Spore-forming bacteria in the dairy chain

To cite this article: T Ledina *et al* 2021 *IOP Conf. Ser.: Earth Environ. Sci.* **854** 012051

Spore-forming bacteria in the dairy chain

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Abstract. Spore-forming bacteria form the most diverse and most complex group of bacteria in terms of their elimination from the dairy chain, due to their ability to form highly resistant spores. As ubiquitous microorganisms, spore-formers can enter the product along the milk-processing continuum from different sources, and subsequently cause spoilage in various types of dairy products. The most important classes of spore-forming bacteria relevant to the dairy industry are Bacilli and Clostridia. Bacilli are responsible mainly for the spoilage and decreased shelf-life of fluid milk, while Clostridia cause late gas blowing in cheese. Spore-forming microorganisms contaminate raw milk primarily at the farm level, with potential for recontamination to occur at various points along the dairy production continuum. The most effective measure in reducing spore load at the farm level is adequate pre-milking teat preparation, while at the dairy plant level, bactofugation and microfiltration are applied. Understanding the ecology of spore-formers can improve application of systematic approaches for controlling the spoilage bacteria in dairy processing systems. Also, novel technologies, such as high-pressure processing, ultrasound treatment, irradiation etc., could provide the dairy industry with the powerful tools to eliminate these bacteria from the dairy chain.

1. Introduction

In recent years food loss and food waste are becoming more relevant global issues. In the European Union, around 88 million tons of food are wasted annually, with an estimated associated cost of €143 billion [1]. Together with retail overstocking and discarding products, microbial spoilage is an important cause of dairy food waste [2].

Among spoilage microorganisms important for the dairy industry, spore-forming bacteria are the most diverse and most complex to eliminate from the dairy chain, mainly due to their ability to transform to dormant state – spores [3, 4]. Spores can survive harsh environmental conditions, such as nutrient deficit, osmotic pressure and temperature deviations, owing to their multilayer structure [5]. While the outermost layer protects the spore from enzymatic attacks, inner layers maintain a dehydrated state and provide additional protection against chemicals [4]. Once the environmental conditions are favourable i.e., primarily when specific nutrients (amino acids, sugars, and purine nucleosides), or some non-nutrient factors become available (calcium dipicolinate, alkylamines, high pressure, heat activation) spores can germinate into a vegetative state [6].

There are many ways to subdivide spore-forming bacteria relevant to the dairy industry: based on their taxonomy, metabolic traits, and ability to grow at different temperatures, or in the presence of oxygen [7]. Taxonomically, they are all members of phylum Firmicutes, which consists of five classes: Bacilli, Clostridia, Erysipelotrichia, Negativicutes and Thermolithobacteria [8, 9]. The most important classes of spore-forming bacteria relevant to the dairy industry are Bacilli and Clostridia [10]. The members of *Bacillus* and related genera and *Clostridium* spp. are ubiquitous in nature, can enter the product along the milk-processing continuum from different sources, subsequently grow at refrigeration temperatures, and significantly affect product safety and quality.

2. Spore-forming bacteria relevant for milk and dairy product quality

Pasteurized milk can be contaminated with spoilage microorganisms via two main pathways: recontamination after pasteurization, mainly with Gram-negative bacteria such as *Pseudomonas* [11], and contamination at the farm level with psychrotolerant spore-forming bacteria that survive pasteurization and subsequently cause spoilage during the refrigeration storage [12]. *Bacillus* spp. and *Paenibacillus* spp. are the main spore-forming psychrotolerant spoilage microorganisms found in pasteurized milk [13, 14, 15].

Genus *Bacillus* is the oldest and most diverse genus of bacteria [10]. Species within this genus have a wide range of physiological characteristics, with some strains able to tolerate extreme temperatures, pH and the presence of salt [16]. The expansive physiology of *Bacillus* spp., has allowed its members to colonize almost all natural habitats, such as soil, air, lake sediments and even extreme environments [10]. As *Bacillus* spp. can be isolated from soil (which was until recently considered as their main habitat) and environments contaminated with soil, their presence on dairy farms is inevitable [16]. It is noteworthy that many *Bacillus* spp. can produce thermotolerant lipolytic enzymes that can lead to milk spoilage. These enzymes have optimal temperatures between 60 and 75°C, which are similar to the temperatures used for thermization and pasteurization in the dairy industry [17]. Also, *Bacillus weihenstephanensis*, a member of the *Bacillus cereus* group, can cause the product defect known as sweet curdling [10].

As traditional culture-based methods could not discriminate between these two genera, *Paenibacillus* spp. were formerly assigned to the genus *Bacillus* [3]. Clear distinction can be made regarding growth dynamics of these two genera during pasteurized milk's shelf-life. While *Bacillus* spp. predominate early in the shelf-life of pasteurized milk (1-10 days post-pasteurization), *Paenibacillus* spp. predominate at the end of shelf-life (14-21 days post-pasteurization) [13, 15, 18]. Although *Paenibacillus* spp. are present at very low numbers in raw milk, they can reach more than 10^6 CFU/ml at the end of the shelf-life of pasteurized milk [3]. This suggests that *Paenibacillus* spp. are better able to grow in milk at 6°C whereas most *Bacillus* spp. cannot, with *Bacillus weihenstephanensis* as the most notable exception [19, 20].

Spore-forming bacteria can also survive thermal treatments during extended shelf-life (ESL) milk production processing [21]. Although there are no international standards that define the thermal treatment necessary for ESL milk production, applied thermal treatments are usually in range 125-130°C for 2-6s [22]. During the production process of ESL milk, all vegetative cells are inactivated, but nevertheless, some spores can survive. *Bacillus* spp. can be present as a spore-forming spoilage microorganism in ESL milk, but *Paenibacillus* spp. emerged as a spoilage microorganism particularly attributed to ESL milk [23]. Rysstad and Kolstad [24] recommended that ESL milk should be stored at ≤6 °C, under which conditions, *Paenibacillus* spp. growth is limited. Furthermore, some spores of *Paenibacillus* spp. can survive temperatures as high as 130°C [25].

Ultra-High Temperature (UHT) milk is a "commercially sterile" product, which is intended to be stored at ambient temperatures for prolonged times. However, some high-heat resistant spores can survive UHT treatment. Subsequent spoilage can occur, especially when the product is stored at temperatures above 40°C. Spores isolated from UHT milk usually belong to *Bacillus sporothermodurans* and *Geobacillus stearothermopillus* [23]. Although these bacteria are present in raw milk at very low counts, heat treatment during UHT milk production favours their germination, outgrowth and subsequent vegetative growth [26]. As some strains are proteolytic, they can cause spoilage of the final product [27]. Other *Bacillus* species and *Paenibacillus lactis* can also occasionally cause UHT milk spoilage [28].

Unlike fluid milk products where aerobic spore-formers predominate as sporogenic spoilage microorganisms, spoilage of cheese is usually attributed to anaerobic spore-formers. The main cheese defect attributed to spore-forming bacteria is late gas blowing [10], usually seen in Dutch or Swiss type cheeses such as Gouda, Comté, Emmental and Beufort [29]. Late gas blowing is characterized by slits, cracks and irregular eyes in cheese, due to excessive production of gas [7]. Bacteria causing late gas defect are referred as butyric acid bacteria, due to their ability to ferment lactate to acetate, butyrate and hydrogen gas [30]. They all belong to *Clostridium* spp. and include the species *Clostridium butyricum,* *Clostridium tyrobutiricum* and *Clostridium beijerinckii*. Butyric acid bacteria reach raw milk through faecal contamination, when cows are fed with poor quality silage that has undergone aerobic deterioration [7]. As acidification in such silage is not sufficient, spores can germinate and grow [31], and subsequently contaminate raw milk. During the aging of cheese, clostridial spores can germinate and grow, as long as lactate is available as a substrate, and environmental conditions (salt concentration) are not limiting for their growth [32]. Even though it is not considered as a butyric acid bacterium, *Clostridium sporogenes* can also cause late gas defects in cheese, owing to proteolytic activity and subsequent gas formation in the anaerobic cheese environment [29].

Aerobic spore-formers *Bacillus polymyxa* and *Bacillus macerans* were also associated with spoilage of Argentinian cheeses [33]. Spoilage of some cheeses was also associated with the presence of *Bacillus* spp. in raw milk and their survival of heat treatment, or post-pasteurization contamination of the product [10].

3. Sources of milk contamination with spore-formers

The literature data indicate that *Paenibacillus* and *Bacillus* spp. are commonly present in raw milk. These microorganisms contaminate raw milk primarily at the farm level (e.g., during milking, raw milk storage and handling on the farm) with potential for recontamination to occur at various points along the dairy production continuum from farm to final product (during transport or at the processing plant). Cow's teats are considered as a major source of spores in raw milk [34], mainly due to contamination from bedding, feed and dust [35, 36, 37, 38, 39]. The members of the genus *Bacillus* are ubiquitous and have been isolated from dairy farm environments, including silage, pasture, soil, bedding material, faces, water and feed [36]. Moreover, a study by Huck et al. [14] has found evidence for the persistence of selected *Paenibacillus* and *Bacillus* subtypes in processing plants. Thus, a recontamination cycle is re-established.

At the farm level, the most common sources of raw milk contamination with *Paenibacillus* spp. are silage and feed concentrate for dairy cows [40].

The resilience of clostridial spores in the dairy farm environment can be explained by the concept of "the clostridial spore contamination cycle", proposed by Pahlow et al. [31]. Crops used for silage become contaminated with clostridial spores through soil and manure when it is used as fertilizer. If the conditions during the silage fermentation are favourable, spores germinate to vegetative cells, and consequently, microbial loads in silage increase. After being ingested by cows, spores pass through animals' gastrointestinal tract, survive gastrointestinal transit, and are excreted with faces. Through faecal contamination of teats, spores reach raw milk. Also, as manure is usually used as an organic fertilizer, crops are once again being contaminated with spores.

Molecular subtyping methods used for differentiation of subtypes within a bacterial species can serve as sensitive tool for tracking contamination sources of spore-forming bacteria in dairy-related environments [41]. Specifically, sequences of *rpoB* gene, encoding for ß subunit of RNA, are used to differentiate strains of spore-forming bacteria through the entire dairy system. Huck et al [12, 14] used this method to determine the relatedness of spore-forming bacteria in different dairy production chain environments (bulk tank milk, trunks, packed products). Some of the allelic types present in packed product were found at the farm level, indicating that those spore-forming bacteria originate from farm and survive pasteurization. Others were present only in the packed product, meaning that contamination, or recontamination occurred at the processing plant. By applying *rpoB* sequencing, Miller et al. [42] determined that spore-forming isolates originating from raw milk and dairy powder samples are significantly different, and therefore, dairy powder producers should focus on reducing spore-forming bacteria not only at the farm level, but also in the processing plant.

4. Strategies to eliminate spore-formers in the dairy chain

Heat treatment is the most widely used processing technology in the dairy industry in order to reduce bacterial counts in milk. However, as spores can survive thermal treatments, the most effective measures for reduction of the spore count should be applied at the farm level [34].

As spore-formers most likely originate from dirt and faeces attached to teats at the time of milking, the most effective measure in reducing spore load is adequate pre-milking teat preparation [34]. Cleaning teats before milking with a moist washable towel and following with drying with a paper towel can reduce spore counts in milk by up to 96% [36]. Special attention should be paid to cleaning of teats during extremely dry or wet weather, when larger amounts of dirt and soil can contaminate teats [34]. Education of employees to properly clean teats, and establishing cleaning protocols for towels (washing with detergent and chlorine bleach, and fully drying towels), can also result in decrease of spores in raw milk [43]. Milking parlours should be washed regularly, but not while the cows are present on the platform [34].

In order to eliminate spore-formers at the dairy plant level, bactofugation and microfiltration are applied. Bactofugation uses high-speed centrifugation in order to separate milk components of different densities [41]. By using this method, between 90 and 98% of spores can be eliminated from milk. This technology was first applied to remove *Clostridium tyrobutiricum* in order to prevent late gas blowing of cheese. Although it is not a common practice, *Paenibacillus* spp. spores can also be eliminated from raw milk intended for high temperature-short time pasteurization by bactofugation. Microfiltration uses semi-permeable membranes in order to separate bacteria and milk components based on the size of the particles. Microfiltration has higher efficiency in spore removal compared to bactofugation, with 99.1% to 99.99% of spores successfully removed from raw milk [44].

The induction of spore germination before a heat treatment could be an efficient method to eliminate spore-formers from milk and ensure product stability. Spores can be triggered to germinate by various nutritive factors (amino acids, sugars, ribosides and potassium ions), surfactants, or physical treatments (mostly hydrostatic pressure) [45]. Once they germinate into a vegetative state, they are easy to eliminate [46]. Thus, the induction of spore germination, followed by inactivation of spores by thermal, or other, processing technologies can be a strategy for control of these bacteria in milk and dairy products.

5. Conclusion

Spore-forming bacteria are an important group of spoilage microbiota in dairy products. Spores are ubiquitous in nature, and consequently, there are multiple points of entry and multiplication for these microorganisms in dairy systems. With reliable tracking methods, we will be able to understand the ecology of spore-formers and successfully integrate a systematic approach for controlling the spoilage bacteria in dairy processing systems. Also, novel technologies, such as high-pressure processing, ultrasound treatment, irradiation etc., could provide the dairy industry with the powerful tools to eliminate these bacteria from the dairy chain.

Acknowledgements

The study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Contract number 451-03-9/2021-14/200143)

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