streptococcus thermophilus*, *Enterococcus* spp., *Lactococcus lactis* subsp. *lactis* and *cremoris, Lactiplantibacillus plantarum*, *Lactobacillus curvatus* in Darfiyeh cheese using culture-dependent methods. Moreover, Dib et al. (2012) isolated 25 different strains in Serdaleh, Laban and Darfiyeh cheese belonging to the species *L. plantarum*, *L. paracasei*, *L. casei* and *Leuconostoc mesenteroides*

Until now, these traditional Lebanese products have only been characterized using cultural methods, which provide a patchy view of the microbiota, whereas new methods for characterizing food ecosystems are now available, based on techniques such as 16S and shotgun metagenomic analyses (Walsh et al., 2022). Approaches based on these techniques have already been used in a study on traditional Brazilian cheeses and provided a global picture of their microbiota, with taxonomic resolution close to the strain level for dominant populations and functional data at the gene level (Kothe et al., 2021). We therefore decided to develop a better understanding of the microbiota of Ambriss, Serdaleh and Labneh El Darff using these new methods to improve our knowledge of their processes, and to thus develop strategies to preserve them and boost their development. To do this, 33 samples of fermented goat milk products from 18 producers were characterized using a combination of culture-dependent and culture-independent analyses, including genomic analyses of isolated strains and metagenomic assemblies, as well as functional analyses based on deep metagenomic profiling. The results were interpreted with regard to the manufacturing processes and physico-chemical characteristics of the products.

3 Materials and Methods

3.1 Development of a producer's survey

A producers' survey was conducted over 50 breeders or producers from the three selected production regions – the Bekaa Valley, the Chouf Mountains and Southern Lebanon – for the artisanal products of Ambriss, Serdaleh and Labneh El Darff, respectively. The questionnaire consisted of 34 questions, covering three main areas: (i) socio-demographic aspects; (ii) breeding activities; and (iii) production activities, including commercialization. The questions included general and socio-economic information about producers, information regarding livestock size, goat breed, breeding type and practices, milk production volumes, as well as the type of dairy product produced, the production season, the raw materials used, production volumes, prices and marketing practices. In addition, a detailed observation of the processing was carried out in order to build a common production flow chart for each product type, showing the existing differences between the producers as well.

3.2 Sample collection, physic-chemical and microbiological analyses

Producers were first selected on the basis of the survey at the different production sites according to two main criteria: their production was small-scale and they had been producing their product for more than 15 years. In total, 34 artisanal samples from 18 producers were collected during two consecutive production seasons in 2018 and 2019 (**Table S1**).

Samples of fermented goat milk products (10 g) were taken aseptically and homogenized for 60 s in a laboratory blender (Blender LB20E, Waring, Torrington, CT, USA) with 90 mL and 20 mL of distilled water, for pH and salinity tests, respectively. The pH was measured using a digital pH meter (GLP 21, Crison instrument, Barcelona, Spain). The salinity content was determined using chloride test strips (30-600 mg/L; QuanTabTitrator, Hach, Loveland, CO, USA). All measurements were performed in triplicate. Samples were preserved in phosphate-buffered saline (PBS 15X, pH 8.7), and stored at -20°C for further analysis.

For culture-dependent analyses, samples were diluted and homogenized in buffer peptone water (2%). They were spread in triplicate on MRS (pH 6.2, Sigma-Aldrich, St. Louis, MO, USA) and M17 (pH 6.5, Sigma-Aldrich, St. Louis, MO, USA) agar plates. Plates were incubated for 48 h, anaerobically for MRS at 30°C and 42°C to allow mesophilic and thermophilic *Lactobacillus* counts, respectively, and aerobically for M17 at 30°C for lactococci.

Specific procedures were applied to isolate *L. lactis* and *Lactobacillus kefiranofaciens* strains. Culture media were supplemented with 2% salt and 10% goat milk for *L. lactis*, or with 25% goat milk and adjusted at pH 5.3 for *L. kefiranofaciens*, and incubated anaerobically at 30°C for 10 days.

Based on their distinct morphology (shape, color and size), colonies were selected and purified by repetitive streaking on the same medium. Isolates were assessed for their catalase activity and Gram coloration according to the criteria described by Kandler and Weiss (1986), as well as for their ability to produce gas from glucose by inverted tubes (Zúñiga et al., 1993). Biochemical identification and carbohydrate fermentation patterns were determined by API 50 CH and API 20 STREP test kits (BioMérieux Inc., Marcy l'Etoile, France) following the manufacturer's recommendations.

In order to identify the different morphotypes, isolates were suspended in 400 µL Tris-EDTA (TE 1X, pH 8.0) buffer in a 2-mL tube. DNAs was lysed using a bead-beater (24 Microtubes BeadBlaster, Thomas Scientific, NJ., USA), with 100 mg zirconium beads of 0.1 and 0.5-mm diameters and homogenized for 20 s at 4.5 m/s. Once extracted, amplification of the coding gene for 16S rRNA was carried out for 30 cycles on a final mix of 25 µL containing the following universal primers: Bact-27F and 1492R, using Taqara DNA Taq polymerase (Almeida et al., 2014). Products were purified using a purification kit (Gen Elute PCR clean up, Sigma, St. Louis, MO, USA). Genotypic identification was carried out by blasting the different sequences of the isolated strains using the NCBI database.

3.3 Genomic DNA extraction and sequencing

For some isolates, DNA was extracted from the bacterial cells according to the protocol described by Kothe et al. (2021). DNA sequencing was carried out on an Illumina HiSeq at GATC-Biotech (Konstanz, Germany) to generate about 5-6 million paired-end reads (150 bases in length). For each strain, the paired-end reads were merged and *de novo* assembly was performed using SPAdes 3.9 (Bankevich et al., 2012). Only contigs with length > 300 bp and coverage > 100 were considered for further study. Annotations were performed with Rapid Annotation using the Subsystem Technology server (Aziz et al., 2012).

3.4 DNA extraction from the products

Samples (0.5 g) were diluted 1:1 (w/v) in guanidinium thiocyanate 4M solution (Sigma-Aldrich, MO, USA) with TrisHCl 0.1M and processed in the same way as for the isolate DNA

extraction. The mixture was then supplemented with 10% N-Lauryl sarcosine (Sigma-Aldrich, MO, USA), vortexed and centrifuged at 4°C and 14,000 *g* for 30 min. Supernatant were discarded and extraction was continued on the emerged pellet, as described by Almeida et al. (2014). DNA was visually evaluated on 0.8% agarose gel and quantified by a fluorometer (Qubit 2.0, ThermoFisher Scientific) supplied with the Qubit dsDNA HS Assay Kit (ThermoFisher Scientific).

3.5 Metagenetic analysis

Bacterial diversity was analyzed by sequencing the amplified region V3-V4 of the 16S rRNA gene using primers V3F and V4R. The PCR was performed with MTP Taq DNA Polymerase (Sigma-Aldrich, St. Louis, MO, USA), and cycling conditions were: 94°C for 1 min, followed by 30 cycles of amplification at 94°C for 1 min, 65°C for 1 min, and 72°C for 1 min, with a final extension step of 10 min at 72°C. Sequencing was performed with the V3 Illumina MiSeq kit, as described in Poirier et al. (2018), and fasta files were generated at the end of the run (MiSeq Reporter software, Illumina, USA).

The quality of the raw data was evaluated with FastQC (Wingett & Andrews, 2018) and the sequences were imported into the FROGS (Find Rapidly OTUs with Galaxy Solution) pipeline (Escudié et al., 2018) to obtain the Operational Taxonomic Units (OTUs). The sequences were filtered by length (480-580 bp) and then pooled into OTUs with SWARM (Mahé et al., 2014) with the distance parameter of 1. Chimeras were removed with VSEARCH (Rognes et al., 2016) and OTUs with at least 0.005% in the whole dataset were retained (Bokulich et al., 2013). OTUs were affiliated with the 16S SILVA 138.1 database (Quast et al., 2013). Alpha-diversity and beta-diversity analyses were performed with R Studio v.3.6.1 using the phyloseq and ggplot2 packages (v1.30.0). Statistics were performed on alpha-diversity using Kruskal-Wallis and linear model regression. DESeq2 was applied to characterize statistically significant differentially abundant OTUs in the different products (Love et al., 2014). The PCoA was plotted with the ward.D2 method and Permanova tests were performed between samples.

3.6 Taxonomic composition by shotgun metagenomics study

Sixteen fermented products (six Ambriss, six Serdaleh and four Labneh) were selected on the basis of the metagenetic results for more in-depth analysis. The metagenomic DNA was sequenced using Illumina HiSeq2500 technology at GATC-Biotech (Konstanz, Germany). Metagenomic reads corresponding to the *Capra hircus* genome were filtered out by reads

mapping against the *Capra hircus* reference genome (LWLT00000000) using Bowtie2 (Langmead & Salzberg, 2012). The proportion of goat reads in the samples was then assessed using samtools flagstat (Li et al., 2009). On the basis of the fastq file generated after host read filtration, we first estimated microbial composition by mapping the samples. Reads were mapped against the representative clade-specific marker catalogue contained in the MetaPhlAn tool v. 3.0.4 (Truong et al., 2015).

In addition, we performed taxonomic profiling using an assembly-based marker gene analysis, which allows non-supervised binning of metagenomes using metaSPAdes v.3.9 (Bankevich et al., 2012). Genes were then predicted using Prodigal (v.2.6.3) and marker genes were extracted using fetchMG, v.1.0 (Sunagawa et al., 2013). Taxonomic assignation was carried out with the COG0012, *ych*F gene. Summary species composition plots were created in R v.3.6.1 using the package ggplot2 v.3.3.2.

3.7 Metagenome-assembled genome (MAG) analyses

Genome binning was performed using MetaBAT2-2.12.1 (Kang et al., 2015), with a minimum contig size of 1,500 nucleotides and the default settings. The quality of the resulting prokaryotic bins was assessed with CheckM (Parks et al., 2015), and MAGs < 80% completeness and/or > 10% contamination were excluded. Several MAG assemblies were refined by additional binning, such as blastn analysis with reference genomes of related species. Contigs were then filtered by percentage of the length covered, identity and coverage levels. Finally, manual curing of questionable contigs (new, low coverage of homology on references, etc.) were performed by blastn and blastx analysis against the nr/nt and nr NCBI databases, respectively. Annotations were performed using the Rapid Annotation with Subsystem Technology server.

3.8 Phylogenomic and functional analyses

The Automatic Multi-Locus Species Tree web server (Alanjary et al., 2019) was used to determine closely related genomes based on core gene alignments of the sequenced genomes and recovered MAGs. The closest species were inferred based on the percentage of average nucleotide identity (ANI) calculated using FastANI, v.1.31 (Jain et al., 2018). The kSNP (v.3.0) was used to perform the phylogenomic analysis using distances based on single nucleotide polymorphism variations for the most representative species present in MAGs (Gardner et al., 2015) with the maximum likelihood option and kmer size of 21. Genomes of the corresponding species were downloaded from Genbank and combined with our MAG to compute this analysis.

Gene content analysis and metabolic reconstruction was performed on SEED using sequence and function-based comparison tools (Aziz et al., 2012). The search for antibiotic resistance (ABR) genes was performed by read mapping against the CARD database (Jia et al., 2017; McArthur et al., 2013) using the PATRIC web server (Antonopoulos et al., 2019). The presence of particular genes in reference genomes, ABR and genes of technological interest was determined using the PATRIC web server, the FoodMicrobiome tool (https://migale.jouy.inra.fr/foodMicrobiome/, and blast analysis. Additionally, FoodMicrobiome was used to detect the subdominant populations of given species with high sensitivity and reliability and to discriminate between subspecies.

3.9 Data availability

Raw sequences were deposited on the European Nucleotide Archive (ENA) under the BioProject ID PRJNA860091 for shotgun metagenomic, amplicon and genomic reads. The MAGs are available in doi: 10.17632/7w4rd76ndj.2.

4 Results

4.1 Goat farmer and dairy producer's survey

According to the results of the questionnaire, 25% of the respondents were identified as both producers and farmers, and 75% as producers only. Production depended on female housekeepers (60%) aged between 40 and 70 years old, and had limited access to the local market place (38%). Most of producers (88%) used only goat milk compared to 4% who used a mixture of goat and cow milk, while the rest used goat Laban in addition to goat milk as the primary dairy material. Producers who reared their own goat herd were able to process larger milk quantities for the three fermented products (135 to 7,500 L/season) than producers who purchased their goat milk (140 to 1,890 L/season). Farming and production parameters for each product are summarized in **Table S1**.

Ambriss, Serdaleh and Labneh El Darff were made with milk from the Black Baladi race that grazes in the Bekaa Valley, the Chouf Mountains and Southern Lebanon, respectively (**Figure 1A**). All production phases were completely manual, the production scale was small to medium (Third Quartile = 216.5 kg/season) and both tools and materials were artisanal. Production takes place during the period when the goats produce milk, i.e., from June to

November. The manufacturing process of Serdaleh and Ambriss lasts up to 6 months and consists of fermenting raw goat milk on a daily basis in terracotta jars, except for 20% of Serdaleh producers who used plastic barrels. The jars were first soaked with salt, periodically refilled with fresh raw milk and salt, with periodic whey evacuation from a hole at their bottom (**Figure 1B and S1**). The manufacturing of Labneh El Darff is conducted in a three-stage process consisting of: (i) Laban production, which is prepared from goat milk inoculated with a previously homemade inoculum; (ii) filling the "Darff" (bag formed by the whole goatskin, but sometimes cloth fabric) with the produced Laban; and (iii) straining of the whey from the suspended Darff during a period of 6 weeks (**Figure 1B and S2**). The manufacturing processes of these products are carried out at ambient temperature.

4.2 Physico-chemical and culture-dependent analyses

4.2.1 Temperature, pH and salinity

Temperature, pH and salinity levels were measured at the time the samples were collected (**Table S2**). The ambient temperature at which the products were developed varied between 28°C and 33°C for Ambriss and Labneh El Darff, but was lower ($p < 0.05$) for Serdaleh (between 20°C to 25°C). The pH values showed little variability within the same product type, unlike salinity, which displayed greater intra-group variability (**Figure 2A)**. The pH values were comprised between 3.01 and 4.80. They were higher ($p < 0.05$) in Ambriss (4.08 to 4.80) when compared to Serdaleh (3.01 to 4.12) and Labneh El Darff (3.62-4.62). The Serdaleh product had the highest level ($p < 0.05$) of salinity (2.69 to 5.04%) compared to Ambriss (0.87 to 2.24%) and Labneh El Darff (1.10 to 1.75%).

4.2.2 Culture-dependent analyses

The examination of culture results shows a significant variability in the different microbial enumerations (**Figure 2B**). We observed a dominance of mesophilic lactobacilli (MRS 30°C, 5.4-6.8 Log CFU/g) over lactococci (M17 30°C, 0.5-5.20 Log CFU/g) in the three products. Thermophilic lactobacilli counts were highly variable in Labneh el Darff (MRS 42°C, 1-6.2 Log CFU/g) compared to its other counterparts.

Overall, 279 strains belonging to 13 species groups were genetically identified by 16S rRNA from 380 isolates. The most commonly cultivated species in the three fermented milks was *Lactiplantibacillus plantarum*. This species appeared to be dominant in Ambriss (38% of isolates in this product) and Serdaleh (77%), and co-dominant with other species in Labneh El

Darff (16%). In Ambriss, we also identified a strong prevalence of the species *E. durans* and *Lacticaseibacillus rhamnosus* (18% of each), in Labneh El Darff, *Levilactobacillus brevis* was the most frequently obtained cultured species (32% of isolates), followed by *L. delbrueckii* and *Lc. lactis* (16% of each) (**Figure 2C).**

4.3 Taxonomic composition using amplicon sequencing

The 16S rRNA amplicon-sequencing analyses were clustered in 209 OTUs, whose abundance was over the threshold of 0.005% in the whole dataset. The rarefaction curves of most of the analyzed samples reached the saturation plateau, indicating that the sequencing depth was sufficient (**Figure S3**).

The alpha-diversity analysis revealed lower richness of species in Labneh El Darff ($p < 0.05$) compared to the other two products (**Figure 3A**). Moreover, the Chao1, Shannon and InvSimpson indices, which measure evenness, confirmed this low diversity. The Principal Coordinates Analysis (PCoA) showed that while Labneh El Darff samples were clearly differentiated, samples of Serdaleh and Ambriss could not be clearly separated and thus likely shared a similar bacterial microbiota (**Figure 3B**). Indeed, the analysis of their taxonomical composition (**Figure 3C** and **Table S3**) showed that *Lactobacillus delbrueckii* was the main species present in Labneh El Darff samples, representing 55 to 82% of the counts, whereas it was undetected or at levels below 0.4% in the samples of Ambriss and Serdaleh. Moreover, the samples of the latter products contained *L. lactis*, *L. kefiranofaciens* and *Lentilactobacillus kefiri* as the major species and, to a lesser extent, *L. diolivorans*-group. Differential analysis of OTUs showed that Serdaleh samples had greater *L. lactis* abundance than Ambriss ($p_{adj} < 0.01$). Remarkably, in the PCoA (**Figure 3B**), these two products appeared to be spread along Axis 1 as a function of the proportion between Lactococcaceae and Lactobacillaceae species. In particular, a group of 12 Serdaleh samples contained *L. lactis* at levels of 58-83%, while the other samples were intermixed on the other part of this axis. Moreover, the 13 samples of Serdaleh collected in 2019 contained low levels (0.67-2.2%) of *Mycoplasmopsis agalactiae* or *M. bovis* (**Table S3**). Finally, three Ambriss samples showed divergent compositions compared to the others: A3-b was dominated by *Lentilactobacillus buchneri*, A4 by the *S. parasuis-sanguinis* group (> 53% of the reads), and A6 by high amounts of species poorly present in other samples such as *L. gasseri* (~36%), *Lacticaseibacillus rhamnosus* (~23%) and Enterobacteriaceae (> 18% of reads). The other species, usually detected at low levels in the various samples, mainly belonged to the

Gammaproteobacteria class, such as *Aeromonas sp.*, *Citrobacter sp.*, *Klebsiella sp.* and *Escherichia-Shigella.*

4.4 Refined microbiota by metagenomics

For a refined taxonomical analysis, 16 samples were selected to perform shotgun metagenomic approaches. Such analyses allow a taxonomic identification up to the species-strain level and the simultaneous assessment of the level of reads corresponding to bacteria and fungi. Moreover, these analyses make it possible to detect eventual virulence factors in pathogenic species, antibiotic resistance genes and genes of technological interest.

Food metagenomic samples may contain animal reads, which may significantly bias the analysis when they are in high abundance. Since the present products are manufactured from goat milk, we tested for the presence of *Capra hircus* reads that were detected at levels ranging from 2.3 to 72% (**Table S4**). They were notably abundant in Labneh El Darff samples where they accounted for more than 50%, as a consequence of the process mode where the product is drained in a goat skin.

4.4.1 Species-level assignments by shotgun met

In a first approach, the MetaPhlAn tool, which identifies species by mapping the metagenomic reads against a genomic database, was used to determine the taxonomic composition of the samples. It yielded 98 bacterial and one eukaryotic species (**Table S5**). Species composition obtained by this analysis was relatively in accordance with those obtained with the 16S rRNA amplicon. Interestingly, it resolved most taxonomical assignation ambiguities for lactic acid bacteria such as *Lentilactobacillus kefiri, Lentilactobacillus diolivorans, Lactiplantibacillus plantarum, Lactobacillus helveticus* and *S. thermophilus*, and also for contaminants such as *S. parauberis* and *M. agalactiae*. It also detected the yeast *Saccharomyces cerevisiae*, but at low levels and in only three samples.

In order to characterize microbiota independently of fixed references, we applied a marker gene analysis from the assembled metagenomes, which yielded 104 bacterial and 15 fungal species (**Table S6**). The overall composition is shown in **Figure 4**. Although the main bacterial species identified here were consistent with the previous analysis made by read profiling, some differences merit highlighting. Whereas MetaPhlAn attributed 35% of the reads of A4 to *S. parauberis*, the marker gene analysis detected only 6.9% of this species and further detected another streptococcal species, *S. parasuis*, which retained 55% of the reads. This species was

also detected at low levels in four other samples, while *S. parauberis* was found in eight samples, generally at low levels. This analysis also confirmed the frequent presence at low levels of several species of Enterobacteriaceae such as *E. coli, K. pneumoniae* and *C. freundii*, and of Enterococcaceae such as *E. faecalis* and *E. faecium.* Lastly, it also established that *L. lactis* subsp. *lactis* was detected in all samples, whereas the *cremoris* subspecies was detected at concentrations ten times lower in S8 and S10-b. A specific analysis by read mapping confirmed the presence of *L. lactis* subsp. *cremoris* at 5-15% lower abundance than that of the *lactis* subspecies in most samples.

Finally, 14 species of yeasts whose genomes are absent from the MetaPhlan database were identified by marker gene analysis. The most frequent and abundant were *Pichia kudriavzevii* and, to a lesser extent, *S. cerevisiae* and *Kluyveromyces marxianus*. Nevertheless, yeast relative abundance was generally below 1%, except in Labneh El Darff where it reached 8%. A more sensitive analysis performed by read mapping (**Table S7**) confirmed the presence of these yeasts at low levels in more samples, as well as that of *Torulaspora delbrueckii.*

4.4.2 MAGs and strain-level analyses of LAB

A total of 62 prokaryotic Metagenome Assembled Genomes (MAGs) of high quality were reconstituted from the metagenome assemblies (**Table S8**). The majority of the MAGs correspond to Lactobacillaceae such as *L. diolivorans* (12), *L. kefiri* (10), *L. kefiranofaciens* (9), *L. plantarum* (5) and *L. gasseri* (1). The two yogurt species *L. delbrueckii* subsp. *bulgaricus* (1) and *S. thermophilus* (1) were also recovered from Labneh El Darff and MAGs of *Lactococcus lactis* subsp. *lactis* (5) were reconstituted from Ambriss and Serdaleh products. In addition, the results included MAGs of the goat pathogen *M. agalactiae* (6) from Serdaleh samples, and of milk contaminants such as *S. parauberis* (3), *S. parasuis* (1), *Escherichia coli* (1), *Klebsiella pneumoniae* (1), *Aeromonas caviae* (2) and *Acetobacter spp.* (2).

Further detailed analysis was performed with the lactococci and lactobacilli MAGs and genome sequences of the most frequent and sometimes dominant species in the three products. Phylogenomic analysis of the *L. lactis* FME150 genome and the five *L. lactis* MAGs was performed with 215 reference genomes of this species, representing strains from its two main subspecies (*lactis* and *cremoris*) and isolated from different environments (dairy, vegetable, animal, etc.) (**Figure S4**). In this analysis, five groups could be highlighted, one of which included all the representatives of the *cremoris* subspecies (brown branches), and three were

formed by (i) industrial dairy strains (in red), (ii) diacetylactis biovariant strains (in blue), and (iii) dairy strains isolated from traditional products (in violet). The last group (green branches), which was the most diverse, contained environmental strains (plants, animals, etc.). The five *L. lactis* MAGs from Ambriss sand Serdaleh were included in this last group (highlighted by external red arrows).

A similar study was done with *L. kefiranofaciens, L. kefiri L. diolivorans* and *L. plantarum* **(Figure S5)**. The *L. kefiranofaciens* FME151 genome and the eight *L. kefiranofaciens* MAGs assembled from Serdaleh (6) and Ambriss (3) metagenomes were compared to seven genomes present in the NCBI database, including those of the two type strains for the subspecies *kefiranofaciens* and *kefirgranum*. These strains were isolated from kefirs of different origins. ANI analysis showed that all strains shared at least 98.1% identity and could be divided into two main groups, one made by kefir strains and the second by Ambriss and Serdaleh strains, where ANI were ~99.2-99.4% within each group and ~98.2-98.4% between groups. Further phylogenomic analyses **(Figure S5A)** showed that two groups could be differentiated within kefir strains, a first one with three strains sharing over 99.9% identity and including the *L. kefiranofaciens* subsp. *kefiranofaciens* type strain, and a second one with four strains that were more divergent between themselves, sharing 99.0 to 99.8% identity and including the *L. kefiranofaciens* subsp. *kefirgranum* type strain. Lastly, Ambriss and Serdaleh strains also formed two groups sharing 99.4 to 99.8% ANI within and 99.1 to 99.3% outside of their group. Analysis of gene composition confirmed and refined these results. First, it showed a very high relatedness of three *L. kefiranofaciens* subsp. *kefiranofaciens* strains from kefir that shared more than 95% of their genes (> 2,325 out of ~2,440 genes), while within each of the other groups, the strains were more divergent between themselves (87% of shared genes in the *kefirgranum* group, 88% in the Serdaleh group and 92% in the Ambriss group). Seventeen EPS-related genes involved in kefiran production present in a 14.4-kb region were present only in the three former strains, whereas only the first part of the operon containing the genes of enzymes involved in EPS regulation and priming was conserved in the other strains. Furthermore, in the latter strains, the genes involved in polymerization, chain length determination and export that could be characterized appeared to be different in each strain, indicating that they produce polysaccharides other than kefiran.

The *L. kefiri* FME71 genome and the ten *L. kefiri* MAGs assembled from Ambriss (3), Serdaleh (6) and Labneh El Darff (1) were compared to the ten reference genomes present in the NCBI database, including seven strains isolated from kefir, two from human gut and one from

yogurt. This analysis showed that all strains shared very high ANI values (> 99.55%), indicating that they were closely related (**Table S9**). Analysis of their phylogenomic tree did not indicate clustering as a function of the origin of the strains (**Figure S5B**). Furthermore, the analysis of the core/pan genome showed that all strains shared ~2,148 genes out of ~2400. The analysis of the specific genes in the different strains indicated that they code mostly for "hypothetical proteins" (50%) and mobile element proteins (25%).

ANI analysis of *Lb. diolivorans* FME74 and of our 12 MAG strains with that of the type strain (isolated from maize silage) and one MAG from an ultra-filtered-milk-permeate fermenting bioreactor showed that these strains could be clustered into four groups (**Figure S5C**), displaying at least ~97.7 to 98.4% ANI . Serdaleh MAGs formed a group of very closely related strains (ANI > 99.9%), Ambriss and Labneh MAGs formed a second homogenous group (ANI > 99.1%), whereas the bioreactor MAG and the type strain were isolated.

The sequences of *L. plantarum* FME91 strain and of the five MAGs obtained from Serdaleh were analyzed with a set of 145 *L. plantarum* strains isolated from different ecosystems, including several types of food, animals, gut, etc. The five MAGs from Serdaleh appeared to be almost identical (ANI > 99.97%), but unrelated to that of FME91 from Ambriss (ANI ~92.7%), and they ranged between 95.7 and 99.7-99.9, with an average of 98.7 with the strains of the set (**Table S9**). An overview of the phylogenic relationship between these strains is given by the tree obtained by kSNP analysis (**Figure S5D**). Strains were dispersed irrespective of their environmental origin. The Ambriss strain FME91 was closely related to those isolated from milk or artisanal dairy products, whereas the Serdaleh strains were closely related to those isolated from meat, fruits and vegetables.

4.4.3 Detection of pathogens by genomic analysis

Marker gene analysis showed that undesirable species were present in several Ambriss and Serdaleh samples. All Serdaleh samples collected in 2019 contained low levels (1-4%) of the goat pathogen *M. agalactiae*. Moreover, zoonotic streptococcal species, namely *S. parauberis* and *S. parasuis*, were detected at 0.5-2% in several samples of Ambriss and Serdaleh and, remarkably, at 7 and 55%, respectively, in Ambriss A4. *S. aureus* was detected at low levels in Serdaleh S10-b. Finally, Enterobacteria such as *E. coli* and *K. pneumoniae* were detected in about half of the samples at levels < 1% (except for *K. pneumoniae* present at 2.7% in Ambriss A1). Deep profiling analysis in the metagenome samples was performed to detect the presence at

low abundance of pathogen species potentially found in dairy products (**Table S7**). No *Brucella abortus*, *Listeria monocytogenes*, *Mycobacterium vaccae* or *Staphylococcus agalactiae* were detected. However, *S. aureus* was detected at low levels in five Serdaleh samples.

Further analyses were performed on these undesirable species, which were sufficiently covered to yield MAGs. An ANI analysis of these MAGs with representative genomes of these species confirmed the taxonomic classification previously obtained by the *ychF* marker. For example, *M. agalactiae*, *S. parauberis* and *K. pneumoniae* MAGs share 99.8, 98.5 and 98.4% ANI with the type strains of their species, *E. coli* MAG; 99.1% with *E. coli* K12 laboratory strain, *S. parasuis* MAG; and 97-98% with four genome references of this species present in the NCBI database.

Gene content analysis was performed with *S. parasuis* MAG_A4, which appears to be dominant in Ambriss A4. In particular, genes involved in lactose metabolism were searched to explain the fitness of this strain in milk fermentation. Among the two systems used for lactose utilization, MAG-A4 contains the Lac-PTS system, including ten genes encoding the Lac-PTS transporter, the phospho-beta-galactosidase, the tagatose pathway, glucokinase, a repressor and a gene of unknown function (LacX). A blast search with these genes provided a hit at 97-99% nucleotide identity on its entire length with an ICESsuJH1308-1 mobile element present in *S. suis* JH1308 and a lower identity with several other *S. suis* strains. However, none of the nine *S. parasuis* genomes available contained these genes. These data show that *S. parasuis* MAG_A4 acquired lactose utilization genes by horizontal transfer, possibly from *S. suis*.

Interestingly, MAGs from *M. agalactiae* were of very good quality, probably due to particular features of their sequences, such as genomic guanine-cytosine content (~29.7%) and di-tri-nucleotide composition, allowing a better binning. In particular, MAGs from Serdaleh S8 and S9 consisted of only four and ten contigs, respectively. ANI analysis indicated that the strains from the Serdaleh samples were very closely related to each other (ANI > 99.9%), as well as to the type strain (ANI ~99.85%; **Table S**). Phylogenomic analysis confirmed the close relationship of *M. agalactiae* Serdaleh MAGs together and with $PG2^T$ (**Figure S6**). Furthermore, gene content analysis indicates that these two MAGs differed from $PG2^T$ by less than 20 genes encoding hypothetical proteins.

4.4.4 Detection of antibiotic resistance genes

A mapping-based approach against a comprehensive collection of antibiotic resistance genes was performed. A total of 18 ABR genes were identified, belonging to seven different classes of antibiotics (**Figure 5**). They were detected in almost all samples, and notably those from Serdaleh had higher levels and more diverse genes than those from Ambriss. Concerning the Labneh samples, only sample L18 presented detectable amounts of ABR. However, it cannot be concluded that L15, L16 and L17 samples did not contain ABR because there were not enough useful reads in these samples due to the presence of goat DNA.

Overall, the most abundantly detected antibiotic classes were aminoglycosides, MLS (Macrolide, Lincosamide, Streptogramin), nucleosides and tetracycline. In particular, Serdaleh samples contained high levels of aminoglycoside (*aad*(6) and APH(3'')-IIIa), MLS (*ermB*), nucleosides (*sat-4*) and tetracycline (*tetS*). The coverage of these genes is similar to that of *L. lactis*, which was the highly dominant species in these samples. Since this level of coverage allows a reliable level of assembly, we searched for contigs carrying these determinants by BLAST analysis in Serdaleh samples. This search pointed out two contigs of 12,360 and 3,370 nucleotides in sample S10-b, with coverages ~2 times that of *L. lactis*, and carrying, respectively, the four first determinants and *tetS*. The first contig is ~98% identical on 76-85% of its length to large plasmids from *E. faecium, E. faecalis* and *Staphylococcus pseudointermedius*. The second contig is identical on its full length to regions of plasmids and genome drafts of various food bacteria such as *L. raffinolactis, L. lactis, S. thermophilus, Carnobacterium divergence* and *Listeria innocua*. The other Serdaleh metagenomes contained contigs of comparable sizes indicating similar events.

The metagenome of Serdaleh S10-b also contained a ~6.6 kb contig carrying the *tetL, tetM* and *cat* genes that was identical on its entire length to regions of plasmids from *E. faecalis*. The coverage of this contig is 40 times lower compared to the two former ones, which is a level corresponding to that of *L. kefiri, L. kefiranofaciens* and *L. diolivorans*. None of the other metagenomes contained these three genes together on one contig. Nevertheless, samples S8, S7-b, S8-b, S9-b and S11-b contained contigs of ~5 and 0.9 kb carrying *tetLM* and *cat* genes, respectively. The coverages of these contigs were significantly different in the other assemblies where *tetLM* coverage was 2 to 10 times higher that of *cat* genes, indicating that their organization might be different from that found in S10-b.

Lastly, in Ambriss A4, *tetM* was found on a ~32 kb contig of S. *parasuis*, *ermB* on a 7 kb contig sharing 90% identity on 3 kb with plasmids of various Lactobacillaceae, *cat* on a ~3 kb contig sharing 93% identity to *L. garviae* plasmids, *aad* and *sat* on a ~23 kb contig sharing 93% identity on ~12 kb (including *aad* and *sat*) of the *S. agalactiae* B111 chromosome and 99% identity on 4-6 kb of *E. faecalis* plasmids and the *S. pseudointermedius* chromosome.

5 Discussion

Traditional Lebanese fermented goat milk products are perceived in Lebanon as having an important historical and cultural heritage, but their microbiota are still poorly characterized. A detailed characterization of 50 producers allowed us to select those who used genuine traditional processes for the production of Ambriss, Serdaleh and Labneh El Darff. The manufacturing processes were established and the physico-chemical characteristics of the products were determined. Our survey also made it possible to define the socio-economic profiles of the producers, which confirmed the danger of extinction of these products, thus making it possible to require a Protected Designation of Origin label. This study explored the microbial biodiversity of 34 samples of three fermented goat milk products from three regions by combining microbiological, genomic and metagenomic approaches.

The samples analyzed here shared similar technical properties for pH, salinity, temperature and microbial counts as those of the same products previously studied (Dimassi et al., 2020; Serhan et al., 2016; Nasbimana et al., 2005; Semaan Hajj et al., 2011), and were therefore probably (Semaan Hajj et al., 2011) representative of these products in Lebanon.

5.1 Microbial originality of traditional Lebanese fermented goat milk products

An overall view of the microbiota is provided by metagenetic analysis, which deciphers species or lower taxonomic levels. This study clearly separated Labneh samples from those of Ambriss and Serdaleh. Labneh contained a relatively simple microbiota, strongly dominated by *L. delbrueckii*, which was almost absent from the other products. Furthermore, it contained *L. diolivorans*, *L. kefiri* and *S. thermophilus* to a lesser extent. The dominance of *L. delbrueckii* is due to the way Labneh is inoculated by Laban, whose composition is that of yogurt made of *L. delbrueckii* and *S. thermophilus* (Chammas et al., 2006). The dominance of *L. bulgaricus* is probably due to its better resistance to acidity than *S. thermophilus* (Béal & Chammas, 2012). Similar observations were made with yogurt and Doogh (also known as Ayran), a traditional Iranian fermented ewe milk product (Davati & Hesami, 2021). The presence of *L. diolivorans*

and *L. kefiri*, which are also found in Ambriss and Serdaleh, might be due to the specificity of the local goat milk. Moreover, the conditioning of Labneh in a goatskin may also influence the microbial composition by releasing nutrients or other metabolic products, as indicated by the very high level of goat DNA obtained from the metagenomic data for Labneh. Interestingly, *L. diolivorans* is usually found in vegetable and fruit fermentation, whereas it is occasionally reported in dairy products (Tamang et al. 2005; C. Zhang et al., 2010; Zheng et al., 2020). To the best of our knowledge, it was isolated from one raw milk in Brazil (Agostini et al., 2018), traditional yogurts in Iran (RoushanZadeh et al., 2014), traditional fermented dairy products in Mongolia (Yu et al., 2011), and detected in metagenomic samples of Koumiss (You et al., 2022; Kozhakhmetov et al., 2014). The present study is thus the first one reporting the genome of *L. diolivorans* isolated from a dairy product.

Ambriss and Serdaleh samples contained, as dominant species, *L. lactis* and *L. kefiranofaciens*, which were almost absent from Labneh, followed by *L. kefiri* and, to a lesser extent, by *L. diolivorans* and *L. plantarum*. Interestingly, this composition was reminiscent of that of dairy kefir, a fermented milk with health properties, which is produced by the inoculation of a stable and complex microbial community made of lactic acid bacteria and yeasts entrapped in gelatinous grains (Blasche et al., 2021; Prado et al., 2015; Nalbantoglu et al., 2014). In this product, the dominance of *L. kefiranofaciens*, which otherwise grows poorly alone in milk, is attributed to the metabolic cooperation it develops with the community, and the synthesis of an exopolysaccharide.

In this respect, growth conditions in Ambriss and Serdaleh, where the microbiota remained essentially in a coagulated milk matrix, displayed strong similarities with the one encountered in the kefir grain, which may thus explain the dominance of this particular *Lactobacillus* and of several sub-dominant lactic acid bacteria. MAGs and genome comparisons of *L. kefiranofaciens* and *L. kefiri* confirm the very close phylogenomic relationship between kefir, Ambriss and Serdaleh strains. Their gene content mainly differed by unknown functions and mobile elements. In *L. kefiranofaciens* strains isolated from Ambriss and Serdaleh and in *L. kefirgranum* subspecies strains, only the first six genes encoding the general functions of initiating and controlling EPS synthesis were conserved, whereas glycosyl transferase conferring the specific structure of EPS units differed. This fact explains why these strains did not produce kefiran, the specific polysaccharide for kefir grain formation. Lastly, metabolic cooperation in Ambriss and Serdaleh certainly differed from that of kefir, as suggested by the low level of

Leuconostoc and *Acetobacter* species, which play an important role in milk fermentation and citrate metabolism (Laëtitia et al., 2014). Nevertheless, the low input of milk on a the coagulated milk matrix gradually offers an environment for this "slow growing species" that is *L. kefiranofaciens* (Blasche et al., 2021; Georgalaki et al., 2021). Since the microbiota of Ambriss and Serdaleh were not inoculated, the environmental origin of *L. kefiranofaciens* in these products still remains unknown. Interestingly, this species was also found to be dominant in several samples of Airag, a traditional Mongolian fermented mare milk made in horse-hide containers (Watanabe et al., 2008) and in Koumiss (M. Zhang et al., 2020), neither of which contain grain.

In the present set of samples, the balance between *L. lactis* and *L. kefiranofaciens* appeared to be the main factor differentiating Ambriss and Serdaleh, the former generally containing lower levels of *L. lactis* and higher levels of *L. kefiranofaciens*. Nevertheless, the samples of these two similarly processed products, originating from two distinct regions, could not be clearly separated since some samples of Serdaleh were also dominated by *L. kefiranofaciens*. Although Serdaleh samples were obtained at lower temperatures, displayed higher salt content, and reached lower pH in comparison to Ambriss samples, these factors do not fully explain this population shift. Alternatively, there may be subtle differences in production methods, such as the quantity of milk added at once, or a potential mixing that modifies the speed of milk infiltration through the product, which may affect the metabolism of the microbial communities in different ways, as discussed earlier. For example, if a significant quantity of the milk poured into the jar is not mixed with the curd, it may favor a rapid planktonic development of *L. lactis*, compared to the slow solid-state development where *L. kefiranofaciens* and *L. kefiri* compete more effectively.

A second important outcome of this study is the detection of uncultivated species as dominant microbiota in Ambriss and Serdaleh. While the initial failure to isolate *L. kefiranofaciens* from the samples can be explained by the fact that this species is tedious to grow and requires specific media and growth conditions (Georgalaki et al., 2021), the lack of recovery of *L. lactis* is less understandable. Indeed, this bacterium is routinely cultured from different environments such as plant or dairy products (Soundharrajan et al., 2021; Li et al., 2020). In this work, we thus tested the hypothesis that *L. lactis* cells were viable but not-cultivable (VBNC) (Oliver, 2010) and would thus require specific conditions to recover growth throughout their transfer from the product to M17 medium. Actually, a transfer of several hours in media

containing goat milk allowed a straightforward isolation of strains of this species. This result indicates that the production process of Ambriss and Serdaleh induced a particular physiological state in *L. lactis* cells that hampers its growth recovery, thus corresponding to the definition of VBNC. Indeed, during the Ambriss and Serdaleh process, various environmental factors (such as pH, salinity and temperature) and the physiological age of the culture could induce the entry of bacterial cells into a VBNC state. Interestingly, the present process is still reversible, which is rarely the case (Dong et al., 2020), and hence offers a reproducible model for the study of the entry into the VBNC state. Further work would be required to understand why *L. lactis* growth recovery was deprived when directly plated on M17.

5.2 Potential safety concerns

A wide metagenomic search for bacteria potentially involved in public health issues indicated the presence of DNA of several kinds of undesirable species. Among hygiene indicators, Enterobacteria were detected at low levels in about half of the samples, as well as *S. aureus*, which was detected at low levels, mostly in Serdaleh samples. These results are comparable to those of a recent study on raw cow milk where, from a total of 195 samples collected from different Lebanese farms, 65% and 17% of the samples were non-compliant with national standards for *Escherichia coli* and S. *aureus*, respectively (Joubrane et al., 2022). Enterobacteriaceae are often reported as a typical bacterial population in raw milk from many mammalian origins (Ramos & Nascimento, 2021). They can also be transmitted to milk and dairy products by fecal contamination during the milking process or because of poor hygiene conditions (Giraffa, 2003). *S. aureus* was highly prevalent in some products and was the most frequent contaminant of milk and goat milk products (Oliveira et al., 2020; Xing et al., 2016). The transmission of *S. aureus* to milk is due to its ability to form biofilms and to remain on surfaces and utensils, its presence being mainly attributed to poor handling during processing (Kümmel et al., 2016).

A striking finding was the presence of several zoonotic streptococcal species such as *S. parauberis* and *S. parasuis*, which were detected at 0.5-2% in several samples of Ambriss and Serdaleh, respectively, and even at 7 and 55% in Ambriss A4. *S. parasuis* is an important pathogen for humans and pigs (Nomoto et al., 2015) and was already isolated as a contaminant in raw camel milk. Intriguingly, in Ambriss A4, *S. parasuis* was strongly dominant over the traditional lactic acid bacteria, which accounted for only 10% of the population. The fact that the

pH of this sample has a value similar to that of the other samples indicates that the strain of *S. parasuis* present in this sample is an acidifying strain. Indeed, our analysis revealed the presence of ten genes encoding a Lac-PTS system in its genome, similar to the one that allows efficient lactose utilization in several lactic acid bacteria such as *L. lactis* (Aleksandrzak-Piekarczyk, 2013). These genes were probably acquired by a horizontal gene transfer from *S. suis*. Adaptation to lactose utilization in milk has already been observed for other non-starter streptococci, in particular, *S. infantarius* in fermented dairy products from East and West Africa (Jans et al., 2013) and in artisanal cheese from Brazil (Kothe et al., 2021).

Serdaleh samples contained low levels (1-4%) of the goat pathogen *M. agalactiae*. This bacterium is one of the causal agents of contagious agalactia, a serious enzootic disease of small ruminants, which notably occurs in the Mediterranean Basin (Kumar et al., 2014). Once it has infected the animal, it disseminates though the blood to different mucosa, including the mammary gland, and can consequently be found in milk. *M. agalactiae* has already been detected in artisanal cheeses via non-cultural techniques (Martín-Platero et al., 2009). Its presence here is, therefore, a sign that the herd in the Chouf region was infected with this pathogen.

Finally, transmissible antibiotic resistance genes, which are considered to be a growing threat to public health (Ghimpețeanu et al., 2022; Yadav & Kapley, 2021), were detected. In particular, Serdaleh samples contained a multi-resistance cassette carrying aad(6) and APH(3'')-IIIa, ermB and sat-4, as well as tetracycline resistance genes. Since these antibiotics are used in prophylaxis for *M. agalactiae*, it is likely that such treatment contaminated the milk and favored the emergence of antibiotic resistant lactic acid bacteria strains.

6 Conclusion

In this study, we analyzed three artisanal Lebanese fermented products (Ambriss, Serdaleh and Labneh El Darff) that are processed from the raw milk of the Black Baladi goat, a local breed. The process consists of the daily introduction of salted milk or Laban into a terracotta jar or a goatskin over several months, together with the draining of the whey, until the container is full. This production remains highly artisanal and offers opportunities for the empowerment of women, but it is in danger of extinction due to the low volumes that are produced, the age of the producers and the absence of marketing. In industrial products that are inoculated with starters or by backslopping, the microbiota rapidly develops in large batches of

milk, whereas in the current products, it develops slowly during percolation of the milk that is added periodically. During batch growth, fast-growing microorganisms are highly favored, whereas in a dense community, slow-growing bacteria can compete more effectively, as is the case with the well-described kefir grain where *L. kefiranofaciens* clearly predominates despite its poor growth performance in milk. Interestingly, kefir is promoted as a probiotic beverage and *L. kefiranofaciens* is presented as a key factor in this attribute (Georgalaki et al., 2021). Although no studies have yet proven this, traditional Lebanese goat milk products are believed to deliver health benefits, and it would thus be interesting to further test this possibility. These products are also interesting as sources of biodiversity because they contain strains of *L. kefiranofaciens* and *L. kefiri*, which are genetically quite different from those present in kefir that are highly conserved.

Furthermore, progress has to be made to ensure the safety of these products from herd prophylaxis to milk chain management via a global action in order to avoid contamination by potential pathogens and the spread of antibiotic resistance genes. Finally, this study also provides key elements related to the microbiology and microbiota functioning of these products that will facilitate the development of strategies to improve these processes and preserve their identity.

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9 Figure legends

Figure 1. Geographical origin (A) and schematic processing diagram (B) of Ambriss, Serdaleh and Labneh El Darff.

Figure 2. Physico-chemical and microbiological characteristics of Ambriss, Serdaleh and Labneh El Darff: Temperature, pH and salinity **(A)**; boxplot representation of microbial counts on M17 and MRS **(B);** and histogram of isolated species **(C)**.

Figure 3. Metagenetic analysis based on OTU data of Ambriss, Serdaleh and Labneh El Darff: Boxplots of alpha-diversity indices **(A);** Principal coordinate analysis among bacterial communities identified by OTUs **(B);** bacterial composition plot depicting the relative abundances of the 20 main OTUs **(C)**.

Colors in **(A)** and **(B)** refer to the different fermented milk products, i.e., yellow for Ambriss, blue for Serdaleh and red for Labneh El Darff.

Figure 4. Relative abundance of microbial composition of Ambriss, Serdaleh and Labneh El Darff.

Values were calculated from the coverage of the *ychF* marker gene assembled in the metagenomes. The asterisk (*) indicates a low depth of the sequences for L15, L16 and L17 samples.

Figure 5. Heatmap showing distribution of the 18 antibiotic resistance genes detected in reads/kb within the 13 metagenomic samples.

MLS: Macrolide, Lincosamide and Streptogramin.

10 Supplementary Material

Figure S1: Flow chart of the Ambriss and Serdaleh manufacturing process

Figure S2: Flow chart of the Labneh El Darff manufacturing process

Figure S3. Rarefaction curves depicting the depth of 16S sequencing and species richness for the data obtained from Ambriss, Serdaleh and Labneh El Darff.

The x-axis represents the sequencing depth (reads) and the y-axis represents the estimated OTU richness detected at the species level.

Figure S4. Phylogenomic tree for *Lactococcus lactis*, highlighting the different groups of *L. lactis.*

The colors of the branches make it possible to visualize cremoris subspecies (brown), environmental sp. lactis strains (green), industrial dairy sp. lactis (red), *diacetylactis* biovariant (blue) and *lactis* sp from traditional dairy products (violet). The internal circle presents the environment from which the strains were isolated (blue: milk; green: vegetable; purple: animal). Ambriss and Serdaleh MAGs and the isolated strain genome are highlighted by external red arrows and names are shaded in gray

Figure S5: Phylogenomic tree for *L. kefiranofaciens* **(A),** *L. kefiri* **(B)**, *L. diolivorans* **(C)** and *L. plantarum* **(D)**.

Figure S6: Phylogenomic tree for *M. agalactiae*.

Table S1: Producer survey results of production parameters, rearing practices and price indicators related to Ambriss, Serdaleh and Labneh El Darff.

Table S2: Physico-chemical parameters and culture counts of Ambriss, Serdaleh and Labneh El Darff samples as a function of their producers and production dates.

Table S3. Percentage (%) of bacterial operational taxonomic units (OTUs) in the samples.

Table S4. Percentage (%) of *Capra hircus* reads aligned in each fermented food metagenome.

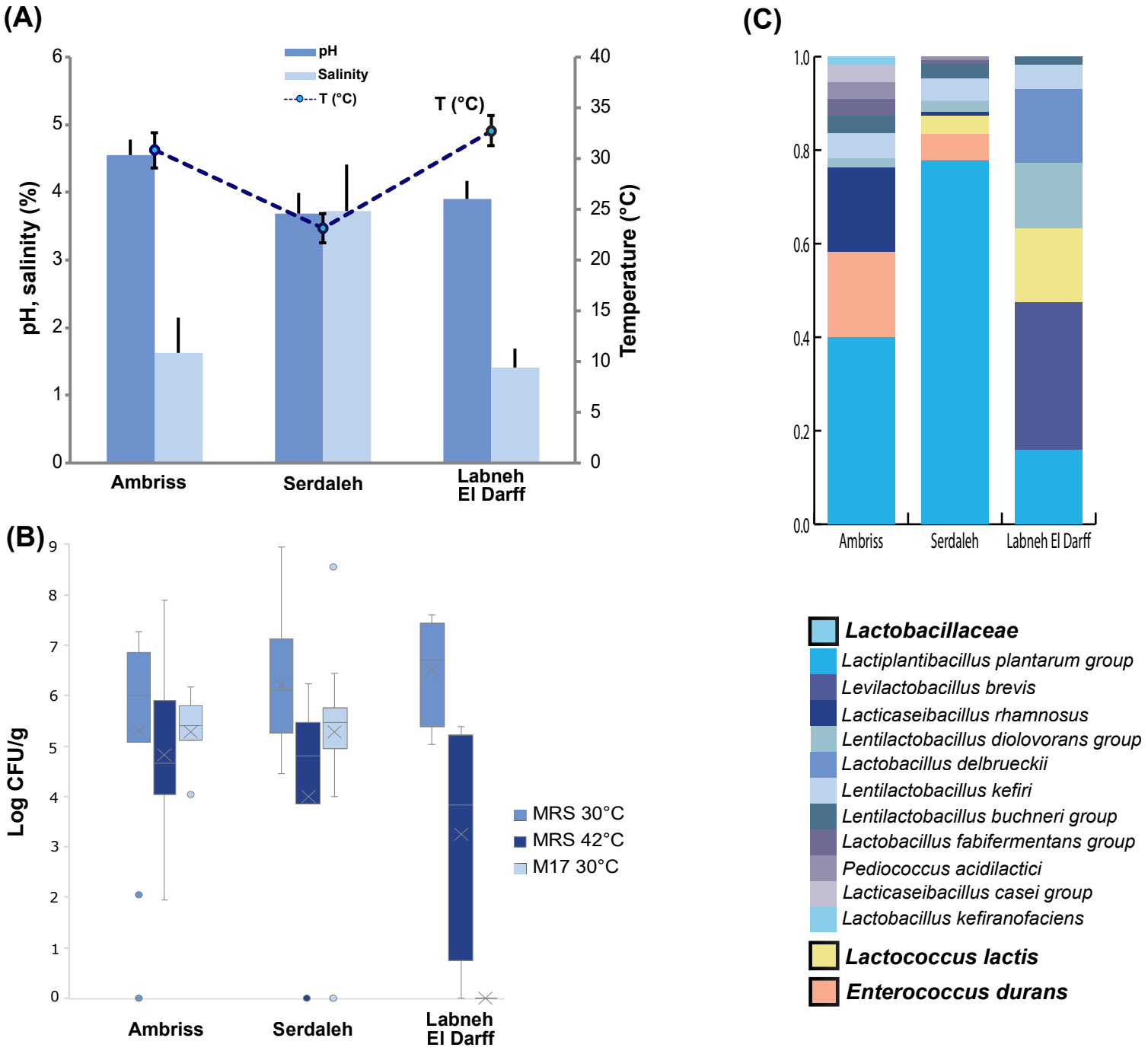
Table S5. Relative abundance of microbial composition of 16 traditional fermented goat milk metagenomes from Lebanon using the MetaPhlAn taxonomic assignment method.

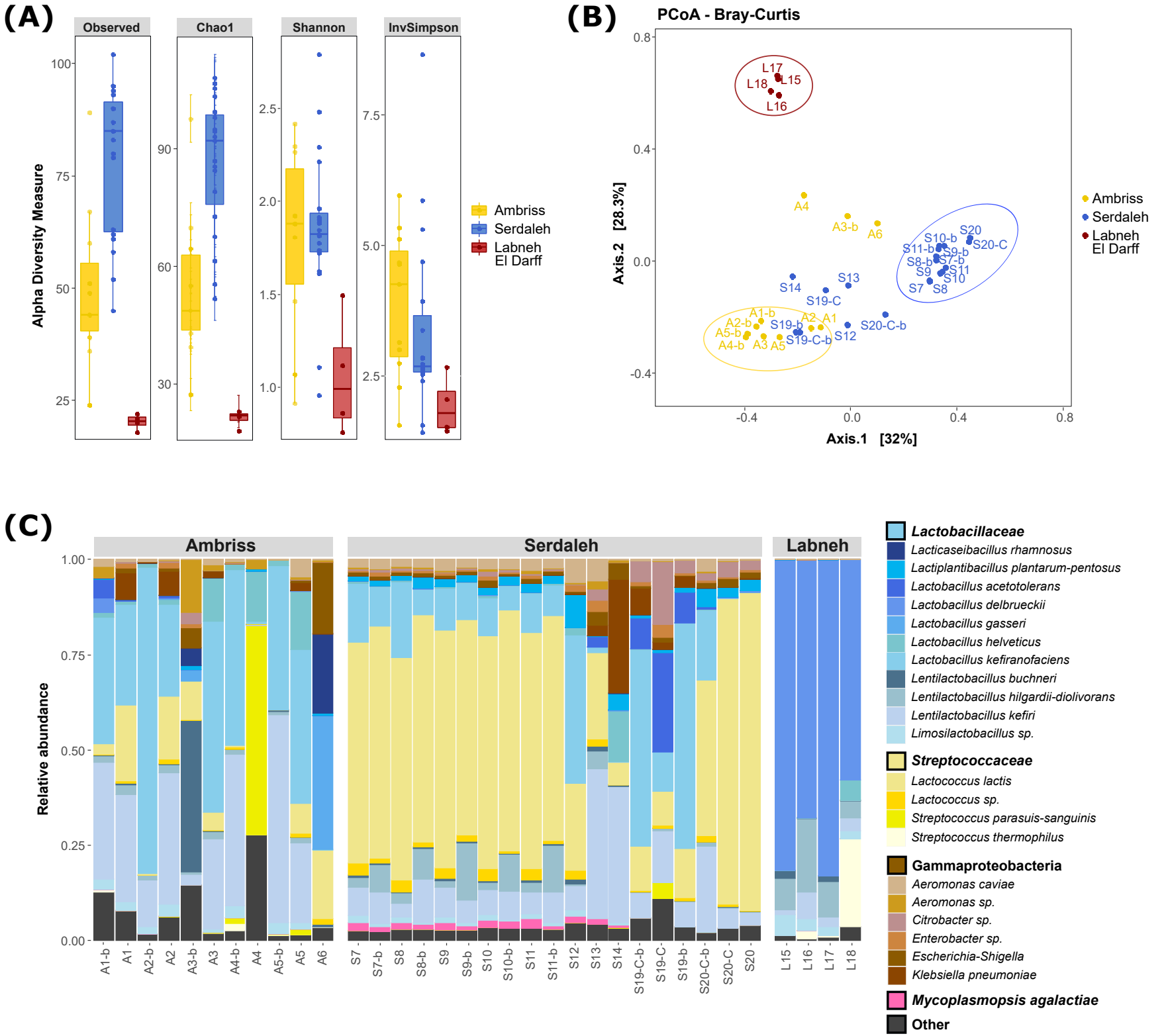
Table S6. Relative abundance of microbial composition of 16 traditional fermented goat milk metagenomes from Lebanon using *ychF* marker gene assignation.

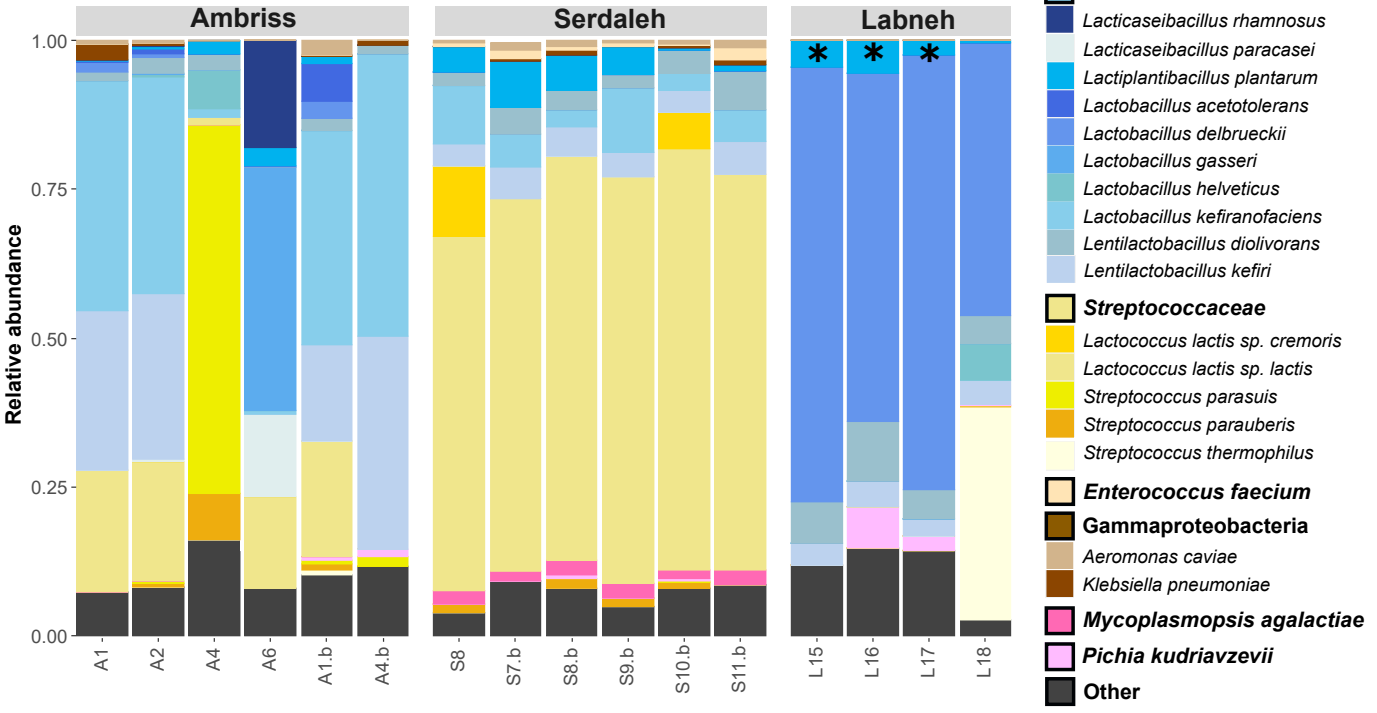
Table S7. Relative abundance of microbial composition of 16 traditional fermented goat milk metagenomes from Lebanon using the *FoodMicrobiome* tool.

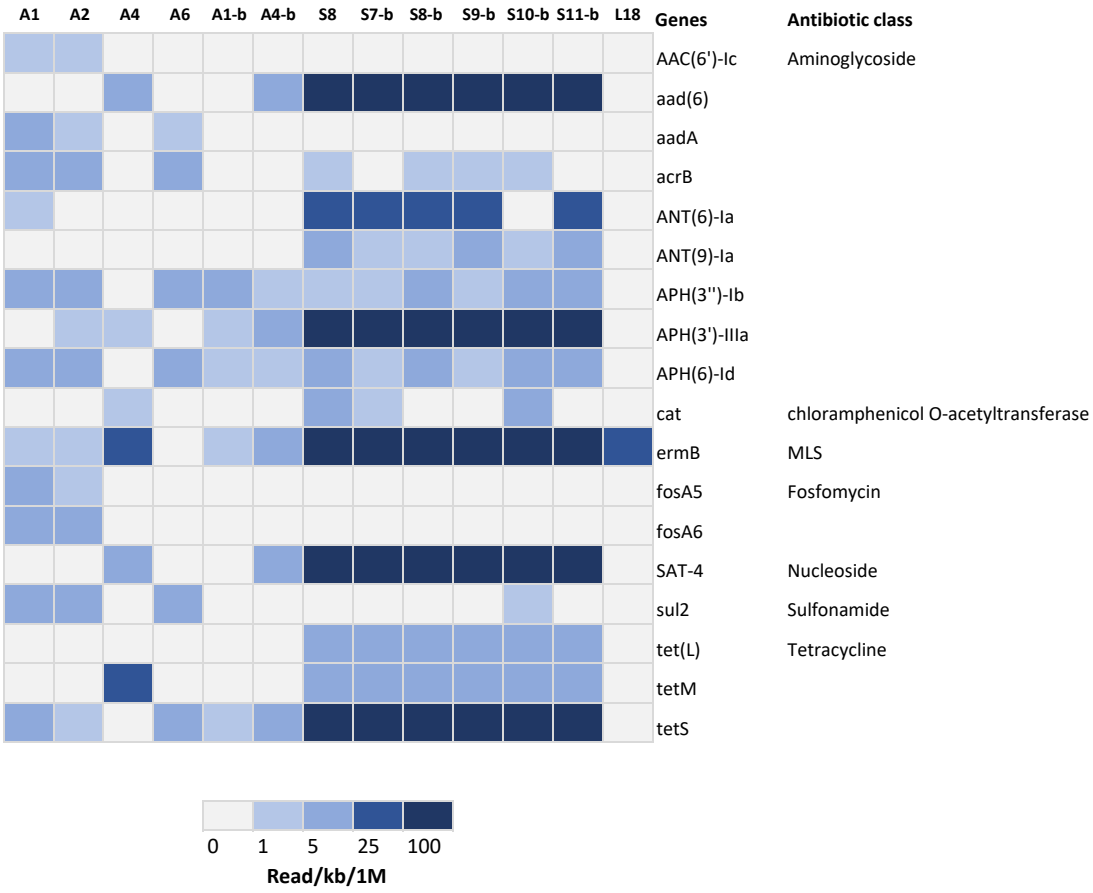
Table S8. Quality of prokaryotic MAGs

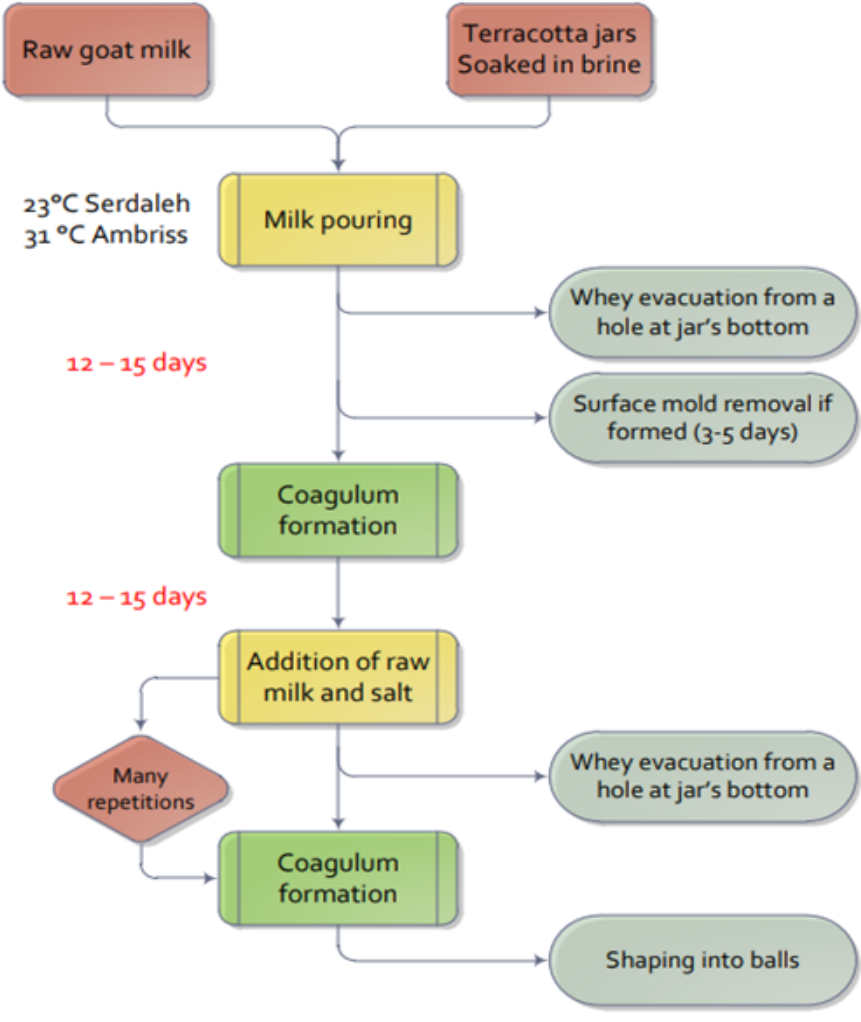
Table S9. FastANI values for MAGs, isolated strain genomes and NCBI reference genomes for *L. lactis, L. kefiranofaciens, L. kefiri, L. diolivorans, L. plantarum, M. agalactiae* and *S. parasuis*.

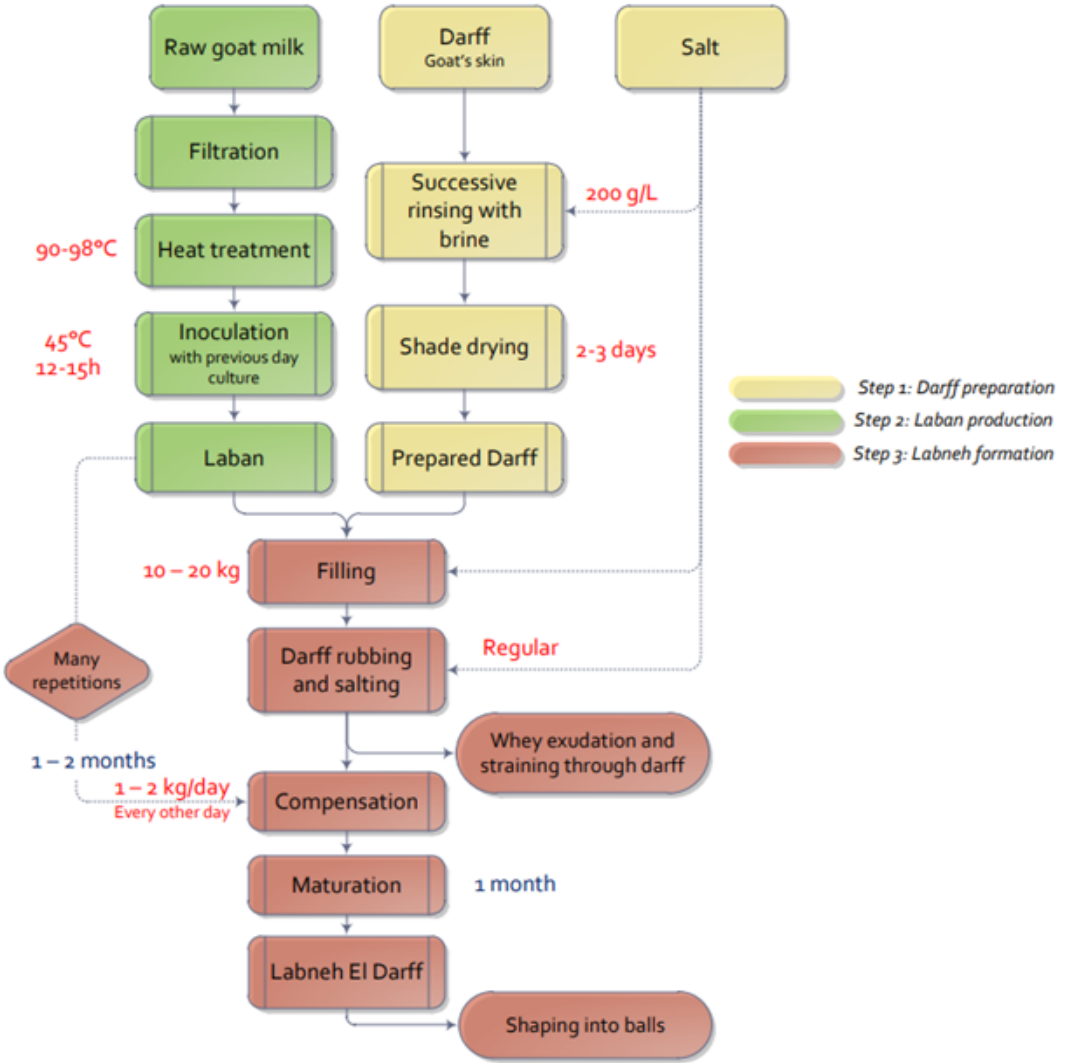

(A)
pH
Salinity
T (°C)
T (°C)
pH, salinity (%)
Temperature (°C)
Ambriss
Serdaleh
Labneh
El Darff
(B)
Log CFU/g
MRS 30°C
MRS 42°C
M17 30°C
Ambriss
Serdaleh
Labneh
El Darff
(C)
Ambriss
Serdaleh
Labneh El Darff
Lactobacillaceae
Lactiplantibacillus plantarum group
Levilactobacillus brevis
Lacticaseibacillus rhamnosus
Lentilactobacillus diolovorans group
Lactobacillus delbrueckii
Lentilactobacillus kefiri
Lentilactobacillus buchneri group
Lactobacillus fabifermentans group
Pediococcus acidilactici
Lacticaseibacillus casei group
Lactobacillus kefiranofaciens
Lactococcus lactis
Enterococcus durans


(A)
Observed
Chao1
Shannon
InvSimpson
Alpha Diversity Measure
Ambriss
Serdaleh
Labneh
El Darff
(B)
PCoA - Bray-Curtis
Axis.2 [28.3%]
Axis.1 [32%]
L17
L18
L15
L16
A4
A3-b
A6
S10-b
S20
S11-b
S20-C
S9-b
S8-b
S7-b
S9
S11
S10
S7
S8
S13
S14
S19-C
A1-b
A2-b
S19-b
A2
A1
S20-C-b
A5-b
S12
A4-b
A3
A5
S19-C-b
(C)
Ambriss
Serdaleh
Labneh
Relative abundance
A1-b
A1
A2-b
A2
A3-b
A3
A4-b
A4
A5-b
A5
A6
S7
S7-b
S8
S8-b
S9
S9-b
S10
S10-b
S11
S11-b
S12
S13
S14
S19-C-b
S19-C
S19-b
S20-C-b
S20-C
S20
L15
L16
L17
L18
Lactobacillaceae
Lacticaseibacillus rhamnosus
Lactiplantibacillus plantarum-pentosus
Lactobacillus acetotolerans
Lactobacillus delbrueckii
Lactobacillus gasseri
Lactobacillus helveticus
Lactobacillus kefiranofaciens
Lentilactobacillus buchneri
Lentilactobacillus hilgardii-diolivorans
Lentilactobacillus kefiri
Limosilactobacillus sp.
Streptococcaceae
Lactococcus lactis
Lactococcus sp.
Streptococcus parasuis-sanguinis
Streptococcus thermophilus
Gammaproteobacteria
Aeromonas caviae
Aeromonas sp.
Citrobacter sp.
Enterobacter sp.
Escherichia-Shigella
Klebsiella pneumoniae
Mycoplasmopsis agalactiae
Other


Ambriss
Serdaleh
Labneh
Relative abundance
1.00
0.75
0.50
0.25
0.00
A1
A2
A4
A6
A1.b
A4.b
S8
S7.b
S8.b
S9.b
S10.b
S11.b
L15
L16
L17
L18
*
*
*
Lactobacillaceae
Lacticaseibacillus rhamnosus
Lacticaseibacillus paracasei
Lactiplantibacillus plantarum
Lactobacillus acetotolerans
Lactobacillus delbrueckii
Lactobacillus gasseri
Lactobacillus helveticus
Lactobacillus kefiranofaciens
Lentilactobacillus diolivorans
Lentilactobacillus kefiri
Streptococcaceae
Lactococcus lactis sp. cremoris
Lactococcus lactis sp. lactis
Streptococcus parasuis
Streptococcus parauberis
Streptococcus thermophilus
Enterococcus faecium
Gammaproteobacteria
Aeromonas caviae
Klebsiella pneumoniae
Mycoplasmopsis agalactiae
Pichia kudriavzevii
Other


A1
A2
A4
A6
A1-b
A4-b
S8
S7-b
S8-b
S9-b
S10-b
S11-b
L18
Genes
Antibiotic class
AAC(6')-Ic
Aminoglycoside
aad(6)
aadA
acrB
ANT(6)-Ia
ANT(9)-Ia
APH(3'')-Ib
APH(3')-IIIa
APH(6)-Id
cat
chloramphenicol O-acetyltransferase
ermB
MLS
fosA5
Fosfomycin
fosA6
SAT-4
Nucleoside
sul2
Sulfonamide
tet(L)
Tetracycline
tetM
tetS
0
1
5
25
100
Read/kb/1M


Raw goat milk
Terracotta jars
Soaked in brine
23°C Serdaleh
31 °C Ambriss
Milk pouring
Whey evacuation from a hole at jar's bottom
12 – 15 days
Surface mold removal if formed (3-5 days)
Coagulum formation
12 – 15 days
Addition of raw milk and salt
Many repetitions
Whey evacuation from a hole at jar's bottom
Coagulum formation
Shaping into balls


Raw goat milk
Darff
Goat's skin
Salt
Filtration
Successive rinsing with brine
200 g/L
90-98°C
Heat treatment
45°C
12-15h
Inoculation
with previous day culture
Shade drying
2-3 days
Laban
Prepared Darff
Step 1: Darff preparation
Step 2: Laban production
Step 3: Labneh formation
10 – 20 kg
Filling
Many repetitions
Darff rubbing and salting
Regular
Whey exudation and straining through darff
1 – 2 months
1 – 2 kg/day
Every other day
Compensation
Maturation
1 month
Labneh El Darff
Shaping into balls

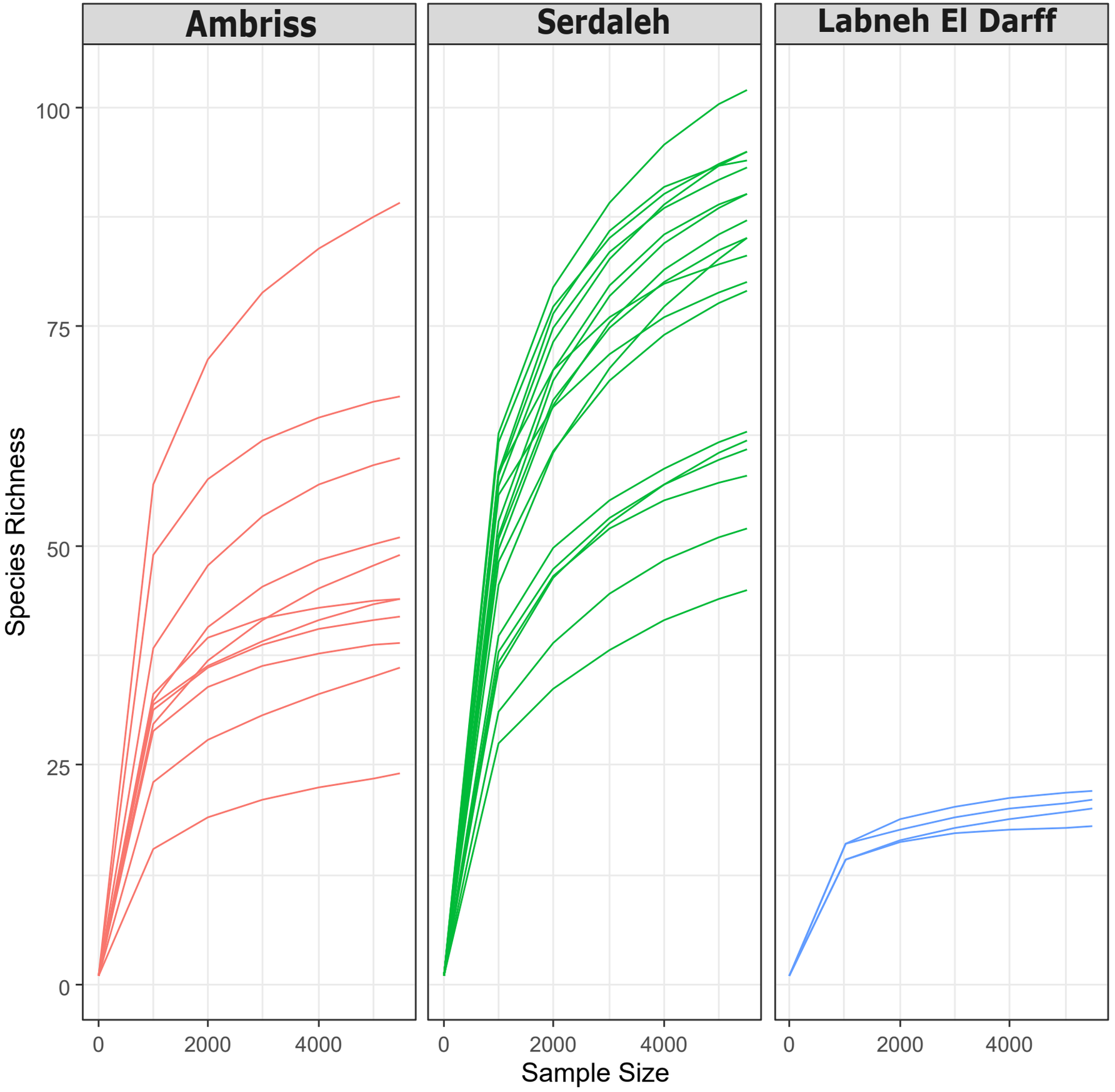
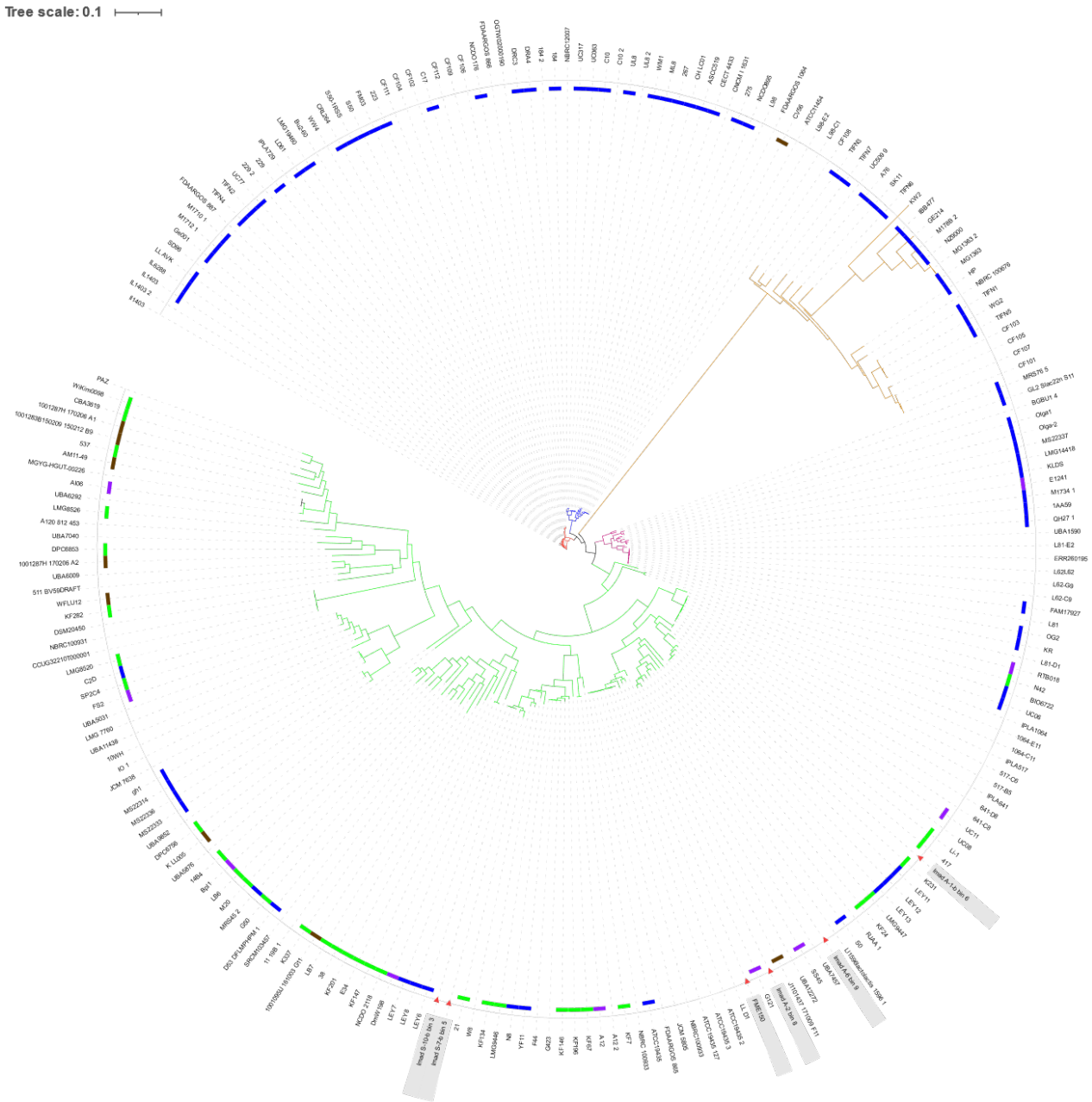

Ambriss
Serdaleh
Labneh El Darff
Species Richness
100
75
50
25
0
0
2000
4000
Sample Size

Fig. S4



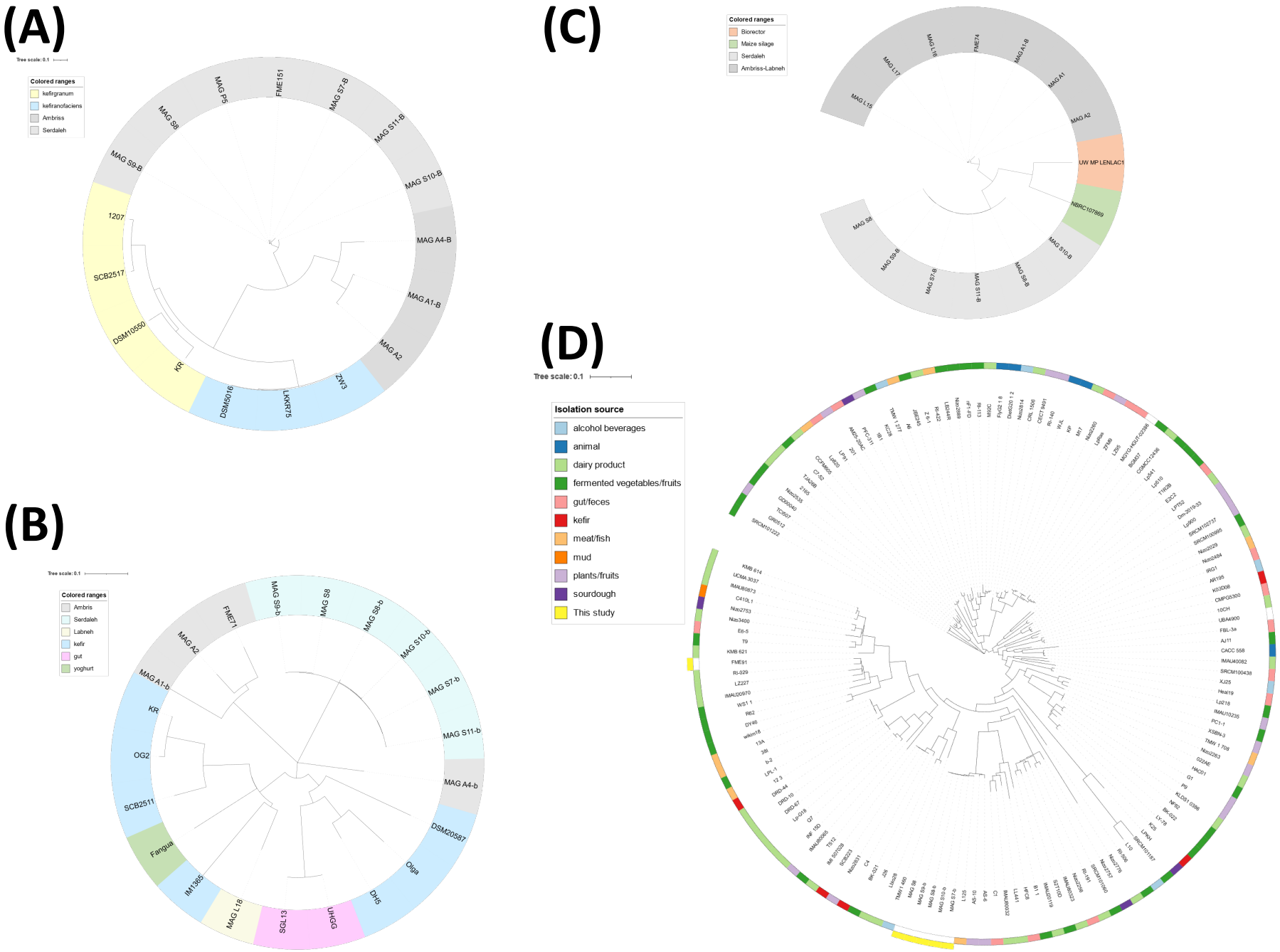

(A)
Tree scale: 0.1
Colored ranges
kefirgranum
kefiranofaciens
Ambriss
Serdaleh
FME151
MAG S7-B
MAG S11-B
MAG S10-B
MAG A4-B
MAG A1-B
MAG A2
ZW3
LKKR75
DSM5016
KR
DSM10550
SCB2517
1207
MAG S9-B
MAG S8
MAG P5
(B)
Tree scale: 0.1
Colored ranges
Ambris
Serdaleh
Labneh
kefir
gut
yoghurt
FME71
MAG A2
MAG A1-b
KR
OG2
SCB2511
Fangua
IM1365
MAG L18
SGL13
UHGG
DH5
Olga
DSM20587
MAG A4-b
MAG S11-b
MAG S7-b
MAG S10-b
MAG S8-b
MAG S8
MAG S9-b
(C)
Tree scale: 1
Colored ranges
Biorector
Maize silage
Serdaleh
Ambriss-Labneh
MAG L16
FME74
MAG A1-B
MAG A1
MAG A2
UW MP LENLAC1
NBRC107869
MAG S10-B
MAG S8-B
MAG S11-B
MAG S7-B
MAG S9-B
MAG S8
MAG L17
MAG L15
(D)
Tree scale: 0.1
Isolation source
alcohol beverages
animal
dairy product
fermented vegetables/fruits
gut/feces
kefir
meat/fish
mud
plants/fruits
sourdough
This study

Fig. S5

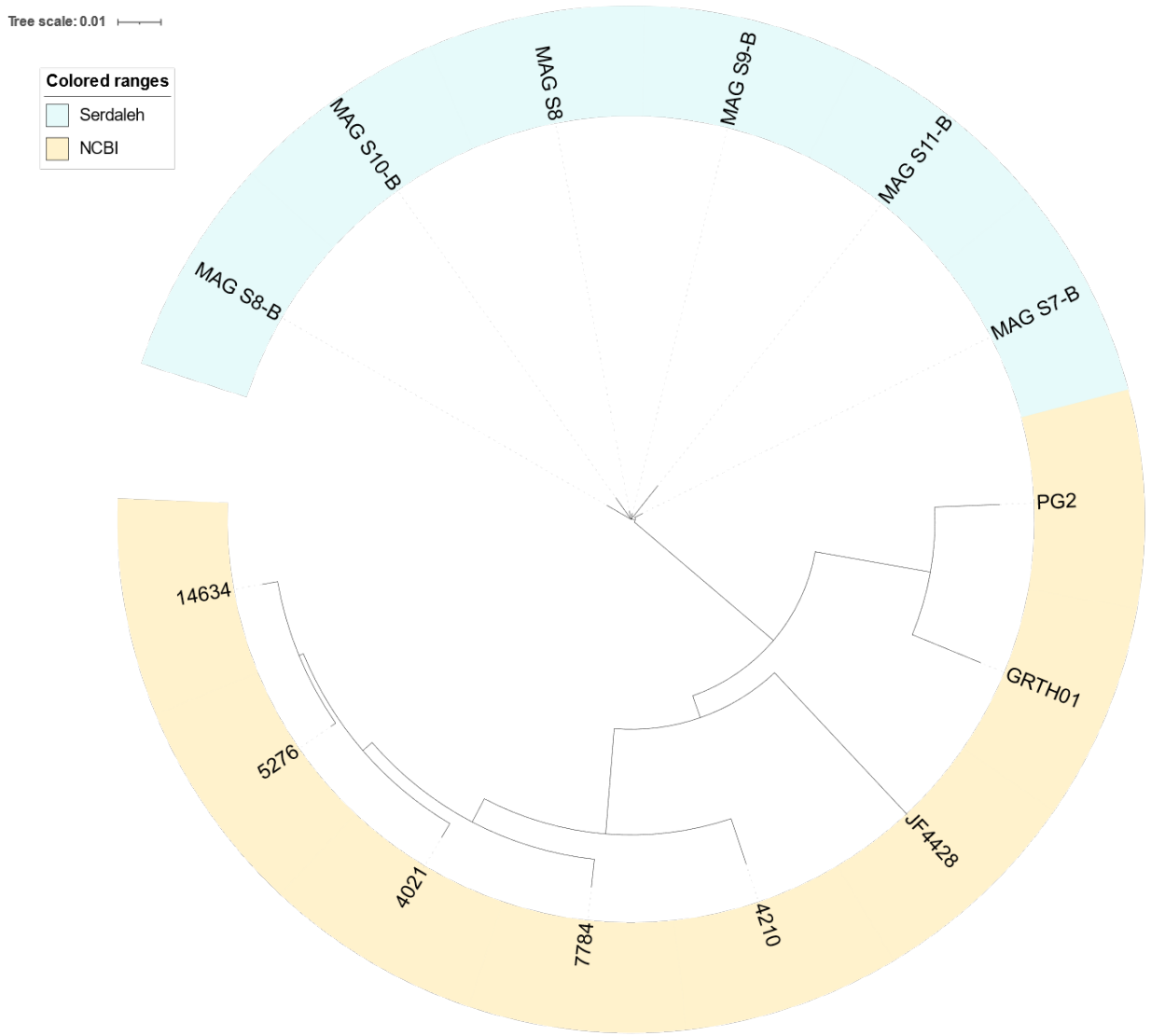

Tree scale: 0.01
Colored ranges
Serdaleh
NCBI
MAG S8
MAG S9-B
MAG S11-B
MAG S7-B
PG2
GRTH01
JF4428
4210
7784
4021
5276
14634
MAG S10-B
MAG S8-B

Fig. S6

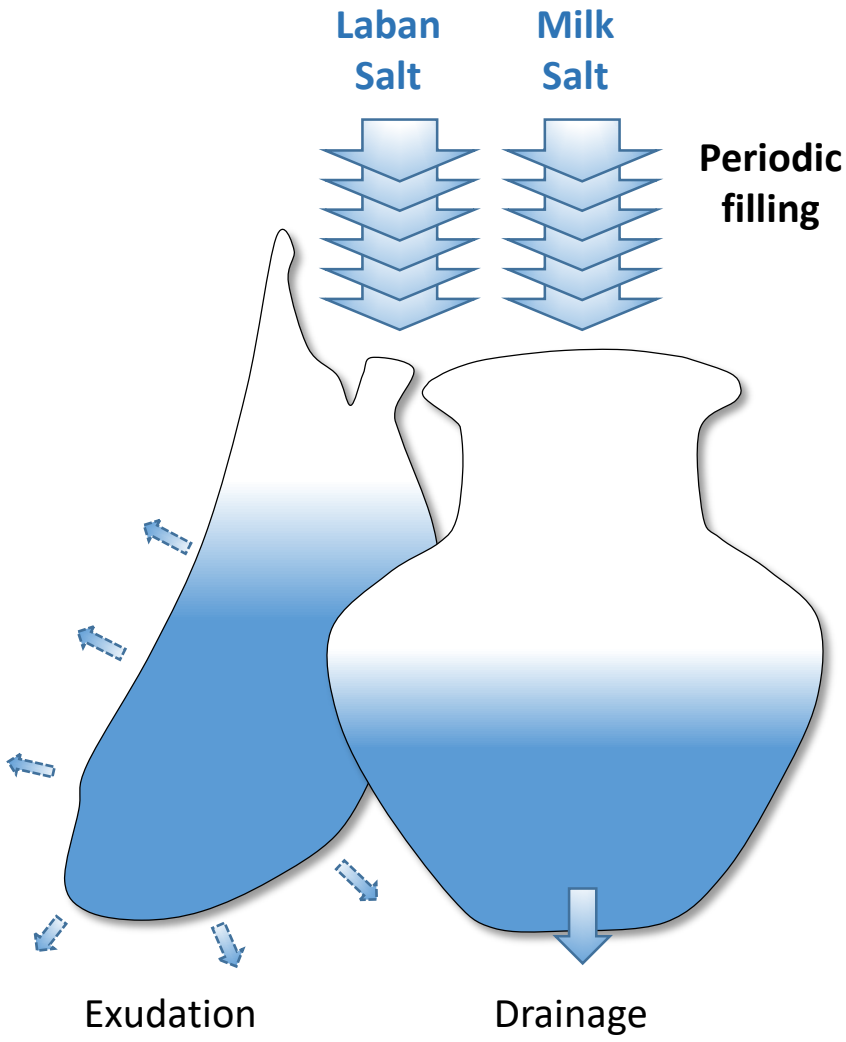
(A)

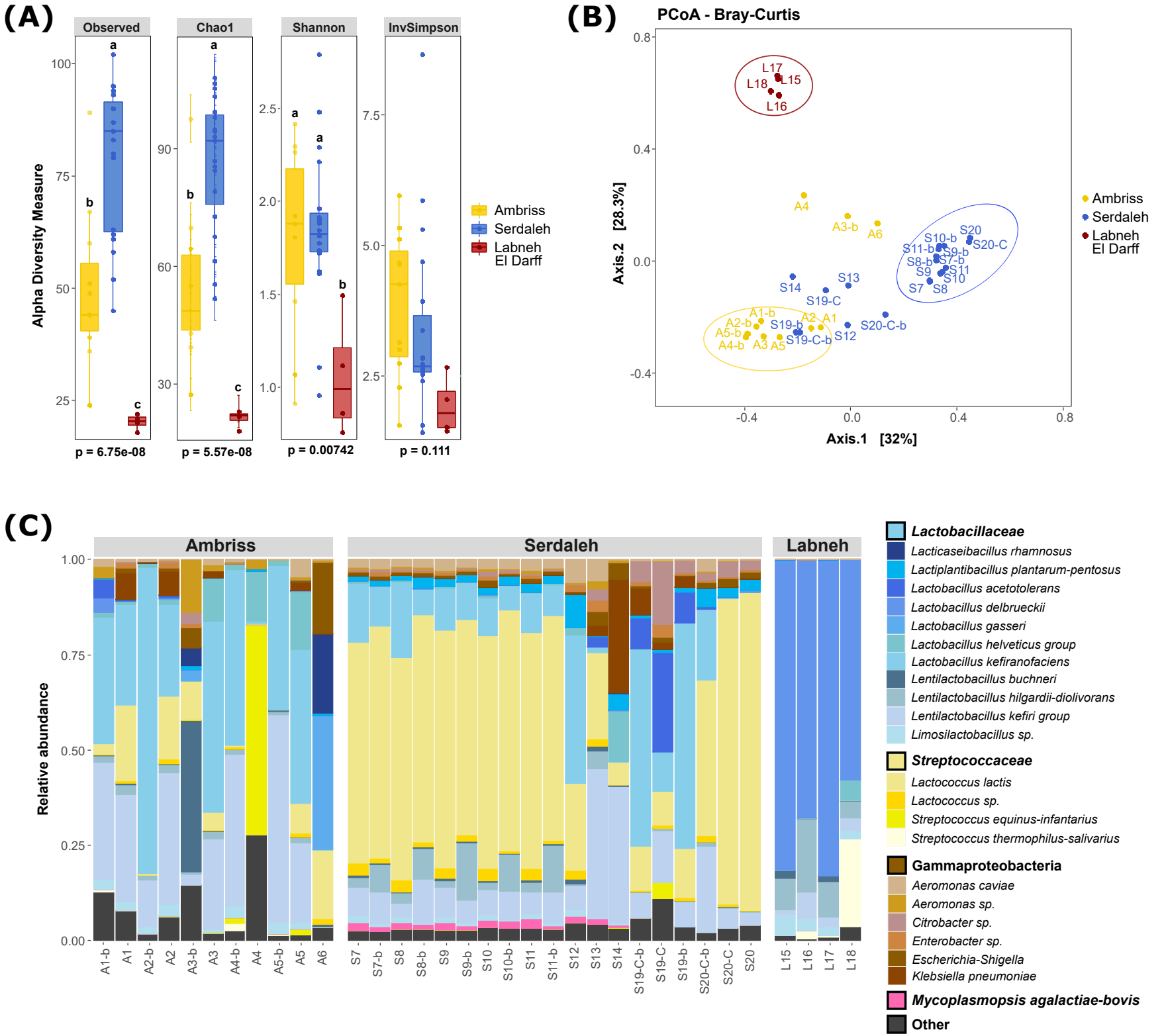
Serdaleh

Ambriss

Labneh El Darff

(B)




(A)
Observed
Chao1
Shannon
InvSimpson
Alpha Diversity Measure
p = 6.75e-08
p = 5.57e-08
p = 0.00742
p = 0.111
Ambriss
Serdaleh
Labneh
El Darff
(B)
PCoA - Bray-Curtis
Axis.2 [28.3%]
Axis.1 [32%]
(C)
Ambriss
Serdaleh
Labneh
Relative abundance
Lactobacillaceae
Lacticaseibacillus rhamnosus
Lactiplantibacillus plantarum-pentosus
Lactobacillus acetotolerans
Lactobacillus delbrueckii
Lactobacillus gasseri
Lactobacillus helveticus group
Lactobacillus kefiranofaciens
Lentilactobacillus buchneri
Lentilactobacillus hilgardii-diolivorans
Lentilactobacillus kefiri group
Limosilactobacillus sp.
Streptococcaceae
Lactococcus lactis
Lactococcus sp.
Streptococcus equinus-infantarius
Streptococcus thermophilus-salivarius
Gammaproteobacteria
Aeromonas caviae
Aeromonas sp.
Citrobacter sp.
Enterobacter sp.
Escherichia-Shigella
Klebsiella pneumoniae
Mycoplasmopsis agalactiae-bovis
Other