Development of new starter cultures for traditional cheeses produced in the mountain areas of the Veneto Region

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Introduction

Natural starter cultures are complex associations of species and strains of lactic acid bacteria which can affect both the cheese-making procedure and the ripening profile. Although natural starters are an important source of strains with desirable technological properties, fluctuations in their composition may result in variable performances. On the other hand, the replacement of natural cultures with defined commercial starters composed of a limited number of strains may lead to cheeses with poor flavour and aroma. The aim of this work was to develop a protocol to produce a new type of starter cultures for traditional cheeses obtained from the freeze-drying of natural milk cultures. These new autochthonous multiple strains culture could be representative of the microbial composition of the natural milk culture and could contribute to the specific and unique characteristics of artisanal traditional cheeses.

Experimental protocol

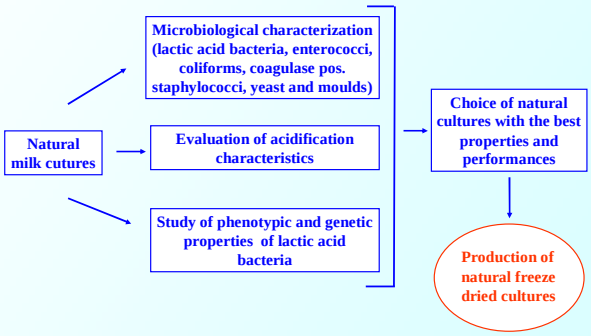

Fig. 1 Experimental scheme followed for the development of freeze-dried natural mik cultures.

For the development of the freeze dried natural milk cultures we followed the approach reported in Fig. 1. Twenty-five natural milk cultures collected in dairy plant or prepared in laboratory using milk coming from different farms located in the mountain areas of Veneto Region were studied for :

1) microbiological characteristics (enumeration of lactic acid bacteria and of spoilage and pathogenic microorganisms on specific media)

2) acidifying activity, in milk incubated at 37°C us ing an automatic system for pH measurement and data acquisition.

3) phenotypic and genetic biodiversity within *S. thermophilus* populations; to this purpose, 10 colonies of presumptive *S. thermophilus* were isolated from each natural culture and evaluated for a) technologically relevant properties such as acidifying and proteolyticc activity, bacteriophage resistance/sensitivity and b) genetic diversity by RAPD-PCR analysis with primers M13 and D11344.

Results

Characterization of natural milk cultures

All the natural milk cultures were characterized by the dominant presence of *S. thermophilus* (10^8-10^9 CFU/ml), whilst thermophilic lactobacilli and enterococci were not detected or were present at low concentrations (10^2-10^3 CFU/ml). Coliforms and coagulase positive staphylococci were not found in all the examined samples; however, 32% of cultures were contaminated by yeasts (max. conc. 190 CFU/ml) and part of them also by Gram-negative spoilage bacteria, belonging to the genus *Pseudomonas.*

Most of the natural milk cultures (78%) were characterized by high acidifying activity in milk with pH <4.4 after 16h incubation and Vm (maximum acidification rate) values <- 12.5 pH millunits min $^{-1}$.

Considerable variation between the *S. thermophilus* strains isolated from the natural milk cultures was observed for acidification and peptidase activities (Arg, Phe-Pro), as well as for their sensitivity/resistance to bacteriophages isolated from the same geographic area. A very significant level of genetic polymorphism was detected by RAPD-PCR (Fig. 2) which revealed the presence of different *S. thermophilus* strains in the different milk cultures and also within the same milk culture (2 to 4 different strains among the 10 colonies considered for each culture).

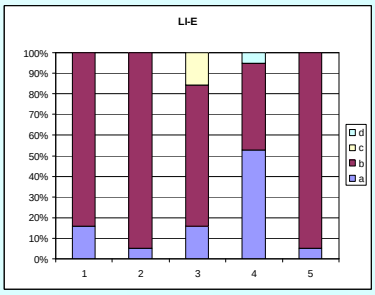

Fig. 2 RAPD-PCR profiles of dominant strains isolated from natural milk cultures.

Production of freeze dried natural milk cultures

Two milk cultures (LI-D and LI-E) characterized by good microbiological and technological properties (absence of spoilage microorganisms, high concentration of S. *thermophilus,* presence of strains with different technological and genetic characteristics, proper acidification ability) were inoculated in reconstituted skim milk and incubated at 44°C till coagulation. The freshly coagulated cultures were then inoculated in 5 L of reconstituted skim milk and incubated at 44°C till an acidity of 8°SH/ 50ml was reached. The cultures were added with cryoprotectors (skim milk + Na-glutammate) and freeze-dried.

In order to compare the composition of the freeze-dried culture with the composition of the original milk culture, 20 colonies of *S. thermophilus* were isolated from each of the following steps and analyzed by RAPD-PCR:

1) Original natural milk culture (step 1)
2) Freshly coagulated culture used as inoculum (step 2)
3) Natural milk culture after reaching 8°SH/50ml (st ep 3)
4) Freeze dried culture (step 4)
5) Freeze dried culture grown in milk and prepared as bulk starter for cheese manufacture (step 5)

The composition in terms of *S. thermophilus* strains is stable in natural milk culture E (Fig.3a) where two main strains, *a* and *b*, are present in all the examined steps. The situation is slightly different in natural milk culture D (Fig. 3b) in which the composition of culture after freeze drying differs with the composition found in the other steps. A new strain (*strain d*) which is probably more resistant to freeze-drying, constitutes the 90% of the freeze dried culture and is not found in any of the other steps. After growth of the freeze-dried culture in milk the typical composition of the natural culture LI-D with the presence of the two dominant strains *a* and *b* was re-established.

Fig. 3a and 3b

Monitoring of *S. thermophilus* strains during propagation of the milk culture.
1,2,3,4,5: steps of production
a,b,c,d,e: RAPD-PCR profile

Cheese production

The two freeze dried natural cultures were used for the preparation of bulk starter and used in the manufacture of traditional semi-hard and hard cheeses. Both the cultures showed high performances during cheese making; sensory analysis of cheeses showed that they present optimal and typical aroma, flavour and texture. These preliminary results suggest that the freeze dried natural milk cultures could be a valid alternative to the natural liquid cultures (which are prepared daily), or to the defined commercial starters.

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