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Symposium review: Lactococcus lactis from nondairy sources: Their genetic and metabolic diversity and potential applications in cheese[1]

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ABSTRACT

The widespread dissemination of species of the lactic acid bacteria (LAB) group in different environments testifies to their extraordinary niche adaptability. Members of the LAB are present on grass and other plant material, in dairy products, on human skin, and in the gastrointestinal and reproductive tracts. The selective pressure imparted by these specific environments is a key driver in the genomic diversity observed between strains of the same species deriving from distinct habitats. Strains that are exploited in the dairy industry for the production of fermented dairy products are often referred to as "domesticated" strains. These strains, which initially may have occupied a nondairy niche, have become specialized for growth in the milk environment. In fact, comparative genome analysis of multiple LAB species and strains has revealed a central trend in LAB evolution: the loss of ancestral genes and metabolic simplification toward adaptation to nutritionally rich environments. In contrast, "environmental" strains, or those from raw milk, plants, and animals, exhibit diverse metabolic capabilities and lifestyle characteristics compared with their domesticated counterparts. Because of the limited number of established dairy strains used in fermented food production today, demand is increasing for novel strains, with concerted efforts to mine the microbiota of natural environments for strains of technological interest. Many studies have concentrated on uncovering the genomic and metabolic potential of these organisms, facilitating comparative genome analysis of strains from diverse environments and providing insight into the natural diversity of the LAB, a group of organisms that is at the core of the dairy industry. The natural biodiversity that exists in these environments may be exploited in dairy fermentations to expand flavor profiles, to produce natural "clean label" ingredients, or to develop safer products.

Key words: *Lactococcus lactis*, niche adaptation, domesticated, environmental

INTRODUCTION

Consumption of fermented dairy foods is an age-old tradition that can be traced back thousands of years. From the spontaneous fermentations of the past to the industrial-scale manufacture of fermented dairy products of the present day, the starter cultures used for the production of fermented dairy foods are of great significance, driving the manufacture and development of the flavor and texture of these products. However, the pressures of the current global market and the desire for new products to meet customer demands can test the limits of microbial performance, resulting in the need for constant development of new starter blends with novel properties. As a result, today's starter cultures are developed mainly by design (Hansen, 2002). Strain discovery pipelines are now a common feature of the research and development units of many commercial culture suppliers and in research labs dedicated to rational strain discovery, non-GMO strain improvements, and their associated processes in industrial application. In addition, the application of state-of-the-art "-omics" technologies has provided sophisticated tools for a more knowledge-based approach to selection of desirable cultures (Mills et al., 2010; Kelleher et al., 2015).

Dairy consumers are willing to experiment with different flavors and ingredients and thus, dairy companies are striving to enhance the differentiation points of their products (Dairy Reporter, 2017). To facilitate this, many companies have looked at culture manipulation as a tool for flavor diversification. This has led to an increasing interest in "environmental" isolates, particularly isolates of *Lactococcus lactis* from plant material or other niches. Adaptation of such strains to the substrates encountered in these environments is expected to result in the development of unique traits and phenotypes that could be exploited in dairy applications. Until relatively recently, such isolates were poorly

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characterized. The aim of this review is to consider the most recent information emerging from studies on these so-called wild or environmental strains, isolated from raw milk, plants, animals, and other nondairy environments. We examine the genomic diversity within the *L. lactis* group, the domestication of well-known strains to the dairy environment, and the metabolic potential afforded to strains by traits known to confer an evolutionary advantage in nondairy environments. In addition, we review studies where environmental strains have been applied in dairy settings and comment on the future perspective of such strains being used commercially for the development of dairy products with novel product attributes.

THE ORIGINS OF MODERN CULTURE SYSTEMS

Modern industrial starter cultures originated mainly from natural contaminants of the raw materials used as substrates in fermentation. The contaminating microbiota was responsible for driving a process of spontaneous fermentation, long before the process of fermentation itself was even understood. The practice of “back-slopping,” where a small amount of whey or cream from one day’s fermentation was used to start the next day’s fermentation, ensured maintenance of the starter (Mullan, 2014). However, a lack of quality and consistency in the resulting fermented products meant that such an approach was not satisfactory for today’s industrial-scale production, and the concept of the defined strain starter culture was realized in the 1930s (Limsowtin et al., 1996). Traditional starter cultures were screened for individual strains with key properties, such as fast growth in milk, insensitivity to bacteriophages, and production of certain flavors and textures. Strains displaying these desired properties were combined as mixed-strain starters, with the strain combinations depending on the application. Although some artisanal fermented food producers still rely on their own undefined culture blends, most large-scale producers now rely on commercially produced, defined culture blends in which the strains have been specifically selected, blended, and cultivated under tightly controlled conditions to ensure an optimized fermentation each time. A recent review on general aspects of starter cultures is available and provides an overview of both traditional and modern culture systems (Parente et al., 2017). What these authors, and others who have discussed the topic, allude to is that the repeated isolation of single strains from undefined mixed starter blends, coupled with the sharing of these strains between laboratories in the early days of culture screening and analysis, has resulted in a relatively small pool of good starter cultures that forms the basis of the modern fermented dairy foods industry (Marshall, 1991; Kelly et al., 2010; Parente et al., 2017). This has reduced the biodiversity of strains from which to choose for novel applications and increased demand for unique strains from diverse sources, with some concerted efforts to mine the microbiota of natural environments, such as raw milk and plants, for strains of technological interest (Klijn et al., 1995; Kelly et al., 1998; Nomura et al., 2006; Alemayehu et al., 2014; Cavanagh et al., 2015).

LACTOCOCCUS LACTIS—THE QUINTESSENTIAL CHEESE STARTER

As primary components of the starter cultures used in fermented food production, members of the lactic acid bacteria (**LAB**) group are of key industrial importance. The major genera used in dairy fermentations include *Lactococcus*, *Lactobacillus*, *Streptococcus*, and *Leuconostoc*. Starter cultures used for the production of cheeses such as Cheddar, Edam, and Gouda typically consist primarily of mesophilic species, predominantly species from the *Lactococcus* genus. Twelve species of *Lactococcus* are currently recognized, along with 6 subspecies (Table 1). Although several species within the genus are derived from nondairy habitats, we are most familiar with the milk- and dairy-associated species. These include *Lactococcus lactis*, *Lactococcus raffinolactis*, and, more recently, *Lactococcus hircilactis* and *Lactococcus laudensis*. The latter 2 species are recently discovered members of the genus isolated from goat and cow milk, respectively (Meucci et al., 2015). The *L. lactis* and *L. raffinolactis* species are recorded in the International Dairy Federation’s Inventory of Microbial Food Cultures (Bourdichon et al., 2012) and have long been associated with fermented dairy foods. Little is known about *L. raffinolactis*, but it has been found as a constituent of complex undefined mesophilic starter blends, and the genome sequence of one such strain was recently elucidated (Meslier et al., 2012). To date, 4 *L. lactis* subspecies have been defined: *lactis*; *cremoris*; *hordniae*, from the leafhopper *Hordnia circellata*; and *structae*, isolated from the intestinal mucus of a brown trout (Schleifer et al., 1985; Pérez et al., 2011).

Strains of the subspecies *lactis* and *cremoris* are central components of the defined strain culture blends used in the commercial production of cheese. The principal role of these strains is to produce lactic acid and contribute to the degradation of milk casein, thus influencing the flavor, texture, and quality of the final product. Although strains of *L. lactis* ssp. *lactis* are often considered fast acidifiers, *L. lactis* ssp. *cremoris* strains are often favored as defined starters as they tend to cause less bitterness and other defects. However, their heat sensitivity compared with *L. lactis* ssp. *lactis*