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Revealing the microbial heritage of traditional Lebanese goat milk fermented products

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BACKGROUND

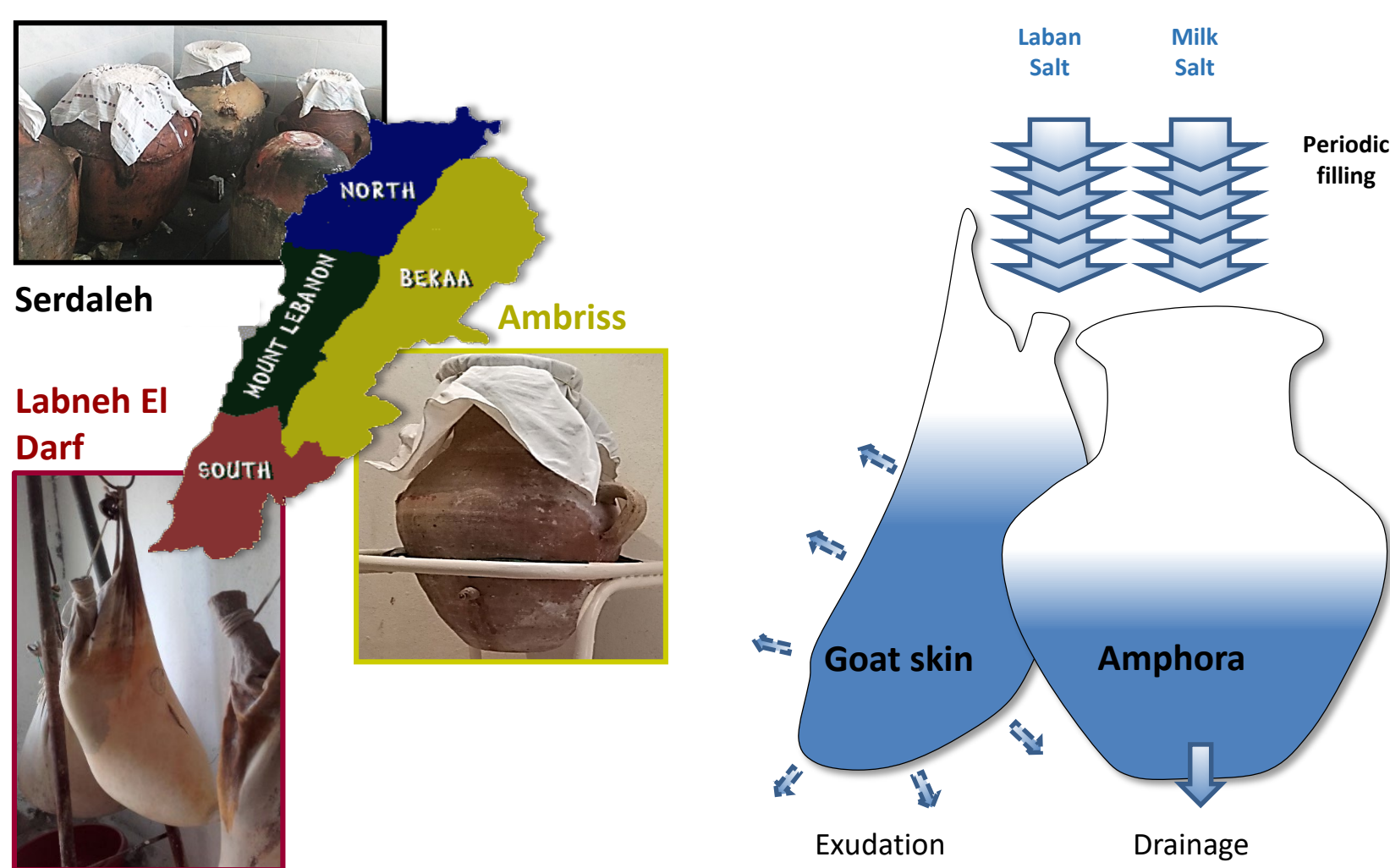


Fig 1. Schematic flow chart for three emblematic products related to their geographical origin

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. The understanding of their microbial ecosystems is becoming necessary and urgent. The aim of this work was to provide an overview of their microbial biodiversity, to isolate and characterize representative strains, and to point out specificities of these microbiota by combining approaches, including assembly- and deep profiling-metagenomics.

We will highlight here the results of "Ambriss" and "Serdaleh" produced respectively in the Bekaa and Chouf regions, by successively filling an amphora with raw goat's milk, draining the whey and salting the surface until it is filled.

WORKPLAN

A survey 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 the three different regions.

Samples were cultured on different media and 279 strains were genetically identified by 16S rDNA. Cheese DNA was extracted for the 16S metagenetic and Shotgun metagenomic sequencing. Bioinformatics was performed as described below.

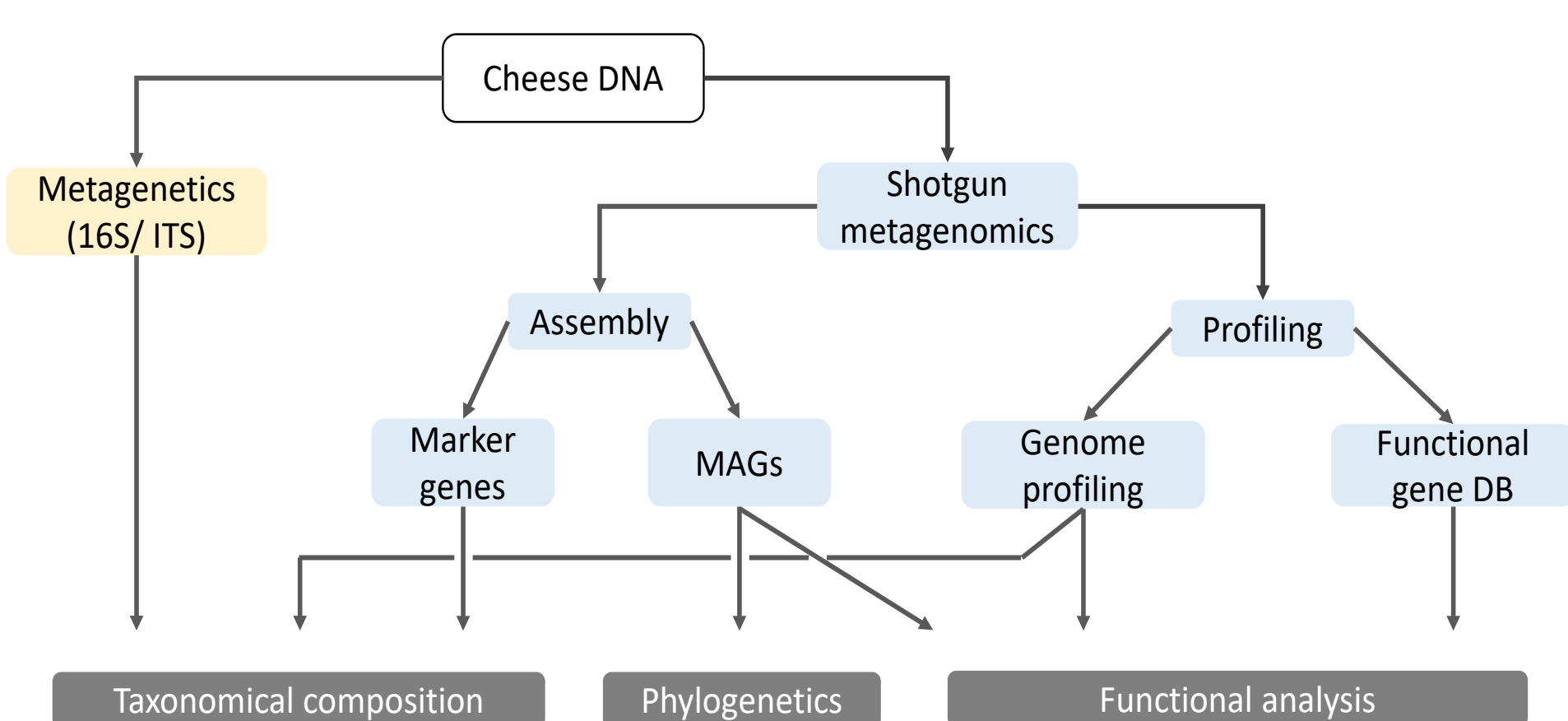


Fig. 2. Overview of methodological approaches used in this study.

RESULTS

Producer's survey

The production is found to be typically 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 to 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 cheese 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 4-6 months	100	75	44.4

Table 1: Survey's results related to production and farming practices

Microbial composition

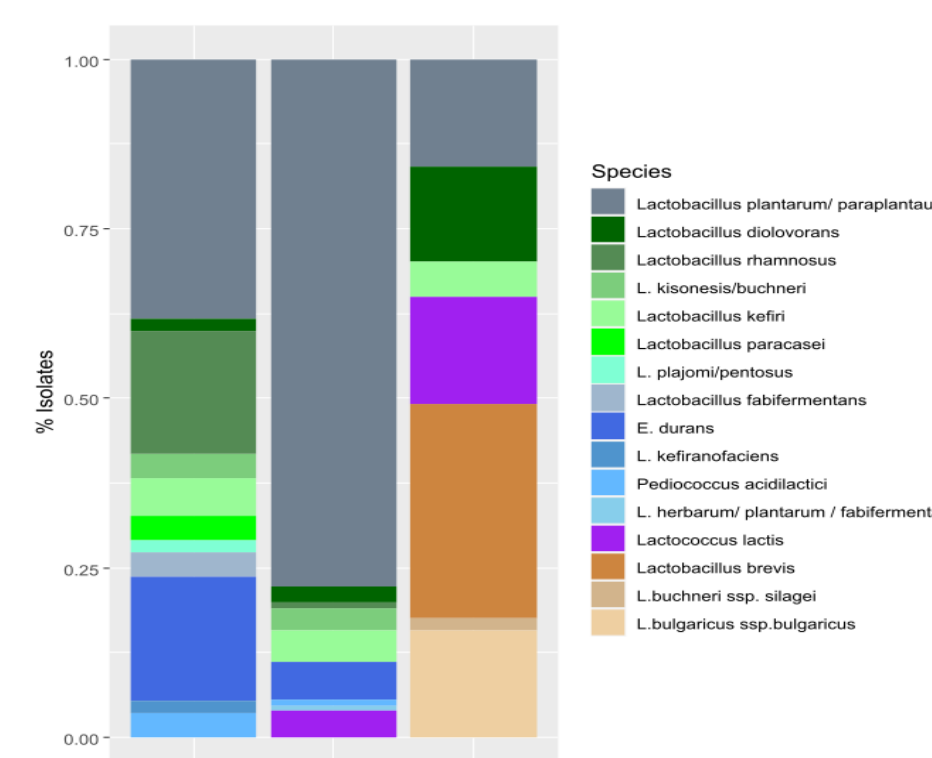


Fig. 3. Composition based on cultures.

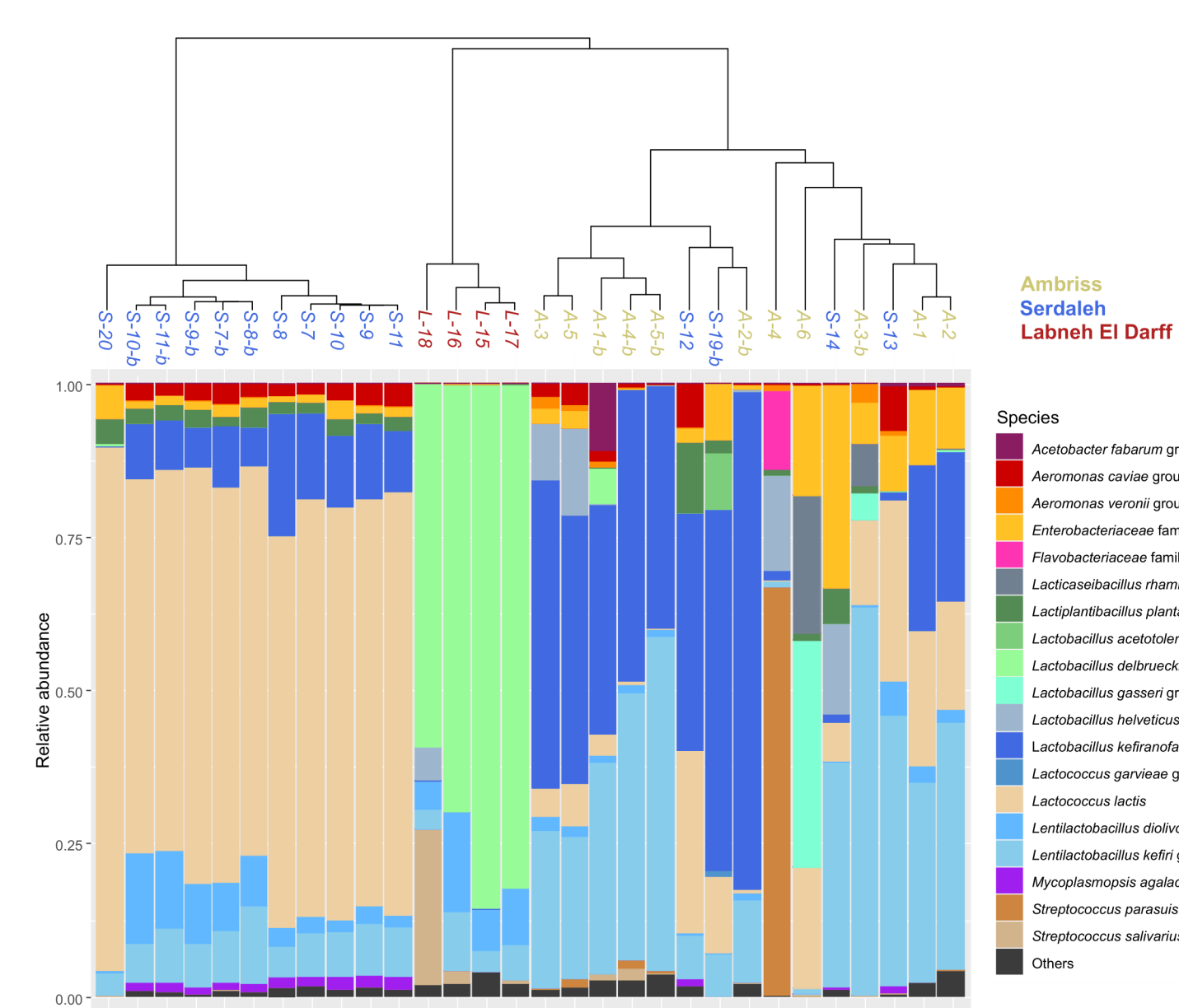


Figure 4 : Composition based on OTUs

Culture-dependent and -independent analyses gave drastically different results. Metagenomics showed that Serdaleh and Ambriss share a similar microbiota but with different proportions of *L. lactis* and *L. kefiranofaciens*, *L. kefir* and *L. diolivorans*. Differences in local conditions (temperature, salt...) may explain the shift in population in these products. This composition is similar to that of kefir, a probiotic product, whose microbiota is driven by a particular structure in polysaccharide aggregates, the kefir grain.

In addition, all Serdaleh samples contained *Mycoplasma agalactiae*, the causing agent of contagious agalactia in sheep and goats.

Refined metagenomic analysis

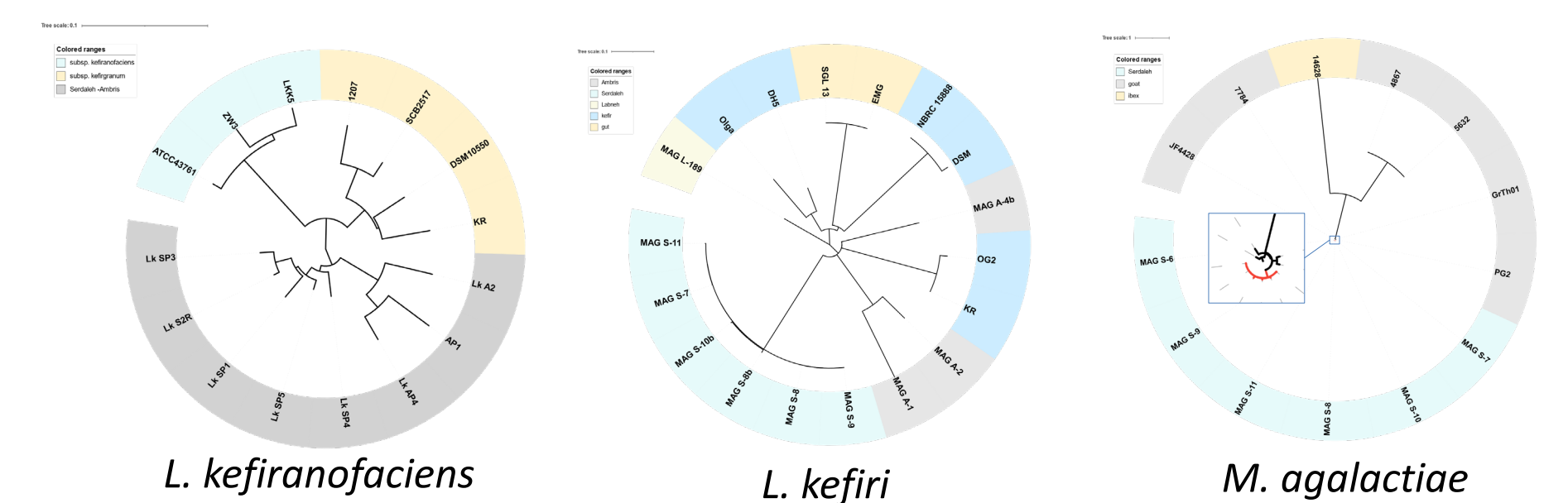


Fig. 5. Example of phylogenetic analysis of product MAGs and reference strain genomes.

Genomic and phylogenomic analyses of the MAGs of different species revealed several particularities. In particular, it shows that *L. kefiranofaciens* strains from Lebanon are genetically diverse compared to those of kefir grains from different origins. Furthermore, this keystone species of the two ecosystems displays functional differences such as the production of a different extracellular polysaccharides and prophages content.

M. agalactiae strains appear to be almost identical, suggesting an epidemic in the Bekaa.

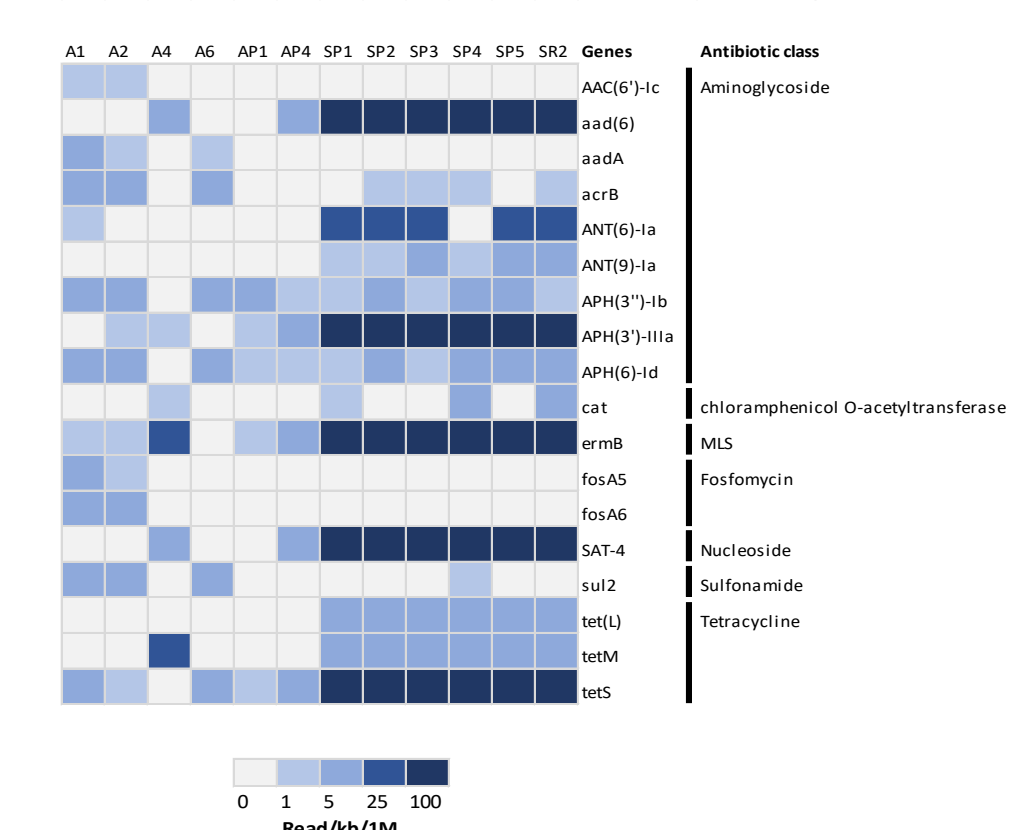


Fig. 6. Antibiotic resistance (ABR) genes detected in the products

ABR genes were detected in most samples, particularly in Serdaleh, whose dominant *L. lactis* strains contain a plasmid with a multiresistance island.

CONCLUSION & PERSPECTIVES

Ambriss and Serdaleh, emblematic products of several millennia and considered part of the Lebanese heritage, share the same microbial composition as kefir, a popular probiotic product worldwide. This fact adds to the interest of preserving their production. However, there are several issues that need to be resolved before further development of these products can take place.

- Additional analyses are required to understand the resilience of the ecosystem established in the amphora, which are used only during goat milk production season.
- Sanitary practices have to be improved for milk production
- Finally, appropriate social and marketing policies should be developed and implemented to support and maintain the main actors of these productions

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