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Review

Anaerobic sporeformers and their significance with respect to milk and dairy products

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ABSTRACT

Sporeforming bacteria are a significant concern for the international dairy industry. Spores present in milk survive heat treatments and can persist during downstream processing. If they are present in sufficient numbers in dairy products they can cause spoilage or lead to illness as a result of toxin production. While many reviews have highlighted the threat posed by spores of aerobic bacteria to the dairy industry, few have focused on problems caused by the array of different species of anaerobic sporeformers (*Clostridium* and related genera) that can be found in milk. This is despite of the fact that members of these bacteria are found throughout the dairy farm environment, and can be toxigenic, neurotoxigenic or spoilage bacteria. This makes the possible presence of *Clostridium* and related spores in bulk tank milk (BTM) important from both a financial and a public health perspective. In this review dairy associated anaerobic sporefromers are assessed from a number of perspectives. This includes the taxonomy of this group of bacteria, the important subgroup of this genus the "sulphite reducing clostridia" (SRC), how these bacteria are detected in milk products, the epidemiological data regarding pathogenic species and strains within the SRC group as well as the influence of farming practices on the presence of SRC in BTM.

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1. Introduction

Sporeformers are Gram positive bacteria belonging to the phylum Firmicutes. Members of this group form spores when subjected to environmental stresses such as nutrient limitation, osmotic pressure or extreme temperature deviations. These spores, which facilitate survival, are resistant to chemicals (Russell, 1990), pH changes (Blocher and Busta, 1983), heat, osmotic shock and ultraviolet light penetration (Roberts and Hitchins, 1969). When conditions again become suitable for growth, spores can germinate to vegetative cells (Russell, 1990). Spores can survive for extended time periods, for example, recoverable spores have been found in dried milk powder from Ernest Shackelton's Cape Royds Hut in Antarctica (Ronimus et al., 2006), and from materials dated to between 25 and 40 Ma ago (Cano and Borucki, 1995). This robust survival strategy, coupled with the toxigenic potential of some sporeformers, makes sporeforming bacteria a major concern for the food industry (Andersson et al., 1995). Spores are frequently associated with silage (Vissers et al., 2007a,b,c), soil (Barash et al., 2010), forage, animal faeces (Princewell and Agba, 1982) and inadequate udder hygiene (Christiansson et al., 1999), which can in turn lead to their presence in bulk tank milk (BTM). As well as being a concern with respect to raw milk products such as raw milk and artisanal cheeses, the heat stability of spores means that they can also be an issue in commercial dairy products, even when the associated milk has been subjected to heat treatments such as thermization and pasteurization (Sugiyama, 1951). Indeed, mild heat treatments, such as thermization, may exacerbate problems by activating spore germination (Griffiths et al., 1988; Hanson et al., 2005). Furthermore, while severe heat treatments such as ultra-high temperature (UHT) and commercial sterilisation are effective at eliminating up to 99.99% of spores (Cox, 1975), these heat treatments significantly alter the flavour of liquid milk (Cogan, 1977). Their ability to survive exposure to sever heat treatments has led to sporeforming bacteria being referred to as "Thermoduric" bacteria (Gleeson et al., 2013). Processes such as bactofugation can be used to reduce the number of spores and total bacteria in milk. Indeed, this processing step has been demonstrated to achieve a >95% reduction in total bacterial load (Kosikowski and Fox, 1968) and to bring about 60% reduction in spore numbers (Su and Ingham, 2000). However, this process is expensive, time consuming and labour intensive (Walstra et al., 2010). Microfiltration is another processing step which can be carried out. This process is restricted to skim milk, as spores are roughly the same size as fat globules in whole milk (Rysstad and Kolstad, 2006). The requirement for milk fat separation to facilitate microfiltration makes this process labour intensive and expensive to carry out (Skanderby et al., 2009). Ultimately, due to their ubiquitous presence in nature and the frequently high levels at which they are found in particular environmental niches on the dairy farm, it is impossible to eliminate the risk of spore contamination of milk. It is, however, possible to reduce this risk through the implementation of good farm management practices (GFMP) and specific processing steps.

This review will provide an initial overview of the spores of particular importance to the dairy industry before specifically focussing on the importance of anaerobic sporeformers, belonging to the genus *Clostridium*, and, even more specifically, spoilage and pathogenic representatives of this group.

2. Different groups of sporeforming bacteria

Sporeformers can be subdivided into different groups based on a number of criteria. These criteria include taxonomy, the specific metabolic capabilities which they possess, their ability to grow at different temperatures or whether or not they can utilise oxygen as a terminal electron acceptor.

2.1. Psychrotrophic thermophilic sporeformers

Sporeforming bacteria belonging to the group psychrotrophic thermophilic sporeformers (PTS) are a particular problem to the dairy industry. Members belonging to this group of thermoduric bacteria, are able to grow at refrigeration temperature. These PTS typically colonizing raw milk after it is excreted from the mammary gland of lactating cows and multiplying in the bulk tank when the milk is chilled (Murphy et al., 1999). Members of the PTS can then survive subsequent heat treatment and processing when in the spore form, and may go on to cause food poisoning, or to limit the shelf life of pasteurized milk and dairy products (Te Giffel et al., 1997). Members of the PTS include *Bacillus* species such as *Bacillus cereus*, *Bacillus licheniformis*, *Bacillus subtilis*, *Bacillus amyloliquefaciens* and some members of the *Clostridium* genus (Cousin, 1982; Sørhaug and Stepaniak, 1997). Two distinct groups of PTS are consistently detected in the dairy production sector i.e., aerobic and anaerobic sporeformers, and an overview of both aerobic and anaerobic PTS is provided below.

2.1.1. Aerobic psychrotrophic thermophilic spore formers

Dairy associated aerobic sporeformers belong predominantly to the genus *Bacillus*, with *Paenibacillus* and other genera that were previously assigned to *Bacillus* (Xu and Côté, 2003; Fritze, 2004) also being of relevance. Of the *Bacillus* spp. implicated in the contamination of dairy produce, *B. cereus* is considered the most important because of the ability of some strains to induce illness. Toxin producing strains of *B. cereus* can cause two types of food poisoning, i.e., emetic and diarrhoeal. The diarrhoeal toxin is produced as a consequence of spore germination and outgrowth in the small intestine, while the emetic toxin is produced by vegetative cells growing in the milk pre-heat-treatment (Kramer and Gilbert, 1989). Recently, it was found that the majority of *B. cereus* group isolates originating from rice were either toxigenic or potentially toxigenic, and that some produced both types of toxin (Oh et al., 2012). From a spoilage perspective, it is notable that many *Bacillus* sp. can produce thermotolerant lipolytic enzymes which can lead to spoilage of milk. These enzymes exhibit optimum activity at temperatures between 60 and 75 °C (Schmidt-Dannert et al., 1997; Chen et al., 2003), i.e., temperatures similar to those used for pasteurization and thermization. *Paenibacillus* sp. are another group of aerobic bacilli associated primarily with the spoilage of milk and milk products (Huck et al., 2008; Ranieri et al., 2009). This genus is heavily associated with the spoilage of milk stored in excess of 10 days and, has previously been found to comprise over 95% of the bacterial population present in milk after prolonged refrigeration (Ranieri et al., 2009, 2012). *Geobacillus stearothermophilus* (formerly *Bacillus stearothermophilus*) is another aerobic sporeforming species of significance for the dairy industry (Burgess et al., 2010). Together with *Bacillus* spp., *G. stearothermophilus* can cause long term persistent contamination of dairy processing facilities, due to their ability to form biofilms on stainless steel surfaces of processing equipment (Flint et al., 1997). It should also be noted that some species belonging to the genus *Bacillus* and other sporeforming genera are facultative anaerobes. This group includes the most commonly isolated thermophilic sporeforming contaminant in the dairy industry *Amoxybacillus flavithermus* (Ronimus et al., 2006; Burgess et al., 2010). Indeed *B. licheniformis* is often the most frequently isolated mesophilic contaminant in raw milk samples (Waes, 1976; Phillips and Griffiths, 1986; Crielly et al., 1994). Some strains of this species have been observed to exhibit accelerated growth in skim milk in an anaerobic environment (Ronimus et al., 2003).

2.1.2. Anaerobic psychrotrophic thermophilic sporeformers

There are also very many anaerobic sporeformers that are problematic for the dairy sector. This group almost is exclusively comprised of current or former members of the genus *Clostridium*, which were first detected in milk and dairy products during the early 20th century (Hussong and Hammer, 1930). With respect to refrigeration temperatures, it is notable that some C. *perfringens* strains have a decimal