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Review Article

Different risk potential of *Bacillus cereus* to the dairy industry – keeping up with the old story

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Abstract: *Bacillus cereus* is one of the major spore-forming bacteria with great genetic diversity in raw milk, dairy raw materials, and finished dairy products, and is widely present in the entire dairy production line. Due to the biofilm formation of *B. cereus* and the heat resistance of its spores, traditional cleaning and disinfection procedures cannot completely remove the spores, resulting in the presence of *B. cereus* in the final dairy products. Although much work has been done to identify *B. cereus* from various dairy samples, an updated knowledge of this spore precursor is still needed. This review describes the prevalence of *B. cereus* from raw milk to commercial dairy products, the biofilm formation of *B. cereus* and its ability to cause spoilage in dairy products, and the possible prevention methods.

Keywords: *Bacillus cereus*; spore-forming bacteria; spoilage enzyme; biofilm formation; dairy quality and safety

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1 Introduction

In recent years, spore-forming bacteria have been detected and screened in dairy products, so spore-forming bacteria in dairy products have received more and more attention, and the requirements for these microorganisms have become more and more stringent around the world[1]. For example, the U.S. Dairy Export Council has strict regulations on the number of both mesophilic (< 1 000 CFU/g) and thermophilic spores (< 500 CFU/g) in milk powder. Controlling the number of spores in dairy products is a huge challenge for the dairy industry due to long processing times and high production volumes that make it difficult to meet these stringent requirements for spore formers[2]. The genus *Bacillus* is one of the major spore-producing bacteria in dairy products and includes several species such as *Bacillus cereus*, *B. licheniformis*, *B. subtilis*, and *B. anaerobicus*[3]. Currently, studies around the globe indicate that bacteria of the genus *Bacillus* predominate in raw cow milk and their main source is the dairy farm environment, where they can escape from pasteurization and ultra-high-temperature (UHT) sterilization during the processing of dairy products and survive and germinate utilizing the nutrients in the milk[4].

B. cereus is a spore-forming bacterium, partially anaerobic, gram-positive bacilli, can be easily isolated from dairy farm environments, and in foodstuffs such as raw milk and various dairy products[5–7]. Spores of *B. cereus* are mainly spread through soil and air, soil contains 50–380 000 CFU/g of *B. cereus* spores, and air contains at least 100 CFU/m^3 of *B. cereus* spores[8–9]. In the 1950s, Hauge[10] discovered that eating food contaminated with *B. cereus* could cause diarrhea. It has been found that *B. cereus* can secrete enterotoxins and emetic toxins, and consumption of foods contaminated by *B. cereus* over 5 (lg (CFU/g)) can cause stomach problems, vomiting, diarrhea, and even death[11]. *B. cereus* has caused foodborne illness outbreaks on many occasions, such as an outbreak associated with *B. cereus* in two secondary schools in Chongqing, China in May 2021, where 198 cases of *B. cereus* infection were reported[12], an outbreak of food poisoning caused by *B. cereus* was reported in Alaska in August 2021, with 64 confirmed cases of food poisoning in 79 people who had eaten improperly stored ham sandwiches[13]. Symptoms experienced by *B. cereus*-infected individuals included abdominal pain, diarrhea, and vomiting. Yu et al.[14] has reported that diarrheal diseases caused by *B. cereus* are usually associated with meat, milk, vegetables, and fish, while the emetic type is likely to be associated with rice products. Previous studies have shown that *B. cereus* remains active after pasteurization and UHT during dairy processing, and that clean-in-place (CIP) regimes for processing equipment fail to remove *B. cereus* biofilm cells even with increased cleaning intensity, and a total of 13 out of 45 *B. cereus* strains were isolated from eleven different sites in a dairy processing line in China[15], which may also lead to bacterial contamination of the final dairy product and finally affect the quality and safety of dairy products[16–17]. Therefore, the health risks posed by *B. cereus* in the dairy industry need to be dealt with quickly and appropriately.

In contemporary society, with the continuous improvement of public awareness of the health hazards of *B. cereus*, a comprehensive understanding of the spread, safety, and quality characteristics of *B. cereus* in the dairy industry and the control measures of potential hazards may reduce the health harm of *B. cereus*. The objective of this review is to focus on elucidating the occurrence of *B. cereus* in the dairy industry, its biochemical characteristics associated with biofilm formation and dairy spoilage, and the potential control strategies.

2 Prevalence and detection of *B. cereus* in the dairy industry

2.1 Prevalence of *B. cereus* in raw milk

B. cereus is widespread in the environment, and its contamination of dairy products can pass through a variety of substrates (including soil, grains, and dairy processing equipment). In addition, this microorganism has a tenacious vitality, forming ubiquitous spores that are resistant to a variety of harsh environments, such as escaping high temperatures and proliferating at low temperatures where nutrients are available[18–19]. Fei et al.[20] collected 300 environmental samples (from the soil, water, feed, air, buckets, milking machines, stalls, bedding, cow surfaces, udders, and employee's hand samples) and 50 raw milk samples from dairy farms, and a total of 56 *B. cereus* strains were detected in the 350 raw milk samples. Therefore, *B. cereus* can enter raw milk through various pathways. A high percentage of *B. cereus* was observed in raw milk samples collected in China (34.38%), Australia (33.33%), Egypt (30.00%), Canada (0.70%), and other countries[21–24]. Variation of the *B. cereus* population in raw milk collected from different countries is influenced by climatic factors, seasonal variations, pasture environment, and geographic differences[25–26]. For example, the relatively high latitude and low temperatures in Canada limit the growth of *B. cereus*[24]. In another study, the number of *B. cereus* spores was higher in late spring and summer, but lower in raw milk samples in fall and winter[18].

2.2 Prevalence of *B. cereus* in the dairy processing environment

It is well known that microbial communities change from a planktonic state to an aggregated state to form a biofilm, which consists of biofilm cells and an extracellular matrix encasing the biofilm cells[27]. Bacteria of the genus *Bacillus* in raw milk can survive and grow under mesophilic and thermophilic conditions and can survive pasteurization, UHT, and CIP procedures in milk processing[28–29]. Then, it is attached to the surface of the processing equipment according to its hydrophobicity[30]. Biofilms are more resistant to antimicrobials and cleaning regimes than planktonic cells[31]. This makes the elimination of *B. cereus* in the dairy industry a major challenge. *B. cereus*, as a genus of *Bacillus*, has a strong biofilm-forming ability and is frequently isolated from the surface of dairy processing equipment. Zou et al.[15] isolated 45 strains from 11 different sampling points in the milk powder processing line, of which *Bacillus* spp. had 22 strains (48.89%), which accounted for the largest proportion; interestingly among the *Bacillus* genus, *B. cereus* had 13 strains, which accounted for the largest proportion of *Bacillus* spp. isolated (59.09%). Moreover, the percentage of gram-positive bacilli increased significantly after pasteurization treatment (from 50% to 61%), and *Bacillus* was the main gram-positive bacterium from dairy processing equipment[29]. Lin et al.[32] has reported that *B. cereus* can be isolated at all stages of UHT milk processing, they isolated 27 strains of *B. cereus* from the UHT milk production line, of which five strains (18.52%) were from silo tanks, eight strains (29.63%) were from ingredient tanks, seven strains (25.93%) were from pasteurization tanks, and three strains (11.11%) from post pasteurization processing step, one strain (3.70%) from filling room air and three strains (11.11%) from finished UHT milk. The above studies clearly showed the potential risks of *B. cereus* contamination in the production line of dairy processing, which can lead to recurrent contamination of dairy products, and this needs to be taken into consideration seriously[30–32].

As a particular survival mechanism, the formation of biofilms by spores further enhances their ability to survive in unfavorable environmental conditions[33]. Spores of *B. cereus* attach to stainless steel more readily than trophoblast cells because they are highly hydrophobic[34–35]. The hydrophobicity of the spore surface produces strong adhesive forces that act as a long-distance marker, which plays a key role in the attachment of *B. cereus* spores to the surface of food and food processing equipment[36]. As a result, even with the extensive washing and cleaning procedures used in the dairy industry, *B. cereus* biofilms remain a constant source of contamination, threatening the safety and quality of dairy products.

2.3 Prevalence of *B. cereus* in final dairy products

Previous studies have demonstrated that *B. cereus* is present in different dairy products such as milk powder, UHT milk, pasteurized milk, and cheese[7,29,32,37]. We collected the prevalence of *B. cereus* in various dairy products (Table 1). For example, *Bacillus* spp. was the most abundant (50.94%) of the bacteria screened in the 13 samples of pasteurized milk and 28 samples of UHT milk collected, and *B. cereus* accounted for the largest proportion (19.27%) of bacteria of the *Bacillus* spp. screened[7], This suggests that *B. cereus* is a ubiquitous environmental strain. In another study, Zhao et al.[16] confirmed the presence of *B. cereus* in pasteurized milk (10 isolates), ultra-pasteurized milk (12 isolates), infant formula (7 isolates), and cheese (12 isolates). Current research has made efforts in the identification of *B. cereus* in final dairy products, but the development of predictive models is still important to assess the behavior of *B. cereus* during storage, transportation, and distribution of different dairy products.

B. cereus has been emphasized as one of the key foodborne pathogens for food poisoning, and indeed, a high incidence of *B. cereus* with hemolysin and enterotoxin genes (*hblA*, *hblC*, *hblD*, *nheA*, *nheB*, *nheC*, *cytK*, *bceT*, *entFM*, and *hlyII*) in commercial

Table 1 Prevalence of *B. cereus* in commercial dairy products.

Type of dairy products	Percentage	Country	Reference
Pasteurized milk and UHT milk	19.27%	China	[7]
Pasteurized milk and powdered infant formula	13/500 for pasteurized milk, 7/500 for powdered infant formula	China	[16]
Liquid milk and milk power	44% for liquid milk, 26.1% for milk power	China	[38]
Cheese	9.8%	Italian	[39]
Cheese	29.48%	Mexico	[40]
Cheese and ice cream	70% for cheese, 20% for ice cream	Turkey	[41]
Powdered infant formula	31.3%	Serbia	[42]
Cheese	30%	Argentina	[43]

dairy products[15,44–45]. In addition to this, due to the misuse of antibiotics in the dairy farming industry, some findings have shown that many *B. cereus* are resistant to *β*-lactam antibiotics such as oxacillin, ampicillin, penicillin, cefepime, and ampicillin[46–47]. Moreover, regarding the phenomenon of multi-drug resistance in *B. cereus*, Yaman[47] has reported that the resistance rate of *B. cereus* isolates to 3 or more antibiotics is 34%, which should attract enough attention from the public. Through horizontal gene transfer, antibiotic-resistant genes are likely to be transferred to the human digestive tract, and people consuming dairy products containing the antibiotic-resistant gene *B. cereus* can cause a huge burden on their health[48].

2.4 Detection of *B. cereus* in final dairy products

Although one can initially identify *B. cereus* by traditional phenotypic and genotypic (16S rRNA sequencing) characterization methods, there is a lack of rapid, efficient, and more accurate methods to detect and identify *B. cereus*. Fernández et al.[49] developed a highly sensitive multiplex polymerase chain reaction (PCR)-based method to target the *p8FPL* and *p806R* gene for simultaneous rapid and accurate identification and detection of *B. cereu*. Oliwa-Stasiak et al.[50] targeting the *motB* gene, primers BCFomp1/BCRmop1 were designed for specific detection of *B. cereus*. Phosphatidylcholine-specific phospholipase C, an isolate of *B. cereus*, is involved in hemolytic activity in cells, and the enzyme is encoded by the *pc-plc* gene. Martínez-Blanch et al.[51] investigated a quantitative real-time PCR method for assessing the spore concentration using the *pc-plc* gene as a target. However, since the spore concentration of *B. cereus* in finished dairy products is too low for direct PCR detection, a cumbersome spore enrichment assay is required before routine testing. In addition, the presence of a large number of ions and fats in milk can inhibit the proper performance of real-time quantitative PCR. Fischer et al.[52] developed an aptamer-based capture scheme for milk analysis, and they realized the specific detection of *B. cereus* spores directly in milk by combining an aptamer (a short-stranded DNA or RNA) with a surface plasmon resonance sensor chip for specific capture of *B. cereus* spores. This technique successfully overcame the interfering effect of complex food matrices on real-time quantitative PCR detection and improved the detection limit of the PCR assay. Other assays such as loop-mediated isothermal amplification, dipicolinic acid detection, and colorimetric detection have also been shown to be rapid and accurate methods for the detection of *B. cereus*[9,53–54].

3 Biofilm formation by *B. cereus* and its underlying mechanism

3.1 Biofilm-forming ability of *B. cereus* strains

As mentioned above, *B. cereus* has been widely isolated in dairy production lines, mainly due to its high ability to form biofilms. In the food industry, biofilms occupy an important ecological niche and are capable of forming spores, which can produce heat-stable spores and enzymes that can lead to spoilage during food processing[55–56]. *B. cereus* biofilms are primarily found at the gas-liquid junction, however, some strains of this bacterium can form biofilms on submerged surfaces, such as on stainless steel pipes and ducts[57]. Biofilm formation is facilitated by the motility of the *B. cereus* flagellum both at the gas-liquid interface and under submerged conditions[58]. These biofilms can easily migrate long distances along the pipelines of dairies and be transported to various stages of processing and even to the finished product, posing a serious health risk for consumers who consume dairy products containing *B. cereus*[57]. Notably, *B. cereus* biofilms are often combined with other microorganisms in the food processing line, which is related to the complex extracellular matrix of *B. cereus*, including extracellular polysaccharides, extracellular proteins, and extracellular DNA[59–60]. The initial attachment of *B. cereus* to food production surfaces creates a preconditioning effect that facilitates the rapid attachment of other bacteria that, without the biofilm formed by *B. cereus*, would be removed by water flow, milk flow, or other physical mechanisms[61]. Dai et al.[7] has reported that 5 of 21 strains of *B. cereus* screened in pasteurized and UHT milk were classified as strong biofilm formers. In the dairy industry, *B. cereus* usually forms biofilms under conditions of flowing milk streams, however, most laboratory studies have used polystyrene microtiter plates and stainless steel sheets to test *B. cereus* biofilm formation under static conditions. This suggests that current laboratory studies should develop a model that mimics actual dairy production to more accurately assess the biofilm-forming ability of dairy-associated strains[48].

3.2 Factors affecting biofilm formation of *B. cereus*

Biofilm formation of *B. cereus* is usually influenced by various factors such as temperature, pH, and carbon sources. Silva et al.[62] studied the effect of different temperatures and pH on biofilm formation by *B. cereus*. It was found that the combination of mild and low pH could effectively inhibit bacterial adhesion and thus inhibit biofilm formation. Zhou et al.[63] investigated the effect of different carbon sources on the biofilm formation of *B. cereus* and showed that incubation of *B. cereus* biofilm in skimmed milk medium at 37 °C for 24 h resulted in more extracellular polymeric substances and a denser structure compared to maltose medium. It is worth noting that the substances contained in dairy products themselves can be utilized by *B. cereus*, thereby improving their ability to form biofilms. Shaheen et al.[64] research has confirmed that the phospholipids in whole milk may be surfactants for biofilm formation in *B. cereus*, facilitating the formation of its biofilm formation.

In nature, microorganisms usually exist in mixed forms, and competitive, symbiotic, and cooperative interactions usually affect the ability to form biofilms and the properties of biofilms[33]. For example, under conditions of bacterial coexistence, the presence of *B. cereus* promoted the growth and multiplication of *B. thuringiensis*, reflecting the existence of synergistic effects between the two bacteria[65]. In another study, *B. cereus* and *Pseudomonas fluorescens*, two common dairy isolates, were observed to exhibit competitive behavior in the formation of a two-species biofilm, and this interaction significantly reduced the number of planktonic and biofilm cells[66].

Biofilm formation by *B. cereus* provides a survival strategy for their escape from the CIP program in dairy processing machinery[67]. Huang et al.[17] searched for an effective method to remove biofilm formed by *B. cereus*. Benzalkonium bromide in combination with CIP was effective in removing *B. cereus* biofilm cells from stainless steel sheets, and the biofilm cells were removed below the detection limit. Other novel approaches such as probiotics and phages have also proven to be effective in controlling biofilm formation in *B. cereus*. For example, the addition of appropriate amounts of metabolites of *Pediococcus pentosaceus* and *Lactobacillus brevis* inhibited biofilm production in *B. cereus* by 50%[68].

3.3 Omics for biofilm studies

Due to the complexity of the biofilm of *B. cereus* in the dairy industry, there is a need to use histological techniques based on current research, including high technologies such as genomics, transcriptomics, proteomics, metabolomics, and even a combination of them, which will greatly benefit biofilm research[69]. In a previous study, Yan et al.[70] conducted a genome-wide study of biofilm formation in *B. cereus*, where they used two genome-wide approaches, transposon insertion mutagenesis, and global transcriptome analyses by RNA sequencing (RNA-seq). The results showed that when *B. cereus* was grown in the biofilm-inducing medium, transposon mutagenesis identified 23 genes that play an active role in biofilm formation, and global transcriptome analyses by RNA-seq showed that carbon metabolism and the fermentative synthesis of various small compounds (e.g., ethanol, lactic acid, acetate, etc.) play an active role in biofilm synthesis at the stage of biofilm generation. Another study also showed the important role of nucleotide biosynthesis in biofilm formation in *B. cereus* by transcriptome analysis[71]. Changes in gene transcription levels in biofilm cells under different sources of *B. cereus* were analyzed by transcriptome[72]. The pathways involved in differentially expressed genes under different conditions of glucose, maltose, lactose, and skimmed milk as carbon sources were mainly glycolysis, pentose phosphate pathway, and tricarboxylic acid cycle. This suggests that different carbon sources affect the carbon cycle in *B. cereus*, which in turn affects the synthesis of extracellular polysaccharides in the biofilm, leading to differences in biofilm production. In another study, gene-based transcriptomics revealed how *L. pentosus* inhibited *B. cereus* sporulation and biofilm formation by regulating the two-component system pathway to induce stress in cells[73]. The results described above provide insights into the characterization of biofilms and offer new ideas for designing effective methods to combat harmful biofilms. Although each of the histological analyses provides useful knowledge, Yuan et al.[74] emphasized that single-omics analyses describe only single data at the nucleic acid, protein, or metabolic level, and do not allow to characterize the detailed and precise mechanisms of biofilm formation, complex interactions in mixed-species biofilms, or their behaviors in response to interfering factors.

4 Spoilage potential of *B. cereus*

Dairy products are one of the most lost food products in the world. It is estimated that about a quarter of all dairy products in the USA are wasted each year during production or retail sales[75]. The presence and growth of *Bacillus* is a major cause of spoilage and waste of dairy products. Numerous studies have confirmed that *B. cereus* has a strong ability to produce protein hydrolases, lipases, and lecithinases[76]. Proteases from *B. cereus* cause age gelation and protein precipitation in UHT milk, which greatly reduces its shelf life[77]. *B. cereus* in dairy products has a strong ability to produce protease, and the activity of protease in dairy products is enhanced with the increase in the number of *B. cereus*[78]. UHT milk was put under 10 and 20 °C for 12 days, and the activity of proteins increased from 0 to 14.40 and 27.90 U/mL, respectively, which indicates that the activity of protease within a certain range is enhanced with the increase in temperature[72]. Therefore, this calls for the necessity of strictly controlling the temperature of transportation and storage of dairy products, as well as the content of *B. cereus* in raw milk. Additionally, it is of concern that some *B. cereus* bacteria have been shown to secrete heat-resistant alkaline protease, an enzyme that remains stable at alkaline pH and temperatures of up to 70 °C[79]. This spoilage enzyme is likely to withstand heat treatment and CIP cleaning during dairy processing and can break down proteins, lipids, and lactose substances in dairy products.

Currently, our understanding of dairy spoilage caused by *B. cereus* is very limited and there are still many mysteries to be solved. How many spoilage enzymes secreted by *B. cereus* are heat resistant? Can they be denatured by conventional heat treatment? Is it possible to develop novel control strategies to reduce *B. cereus*-induced dairy spoilage from the on-farm milking process to the production line?

5 Potential strategies for controlling *B. cereus*

Strategies to reduce spoilage of dairy products caused by *B. cereus* have long focused on heat treatment processing and prevention of secondary contamination through cleanliness and sanitation of the processing environment. However, *B. cereus* spores are highly resistant to heat treatment and it has been demonstrated that spores formed in biofilms are more thermally stable than those formed from planktonic cells[80]. The ubiquity of *B. cereus* spores, coupled with the ease with which *B. cereus* forms biofilms during dairy processing, makes it very difficult to prevent the presence of *B. cereus* in finished dairy products. Here, we discuss several other effective methods that can be used to minimize *B. cereus* contamination in dairy products.

5.1 Biocontrol methods

As people's standard of living improves and consumers demand more and more clean labeled foods, preserving dairy products with natural antimicrobials is a very desirable way to prevent dairy spoilage. Chitosan is a naturally occurring, non-toxic polysaccharide that can be used in a wide range of applications in biomedicine, water treatment, the food industry, agriculture, and cosmetology, it is usually derived from chitin, the main structural component of the exoskeleton of crustaceans[81–82], which is composed of a linear copolymer compromising β-1,4-linked 2-amino-2-deoxy-β-*D*-glucose and units of *N*-acetyl-*D*-glucosamine[83]. Valdez et al.[84] confirmed that the bactericidal ability of insect chitosan against *B. cereus* depended on the temperature and the concentration of the culture, at a temperature of 10 °C, and 180 and 150 μg/mL of chitosan had the bactericidal ability, but at 20 and 30 °C, 180 and 150 μg/mL of chitosan were only bacteriostatic and did not have bactericidal effect. In another study, higher molecular weight chitosans (628 and 100 kDa) caused the death of the vegetative form of *B. cereus* by preventing the uptake of nutrients but did not affect the spores since these can survive for extended periods without nutrients. On the other hand, lower molecular weight chitosans (< 3 kDa) induced more visible damages in its vegetative form by the penetration of the cells by the chitosans[85].

Lysozyme is a natural enzyme with antimicrobial activity that is an intrinsic component of the human immune system and has low toxicity; it can be considered a natural antimicrobial agent used in the food and pharmaceutical industries. It exerts antimicrobial activity against bacteria, especially gram-positive bacteria, by hydrolyzing the 1,4-β-bond between *N*-acetylcytidylic acid and *N*-acetylglucosamine in the bacterial cell wall[86–87]. Lysozyme has been declared generally recognized as safe (GRAS) by the U.S. Food and Drug Administration for use in cheese as a class 1 enzyme and has been designated as a food by the Joint United Nations Food

Agriculture Organization/World Health Organization Expert Committee on Food and Drugs, and has been shown to be a natural agent for preventing spoilage of cheese; in addition, the European Additives Directive also approves the use of lysozyme as a preservative for controlling spoilage of cheese[81]. Lysozyme from eggs is considered to be the main industrial source for practical applications because of its lower cost and availability compared to other sources, with 0.3–0.4 g of lysozyme per egg (3.5% of the total egg white protein)[88]. Abdou et al.[89] reported that the use of lysozyme at 100 μg/mL was effective in inhibiting the growth of *B. cereus*, but was not able to kill all *B. cereus*. This suggests that lysozyme can be a candidate for controlling *B. cereus*, but the exact concentration of its addition in dairy preservation is not clear, which requires further research. In addition to the direct addition of lysozyme to dairy products for dairy preservation, lysozyme can also be used as an antimicrobial agent in packaging to minimize microbial contamination of finished dairy products[90].

5.2 Physical methods

Milk bactofugation is a technical method for removing heat-resistant microorganisms during pasteurization in milk growth lines. *B. cereus* is one of the most important spoilage microorganisms in the dairy environment, and its spores are highly resistant to heat and can survive routine pasteurization in the dairy industry. Milk bactofugation serves to reduce spore levels in milk by 94%–98%[91].

In another study, pasteurization alone or in combination with ultrasonic treatment inactivated heat-resistant *Bacillus* spp. and completely eliminated *Bacillus* cells beyond 6 (lg (CFU/mL)) in skim milk, and ultrasonic treatment physically disintegrated the *Bacillus*-forming bacteria. which suggests the potential application of continuous flow ultrasonication as an aid to spore inactivation along with pasteurization[92]. The study of Lü et al.[93] showed that the combination of ultrasound, heat, and pressurization had a more significant effect on the removal of *B. cereus* spores, which could reduce the spores of *B. cereus* by 3.13 (lg (CFU/mL)) within 30 min, and the spores that survived the combination of the three methods were severely damaged in their cellular structure, and these results indicated that ultrasound combined with heat and pressurization had a significant sporicidal effect on *B. cereus* spores, which may be a promising green sterilization technology. These results indicate that ultrasound combined with heat treatment and pressurization has a significant sporocidal effect on *B. cereus* spores and may be a promising green sterilization technique.

Microwave volumetric heating is an emerging and popular technology that ensures a specific heat load, reduces the energy exposure time, improves the quality of dairy products, and is an alternative to pasteurization for industrial applications. Roohi et al.[94] have studied the effect of microwave volumetric heating on *B. cereus* was investigated when carried out at different powers of 400 and 600 W. The effect of microwave volumetric heating on *B. cereus* was studied. The results showed that the technique significantly reduced the number of *B. cereus*, with half of *B. cereus* spores inactivated at powers up to 600 W. However, the power of 600 W did not reduce the number of *B. cereus* below the detection limit, which requires one to explore more appropriate microwave volumetric heating power to inhibit the growth and reproduction of *B. cereus*.

Another physical method that may reduce *B. cereus* contamination is high-pressure treatment. High-pressure treatment is a method that can reduce the number of microorganisms without compromising the quality of the dairy product[69]. van Opstal et al.[95] had a mild pressure and heat treatment to inactivate *B. cereus* spores in milk, and the results showed that 200 MPa, 45 °C treatments for 30 min, and then increasing the temperature to 60 °C, the number of *B. cereus* spores was reduced by more than 6 (lg (CFU/mL)), and that this condition did not lead to quality problems such as curdling of the milk.

5.3 Hurdle technology

Hurdle technology refers to the combination of two or more sterilization techniques that attack bacteria in different ways to achieve the effective removal of microorganisms from the dairy processing environment[96]. A previous study showed that the combination of cell wall hydrolase Lys14579 and cinnamic aldehyde had a synergistic effect on the inhibition of *B. cereus*. After 4 h of treatment with the combination of cell wall hydrolase Lys14579 (12.5 μg/mL) and cinnamic aldehyde (100 μg/mL), the planktonic cells of *B. cereus* were inactivated in all 6 (lg (CFU/mL))[97]. In addition, Pina-Pérez et al.[98] has reported that the combination of high-pressure and natural antibacterial substances has a significant inactivation effect on the removal of *B. cereus*. cinnamon, anise, vanilla, and cocoa at 300 MPa with 700 mg/L inactivated 8 (lg (CFU/mL)) of *B. cereus*.

5.4 Other methods

The adhesion of biofilms to the surface of processing equipment often has a lot of adverse effects on the safety and quality of dairy products, although people have made a lot of efforts on the subject of combating dairy biofilms, there is still a lack of effective methods to completely solve the problems related to biofilms in the dairy industry. Pechook et al.[99] prevented the adhesion of surface biofilm by self-assembling the superhydrophobic surface formed by paraffin and wax fluoride crystals. The results showed that the adhesion inhibition rate of the surface biofilm of *B. cereus* is as high as 99.9%. Huang et al.[100] investigated that the use of nickel polytetrafluoroethylene (Ni-PTFE) coated steel could effectively reduce the biofilm adhesion of *B. cereus* by more than 2 (lg (CFU/cm^2)), and the biomass residues of biofilm on normal stainless steel after CIP cleaning with water and alkaline detergent were 3 and 1 (lg (CFU/cm^2)) respectively, but the biofilm biomass of Ni-PTFE coated steel was reduced to below the detection limit after the CIP cleaning. The results of the above studies show that surface-modified cleaning operations are more efficient, time-saving, labor-saving, and cost-saving. It can be used as an alternative anti-biofilm technology in the dairy industry to combat bacterial contamination and improve the microbiological quality of final dairy products.

It is important to note that *Bacillus* is highly biodiverse and widespread. It is usually found in small-scale dairy farms without good hygiene practices, which may lead to lower quality of the final dairy product. From a practical point of view, there is a need to increase the hygiene awareness of dairy farmers and to implement good production practices in dairy farms to reduce the number of *Bacillus*, and to achieve the microbial content of raw milk to the testing standards. For example, in terms of bedding used on dairy farms, livestock feces would serve as the organic matter on which *B. cereus* thrives, and urine would raise the pH to promote the *B. cereus* growth[101]. Therefore, timely replacement of bedding can reduce the risk of contamination of dairy products with *B. cereus*.

In addition, the implementation of predictive microbial growth modeling is important for the dairy industry to control microbial growth, improve the quality of dairy products, and extend the shelf life of dairy products. For example, Sutherland et al.[102] used response surface modeling to analyze how pH, temperature, sodium chloride concentration, and CO_2 concentration affect the growth of *B. cereus* and assessed the spoilage of dairy products due to *B. cereus*.

Despite significant efforts to develop control strategies for *B. cereus*, these emerging technologies are still not widely used in the dairy industry. When applied in practice, care should be taken to ensure that these methods do not alter the organoleptic and nutritional properties of dairy products in a way that is unacceptable to consumers. This has been neglected but should be considered in future research. Attention should also be paid to whether the methods are in line with the green concept to ensure the sustainability of the dairy industry in the future.

6 Conclusion

B. cereus is widely present in dairy production. It has gradually been recognized as one of the concerns of the dairy industry. It is capable of sporulation and is commonly found in raw milk, raw dairy products, final dairy products, and dairy processing environments. The main source of this bacterium may be the farm environment, and its propagation during dairy processing may increase the risk of bacterial contamination in dairy products. However, there is a lack of sufficient knowledge to confirm the above hypothesis. The role of *B. cereus* in dairy contamination has been increasingly recognized. In order to adapt to the bactericidal steps in dairy processing, *B. cereus* has evolved many specific survival strategies (such as spore production and biofilm production), and is also the cause of dairy spoilage and foodborne diseases. This underscores the importance of exploring effective methods to control *B. cereus* in the dairy industry. However, the current studies remain mostly at the laboratory stage and do not simulate real dairy processing conditions in industrial production, for example, without taking into account the fluidity of milk in industrial production. In addition, the benefits of *B. cereus* have been demonstrated, but there is a growing need to evaluate the safety of *B. cereus* strain on a case by case basis.

Conflict of interest

The authors declare no conflict of interest.

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