

Aosta Valley: a case study of microbial biodiversity

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INTRODUCTION

Valle d'Aosta is inserted in the Alpine mountains which incorporated several regions of the European Union and Switzerland. The Alpine context represents a notable mine of biodiversity, both animal and vegetable. In particular, thanks to a unique agricultural management, a precious reservoir of microbial biodiversity is present and is maintained, especially in the agri-food supply chains. Since 1996, the Institut Agricole Régional has launched research projects for the selection of native starter microorganisms in order to obtain fermented products typical of the Aosta Valley environment.

VALLE D'AOSTA FONTINA PDO CHEESE

Fontina is a full-fat, semi-cooked, washed-rind PDO cheese, produced from raw cow's milk which is traditionally made in Aosta Valley. Fontina PDO Production Regulations provide for the addition of native starter cultures selected in the Fontina production area.

THE EXPERIMENTAL PROTOCOL:

- isolation of hundreds of strains belonging to the lactic microflora selected from dairy processes carried out in 22 Aosta Valley mountain pastures, in which commercial ferments had never been used;
- preservation of isolates in a lactic acid bacteria strain collection at the Institut Agricole Régional;
- genetic identification, genotypic, phenotypic and technological characterization of lactic strains isolates;
- selection of strains belonging to the species *Streptococcus thermophilus*, *Lactobacillus delbrueckii lactis* and *Lactococcus lactis*, for a bacteria formulation in the form of freeze-dried starter culture for direct inoculation;
- trials of the starter formulation in dairies and mountain pastures and microbiological monitoring of vat milk and cheese microbiota during ripening, assessing the hygiene quality of the products;
- molecular analysis in order to evaluate the colonization of the starter during cheese ripening, using random amplified polymorphic DNA (RAPD) fingerprinting;
- study of the typicality of the cheeses obtained through the final tasting of the wheels and evaluating its compliance with Fontina PDO Production Regulations.

YOALP® FERMENTED MILK

YoAlp® is a fermented milk with the characteristics of a yogurt created with a mixture of lactic acid bacteria selected in the Aosta Valley. It is a unique product, made from Aosta Valley cow's and sheep's milk that exploits the raw materials defending the typicality of the mountain area.

THE EXPERIMENTAL PROTOCOL:

- analysis of 43 autochthonous bacterial strains, belonging to *Streptococcus thermophilus* and *Lactobacillus delbrueckii* species, from the strain collection of lactic acid bacteria of the Institut Agricole Régional;
- protechnological characterization of the individual strains according to their potential use for a fermented milk;
- phylogenetic investigation of strains: specific identification by sequencing of the 16S rDNA, and intraspecific identification by sequencing-based MLST analysis;
- formulation of 10 experimental blends (formed by *Streptococcus thermophilus* and *Lactobacillus delbrueckii* species) with different genetic profiles and industrialization trials on a pilot scale with different types of milk and beverages of vegetable origin;
- tasting of the products obtained compared with a commercial starter;
- selection of the best formulation with two strains of *S. thermophilus* and a strain of *L. delbrueckii*. The lactobacillus strain does not belong to the *Bulgaricus* subspecies but it has a high homology with *L. delbrueckii ssp. lactis*;
- freeze-drying strains for direct inoculation.

MICROBIAL BIODIVERSITY

VALLE D'AOSTA FROMADZO PDO CHEESE

Valle d'Aosta Fromadzo PDO is a semi-hard cheese obtained from cow's milk, and protected denomination of origin (PDO) in Aosta Valley. Its lactic microflora was isolated and analyzed starting from traditional spontaneous fermentations without the addition of commercial starter cultures.

THE EXPERIMENTAL PROTOCOL:

- isolation from milk and cheese samples taken from mountain pastures in the Valle d'Aosta Fromadzo PDO production area for create a the dairy microflora strain collection to be safeguarded;
- genetic identification, phenotypic and biochemical characterization of the isolated strains (acidifying power, proteolytic and inhibitory activity against *Listeria monocytogenes*, *Staphylococcus aureus* and *Escherichia coli*);
- monitoring the comparative processing of the same batch of milk and the subsequent cheese in different lactic acid bacteria formulations composed with different strains of *Lactococcus lactis* and *Lactococcus cremoris*;
- monitoring by analysing both the microbial and chemical composition of both the vat milk before and after starter inoculation and the relative cheese during its various ripening stages;
- molecular analysis in order to evaluate the colonization and the persistence of the added starter cultures by extracted DNA in cheese paste, using the rep-PCR (Repetitive Extragenic Palindromic);
- tasting of the cheeses to evaluate organoleptic characteristics differences between the experimental formulations;
- selection of the starter formulation with the best technological performance and freeze-drying.

YEASTS IN BREWING

The practice of inoculating selected strains of *Saccharomyces cerevisiae*, according to parameters of technological interest, for a guided fermentation of the musts is widespread. Their use allows to control fermentation, ensuring good protection against oxidation and contamination of unwanted microorganisms.

THE EXPERIMENTAL PROTOCOL:

- collection of 38 grape samples in different grape varieties from 26 wine-growing areas of Valle d'Aosta;
- mashing of grape samples in the laboratory, isolation and conservation of 394 yeast strains;
- morphological, biochemical identification and genotypic characterization by analysis of mitochondrial DNA restriction profiles of 81 *Saccharomyces cerevisiae* isolates;
- selection of 9 strains of *S. cerevisiae* with different genetic profiles;
- oenological characterization of the 9 indigenous strains by microvinification tests on synthetic must under controlled laboratory conditions;
- experimental microvinification in the wine cellar of 9 *S. cerevisiae* strains as starters for the vinification of Aosta Valley wines;
- possible use as starter for beer, mead and bakery.

Development of a starter of native lactic acid bacteria for the production of YoAlp[®] Aosta Valley fermented milk

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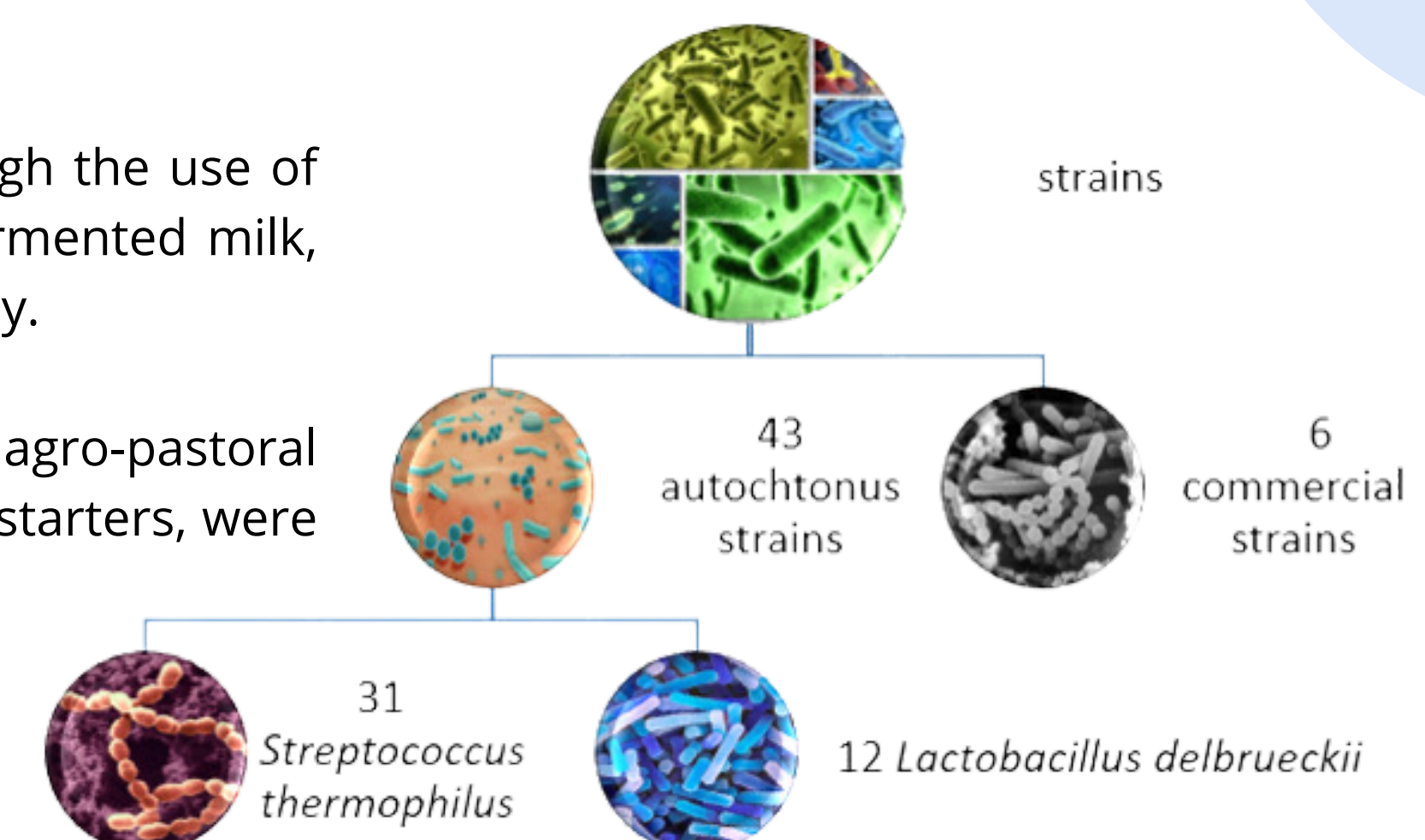
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INTRODUCTION

The research activity had the aim of enhancing the raw materials and microbial biodiversity of the Aosta Valley, through the use of native lactic bacteria belonging to the Institut Agricole Régional's bacteria strains collection for the production of a fermented milk, recognized YoAlp®, with the characteristics of a yogurt, increasing interest in a dairy product entirely from the Aosta Valley.

A total of 43 lactic acid bacteria strains, isolated over the years from lactic microflora typical of the Aosta Valley agro-pastoral environment such as mountain pastures and small dairies that traditionally process milk without the use of commercial starters, were studied.



RESULTS

- Analysis of the 16 rDNA portion identified 12 strains of *L. delbrueckii* and 31 strains of *Streptococcus thermophilus*.
- Identification of 2 autochthonous strains distinct of *L. delbrueckii* from commercial strains and other strains present in GenBank.
- Identification of 13 autochthonous strains of *S. thermophilus* distinct from commercial strains and other strains present in GenBank (Fig. 1).
- Strains phylogenetically closest to *L. delbrueckii ssp. indicus* (Ldb B) and *L. delbrueckii ssp. lactis* (Ldb A) (Fig. 2).

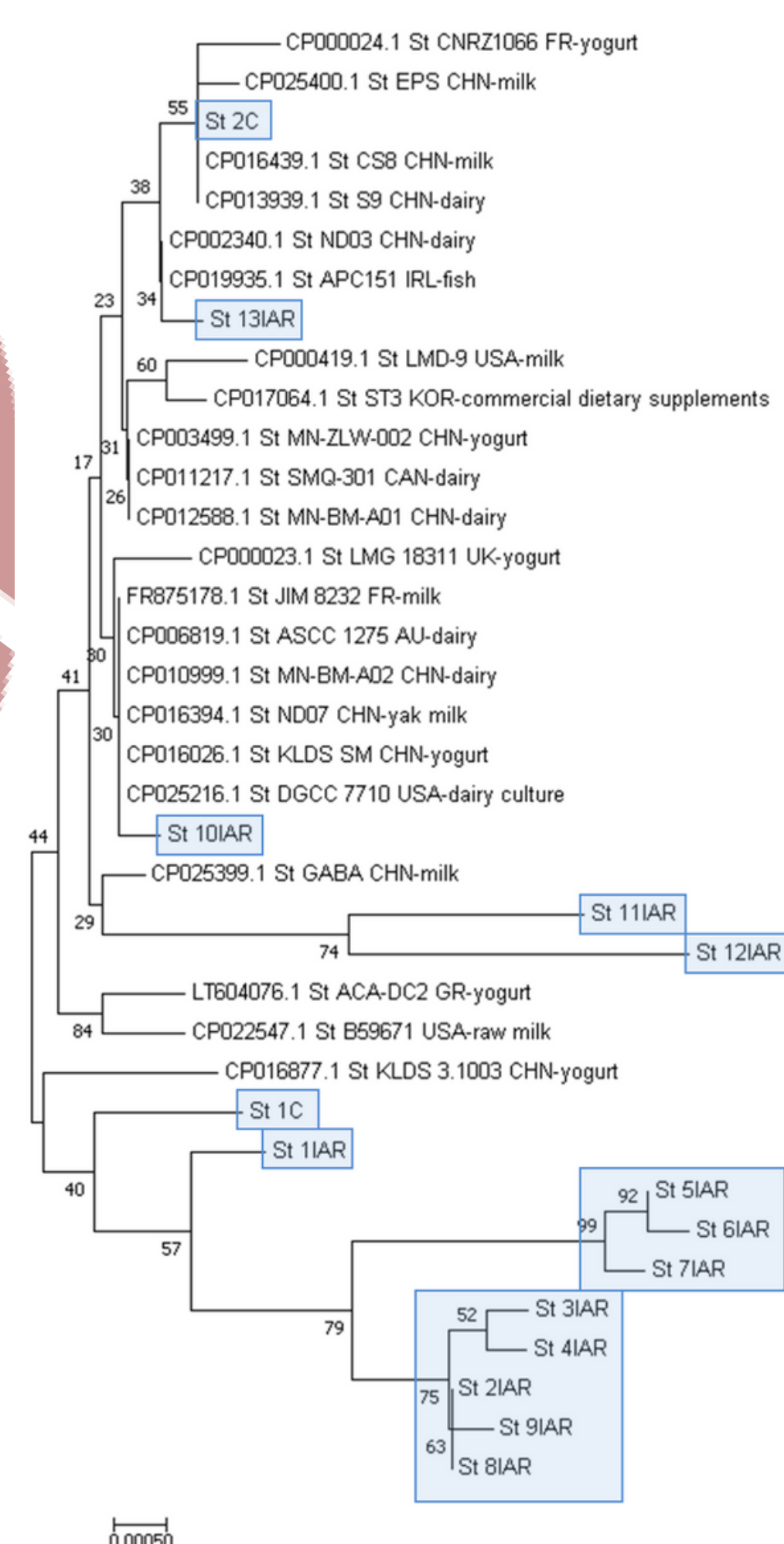


Fig. 1 Dendrogram of *S. thermophilus* strains based on distance matrix from six concatenated loci (for a total of 2576 bases) and using the Neighbor-Joining method. The Bootstrap test (500 replicates) is shown beside each branch. Evolutionary distances were calculated using the Kimura 2-parameter method.

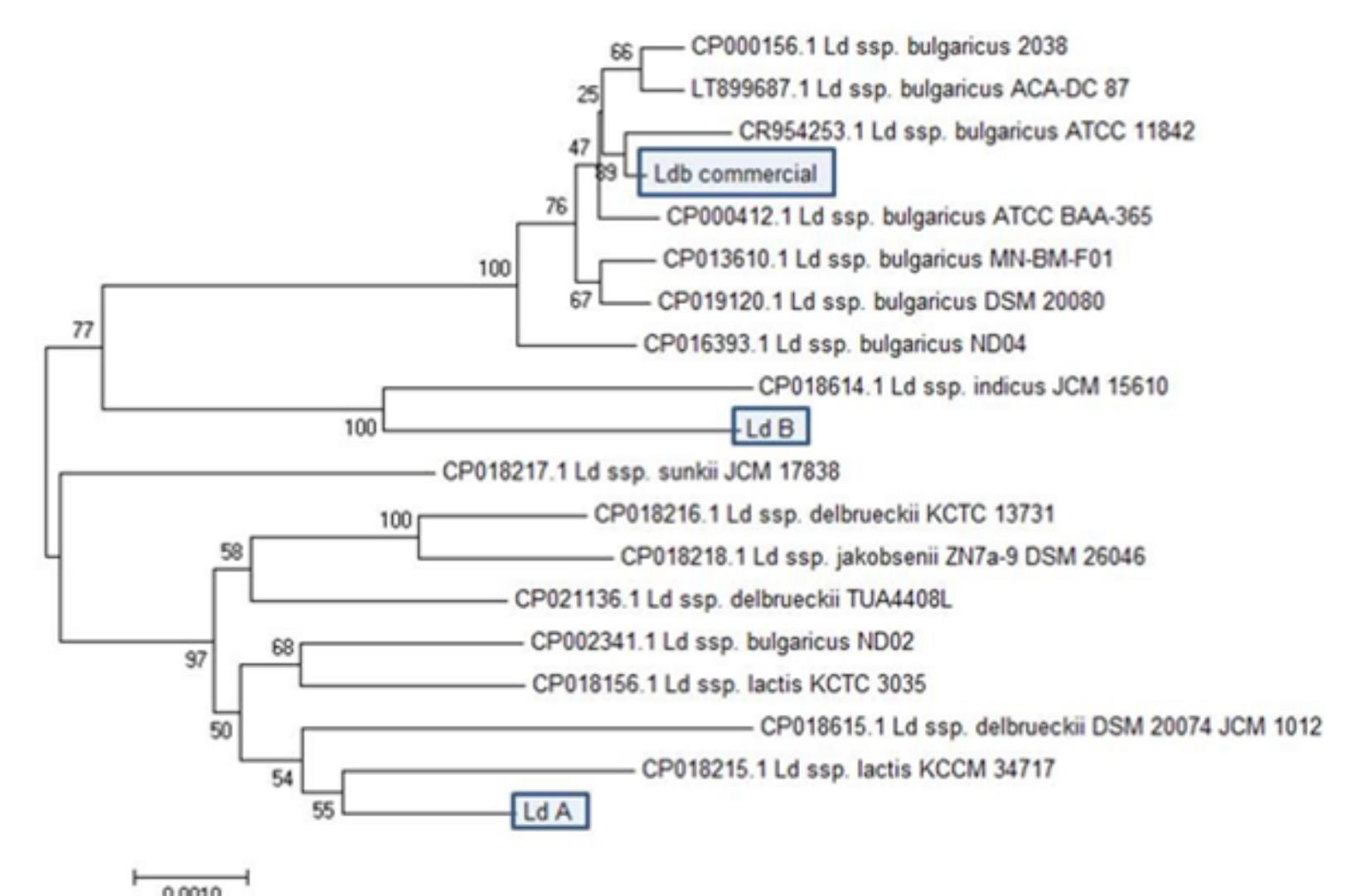
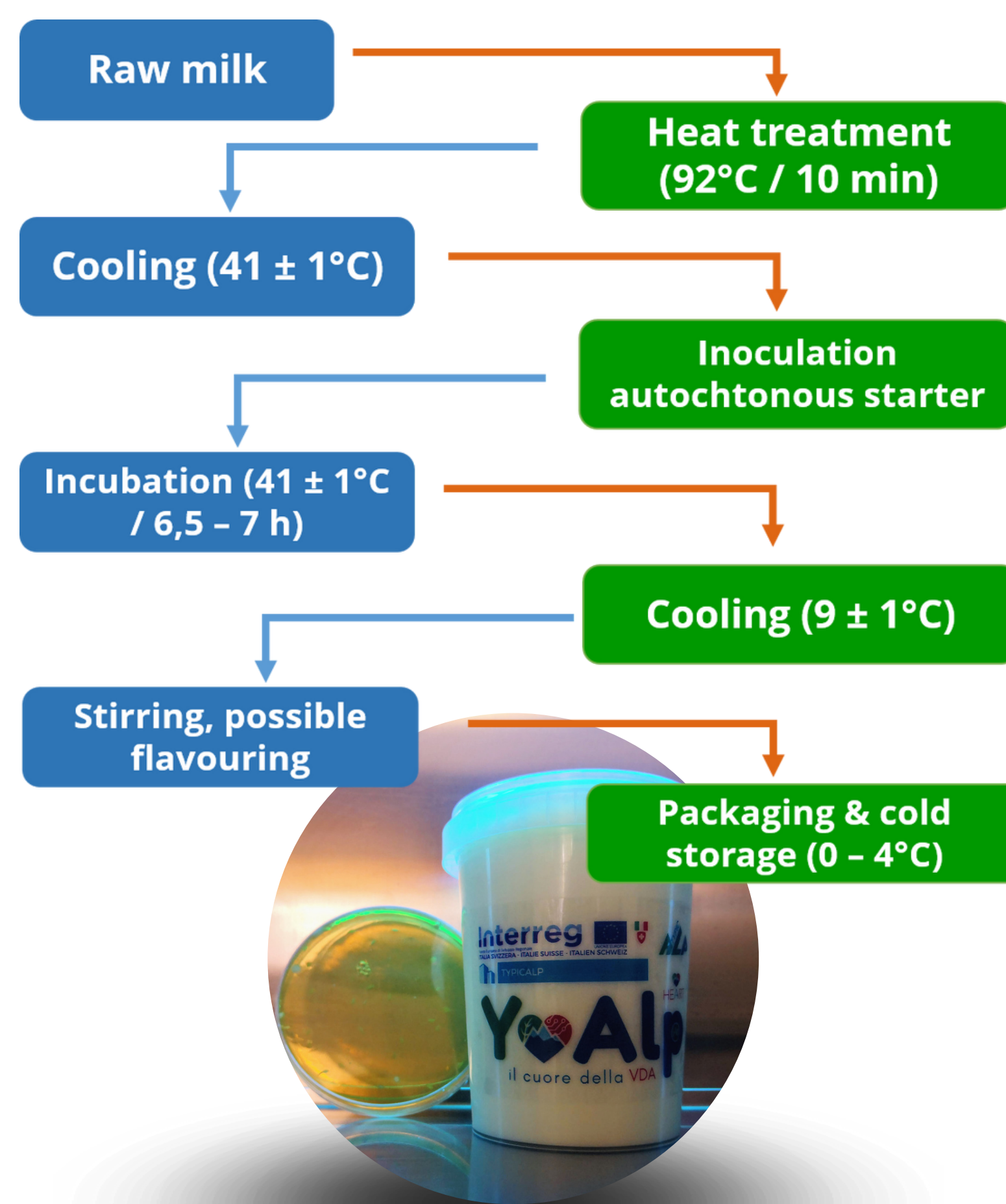


Fig. 2 Dendrogram of *L. delbrueckii* strains based on distance matrix obtained from the analysis of sequences concatenated from nine loci (for a total of 5362 bases) and using the Neighbor-Joining method. The bootstrap test (500 replicates) is reported next to each branch. Evolutionary distances were calculated using the Kimura 2-parameter method.

ID IAR	LAB	Lactic acid g/L 6,5 h	Acidification start time (minutes)	pH 6,5 h	Aspect of the clot	Acetaldehyde g/L 6,5 h
M17Pet2'96-20/7A/24	ST-LB		>360		liquid	
MTH17FE4-20/7A/24	ST-LB	4,4	>360	5,3	liquid	0,00265
MTH17V6-20/7A/24	ST-LB		>360	4,52	compact	
MTH17FV5-20/7A/24	ST-LB	7,56	231,1	4,01	compact	0,00708
MTH17C8-20/7A/24	ST-LB		295,1	5,01	liquid	
M17Pet2'96-66QU1MRS/60	ST-LB	6,52	199,6	4,23	compact	0,00175
MTH17FE4-66QU1MRS/60	ST-LB		>360	5,82	liquid	
MTH17V6-66QU1MRS/60	ST-LB		>360	5,11	liquid	
MTH17FV5-66QU1MRS/60	ST-LB	5,54	230,7	4,45	compact	0,00052
MTH17C8-66QU1MRS/60	ST-LB		>360	5,68	liquid	
Commercial starter	ST-LB	6,33	125,9	4,51	compact	0,00563

Fig. 3 List of blends tested on fresh pasteurized milk and selected pairs with the best technological characteristics (green lines).

- 3 pairs of ST - LB (M17Pet2'96-66QU1MRS/60, MTH17FV5-20/7A/24 e MTH17FV5-66QU1MRS/60) presented the best technological application characteristics and interesting starter potential (Fig. 3).
- On pasteurized milk, these cultures have shown high acidifying power, to which are corresponded significant quantities of lactic acid and acetaldehyde which are essential for obtaining a compact clot and the production of aromatic compounds (Fig. 3 and Fig. 4).

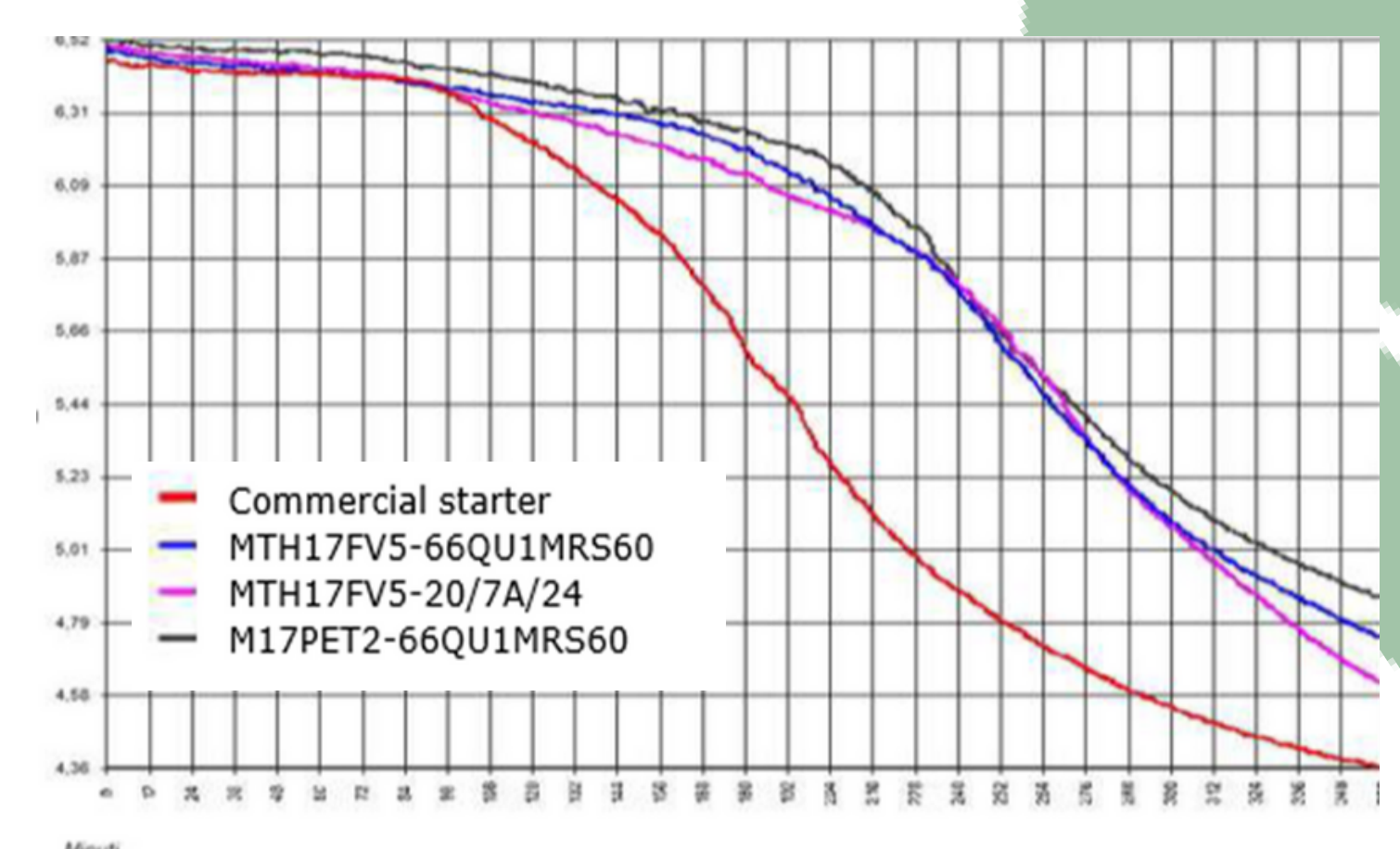


Fig. 4 Acidification curves.

CONCLUSIONS

The results of the molecular analyzes and the technological evaluation of the strains made it possible to identify two strains of *L. delbrueckii* (20/7A/24 and 66QU1MRS/60) and two strains of *S. thermophilus* (M17Pet2'96 and MTH17FV5) suitable for the production of a fermented milk. After freeze-drying only two strains of *S. thermophilus* (M17Pet2'96 and MTH17FV5) and a strain of *L. delbrueckii* (66QU1MRS/60) were found to be suitable for producing a freeze-dried blend. The *Lactobacillus* strain does not belong to the *Bulgaricus* subspecies but it has a high homology with *L. delbrueckii ssp. lactis*. The culture showed high technological performances and ensured a high vital microbial load during product storage. The mixture presented a high acidifying power and a typical aroma, flavour and a compact clot. Overall, the blend also showed excellent technological properties on different types of milk (sheep, goat, skimmed, lactose-free), even on vegetable drinks and in presence of honey.