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Review

Culture-independent methods for identifying microbial communities in cheese

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ABSTRACT

This review focuses on the culture-independent methods available for the description of both bacterial and fungal communities in cheese. Important steps of the culture-independent strategy, which relies on bulk DNA extraction from cheese and polymerase chain reaction (PCR) amplification of selected sequences, are discussed. We critically evaluate the identification techniques already used for monitoring microbial communities in cheese, including PCR-denaturing gradient gel electrophoresis (PCR-DGGE), PCR-temporal temperature gradient gel electrophoresis (PCR-TTGE) or single-strand conformation polymorphism-PCR (SSCP-PCR) as well as some other techniques that remain to be adapted to the study of cheese communities. Further, our analysis draws attention to the lack of data available on suitable DNA sequences for identifying fungal communities in cheese and proposes some potential DNA targets.

Contents

1. Microbial communities in cheese

Cheese is produced throughout the world and more than 1000 varieties (Sandine and Elliker, 1970) with different forms and flavours exist. Cheesemaking is conjectured to date back to some 8000 years ago originating from the Middle East (Fox et al., 2000) where the first fermented milk-based foods were made. Four basic ingredients are required to produce most cheeses: milk, rennet, salt and microorganisms. These four ingredients are processed through different steps such as acidification, coagulation, syneresis and ripening (see Fox et al., 2000). Each unique combination of ingredients and processing parameters leads to a specific type of cheese with unique properties. Fox et al. (2000) mentioned how fascinating it is that "such a diverse range of products can be

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produced" from "basically similar raw material". The composition and activity of the microflora is the least controllable of all the parameters. The microflora is made up of (i) starter lactic acid bacteria that are involved in acid production during cheese manufacture and that also participate in the ripening processes to various extents; and (ii) non-starter lactic acid bacteria, other bacteria, yeasts and filamentous fungi that form the secondary microflora, which plays a significant role during ripening (Fox et al., 2000). Both starter and secondary flora modify the physical and chemical properties of cheese, contributing to and reacting to environmental changes that occur during the manufacture and ripening of cheese. Coppola et al. (2007) distinguished seven technological production phases of cheese that may constitute selective pressures for the microbial species that play an important role in community succession occurring throughout cheese manufacture and ripening. The fundamental features that influence the dynamics of the cheese ecosystem have been described by Beresford et al. (2001). They include (i) physical features such as moisture, salt concentration, pH or redox potential which change during cheese manufacture and are themselves influenced by the microflora, and (ii) biological features such as those resulting from the interactions between microorganisms. The characteristics of a given cheese therefore depend on microflora dynamics. Although food microflora is undoubtedly not as diverse as environmental microflora, such as those found in the soil (Garbeva et al., 2004), cheese microbial populations still remain difficult to control due to their complex dynamics and to their interactions (Beresford et al., 2001). Knowledge of the structure and dynamics of the whole microbial community of cheese would promote better understanding of how cheese characteristics vary with respect to microbial growth and metabolism. For instance, greater control over microflora composition would make it possible to better select for specific organoleptic properties or to prevent quality defects or spoilage. For these reasons, cheese bacterial and fungal communities have already been partially identified using traditional methods and, to a lesser extent, molecular techniques.

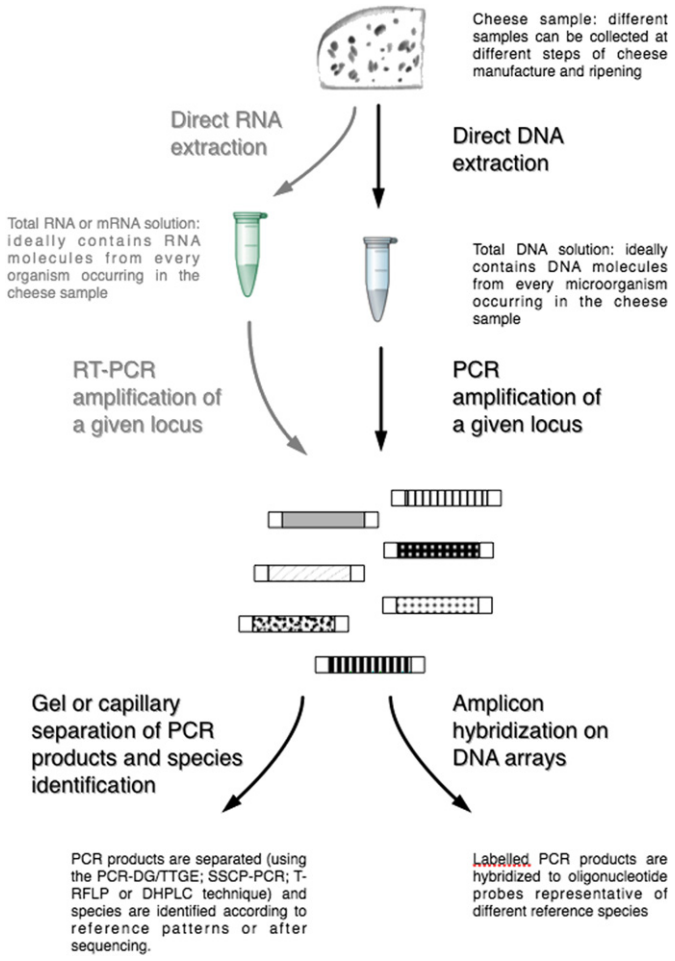

Fig. 1. Flow diagram of the application of nucleic acid-based culture-independent methods to the study of microbial communities in cheese.

2. Towards culture-independent methods

As in other fields in microbiology, species identification in cheese can be assessed through the use of either culture-dependent or culture-independent methods. Culture-dependent methods consist of isolating and culturing microorganisms prior to their identification according to either morphological, biochemical or genetic characteristics. Different cultures can even be bulked and analysed using global analysis methods, such as those as described below for culture-independent methods (Ercolini et al., 2001, 2004). These methods have already shed light on the structure of microbial populations during cheese manufacture (Andrighetto et al., 1998; Fitzsimmons et al., 1999; Mannu et al., 2000; Berthier et al., 2001; Dasen et al., 2003). However, culture-dependent methods are time-consuming, due to long culture periods and elaborate culture techniques. They are therefore not amenable to monitoring community dynamics during cheese manufacture and ripening. Moreover, species occurring in low numbers are often out-competed *in vitro* by numerically more abundant microbial species (Hugenholtz et al., 1998) and some species may be unable to grow *in vitro* (Ward et al., 1990, 1992; Head et al., 1998). Hence, if culture conditions are poor and the number of isolates too low, the culture collection will not be representative of the community and the actual microbial diversity will be misinterpreted.

Community-level studies are relying more and more on culture-independent methods based on the direct analysis of DNA (or RNA) without any culturing step. These methods are based on protocols where total DNA (or RNA) is directly extracted from the substrate. Coupled with a global analysis, these methods make it possible to study the total diversity from the bulk extract in a single step. As they are fast and potentially more exhaustive, these methods are well suited for analysing microbial communities over time and may provide the possibility of exploring cheese microflora dynamics in detail. Most of these methods use polymerase chain reaction (PCR) amplification of total DNA. The PCR amplicons from different species are discriminated by using gel or capillary separation or by hybridization to specific probes (Fig. 1). However, these methods have potential biases, which will be discussed below.

3. 'Pick'em all

As previously mentioned, the isolation step of culture-dependent methods introduces biases because some species are unable to grow under the selected experimental conditions. Culture-independent methods typically aim at collecting DNA from the whole community to overcome this bias. Nevertheless, technical issues may arise: DNA may not be recovered from all genotypes or PCR amplification may be inaccurate. Some genotypes may remain undetected due to low species abundance in the substrate, low species availability due to insufficient homogenization of the matrix, inadequate cell lysis that prevents release of nucleic acids, or inhibition of PCR amplification.