

Interim report on the comparison of microbial diversity and population dynamics by culture-independent SSCP method, FTIR methods and classical methods

The study aims at comparing microbial diversity and population dynamics by culture-independent SSCP method, FTIR methods and classical methods. Thus, the composition of microbial consortium studied for their antilisteria activities under task2 of WP2A was analyzed and the population dynamics of these consortia at the surface of cheeses was monitored.

There was a good agreement between the RFLP/SSCP and classical methods for the identification of LABs, as well as with FTIR method provided that APT agar is used in standardized conditions. PCR-SSCP results and FTIR identifications differed mainly for coryneform and Gram-negative bacteria. The results also highlighted the possible assignment to multiple species or genera for one FTIR or SSCP pattern. For instance, by SSCP analysis several species in the same genus could be discriminated (*Corynebacterium casei*, *Leucobacter*, *Microbacterium casei*, lactic acid bacteria) while co-elutions were observed with several species of the same genus (*Brevibacterium sp*, *Arthrobacter sp.*) or not (*Enterococcus faecalis* and *Serratia proteomaculans*). For more accurate and reliable identification, methods based on physiological and genomic characterization and allowing identification at different taxonomic levels (genus or species levels) should be combined.

For instance, classical or RFLP methods may be used first to assign the isolates to a genus, and FTIR or SSCP may be used in a second step to confirm this identification.

The diversity of the microbial community from the surface of TR15 Saint-Nectaire cheese (cf. Deliverable D2A.2.4) was independently investigated by FTIR or molecular (RFLP/sequencing) methods. The distribution of isolates as described by the two approaches differed mainly by the balance between lactic acid bacteria (42% of the isolates identified by RFLP/sequencing and 10% of the isolates identified by FTIR) and Gram-positive catalase-positive bacteria (coryneforms, *Staphylococci*) (20% of the isolates identified by RFLP/sequencing and 54% of the isolates identified by FTIR). These differences could be explained by the selectivity of the different culture media used by each partner (seven media used for identification by RFLP and 16S rDNA sequencing *versus* one for FTIR analysis) and the potential discrepancies between the methods used for identification of the isolates.

By counting on different media, it was possible to monitor the dynamics of bacterial populations (lactobacilli, *Enterococcus*, *Leuconostoc pseudomesenteroides*, *S. pulvereri*, *Arthrobacter* and *Brachybacterium*) and yeast populations (*Candida sake*, *Yarrowia lipolytica* and *Debaryomyces hansenii*) of the complex microbial consortium TR15 and of reconstituted consortia at the surface of Saint-Nectaire cheese during ripening (deliverable D2A.2.4). The plate count method and SSCP analysis on the one side, and FTIR and SSCP analysis on the other side, were in agreement for revealing the dynamics of dominant populations (Gram-negative bacteria, *Staphylococcus...*) in TR15 native and reconstituted consortia at the surface of Saint-Nectaire, and in TF23 and TF25 consortia after successive propagations in the absence and in the presence of *Listeria*. The less dominant populations (*Lactobacillus*, *Enterococcus*) detected by culture methods on various selective media were not found by SSCP analysis since their levels were more than 2 Log lower than those of the dominant one.