Bacillus spp. Responsible for Spoilage of Dairy Products

Burcu Marangoz[1,*], Sibel Kahraman[1], Kamil Bostan[2]

Abstract
This review's subject is *Bacillus* genus involved in the spoilage of dairy foods. Despite the improvement of production processes and hygiene applications, spore-forming bacteria are still an emerging problem in milk and dairy products. Spore-formers have adaptable spores, and thus they are prevalent in nature and food processing lines. Spore formation allows these bacteria to survive under heat treatments. Among the spore-forming bacterias, the genus Bacillus is of particular importance in dairy industry.

Keywords: *Milk, Dairy Products, Bacillus spp., Spoilage in Dairy Products*

Introduction

In 1950s, introduction of cooling and cold storage techniques to raw milk was an obligatory technological step, and thus bacteriological quality of milk and dairy products has been significantly improved. After this, the acidification of raw milk caused by the growth of mainly lactic acid bacteria and/or other mesophilic bacteria was almost completely stopped.

The EU standard for high quality raw milk requires the total count of mesophilic aerobic bacteria to be lower than 30,000 cfu/mL, and the count of psychrotrophic bacteria to be lower than 5,000 cfu/mL (Kumarsan et al., 2007).

However, the extended storage times of raw milk at low temperatures (2-6 °C) have a significant effect on the composition of the natural microbial population present in milk. Thus, in cooled raw milk, initially dominant Gram-positive mesophilic aerobic bacteria are replaced by psychrotrophic bacteria. For these reasons, psychrotrophic bacteria usually account for more than 90% of the total microbial population in cooled raw milk.

Psychrotrophic bacteria associated with milk and dairy products include *Pseudomonas* spp. and *Bacillus* spp. These are the most commonly isolated psychrotrophic bacteria associated with milk and dairy products, microorganisms that come from degraded raw milk or heat-treated milk (Meer et al., 1991). The main concern about the Bacillus spp. is their spoilage activity effecting product acceptance and product shelf-life. They can form endospores and can thus survive during heat treatments commonly used to process raw milk.

The genus Bacillus contains a varied array of Gram-positive, aerobic and facultative anaerobic endospore- forming rods. Bacillus spp. are found in a wide range of habitats. Bacillus genus belongs to the Bacillaceae family and is gram-positive (some variants are variable), aerobic or facultative anaerobic, spore-forming and rod-shaped bacteria (Kalkan and Halkman,2006). Spore formation ensures that this bacterium is resistant and is not affected by processes such as pasteurization. Therefore, *Bacillus pumilus, B. licheniformis, B. subtilis and Bacillus megaterium* are found in some of the heat-treated products as the spoilage microflora.

Xu and Côté (2003) reported that predominant spore-formers are *Bacillus* genus with *Paenibacillus* in dairy products.

[1] *Istanbul Aydın University, Faculty of Engineering, Food Engineering Department, Istanbul, Turkey*
[2] *Istanbul Aydın University, Faculty of Fine Arts, Gastronomy and Culinary Arts Department, Istanbul, Turkey*
[*] *Correspondence author: B.Burcu Marangoz: mrngzburcu@gmail.com*

Bacillus pumilus, *B. licheniformis*, *B. subtilis*, and *B. megaterium* have been related to spoilage of acidic food products (Hanlin,1998).

Bacillus licheniformis, B. cereus, B. subtilis, B. Mycoides and *B. Megaterium* are the most prevalent spore-formers in dairy products (Ledenbach and Marshal, 2009). The ability to form spores, make the genus *Bacillus* an important threat for the dairy industry.

Raw Milk

Raw milk is the general source of spore-forming bacteria. The basic microflora of raw milk consists of the *Bacillus* genus members (Kable et. al, 2016; Magnusson et al., 2007). Among the spore-forming bacteria, the incidence of *Bacillus* species is approximately 95% in raw milk. Griffiths (1990) found that *Bacillus* species may be present at a level of 10^5 cfu / mL after 7 days of storage at 6 ° C. Several studies have shown that more than 5000 spores/mL can exist in fresh milk (Mikolajcik et al., 1978).

In another study, Lücking et al. (2013) studied the spoilage flora of milk and they found 43 species. The *B. cereus* group and *B. licheniformis* were the predominant species.

Bacillus species can produce extracellular proteolytic enzymes which degrade milk components. These components can cause off-flavor and quality defects in milk (Champagne et al., 1994; Ternstrom et al., 1993).

Pasteurized Milk

HTST (High Temperature Short Time) pasteurization is an effective method which is performed at 72 ºC for 15 s. Spores of psychrophilic, mesophilic and thermophilic species can survive from this process. Ivy et al. (2012) reported that the genus *Bacillus* has shown the predominance >30%) among psychrophilic aerobic spore-formers in raw and pasteurized milk. Among the mesophilic species, *Bacillus subtilis* and *Bacillus licheniformis* are the most prevalent strains. The *B. cereus* group is the major one for psychrophilic species.

Sterilized Milk

Ultra High Temperature (UHT) treatment is generally applied by heating milk between 135-145 ºC for 1-10 s. The UHT process is planned to destroy approximately all microorganisms comprising spores. But some of these spores can survive this process and they can proliferate if they find adequate conditions.

Bacillus licheniformis, Bacillus cereus, Bacillus coagulans, Bacillus sporothermodurans and *Bacillus sphaericus* are the main *Bacillus* species isolated from UHT milk (Pettersson et al., 1996; Rombaut et al., 2002; Aouadhi et al., 2014). In another study carried out in Tunisia, the distribution of *Bacillus* species in UHT milk was 33% and *Bacillus licheniformis* was found to be the predominant strain in UHT milk samples (Kmiha et al., 2016).

Cheeses

While the occurrence of *Clostridium* species in cheeses from different geographic areas has been widely investigated, there is no adequate research relating to *Bacillus* species.

In the study performed by Iurlina et al. (2006,) B. *pumilus* was identified in both Port Salut and Quartirolo cheeses with an incidence of 50% and 25%, respectively. They reported Port Salut Argentino cheeses showed an incidence of *B. cereus* in 50% of the 30 samples. Meanwhile, they determined positive proteolytic and lipolytic activities in their isolates that could be associated with the spoilage of cheeses.

Cosentino et al. (1997) studies on 183 cheese samples collected in Sardinia to detect the frequency level of *Bacillus* spp. contamination. They found the incidence of positive samples at a ratio of 78% in Ricotta cheese and 69% in processed cheese. Their results showed that *B. cereus* was the predominant species in the cheese samples. *B. sphaericus* was the second prevalent species in Ricotta cheese samples while *B. brevis* was prevalent in processed cheese samples.

Milk Powder

The primary bacteria causing the spoilage of milk powder are thermophilic bacilli. Thermophilic bacilli are not pathogenic but when milk powder is used, spores can germinate in the product. This may induce acid production, enzyme production and significant off-flavors in the product (Chopra and Mathur 1984; Chen et al. 2004). Spores can survive at high temperatures during the process and the low water activity, the cleaning-in-place (CIP) system and the long-term storage of the final product.

Rueckert et al. (2004) studied the distribution of thermophilic spores in milk powder for infants in China. *B. licheniformis* was the most frequent species found in the samples. These samples have spore contamination generally less than 1000 CFU g^{-1}. Other bacilli that are also found in milk powder are the facultative thermophiles such as *Bacillus licheniformis*, *Bacillus coagulans* and *Bacillus subtilis*. These are usually present at low levels (Crielly et al. 1994; Ronimus et al. 2003).

Conclusion

Spoilage of milk and dairy products through *Bacillus* spp. causes high economic losses in the dairy industry. The species isolated in dairy product, especially from milk and cheese, have a high spoilage potential.

Bacillus spp. have proteolytic and lipolytic activity and this may cause quality losses such as sweet curdling of milk, off-flavors and bitterness in cheese and textural defects in cultured dairy products. In order to prevent and control of contamination, many research studies are focused on the possible contamination sources. Thus, to understand and control the effects of spore forming, spore germination and food-spoilage mechanisms need to be well established.

In conclusion, this review provides food microbiologists with an overview of *Bacillus* species responsible for dairy product spoilage.

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