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Molecular evaluation of microbial diversity occurring in different types of Mozzarella cheese

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Aims: The microbial community of different types of unripened Pasta Filata cheese was investigated by culture-independent methods with the aim of rapidly achieving knowledge about cheese microbiota and discriminating traditional and industrial cheeses.

Methods and Results: The microbial DNA extracted directly from the samples was used as a template in PCR experiments to amplify the 16S–23S rDNA spacer region and the V3 region of the 16S rDNA. Conventional electrophoresis of the amplified spacers allowed known classes of these DNA fragments belonging to genera and species of lactic acid bacteria to be distinguished. Denaturing gradient gel electrophoresis analysis of V3 amplicons was supported by reference cultures of LAB used as markers.

Conclusions: Both molecular approaches furnished the expected information about microbial diversity and were quite valid for discriminating industrial, semi-artisanal or traditional cheeses, characterized by increasingly complex DNA profiles.

Significance and Impact of the Study: Both methods could be used for legal purposes when products obtained through prescribed manufacturing regulations are to be analysed.

INTRODUCTION

Mozzarella is the most popular member of the 'Pasta Filata' cheeses. At present, in Europe, the following are produced: water-buffalo Mozzarella cheese from Campania (EC Rule no. 1107/96), Mozzarella from cow's milk, and another very common cow's milk product in Italy called 'Fior di latte'. Apart from the different origins of the milk used, these unripened dairy products are manufactured either according to traditional procedures (raw milk inoculated with natural whey or milk cultures, raw milk ripened under special conditions, without starter addition) or by using pasteurized milk and commercial starter cultures of lactic acid bacteria (LAB). Moreover, Mozzarella cheese is also know to be produced by direct acidification with lactic acid, citric acid or glucono-δ- lactone (Parente and Moschetti 1997).

Within the complex bacterial community of traditional raw milk cheeses, lactic acid bacteria are considered to be the dominant microflora, but many genera and species of these micro-organisms are necessarily involved in the curd ripening process in order to assure the typical taste and aroma of the final cheese through appropriate acidifying, proteolytic and flavouring activities (Coppola *et al.* 1988, 1990). The variety of such organisms in cheeses depends on the starter used and could therefore represent a good marker to discriminate traditional from industrial products.

Research on the microflora of dairy products relies on the isolation, on suitable substrates, of the cultivable organisms that are not always representative of the complex ecosystem. It has been reported (Ward *et al.* 1990; Engelen *et al.* 1998) that only a small fraction of micro-organisms is analysed by conventional methods and often, the isolated strains do not seem to represent the real spectrum of micro-organisms and their genes active in the habitat of choice. By contrast, culture-independent methods can provide a more realistic view of microbial diversity in the ecosystem, as recently demonstrated in lactic acid-fermented maize dough by Ampe *et al.* (1999).

To analyse microbial communities without their cultivation, denaturing gradient gel electrophoresis (DGGE) (Fischer and Lerman 1979) by sequence-specific separation

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