

Aosta Valley: a case study of microbial biodiversity

Simona ZENATO, Rita PRAMOTTON, Michel OREILLER, Sabina VALENTINI, Luca VERNETTI-PROT

Institut Agricole Régional - Regione La Rochère1/A, 11100 Aosta, Italia

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;
- 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 *S. thermophilus* and *L. 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 *L. 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 cheese made with partly skimmed cow 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 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 differences between the experimental formulations;
- selection of the starter formulation with the best technological performance and freeze-drying.

YEASTS

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.