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Invited review: Microbial evolution in raw-milk, long-ripened cheeses produced using undefined natural whey starters

Monica Gatti,*†[1] Benedetta Bottari,* Camilla Lazzi,* Erasmo Neviani,*† and Germano Mucchetti*†
*Department of Food Science, University of Parma, Parco Area delle Scienze, 48/A, 43124 Parma, Italy
†Centro Multidisciplinare Interdipartimentale Lattiero Caseario (MILC), University of Parma, Parco Area delle Scienze, 85/A, 43124 Parma, Italy

ABSTRACT

The robustness of the starter culture during cheese fermentation is enhanced by the presence of a rich consortium of microbes. Natural starters are consortia of microbes undoubtedly richer than selected starters. Among natural starters, natural whey starters (NWS) are the most common cultures currently used to produce different varieties of cheeses. Undefined NWS are typically used for Italian cooked, long-ripened, extra-hard, raw milk cheeses, such as Parmigiano Reggiano and Grana Padano. Together with raw milk microbiota, NWS are responsible for most cheese characteristics. The microbial ecology of these 2 cheese varieties is based on a complex interaction among starter lactic acid bacteria (SLAB) and nonstarter lactic acid bacteria (NSLAB), which are characterized by their different abilities to grow in a changing substrate. This review aims to summarize the latest findings on Parmigiano Reggiano and Grana Padano to better understand the dynamics of SLAB, which mainly arise from NWS, and NSLAB, which mainly arise from raw milk, and their possible role in determining the characteristics of these cheeses. The review is presented in 4 main sections. The first summarizes the main microbiological and chemical properties of the ripened cheese as determined by cheese-making process variables, as these variables may affect microbial growth. The second describes the microbiota of raw milk as affected by specific milk treatments, from milking to the filling of the cheese milk vat. The third describes the microbiota of NWS, and the fourth reviews the knowledge available on microbial dynamics from curd to ripened cheese. As the dynamics and functionality of complex undefined NWS is one of the most important areas of focus in current food microbiology research, this review may serve as a good starting point for implementing future studies on microbial diversity and functionality of undefined cheese starter cultures.

Key words: raw milk cheese, microbial evolution, natural whey starter

INTRODUCTION

In the early twentieth century, the industrialization and commercialization of fermented food necessitated the need to turn traditional fermentation processes into controlled and rationalized food processing. Moreover, the food and beverage industry is rediscovering fermentation as a crucial step in product innovation (Hugenholtz, 2013). With this aim, fermentation research in food microbiology has focused on the isolation and phenotypic characterization of microbes originating from natural sources, such as spontaneous fermentation. This approach has delivered defined single and mixed-strain cultures suitable for industrial applications (Kutahya and Smid, 2012). However, the production of selected dairy starter cultures has been met with several obstacles, such as limitations in carbon and nitrogen source availability, salt stress during brining, temperature fluctuations, pH changes, and phage predation during cheese fermentation (Kutahya and Smid, 2012). Sensitivity to those factors can be essential for proper flavor formation. The robustness of the starter culture during cheese fermentation is enhanced by the presence of a rich consortium of microbes. Diversity in the starter culture is determined by different phenotypic characteristics linked to the coexistence of different genomic lineages of microbes. The genetic diversity present in wild strains includes a variety of features that are relevant to the improvement of starter cultures (Smid and Hugenholtz, 2010), and this diversity is selectively propagated as a result of adaptation by the microbes to dynamic environments.

For these reasons, the study of the dynamics and functionality of complex undefined starter cultures is one of the most important areas of focus in current food microbiology research. The dynamics of the biochemical activities, growth, survival, and death of microorganisms in fermented foods are the result of interactions between microbes and their microenvironment. This microenvironment exists in an unsteady state due to

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[1]Corresponding author: monica.gatti@unipr.it

chemical and physical changes partly resulting from the microbes' own metabolic activities, which can be further manipulated by the sequence of operations in the food technology industry.

The fundamentals of the microbial ecology of long-ripened cheese produced from raw milk using undefined whey starter cultures are based on complex interactions among starter lactic acid bacteria (**SLAB**) and nonstarter lactic acid bacteria (**NSLAB**), which are characterized by their different abilities to grow in a changing substrate. The various steps of cheese-making induce heat-related, acidic, osmotic, and oxidative stresses on microorganisms and are responsible for alterations in heat load, pH, water activity ($\mathbf{a_w}$), and redox potential gradients in the matrix (Beresford et al., 2001). As a consequence, SLAB viability is reduced within a few hours or a few days from the start of the cheese-making process, at which point a sizable fraction of the starter cells undergo autolysis, releasing their enzymes into the matrix. In contrast, NSLAB, which are able to grow using energy sources other than lactose and are more resistant to environmental stresses, grow slowly and become the dominant microflora of the ripened cheese. The ratio between SLAB and NSLAB is modulated by the type of culture added to the cheese milk, the cheese-making conditions, and the duration of cheese ripening.

Grana Padano (**GP**) and Parmigiano Reggiano (**PR**) cheeses are typical extra-hard, cooked Italian Protected Designation of Origin (**PDO**) cheeses characterized by a very long ripening period (a minimum of 9 and 12 mo for GP and PR, respectively) of up to 20 to 24 mo and often more than 30 mo in the case of gold-label PR cheese.

This long ripening period is common to other minor Italian cheeses produced in the mountainous areas of northern Italy, such as Bagoss from Bagolino (Brescia) and Nostrano Valtrompia PDO (Mucchetti and Neviani, 2006). Several other Italian cheeses such as Asiago d'Allevo, Montasio, Pecorino Romano, and Fiore Sardo PDO are ripened for 1 yr or more (Gobbetti, 2004), although the typical ripening time for these cheeses is substantially less.

More famous long-ripened cheeses from Europe such as Swiss cheeses, Emmental, Gruyere, Sbrinz, Hobelkäse, and Comté are usually ripened for 6 to 48 mo (Depouilly et al., 2004; Harbutt, 2009; Turgay et al., 2011). Some other cheese varieties such as the Scandinavian Västerbottenost, Prästost, Herrgärdsost, Danbo, or Jarlsberg cheeses or the Low Countries' Postel and Leisde Kaas, or some varieties of old Edam and old Gouda cheese are ripened up to 36 mo (Harbutt, 2009). West Country Farmhouse Cheddar PDO cheese, produced in the United Kingdom, is aged for a minimum of 9 mo, with extra mature or vintage varieties being aged for more than 18 mo.

Natural starters, particularly natural whey starters (**NWS**), are the most common undefined cultures currently used to produce different varieties of cheeses, including PR, GP, Provolone Valpadana, Mozzarella di Bufala Campana, and Pecorino Romano cheeses in Italy and Comté and Gruyere cheeses in France and Switzerland.

Parmigiano Reggiano and GP cheeses are unmatched among world cheeses, and this review aims to summarize the latest findings on these 2 varieties to better understand the dynamics of SLAB and NSLAB and their role in determining the characteristics of these cheeses. This knowledge may serve as a good starting point for implementing future studies on microbial diversity and functionality of undefined cheese starter cultures.

PARMIGIANO REGGIANO AND GRANA PADANO: MAJOR CHARACTERISTICS AND CHEESE-MAKING TECHNOLOGY

Chemical Composition of Ripened Cheeses

Parmigiano Reggiano and GP cheeses have many common characteristics and some distinct properties. Although the similarities between these cheeses are defined by their similar cheese-making technologies, the differences are largely determined by the methods of milk collection, milk management before coagulation, and by the ripening conditions.

The moisture content of PR and GP cheeses ranges from 28 to 35% and varies according to ripening time; the ratio of fat to protein in PR and GP is quite variable and may be 0.94 ± 0.02 according to cheese milk characteristics. It is notable that PR and GP cheeses are lactose- and galactose-free, and the lactic acid content is approximately 1.5%. Both isomers of lactic acid are present, with an L-to-D ratio of approximately 1.1 (Careri et al., 1996; De Dea Lindner et al., 2008).

Some minor carbohydrates, such as glycosaminoglycans, have been found in PR at levels of 1.5 to 3.0 mg/kg (Coppa et al., 2012). Citric acid was found at levels ranging from 8 to 93 mg/100 g, with an average value of 50 mg/100 g in PR (Careri et al., 1996; Coppola et al., 2000; De Dea Lindner et al., 2008). Organic acids derived from bacterial fermentation, such as acetic and propionic acid, are also present in low levels, usually <100 and 0.5 mg/100 g, respectively, in PR (Tosi et al., 2008). A threshold of 2 mg of propionic acid/100 g of cheese was proposed by Bacci et al. (2002) as a marker for evidence of a defect in PR cheese. In contrast, butyric acid can be generated during fermentation or lipolysis, and its threshold for nondefective PR cheeses