5
Discussion

5.1. Prevalence and characterisation of *Bacillus cereus* in dairy environment

Bacillus cereus is ubiquitous in environment, however underestimated as a foodborne pathogen. It is important to understand the behaviour of *B. cereus* in the dairy environment, since it can grow at a low temperature and play a role in limiting the products' shelf-life and causing potential human health hazards. This study was undertaken to establish its presence in marketed milk and dairy products and during processing, and also to assess potential hazard posed to consumers in the district of Darjeeling. Further, investigation was aimed at optimising existing cleaning-in-places (CIPs) and designing alternative CIP regimes.

Bacillus cereus occurred in six out of eight different dairy products marketed in the district. In case of pasteurised/sterilised milk, 55% of the samples contained *B. cereus* at a level up to 4 log cfu ml^{-1}. In a similar study in Denmark, 47% of the pasteurised milk samples were found to be contaminated by *B. cereus* at a level of 3–5 log cfu ml^{-1} (Larsen and Jørgensen, 1997). In another study in the Netherlands, 35% of the pasteurised milk samples contained *B. cereus* at a level of 1-4 log cfu ml^{-1} (te Giffel *et al.,* 1996). In Poland, Bartoszewicz *et al.* (2008) reported pasteurised milk to contain relatively low counts (1-2 log cfu l^{-1}). The difference in percentage of contaminated samples and level of contamination in different studies may be attributed to the degree of post-pasteurisation contamination and/or storage temperature abuse and seasonal variation in sampling. In case of milk powder, 52% of the samples contained *B. cereus* at a level of 2–3 log cfu g^{-1}. As milk powder

contains an elevated level of carbohydrates and minerals, which can promote *B. cereus* cell proliferation and enterotoxin production when they are reconstituted and held at ambient temperature for extended periods, even low levels of *B. cereus* in milk powder can act as potential vehicles for foodborne diseases (Reyes *et al.*, 2007). In Chile, Reyes *et al.* (2007) found 10% of milk powder samples contaminated with *B. cereus* at a level up to 3 log cfu g^{-1}. The high incidence of *B. cereus* in milk powder samples in the present study is likely due to monospecies biofilm formation on the milk evaporators which can be a source of recurrent contamination of the final product, as stated by Burgess *et al.* (2010). The results were in agreement with a previous report where 60% of the milk powder samples were found contaminated with *B. cereus* (te Giffel *et al.,* 1996). In case of ice cream, 40% of the samples contained *B. cereus* at a level as high as 8 log cfu ml^{-1}. In a similar study made with the samples from retail outlets in Mumbai, India, the organism was prevalent in 40% of the unpackaged samples and 27% of the packaged samples, at a level of 1–3 log cfu ml^{-1} (Warke *et al.,* 2000). The high prevalence and population level of *B. cereus* may be attributed to post-production handling of the products and temperature abuse, which is likely to occur during frozen storage or transportation and unhygienic conditions prevailing during distribution or sale in the ice cream parlours. Thirty-three percent of the cheese samples analysed contained *B. cereus* at a level of 2–6 log cfu g^{-1}, while Molva *et al.* (2009) reported only 12% of Turkish cheese samples to be contaminated with *B. cereus*. This difference might be attributed to type of cheese samples analysed and post-production contamination. In case of butter, 20% of the samples were found to be contaminated with *B. cereus* at a level of 3-4 log cfu g^{-1}. This may be due to the use of contaminated milk or due to biofilm formation by *B. cereus* on centrifugal separators and recycle loops in butter manufacturing plants and subsequent contamination of finished products. In khoa and curd samples analysed, *B. cereus* was not detected. Khoa is a partially desiccated milk which is prepared by condensing milk through regular heating (90–95 ºC) till total solid reaches 65–70% (Bhatnagar *et al.,* 2007). Heating and dehydration during the preparation of khoa and low pH (3.5–4.5) in curd and various organic acids, peroxides and antibacterial agents produced by lactic acid bacteria during fermentation might be the likely cause for its absence. A high level of *B. cereus* in ice cream and cheese is a matter of great public health concern as its level reported in food poisoning is 2–8 log cfu g^{-1} or ml^{-1} (Beattie and Williams, 2000). This is because a food exceeding 4–5 log cells g^{-1} or ml^{-1} is considered unsafe for consumption (Notermans *et al.,* 1997).

For the growth of *B. cereus* isolates, a large temperature range was observed, indicating a wide diversity and ecotype. The majority (74%) of the strains were able to grow at $\leq$7 ºC. To be considered as psychrotrophic, an organism should be able to grow at 7 ºC or less (te Giffel *et al*., 1995; Francis *et al.,* 1998). Presence of such a large number of psychrotropic strains in dairy environment is of major concern mainly because of their potential for growth, spoilage and toxin production in chilled products, such as milk and dairy products (Anderson Borge *et al.,* 2001). Other studies also showed pasteurised milk and refrigerated food to frequently harbour psychrotrophic strains of *B. cereus* (te Giffel *et al*., 1997; Svensson *et al*., 2004). Thirty-four percent of the *Bacillus cereus* isolates from milk powder were able to grow up to 50 ºC. This may be attributed to adaptation or selection of thermotolerant strains during drying and heating process generally used to make milk powder.

All the *B. cereus* isolates were multi-drug resistant. Each of those was resistant to at least five different antibiotics used. Most of the isolates were resistant to β-lactams (ampicillin, carbenicillin, cephalothin and pencillin G), but susceptible to protein synthesis inhibitors. Only 16% of the isolates initially enriched on *Bacillus cereus* selective agar (containing 100 U polymyxin B l^{-1}) were resistant to a higher concentration of polymyxin B (300 U $disc^{-1}$). However, all the 48 isolates of *B. cereus* from legume-based fermented food products were resistant against this higher concentration of polymyxin B (Roy *et al.,* 2007). As expected, all the isolates were resistant to metronidazole. An investigation on antibiotic-resistance profiles of *B. cereus* isolates from different food products in Morocco showed that the isolates were resistant to ampicillin, tetracycline and penicillin, but susceptible to chloramphenicol and erythromycin (Merzougui *et al*., 2014). Thus, emergence of multi-drug resistance among foodborne bacterial pathogens can be a major health concern.

Enzymes, such as protease, lipase and amylase significantly contribute to the reduction of shelf-life of processed milk and dairy products by degrading milk components and additives (Chen *et al.,* 2003; Datta and Deeth, 2003). In the present study, 97%, 96% and 63% of the isolates produced protease, lipase and amylase, respectively, and 60% of the isolates produced all the three enzymes. This indicates potentiality of

majority of the isolates for spoilage of dairy products, which in turn can be responsible for the reduction in shelf-life of the products. The high spoilage potential of *B. cereus* isolates is also emphasised by a previous work, analysing spore-formers isolated from dairy processing environments for spoilage-associated enzyme activities, where all the tested strains showed a high proteolytic activity (Lücking *et al*., 2013). In another study on legume-based fermented food products, 50% of the 48 isolates of *B. cereus* were capable of producing one of these enzymes and 23% of producing all the three enzymes (Roy *et al.,* 2007). Interestingly, in the present study, 37% of the isolates were amylase negative. According to previous reports (Agata *et al.,* 1996; Valero *et al.,* 2002), the inability to hydrolyse starch has been indicative of emetic subtype. This indicates a possible high prevalence of emetic subtype in dairy products analysed in the present study.

The protease and lipase produced by the representative isolates of *B. cereus* were thermostable. Thermal stability of these enzymes from *B. cereus* has been reported previously (Chen *et al.,* 2004; Akanbi *et al.,* 2010). The presence of thermostable protease and lipase increases spoilage potentiality of the isolates as these enzymes retain their activity even after heat treatments, such as pasteurisation and spray drying. Thermostable enzymes can withstand milk heat treatments, remain active in dairy products, and can provoke changes in texture up to structural defects and typical off-flavours. Well-known are the 'bitty cream' and 'sweet curdling' defects, caused by the lecithinase and proteolytic activity of *B. cereus*. Lipolysis causes bitter taste of dairy products, making them unacceptable to consumers and lead to a significant economic loss and/or reputational damage of food companies.

Haemolysin is a three-component enterotoxin produced by *B. cereus*, which is one of the potential virulence factors in *B. cereus*-mediated diarrhoea (Beecher *et al.,* 1995). Ninety-three percent of the *B. cereus* isolates exhibited β-haemolysis which was a discontinuous pattern in blood agar. This is as a result of a mutually inhibitory effect of B and L1 components and the slow reaction between the B component and the erythrocyte membrane (Stenfors Arnesen *et al.,* 2008). This is in consistence with the report of β-haemolytic activity exhibited by 92% of the *B. cereus* isolates from food ingredients and products in Brazil (Chaves *et al.,* 2011).

Majority (97%) of the isolates were positive in the Tecra antibody test for the production of diarrhoeal enterotoxin. Results were in consistence with the earlier reports, where 96% of the isolates from various food products and 74% of the isolates from dairy production chain were Nhe positive (Moravek *et al.*, 2006; Svensson *et al.,* 2007). Semi-quantitative production index indicated 94% of the isolates were high producers (index 4-5) of NheA. The prevalence of high producers of Nhe among dairy isolates is of significance as Moravek *et al.* (2006) found cytotoxicity on Vero cells to be dominated by Nhe, indicating a high diarrhoeic potential of the toxin.

Majority (72%) of the isolates were able to form biofilm even at 4 ºC. Biofilm formation by *B. cereus* isolates from dairy origin has been previously reported by Shaheen *et al.* (2010). In another study, 98% of the 56 isolates of *B. cereus* from foods and clinical specimens were able to form biofilm (Wijman *et al.,* 2007). As bacteria within biofilms are more resistant to antimicrobial agents and cleaning, it is more difficult to eliminate biofilm cells than planktonic ones (Faille *et al.,* 2001; Hornstra *et al.,* 2007). Thus, biofilm formed by the cells in dairy processing lines can be responsible for recurrent contamination and spoilage of dairy products or facilitate transmission of diseases.

The principal component (PC) analysis allowed classifying correlated variables (production of exo-enzymes, biofilm and haemolysin) into two types of adversities (spoilage and food poisoning). It is evident that majority of the isolates from cheese, butter, ice cream and a few from milk and milk powder were dominant in the positive side of PC1 and closer to variables, such as biofilm, amylase, lipase and protease production. On the other hand, majority of the isolates from milk powder were grouped in the positive side of PC2 which mainly consists of variable haemolysin. Many milk isolates were predominant in the PC1 and PC2 negative sides, and thus characterised by low production of enzymes and biofilm. Agglomerative hierarchical clustering (AHC) resulted in four heterogeneous clusters.

In the study for prevalence of *B. cereus* along the dairy processing line, 35% of raw milk samples collected from silos were found contaminated with *B. cereus*. The prevalence of positive samples increased up to 40% during the processing of pasteurised milk. In a similar study, 35% of the raw milk samples and 70% of the pasteurised milk samples were found contaminated with *B. cereus* when samples were collected from dairy

processing plant (te Giffel *et al*., 1996). The one-day in-depth sampling study indicated that contamination of milk by *B. cereus* may occur in dairy plants. *Bacillus cereus* was also isolated from surfaces of the pasteurised milk storage chilling tanks. Thus, it seems likely that the source of contamination for pasteurised milk was present in the production line, possibly in the pasteurised milk storage tank. The presence, adhesion and biofilm formation of *B. cereus* strains on stainless steel surface of dairy tanks may represent a mechanism for survival and dispersal of spores with rinse water from one location to another (Shaheen *et al.*, 2010).

5.2. *In vitro* model study for biofilm formation by *Bacillus cereus* in dairy chilling tanks

Presence of *B. cereus* biofilm in dairy processing line, chilling tanks in particular, can be a source of post-pasteurisation contamination. An *in vitro* model was designed to study biofilm formation by *B. cereus* PT4, isolated from a chilling tank where pasteurised milk was stored. The selected strain was able to form biofilm even at 4 °C. The study on different simulated conditions indicated that the *B. cereus* cell count in the biofilm developed on the surface of stainless steel chilling tanks could reach up to 6 log cfu cm^{-2}, if inadequately cleaned tanker was left to stand empty at room temperature. In a study simulating the surface of a raw milk tanker, bacterial cells found in the biofilm reached up to 8 log cfu cm^{-2} (Teh *et al.,* 2012). This is a matter of concern, as the presence of biofilm on internal surfaces of chilling tanks can lead to sporulation within biofilm. As environmental conditions in biofilm affect sporulation and heat resistance, these spores pose quality issues and safety risk either by directly contaminating food through contact or germinate on the surfaces of equipment to form new biofilms (Faille *et al.*, 2014; Hayrapetyan *et al.*, 2016). Enzymes produced in the biofilms on the internal surfaces of chilling tanks can be responsible for spoilage of milk (Teh *et al.,* 2012, 2014).

5.3. Optimisation of *Bacillus cereus* biofilm removal by alkali-based cleaning-in-place

The spores of *B. cereus* possess a pronounced ability to adhere to stainless steel surface, a common food processing material (Peng *et al*., 2002). *Bacillus cereus* biofilm can be a recurrent source of food spoilage and outbreaks of food poisoning. Thus, biofilms have been a major food safety concern for dairy industry. The technique used for biofilm cell removal in dairy industry mainly consists of CIP regimes which commonly consist of alkali and acid washes (Chisti, 1999). Effectiveness of CIP regimes against *B. cereus* biofilm is not reported extensively. Peng *et al.* (2002) reported requirement of a long-hot CIP for an effective removal of *B. cereus* biofilm. Efficacy of CIP regimes greatly depends on exposure time, temperature and concentration of cleaning agent (Bremer *et al*., 2002; Parkar *et al*., 2004). Thus, for designing an effective CIP, optimisation of these parameters is essential. The caustic step in CIP regimes is believed to be predominantly responsible for biofilm removal (Chisti, 1999). So, use of response surface methodology (RSM) to optimise parameters influencing biofilm cell removal during caustic step is of significance. All the variables used in the present study significantly contributed to biofilm removal, and an interaction between time and temperature was the main influencing factor. A linear increase in biofilm removal was observed when exposure time and temperature were increased. Maximum removal was predicted when the biofilm was exposed to 15 g NaOH l^{-1} for 30 min at 65 °C, where 2.55 log reduction cm^{-2} in biofilm cell count was achieved. Thus, RSM was successfully deployed to obtain conditions which influenced efficacy of the caustic step. The RSM results were used to design an optimised CIP regime which consisted of 15 g NaOH l^{-1} for 30 min at 65 °C - water rinse – 10 ml HNO_3 l^{-1} for 10 min at 65 °C- water rinse. Effectiveness of reference CIP (10 g NaOH l^{-1} for 10 min at 65 °C- water rinse – 10 ml HNO_3 l^{-1} for 10 min at 65 °C - water rinse) was compared with that of optimised CIP against 24 h-old biofilm. It was found that the reference CIP achieved 3.29 log reduction cm^{-2} in the number of *B. cereus* cells recovered, as compared to control. This result was in consistence with the findings of Bremer *et al.* (2006) who reported 2 log reduction cm^{-2} in biofilm cells after reference CIP regime. Difference in the reduction of cells recovered may be attributed to the fact that Bremer *et al.* (2006) used biofilm formed by consortium. The optimised CIP achieved 4.77 log reduction of biofilm cells cm^{-2}. From crystal violet staining of coupons it was evident that not only biofilm cells were inactivated by optimised CIP, but biofilm matrix also got removed from coupons. Thus, the optimised CIP in the present study effectively removed biofilm. The concentration of NaOH used was within

the permissible limit and usually used in dairy industry for CIP; 10-50 g NaOH l^{-1} is used for plate-type heat exchangers and 10-20 g l^{-1} for general cleaning (Flint *et al.,* 1997). An effective CIP regime should achieve maximum removal of biofilm cells within the shortest possible time and at a low temperature. The optimised CIP regime achieved a significant increase in log reduction of biofilm cells (4.77 cm^{-2}). Parker *et al.* (2004) reported the use of 20 g NaOH l^{-1}, 18 ml HNO_3 and a temperature of 75 °C for the removal of *Bacillus flavothermus* biofilm. The optimised CIP achieved a significant reduction in biofilm cells at lower concentrations of NaOH (15 g l^{-1}) and HNO_3 (10 ml l^{-1}), and at a lower temperature (65 °C). As evaluation of biofilm status and development of an effective CIP regime is part of HACCP plan development and ISO:9000 specifications for food processing industry to make them more meaningful (Sharma and Anand, 2002), the optimised CIP regime established can be an effective tool for *B. cereus* biofilm cell removal.

5.4. Optimisation of *Bacillus cereus* biofilm removal by enzyme-based cleaning-in-place

Alkali and acid treatments, practiced in dairy industry to achieve standard CIPs, are not always sufficient for removing biofilms (Antoniou and Frank, 2005). So, an effective alternative can be a use of enzymes to breakdown extracellular polymeric substances (EPS) network and remove biofilms (de Carvalho, 2007). Since proteases were more efficient in removing cells of *B. cereus* biofilms than polysaccharidases (Lequette *et al.,* 2010), RSM was deployed to study the influence of individual factors and their interaction on *B. cereus* biofilm removal using protease only. Biofilm removal increased with the increase in pH, indicating pH had a major role in biofilm removal. This was substantiated by *ANOVA* which showed that pH significantly (P <0.05) affected biofilm removal. EPS is insoluble at an acidic pH and responsible for increase in compactness of biofilm, making it more resistant to cleaning (Dogsa *et al.,* 2005). Alkaline pH reduces biofilm cohesiveness and facilitates removal of biofilm by increasing solubility of EPS and inducing swelling of EPS network (Lequette *et al.*, 2010).

Since biofilm formation and removal are greatly influenced by the physicochemical properties of the attachment surface (Donlan, 2002), the results of the microtiter plate assay were compared with those of the biofilms developed on stainless steel coupons in skim milk which mimics the actual environment in dairy industry. The type of assay used to study biofilm formation is of great importance, since *B. cereus* shows a preference to form biofilms at an air-liquid interface (Wijman *et al*., 2007). Using submerged assays might lead to an underestimation of the possible number of biofilm cells in a system. Factors contributing to the formation of biofilms at the air-liquid interface may involve oxygen availability at the surface, causing aerotaxis of *B. cereus* towards oxygen (Laszlo *et al.*, 1984). Thus, biofilm formation was carried out by placing coupons at an air-liquid interface. The biofilms developed on coupons were more resistant than those in microtiter plates. This may be attributed to the fact that stainless steel provides more favourable conditions for *B. cereus* biofilm formation and maturation compared to polystyrene (Hayrapetyan *et al*., 2015).

In dairy industry, an effective CIP is essential as it greatly affects the final product quality (Bremer *et al.,* 2006). Since conventional CIP using chemical agents do not provide satisfactory hygienic results, enzymic control of biofilms would present a prospective alternative (Lequette *et al.,* 2010). The RSM results were used to design an optimised protease CIP (1.0 U ml^{-1} protease in pH 8.5 buffer at 60 ºC for 20 min - water rinse - 10 ml HNO_3 l^{-1} at 65 ºC for 10 min - water rinse). The optimised protease CIP was able to completely remove *B. cereus* biofilm cells from coupons, while non-optimised protease treatment (pH 10-45 °C - 30 min) caused a reduction of 0.92 log *B. cereus* BC98/4 cells cm^{-2} in biofilm (Lequette *et al.*, 2010). The optimised protease CIP not only removed biofilm cells completely, but also removed biofilm matrix significantly (P <0.05), as compared to both reference and optimised alkali CIPs. NaOH could not remove biofilm matrix so effectively. Cleaning regime should break-up or dissolve the EPS matrix associated with biofilm, so that disinfectants can gain access to bacterial cells (Simões *et al.* 2006). Compared to other CIPs, the optimised protease CIP had an added advantage of a significantly reduced (near neutral) pH level. Thus, a significant benefit could be achieved by replacing caustic-based cleaning solutions with enzymes. This is because, in contrast to concentrated NaOH and other caustic detergents, enzymes are non-corrosive and their use leads to reduced rinsing volumes and easier disposal without neutralisation (Boyce *et al.,* 2010). To apply these results, further industrial-scale studies and economic feasibility are warranted.

5.5. Quantitative risk assessment of human exposure to *Bacillus cereus*

Incidence and level of *B. cereus* contamination in milk have been reported by various researchers and those have been found to be associated with different foodborne outbreaks (Boxall and Ortega, 2003; EFSA, 2005). Thus, the presence of *B. cereus* in pasteurised milk is regarded as a potential microbial hazard. Milk and dairy products are purchased from retail outlets and subsequently stored under different conditions prior to consumption. So, a risk assessment study at consumer level is of paramount importance.

Thirty percent of the pasteurised milk samples in 2–4 h-old stored packages from 50 household refrigerators in the present study were found to be contaminated with *B. cereus*, and the level of contamination was 3–5 log cfu ml^{-1}. The presence of such a high prevalence of *B. cereus* in pasteurised milk is a matter of concern as milk is usually stored in households for more than two days. The critical limit for *B. cereus* is 4 log cfu ml^{-1} (Notermans *et al.,* 1998), since the infective dose of *B. cereus* to cause foodborne illnesses is 5–8 log cfu g^{-1} or ml^{-1} (Notermans *et al.,* 1997; Granum and Baird-Parker, 2000). However, in the context of a dose–response model, this level cannot be considered as threshold for illness. It is used as an alternative since the development of dose–response models for toxigenic spore-forming microorganisms is complex.

The results of Monte Carlo simulation showed that the 95th and 99th percentiles of the load of *B. cereus* in stored milk were 3.82 and 4.16 log cfu ml^{-1}, respectively, and only 1% of the stored milk had contamination of less than 2 log cfu ml^{-1}. In a study in the Netherlands, 40% of the pasteurised milk samples stored in the household refrigerators were found contaminated with *B. cereus* (te Giffel *et al.,* 1997). Storage temperature in the refrigerators ranged from 3.54 °C to 12.84 °C with the mean of 8.2 °C, having probability of only 5% refrigerators operating at 5 °C. In an investigation in Greece, 25% of the 136 domestic refrigerators and 13.6% of the 228 supermarket refrigerators were found to be operating at temperatures higher than 10 °C (Sergelidis *et al.,* 1997). In a survey in Sweden, on the top shelf, the mean temperature was found to exceed 8 °C in more than 37% of the cases and on the middle shelf, it exceeded 8 °C in 11% of cases. Almost 33% of the bottom shelves tested had a temperature higher than 8 °C (Marklinder *et al.,* 2015). Results indicate that there are chances that milk and dairy products are being stored at higher temperatures; this may be due to over stacking of refrigerators, preventing proper circulation of chilled air.

Only 1% of the stored milks was found to be used within the day of purchase, while 45% within 2 days and to the maximum within 3 days of purchase. In a similar study in Slovakia, the storage time in domestic refrigerators was reported to vary from 1 day to 11 days, with the mean of 3.11 days (Acai *et al.,* 2014).

Quantitative exposure assessment provides numerical estimates of exposure and requires development of mathematical models in which relationship between factors affecting exposure can be studied (FAO/WHO, 1995). A predictive model was developed using RSM to study individual effects and interaction of three risk factors (storage time, storage temperature and load of *B. cereus* cells) on the final cell population in milk. The model predicts *B. cereus* population will reach the threshold level (>4 log cfu ml^{-1}) after 47.5 h, 45.5 h, 41.6 h, 35.3 h, 25.3 h and 24 h at 7 ºC, 8 ºC, 9 ºC, 10 ºC, 11 ºC and 12–13 ºC, respectively, when the load of *B. cereus* cells in milk is 3 log ml^{-1}. Thus if milk is stored for more than 24 h in refrigerators, the chance of consumers being exposed to *B. cereus* more than the threshold level is likely to occur which can lead to foodborne illness. Results showed that for safer pasteurised milk consumption, a lower initial load from industry part and a better temperature control and sanitation of domestic refrigerators can be effective measures to control *B. cereus*-associated hazard.

From the distribution study it is evident that there are chances that 50% of the consumers may be exposed to a high level (4.5 log cfu ml^{-1}) of *B. cereus* cells if milk is stored for more than two days. Actual scenario can be even worse as the risk associated with per capita intake of 322 g (NDDB, 2015). A similar study in the Netherlands reported 11% of portions of the milk consumed contained >4 log *B. cereus* cells ml^{-1} (Notermans *et al.,* 1997). Another study in Slovakia reported 14% of pasteurised milk to contain *B. cereus* at a level of >4 log cfu ml^{-1} (Acai *et al.,* 2014). Thus, *B. cereus* was identified as a microbiological risk in pasteurised milk presently stored in domestic refrigerators. The temperature control of refrigerators is important to prevent the growth of bacteria. To maintain a low refrigerator temperature, one should leave enough space in the refrigerator to allow the cool air to circulate. In addition, consumers should maintain proper hygienic

conditions to prevent cross contamination in the refrigerator. This study can be a valuable tool for risk management with a comprehensive picture of the key factors in the system of interest. In addition to an initial low spore count, cooling after pasteurisation and limited exposure to storage time-temperature, as set by such predictive and probabilistic modellings, will help to control the growth of this pathogen below the critical limit.

Thus, to limit the presence of *B. cereus* in dairy processing environment, there is need to gain a better insight into the whole contamination flow of endospore-formers originating from soil as well as in the conditions permitting their proliferation. Good manufacturing practices (GMPs) in farms during the production and storage of milk should be implemented and strategies aiming at reducing the population of spore-forming bacteria in raw milk should be reinforced. Better implementation of HACCP in dairy processing lines should be given importance so that the initial load in finished products could be minimised. CCPs, such as storage temperature and time, should be properly defined. More research on better understanding of the structure of *B. cereus* biofilms in the context of milk processing environment is needed to develop better CIP regimes for eliminating biofilm from dairy processing lines. Furthermore, optimisation of the existing cleaning processes and development of novel and effective strategies are of great importance to the dairy industry, as these may lead to quality improvements of products and processes. Future research could also focus on coating strategies to reduce microbial attachment on dairy equipment and on food grade quorum inhibitors as an intervention strategy which can offer new opportunities for the dairy industry in the coming years.