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Metagenomics reveal the specificities of the microbiota of three traditional Lebanese goat milk fermented products

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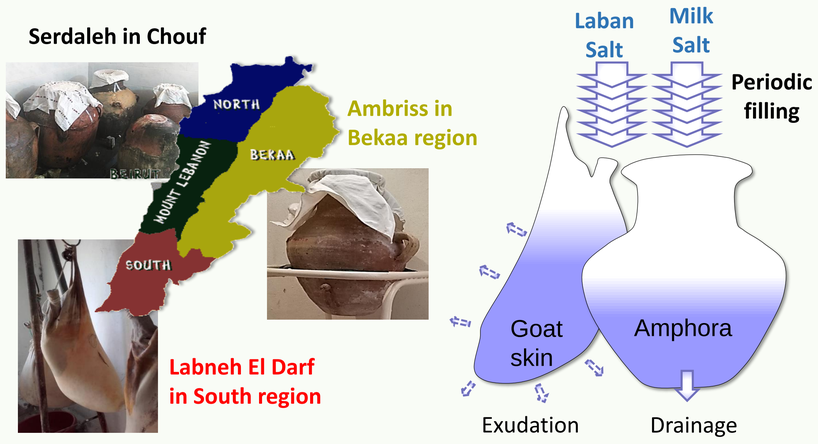
Introduction

In Lebanon, the production of traditional fermented goat dairy products is carried out at small scale, and only in a limited number of 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). Therefore, the investigation of their microbial system is becoming urgent matter. As a response, three products were selected for this study, namely Ambriss, Serdaleh and Labneh El Darff. This study aims to reveal their characteristics and microbial diversity in order to preserve their biological resources for further evaluation for their technological properties.

Materials and Methods

Product selection, production and producer's survey

A questionnaire regarding farming and production practices was conducted on 50 producers, from which 15 samples of Serdaleh, 11 of Ambriss and 4 of Labneh El Darff were collected across three different regions.



Microbiological, Genomic, Metagenetic and Metagenomic analyses

Samples were cultured on different media while their DNA was extracted for the 16S metagenetic and Shotgun metagenomic analyses. From 380 isolates, 279 strains were genetically identified by 16S rDNA. Metagenomic microbial composition was estimated (i) by mapping the reads against the representative clade-specific marker catalog using the MetaPhlAn tool and (ii) by using YchF gene marker for binning of the assembled metagenomic contigs..

Figure 1. Schematic flow chart for the three products related to their geographical origin on the Lebanese map.

Results and discussion

Producer survey

According to the survey, the production is very artisanal, mainly depending on women housekeepers (60%) aged between 40 and 78 years old and displays limited access to local market place (38%).

	Ambriss	Serdaleh	Labneh El Darff
Producers dedicated only to 1 dairy product (%)	80	51.61	100
Extensive goat Farming system (%)	50	66.70	66
Milk processed by farmers-producers (L/year)	160-3200	220-7200	135-1350
Milk processed by producers only (L/year)	140-1890	110-1250	135-850
Average yearly production (Kg)	108.8	411.9	308.3
Production yield (Kg/100L milk)	19.44	18.89	16.35
Producers (%) implementing a processing period of 1-3 months	0	15	55.6
Producers (%) implementing a processing period of 4-6months	100	75	44.4

Table 1: Surveys' results related to production and farming practices of the three products

Culture-dependent approaches

The examination of culture results showed an important variability in the different enumerations. A dominance of mesophilic lactobacilli (5.4 – 6.8 Log CFU/g) over lactococci (0.5 – 5.2 Log CFU/g) was observed in the three products. Thermophilic counts (MRS at 42 °C) were highly variable in Labneh El Darff (1 – 6.2 Log CFU/g) compared to its other counterparts. Plate counts of lactobacilli at 30 °C were more pronounced ($P < 0.05$) in Labneh El Darff (6.8 Log CFU/g) than those recorded for Ambriss and Serdaleh (5.4 and 6.0 Log CFU/g respectively).

Species determination by 16S sequencing of the isolates showed that *L. plantarum was* a dominant species in Ambriss (38%) followed by *L. rhamnosus* and *Enterococcus durans* (18% for each). Serdaleh presents 10 different species similar to Ambriss, in which *L. plantarum* was predominant (63%), whereas in Labneh El Darff, *Lb. brevis w*as the most abundant followed by *L. bulgaricus* subsp*. bulgaricus,* and *Lactococcus lactis* (16% for each).

Culture-independent approaches

The estimation of microbial complexity by metagenetic study, based on the 4 indices of alfa diversity (Figure 2) showed that Labneh El Darff presents a low bacterial diversity ($P < 0.05$) and is dominated by *Lactobacillus delbrueckii* subsp*. bulgaricus,* originating from Laban.

Exploring beta diversity, Serdaleh and Ambriss presented 2 major clusters sharing a similar microbiota mainly composed of Lactobacilli. .

Combined analysis with metagenomics showed that Serdaleh has a distinct homogenous clade dominated by *Lactococcus lactis* subsp *lactis* (> 65% of reads) followed by the species *L. kefiranofaciens, L. kefiri* and *L. diolivorans*. Sample A-4 was dominated by *Streptococcus parasuis* group while sample A-6 showed high amount of *L. gasseri* (~36%), *L. rhamnosus* (~23%) and Enterobacteriaceae (Figures 2 and 3). All Serdaleh samples were contaminated by *Mycoplasmopsis agalactiae* and a low level of *Listeria monocytogenes* was detected in 4 Ambriss and 2 Serdaleh. *Capra hircus* reads, corresponding to goat DNA were shown in abundance in all Labneh samples, probably originating from goat's skin during processing.

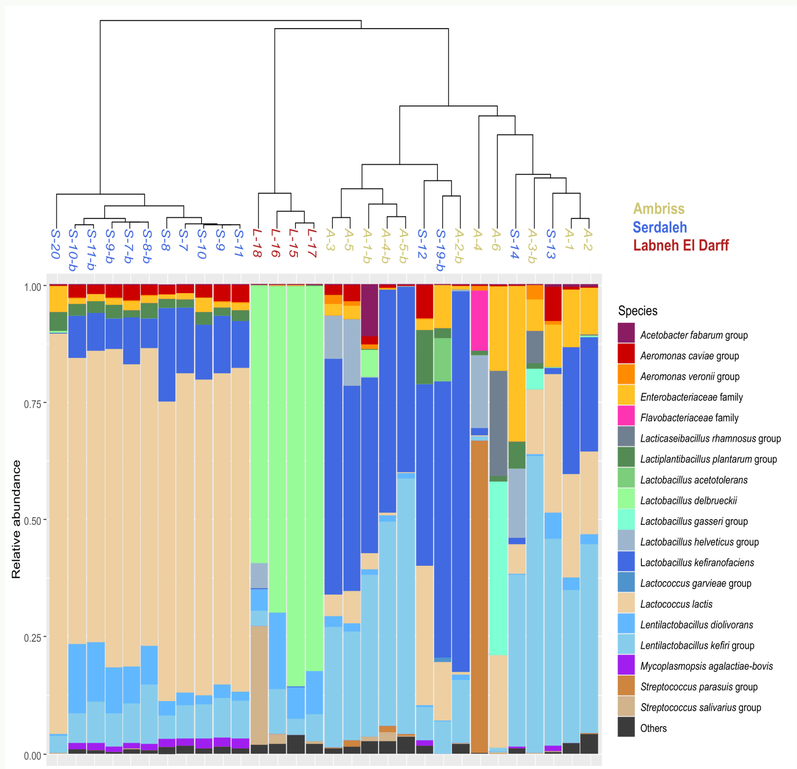


Figure 3 : Taxonomic composition plot of the relative abundance of the 20 most abundant bacterial OUTs assigned at the species level in Ambriss, Serdaleh and Labneh El Darff products.

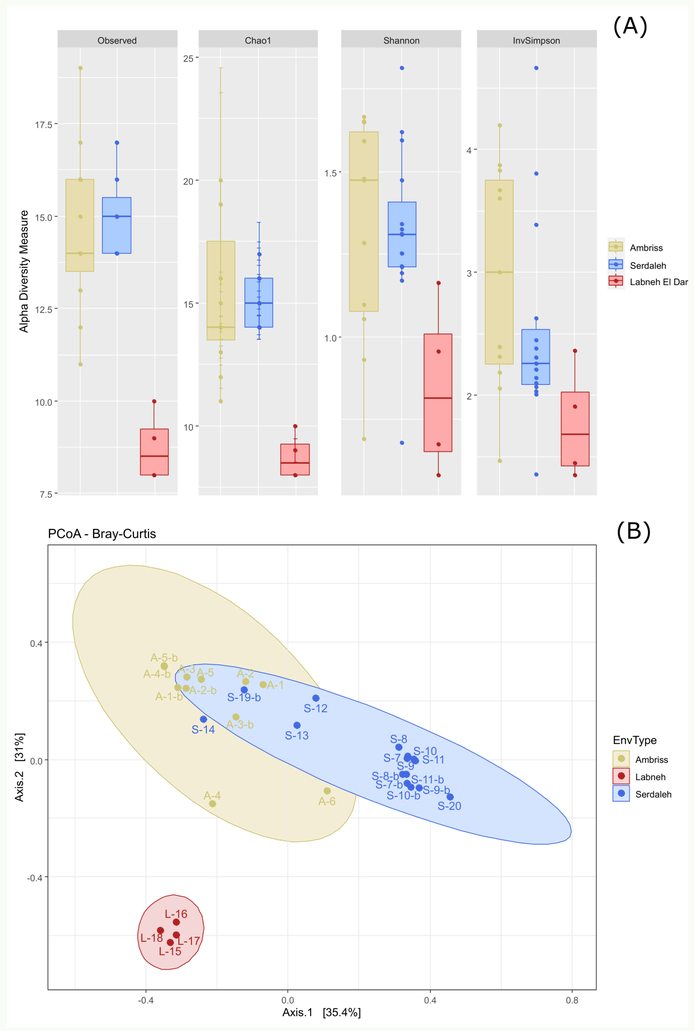


Figure 2 : Box-plot of alfa-diversity and indices (A) and Principal Coordinates Analysis (B) among bacterial communities identified in the three products.

Conclusions

Using both culture-dependent and independent approaches, we demonstrated that Serdaleh and Ambriss possessed an original living microbiota that was present to a lesser extent in Labneh El Darff. The first two products share an association of *Lactococcus lactis* and three *Lactobacillus* species, with different proportions as they originate from 2 distinct regions, even if they share similar ways of processing. The detection of contaminating bacteria in some products focuses the attention on the implementation of Good Manufacturing Practices and Good Hygienic Practices in such artisanal productions. In addition, a commercialization policy is needed to develop other social blocks that enable housekeepers to be involved.

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