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Review

Psychrotolerant *Bacillus cereus*: An emerging pathogen from foodborne diseases

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Abstract

Bacillus cereus is a foodborne pathogen which has become a concern to food industries due to its ability to produce spores. The high resistance of the spores against heat, radiation, and chemical agents allows them to survive much longer during food processing and sanitising treatments, and causes recontamination of the products. Furthermore, the emergence of psychrotolerant *B. cereus* species able to grow and proliferate at refrigeration temperatures has raised concerns for food industries as it shows enhanced germination at low temperatures which makes the problem associated with chilled and minimally processed foods much more complicated. Temperature discrepancies often occur during transportation and storing of chilled foods at retail and consumer's homes, which provide more favourable conditions for the spores to germinate into active cells. The present review therefore highlights the current scientific knowledge associated with this pathogen, including an introduction on the characteristics, classification, sources, virulence, and foods associated with it, as well as the clinical syndromes and preventive measures to control and mitigate foodborne diseases it causes.

Introduction

Over the last few decades, foodborne diseases have become a major public health issue. It was reported that there were more than 250 known foodborne diseases globally, and it was estimated that 48 million people get sick, 128,000 are hospitalised, and 3,000 die from foodborne diseases, each year (CDC, 2019). *Bacillus cereus* is among the primary pathogens of concern, and associated with public health. This bacterium is largely found in the environment, *e.g.*, soil, grass, and other vegetation. *B. cereus* is a major concern for the food industry as it is not only associated with spoilt food, but also food poisoning. The high resistance of *B. cereus* spores against heat, radiation, and chemical agents, as well as their ability to form biofilms, allow them to survive during food processing and sanitising treatments, and cause recontamination of the food products, which ultimately results in product shelf-life reduction. The emergence of a new strain of psychrotolerant *B. cereus* which is able to grow and proliferate at low temperatures makes the problem associated with chilled and minimally processed products even difficult to tackle.

Bacillus cereus: characteristics and classification

Bacillus cereus is a Gram-positive spore-forming, facultative anaerobic, rod-shaped bacterium. The vegetative cells are quite large, typically ranging from 1.0 to 1.2 µm, by 3.0 to 5.0 µm, and existing in chains (Adams and Moss, 2008). This mesophilic bacterium can grow over a wide range of temperatures (10 to 48°C) (Vilas-Boas *et al.*, 2007), with the optimum temperature ranging between 25 to 35°C (Morita, 1975; Drobniewski, 1993; Jenson and Moir, 1997; Lechner *et al.*, 1998). This bacterium grows within the pH range of 4.3 to 9.3 (Raevuori and Genigeorgis, 1975), while the minimum range of water activity (a_w) for its growth is between 0.912 and 0.950 (Jenson and Moir, 1997).

This bacterium is ubiquitous in nature, and commonly found in soil, vegetation, and frequently in foods of plant and animal origins. A wide variety of foods have been found to be associated with it which include dairy products, meats, infant foods, rice dishes, vegetables, spices, and cereals (ICMSF, 2005; Ray and Bhunia, 2008; Bilung *et al.*, 2013; 2017; Gdoura-Ben Amor *et al.*, 2018). Due to its ubiquitous nature, *B. cereus* can easily reach any types of fresh and processed food products, including food

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production and processing equipment, at any points of the food supply chain, and through various mechanisms (Figure 1) (Ehling-Schulz *et al*., 2019). It was suggested that when the nutrients are present and conditions are favourable for growth, *B. cereus* which exist as spores in soils, will germinate and grow. Subsequently, it will reach foods of plant and animal origins, and ultimately enter humans and other mammals through ingestion, inhalation, and breaks in the skin. It was reported that the bacterium could infect some animals, and cause a range of debilitating symptoms, and even death. Nevertheless, sufficient evidence point out that under normal conditions, it is unlikely to be a serious hazard to healthy livestock. So, affected animals could rapidly recover upon antibiotic treatment (Ehling-Schulz *et al*., 2019).

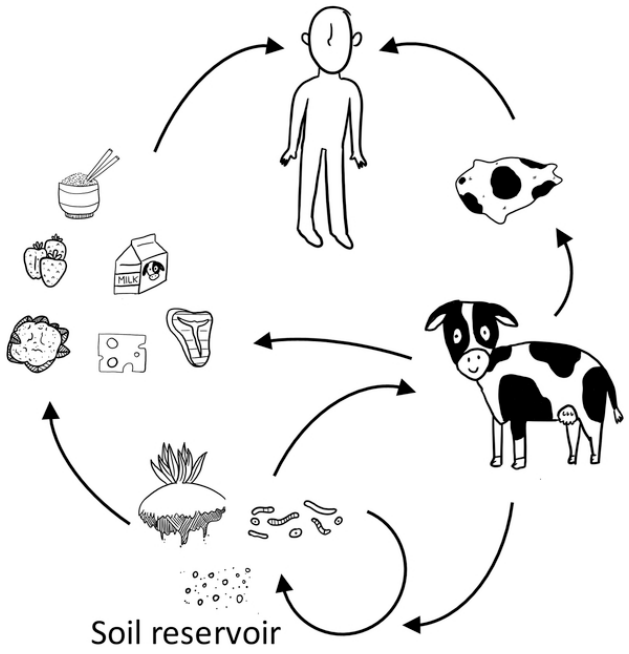

Figure 1. Transmission of the *B. cereus* group species from the soil reservoir to humans via food and textile production (adapted from Ehling-Schulz *et al*., 2019).

The classification of the *B. cereus* group to determine the species of the genus has been a lengthy discussion over the last few decades due to its highly similar properties among the members of the *B. cereus* group. The GC-content (or guanine-cytosine content) of the *Bacillus* species chromosome was reported to range between 32 to 69% which indicates a substantial genetic diversity. According to the widely used classification system which is based on the physiological characteristics, initially, there were six species in the *Bacillus cereus* group, *i.e.*, the *B. cereus sensu stricto* (*B. cereus*), *B. anthracis*, *B. thuringiensis*, *B. mycoides*, *B. pseudomycoides*, and *B. weihenstephanensis* (Lechner *et al.*, 1998; Granum, 2002; Jensen *et al.*, 2003; Vilas-Boas *et al.*, 2007; Montville and Matthews, 2008). Five additional species, *i.e.*, the *B. cytotoxicus*, *B. toyonensis*, *B. gaemokensis*, *B. manliponensis*, and *B. bingmayongensis* were later added to the group (Jung *et al.*, 2011; Guinebretière *et al.*, 2013; Jiménez *et al.*, 2013; Liu *et al.*, 2014).

A close genetic relationship was observed between the *B. cereus* group members (Helgason *et al.*, 2000) as evidenced by highly similar 16S and 23S rRNA sequences (Vilas-Boas *et al.*, 2007; Montville and Matthews, 2008), although there were also some distinguishing characteristics among them. *B. cereus sensu stricto* is known as a food poisoning microorganism which has been reported to be associated with several outbreaks (van der Zwet *et al.*, 2000; Stalheim and Granum, 2001; Dierick *et al.*, 2005; Glasset *et al.*, 2016). *B. anthracis* is recognised as a dangerous animal and human pathogen, and the causal microorganism for anthrax. The ability of this bacterium to survive at extreme conditions for long periods of time is one of the reasons for it to be used in biological warfare (Spencer, 2003; Montville and Matthews, 2008). *B. thuringiensis*, on the other hand, is used as biological insecticide for crops. *B. mycoides* and *B. pseudomycoides* are grouped under the taxon *B. cereus* as they are genetically closely related. However, they are considered phenotypically different from *B. cereus* based on their rhizoidal colony shape and fatty acid composition (Nakamura, 1998; Pruß *et al.*, 1999; Raddadi *et al.*, 2005). *B. weihenstephanensis* is a new species in the *B. cereus* group, and most frequently associated with spoilt milk which has become somewhat of a concern for refrigerated dairy products. As is the case with *B. anthracis* and *B. cereus*, *B. cytotoxicus* is also a well-known human pathogen, and characterised by high amount of iso-$C_{15:0}$ and low amount of iso-$C_{13:0}$ as compared to the other members of the *B. cereus* group (Guinebretière *et al*., 2013). *B. toyonensis* is non-pathogenic, and commonly found in the natural environment and gastrointestinal tract. It was initially identified as *B. cereus* var. *toyoi*. However, as it showed significant genomic differences from the other *B. cereus* group strains, it has been proposed and added as a novel species (Jiménez *et al*., 2013). Three *B. cereus* group strains, *i.e.*, the *B. gaemokensis*, *B. manliponensis*, and *B. bingmayongensis*, which represent the novel species

within the *B. cereus* group, have been proposed, but have not been published.

Bacillus weihenstephanensis, a potentially pathogenic psychrotolerant B. cereus group strain

An emergence of the psychrotolerant *B. cereus* strain was recently reported. This strain is distinguished from the mesophilic strains because of its ability to grow at low temperatures (4°C), but not at 43°C like the other *B. cereus* strains (Lechner *et al.*, 1998). It has a specific signature sequence in its 16S rRNA and cold shock protein genes (*cspA*) which are not found in mesophilic strains (Francis *et al.*, 1998; Pruß *et al.*, 1999). This *cspA* is associated with the ability of the *B. cereus* strain to synthesise the so-called cold-shock proteins, as a response to the cold-shock treatment. This novel species of *B. cereus* is called *B. weihenstephanensis* (Lechner *et al.*, 1998). It is noteworthy that not all psychrotolerant *B. cereus* strains belong to the species *B. weihenstephanensis* (Stenfors and Granum, 2001). Only psychrotolerant *B. cereus* strains which are able to grow at 4°C, but not at 43°C, and have specific sequences in the 16S rRNA and the *cspA*, can be classified as *B. weihenstephanensis*.

Spore structure, sporulation, germination, and outgrowth

B. cereus can be present in two different forms, *i.e.*, bacterial spores and vegetative cells (Figure 2).

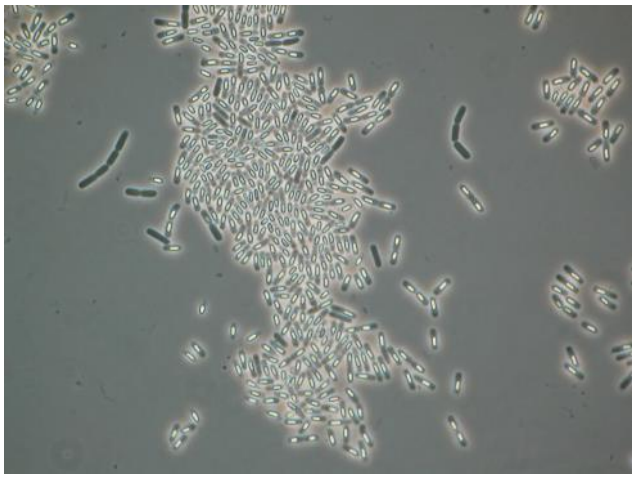

Figure 2. Vegetative cells and spores (inside and outside the cells) of *B. cereus* NVH 0075-95 (personal collection).

Due to the depletion of nutrients, the vegetative cells will transform into spores through a process called sporulation (Piggot and Hilbert, 2004). Germination occurs when the conditions, are again, favourable for growth (Setlow, 2003). Germination leads to the loss of the properties of the phase-bright dormant spores, such as heat resistance, when they turn into the so-called phase-dark spores under phase-contrast microscopy (Moir *et al.*, 1994; 2002; Moir, 2003). Germination is followed by cell enlargement and cell division which are termed as outgrowth (Hansen *et al.*, 1970; Montville and Matthews, 2008). The overall life cycle of *B. cereus* is presented in Figure 3.

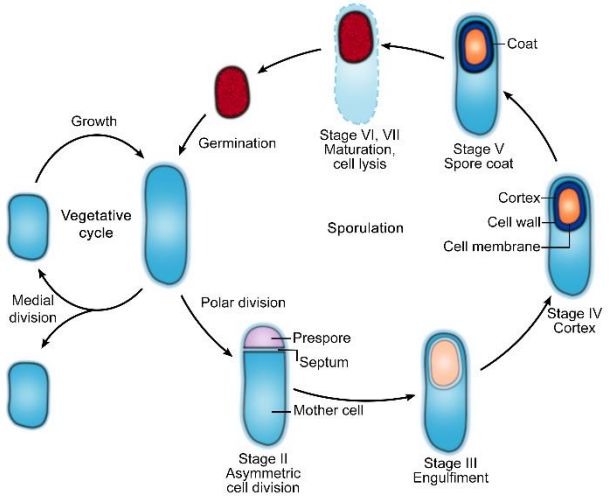

Figure 3. *B. cereus* life cycle (adapted from Errington, 2003).

Spore structure

Spores are metabolically dormant, and the structure and physiology of the spores are totally different from those of the vegetative cells (Montville and Matthews, 2008; Ray and Bhunia, 2008). The spore structures (from the inside to the outside) consist of core, inner membrane, cortex, outer membrane, and coats (Foster and Johnstone, 1990; Setlow, 2003; Ray and Bhunia, 2008). In some species, an additional layer called the exosporium is present (Figure 4).

The spore core is the most inner part of a spore. It is the most important part of a spore, as all the cellular components such as DNA, RNA, ribosomes, enzymes, proteins, dipicolinic acid (2,6-pyridinedicarboxylic acid, DPA), and Ca^{2+} accumulate in the spore core (Montville and Matthews, 2008). The pH and water content in the spore core is much lower than in vegetative cells, *i.e.*, at a pH of 6.3 to 6.5 (Setlow and Setlow, 1980), and 30 to 50% (Setlow, 2000; Montville and Matthews, 2008), respectively. This low water content enables the spore to be metabolically dormant, resistant

(Beaman and Gerhardt, 1986; Popham *et al.*, 1995), and able to survive for extremely long periods (Algie, 1984). DPA (a spore specific compound) forms a complex with a divalent cation (mostly Ca^{2+}), which constitutes 10% of the total spore weight (Setlow, 2003; Moir, 2006), and contributes largely to the spore heat resistance (Mallidis and Scholefield, 1987; Paidhungat *et al.*, 2000; de Vries *et al.*, 2004a; Kort *et al.*, 2005). Unique small acid soluble proteins (SASP), which are bound to the spore's DNA, play a major role in spore resistance (Mallidis and Scholefield, 1987; Paidhungat *et al.*, 2000; Kort *et al.*, 2005), especially when dealing with UV light (Setlow, 2001; Montville and Matthews, 2008). It is able to protect the DNA during spore dormancy. SASP contributes 10 to 20% of the total spore protein (Montville and Matthews, 2008).

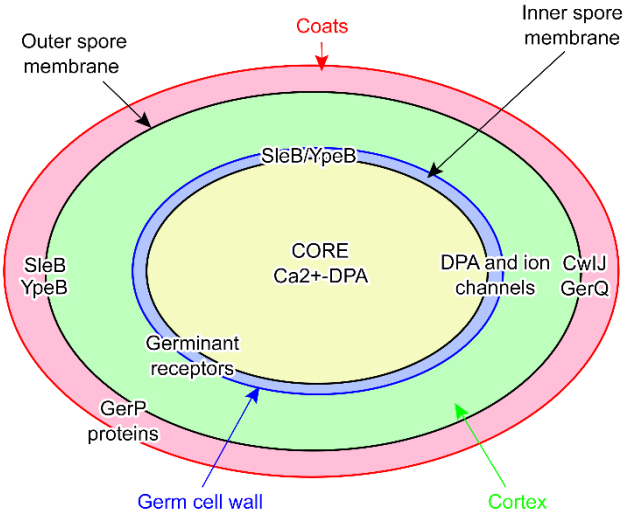

Figure 4. Spore structures (adapted from Setlow, 2003).

The inner membrane is a highly permeable barrier surrounding the spore core. This membrane has a great effect on the first stage of germination, since the spore germination receptors are located in this membrane (Paidhungat and Setlow, 2001). This membrane then becomes the cell cytoplasmic membrane when the spores germinate (Ray and Bhunia, 2008). Between the inner membrane and the cortex is the germ cell wall which becomes the cell wall of the future vegetative cells (Ray and Bhunia, 2008).

The cortex is a thick peptidoglycan layer around the germ cell wall which plays an important role in the spore dormancy as well as its resistance by maintaining the dehydration of the spore core (Algie, 1984; Atrih and Foster, 2001). As the spores germinate, the cortex is degraded by the cortex lytic enzymes (Moriyama *et al.*, 1996; Hu *et al.*, 2007) which are already present in the inner layer of the dormant spore coat (Bagyan and Setlow, 2002; Chirakkal *et al.*, 2002).

Surrounding the cortex is the outer membrane. Some studies reported that the spore outer membrane is impermeable, even to small molecules. This outer membrane impermeability contributes to the spore properties, especially its resistance toward chemicals (Genest *et al.*, 2002; Russell, 2003). However, other studies mentioned that the spore outer membrane is permeable enough, such that eliminating this membrane will not give any effect on the spore properties (Setlow, 2000; Nicholson *et al.*, 2002).

The outer membrane is surrounded by a coat. The spore coat provides protection for the spores against UV rays, chemicals, and lytic enzymes (Riesenman and Nicholson, 2000; Nicholson *et al.*, 2002; Driks, 2002; Setlow, 2006). The coat is composed of layers of proteins (Henriques and Moran, 2007; Ghosh *et al.*, 2008) which partly contribute to the spore germination (Moir, 1981; Kutima and Foegeding, 1987; Aronson *et al.*, 1989; Moir *et al.*, 2002). In contrast to the Ca^{2+}-DPA, high energy compounds such as deoxynucleoside triphosphates, ribonucleoside triphosphates, and acyl-CoA are present in the spore coat in very small amounts (Montville and Matthews, 2008).

The coat is surrounded by loosely attached exosporium (Henriques and Moran, 2007). Not all species of spore-forming bacteria have an exosporium. It is present in *B. thuringiensis*, *B. cereus* (Ray and Bhunia, 2008), and *B. anthracis* (Redmond *et al.*, 2004), but not in *B. subtilis* (Koshikawa *et al.*, 1989). However, an exosporium-like outer layer was previously reported to be present in *B. subtilis* (Sousa *et al.*, 1976). The exosporium is suggested to be correlated to the spore hydrophobicity and adherence (Koshikawa *et al.*, 1989; Charlton *et al.*, 1999; Tauveron *et al.*, 2006; Brahmbhatt *et al.*, 2007; Ghebrehiwet *et al.*, 2007).

Sporulation

Spore formation by spore-forming bacteria is triggered by several factors. The cells enter the sporulation stage, mainly as a result of starvation and a lack of nutrients due to high cell population density (Errington, 1993; Piggot and Hilbert, 2004). After exponential growth, the cells enter the stationary phase, followed by the onset of the sporulation

process, as observed previously for other *B. cereus* strains. Furthermore, sporulation capacity is also strain-dependent (de Vries *et al.*, 2004a).

The process of spore formation (sporulation) takes place in several stages, and involves more than 400 genes (Wolska *et al.*, 2007). The schematic overview of the stages during spore formation is presented in Figure 5. The brief description on sporulation given herein was derived from previous reviews (Hilbert and Piggot, 2004; Piggot and Hilbert, 2004; Wolska *et al.*, 2007).

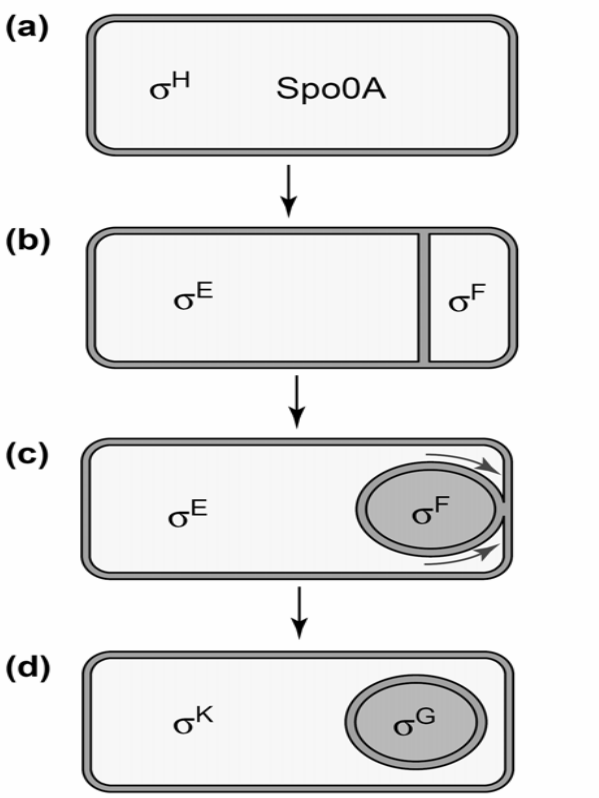

Figure 5. Morphogenesis and gene regulation during spore formation. **(a)** Activation of Spo0A and σ^H in the pre-divisional cell leads to asymmetric division and **(b)** early compartmentalised gene expression with σ^F becoming active in the pre-spore and σ^E in the mother cell. **(c)** A series of proteins produced in the mother cell degrade the asymmetric septum and trigger migration of the membrane around the pre-spore, a process called engulfment, represented here by the curved arrows. **(d)** When the membranes fuse at the pole of the cell, the pre-spore is released as a protoplast in the mother cell, and a second round of compartmentalised gene expression occurs, with σ^G becoming active in the pre-spore and σ^K in the mother cell. These delayed factors activate the transcription of the genes that build the structural components of the spore that provide its resistance qualities (adapted from Piggot and Hilbert (2004).

Sporulation is initiated by the transcription factor Spo0A, and its activity is regulated by phosphorylation. Spo0A is seen as the master sporulation response for the regulator in sporulation, which can either activate or repress the transcription of genes in the initial stages of sporulation (Hilbert and Piggot, 2004; Piggot and Hilbert, 2004). The activation of the alternative σ factor σ^H is also needed in the initial stages of sporulation (Piggot and Hilbert, 2004). As a result of the activation of the Spo0A, the cells divide and form two unequally/asymmetric-sized daughter cells (mother cell and forespore). Subsequently, the two different early compartmentalisation factors are activated immediately after completion of the sporulation division, *i.e.*, the σ^E in the mother cell and σ^F in the forespore. The engulfment of the forespore by the mother cell takes place as the result of activation of the σ^F and σ^E. The late stage of sporulation is regulated by the σ^K in the mother cell, and the σ^G in the forespore. The σ^K factor is responsible for the formation of the spore coat (Henriques and Moran, 2007) and its maturation (Fan *et al.*, 1992). The σ^G factor is involved in the insertion of germination receptors in the inner membrane of the spore (Henriques and Moran, 2007). The activation of these two σ factors results in the next morphology alteration, *i.e.*, lysis of the mother cell and release of the spore.

Germination

Germination is a process in which the dormant spore converts back to a vegetative cell, through a series of degradative and biosynthetic steps (Setlow, 2003). Spore germination is triggered by nutrients (Barlass *et al.*, 2002), and other non-nutrient agents, such as high pressure (Wuytack *et al.*, 1998; Furukawa *et al.*, 2004; Lopez-Pedemonte *et al.*, 2003; Paidhungat *et al.*, 2002; Black *et al.*, 2007), pH (Broussolle *et al.*, 2008), lysozyme (Setlow, 2003), and cationic surfactants (Setlow, 2003). During dormant periods, although metabolically inactive, the dormant spore always monitors its environment. When the conditions are appropriate and favourable for growth, the spore will germinate, grow out, and finally will be converted back to an active vegetative cell (Setlow, 2003). The schematic overview of the steps that take place during spore germination are described in Figure 6.

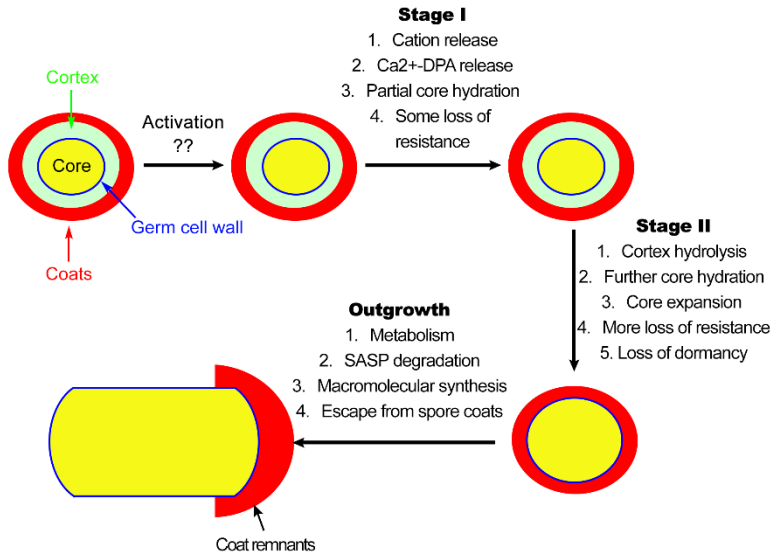

Figure 6. Germination process in *B. cereus* (adapted from Setlow, 2003).

Germination in *B. cereus* is initiated by nutrient germinants binding to the germinant receptors located in the spore inner membrane (Setlow, 2003; Moir, 2006). These germinant receptors are specific for each nutrient germinant (Paidhungat *et al.*, 2000; 2001; Paidhungat and Setlow, 2001; Fisher and Hanna, 2005; Moir, 2006). The germinant receptors for the *Bacillus* species are encoded by the *gerA* family of the operons (tricistronic *gerA*, *gerB*, and *gerK*) (Moir *et al.*, 1994). There are at least seven germinant receptors present in *B. cereus* ATCC 14579, noted as *gerG*, *gerI*, *gerK*, *gerL*, *gerQ*, *gerR*, *and gerS* (Hornstra *et al.*, 2006). Upon the binding of the ligand, the receptor triggers the release of the spore core large depot of the DPA, along with its associated divalent cations, mainly Ca^{2+}, from the spore core (Setlow, 2003), resulting in spore core hydration, followed by the loss of the spore's resistance, as well as its refractility (the spores become phase-dark) (Paidhungat *et al.*, 2000; de Vries *et al.*, 2004a). The release of the Ca^{2+}-DPA also corresponds to the increase in the electrical conductivity of the medium in which the spores are suspended (Liu *et al.*, 2007). Subsequently, after the binding of germinants to their receptors and the release of the DPA, the activation of the cortex-lytic enzymes (CLEs) takes place and leads to the hydrolysis of the spore cortex peptidoglycan (Makino and Moriyama, 2002; Setlow, 2003; Moir, 2006). Two major cortex lytic enzymes in *B. subtilis* are CwlJ and SleB. It was suggested that SleB is much more stable to heat inactivation, as compared to the CwlJ (Moir, 2006). The degradation of the spore cortex causes core expansion and further core hydration, thus resulting in the loss of spore dormancy. The next stage of the germination process is outgrowth.

Outgrowth

Outgrowth is a process in which the germinated spores convert into an active growing vegetative cell (Setlow, 2003). This process occurs after spore germination. At this stage, biosynthetic processes such as RNA, protein, and finally DNA syntheses, begin. Subsequently, the germinated spores become enlarged and proliferated as a result of the metabolic activities, synthesised macromolecules, and activated enzymes (Setlow, 2003; Montville and Matthews, 2008). As reported by Trunet *et al*. (2017), there are many techniques and methods which have been implemented to monitor the spore germination, outgrowth, and growth processes either at the population or single cell levels. These include culture-dependent methods, indirect measurements, microscopy, flow cytometry, and molecular methods.

Bacillus cereus and food spoilage

B. cereus has been identified as a major cause for food spoilage (Blackburn, 2006). Its ubiquitous nature has made this bacterium to be easily found in various foods. Although the cells of the bacterium are sensitive to heat and/or sanitising agents, and inactivated under normal conditions of pasteurisation, problems can arise from the spores produced by the cells. Destroying the cells by sterilisation is not difficult, but the spores they produce will still survive.

The resistance of the spores often causes serious problems for the food industry (Blackburn, 2006; Montville and Matthews, 2008; Jessberger *et al.*, 2020). Moreover, the hydrophobicity of the spores which allows them to adhere to the food processing equipment (Andersson *et al.*, 1995), as well as the ability to form biofilms, cause continuous recontamination in food processing (de Vries *et al.*, 2004b).

B. cereus has been frequently reported as a major contaminant related to food spoilage. Many studies have isolated and identified *B. cereus* from diverse spoilt food products such as milk (Helmy, 1984; Dufrenne *et al.*, 1995; Christiansson *et al.*, 1999; Pacova *et al.*, 2003; Valero *et al.*, 2007; Bartoszewicz *et al.*, 2008; Rossi *et al.*, 2018; Ubong *et al.*, 2020); egg products (Baron *et al.*, 2007), and rice (Sarrías *et al.*, 2002; Dierick *et al.*, 2005; Haque and Russell, 2005; Rodrigo *et al.*, 2021).

Furthermore, the emergence of a psychrotolerant *B. cereus* species has created a new problem for the food industry. *B. cereus* is mainly related to the spoilage of chilled food. Spoilage in the chilled foods industry has been frequently reported. It has been attributed to the presence of the psychrotolerant *B. cereus* strains (Christiansson *et al.*, 1989; van Netten *et al.*, 1990; Granum *et al.*, 1993; Dufrenne *et al.*, 1995; Te Giffel *et al.*, 1997; Andersen Borge *et al.*, 2001; Pacova *et al.*, 2003; Baron *et al.*, 2007; Webb *et al.*, 2019; Park *et al.*, 2020).

Foodborne illnesses associated with Bacillus cereus

The problems caused by the presence of *B. cereus* in food is not only limited to food spoilage. Its ability to produce toxins is much more hazardous for consumers. *B. cereus* is a known agent of foodborne illness for humans (Blackburn, 2006; Adams and Moss, 2008; Ray and Bhunia, 2008) as it can produce toxins which can cause two different types of food poisonings, *i.e.*, enterotoxins that cause diarrhoea, and an emetic toxins that cause vomiting (Granum and Lund, 1997). The comparison between these two types of foodborne illnesses is presented in Table 1.

Table 1. Comparison of the two types of foodborne illnesses associated with *B. cereus*.

Characteristic	Diarrhoeal syndrome	Emetic syndrome
Cause	Enterotoxins produced by bacteria	Cereulide, a heat-stable toxin produced by bacteria
Where the toxin is produced	In the small intestine of the host	Within a food matrix prior to consumption
When the toxin is produced	During growth of the bacteria in the small intestine	During growth of the bacteria in the food
Syndrome	Watery diarrhoea, abdominal cramping	Nausea, vomiting, abdominal cramping
Incubation period and recovery time	Has a longer incubation period (8 - 16 h) and recovery time (12 - 14 h, can continue for several days)	Has a short incubation period (1 - 5 h) and recovery time (6 - 24 h)

The diarrhoeal syndrome is caused by enterotoxins produced during the growth of the microorganisms in the gastrointestinal tract. There are three complex enterotoxins produced by *B. cereus* which have been characterised as haemolysin BL, non-haemolytic enterotoxin Nhe, and cytotoxinCytK (Granum and Lund, 1997; Kotiranta *et al.*, 2000; Ehling-Schulz *et al.*, 2004). When the spores of *B. cereus* are ingested, these spores can germinate and subsequently grow and produce toxins in the small intestine, which lead to the diarrhoeal syndrome. This poisoning is characterised by abdominal pain, diarrhoea, and sometimes nausea. It has an incubation period of 8 to 16 h after contaminated food is eaten (Granum and Lund, 1997; Griffiths and Schraft, 2002). The duration of the illness is 12 to 24 h. The foods associated with this type of poisoning are meat and vegetables dishes, sauces, pastas, desserts, and dairy products.

The emetic syndrome is caused by the ingestion of the heat-stable emetic toxin (cereulide, a cyclic peptide of 1.2 kDa) (Granum and Lund, 1997) produced by *B. cereus* in food before it is consumed. This type of poisoning is characterised by nausea and vomiting. The incubation time of this poisoning is 0.5 to 5 h after consumption of the contaminated food (Granum and Lund, 1997). The duration of illnesses is 6 to 24 h. Products made from rice are commonly

associated with this type of food poisoning. But other food has also been reported to be associated with the emetic syndrome, including starchy foods (such as potatoes and pasta), food mixtures (such as sauces, puddings, soups, casseroles, pastries, and salads), and cheese products (Griffiths and Schraft, 2002).

Although the diseases caused by this bacterium is mild and has a short duration as compared to other foodborne diseases, the occurrences of the cases are quite frequent. Furthermore, the foodborne illnesses caused by *B. cereus* are often underreported (Griffiths and Schraft, 2002; Montville and Matthews, 2008).

Bacillus cereus in food industries

The consumers' demand for fresh and natural foods has seen an increase (ICMSF, 2005). For this reason, mild processing has become the best choice for the processing technology applied by the food industries (Bozoglu *et al.,* 2001; Bell *et al.,* 2005). Mild processing allows the production of better taste, texture, colour, quality, and more convenient products. To extend the shelf-life of the mild processed products, the products should be stored at low temperatures (Adams and Moss, 2008; Ray and Bhunia, 2008). However, storing at low temperatures does not only prevent or reduce the growth of microorganisms, but it also enhances the emergence of new microorganisms which are tolerant to low temperatures (Blackburn, 2006). Also, spores from spore-forming bacteria which are not killed during mild food processing, could survive at low temperatures (Montville and Matthews, 2008).

B. cereus has become a major concern for the food industry due to its ability to produce spores. The high resistance of the spores against heat, radiation, and chemical agents allows them to survive during food processing and sanitising treatments (Pirttijärvi *et al.,* 2000), and causes recontamination of the products, which ultimately results in product shelf-life reduction. Moreover, the emergence of psychrotolerant *B. cereus* strains, which are able to grow and proliferate at low temperatures, makes the problem associated to the chilled products much more complicated (Webb *et al*., 2019; Park *et al*., 2020). For many chilled products, temperature fluctuations occur during transportation and storing at retailers, and in consumer's homes (ICMSF, 2005; Blackburn, 2006). These conditions are favourable for the spores to convert into active cells.

Preventive measures

Due to its ubiquity in the environment, *B. cereus* is easily spread to many types of fresh and processed products. There are several preventive measures that can be carried out in order to mitigate foodborne diseases associated to *B. cereus*. Rodrigo *et al*. (2021) summarised the most important preventive measures of *B. cereus* spores and vegetative cells into three different stages. The first stage is controlling the initial microbial load, which can be done by cleaning of the equipment or machines where food products circulate in the industry. It is essential to have a low initial concentration of *B. cereus* in raw materials, and an adequate design of processing equipment to prevent the growth of *B. cereus*. Secondly is inactivation and destruction of *B. cereus* vegetative cells, and where appropriate, bacterial spores, which can be conducted by the use of preservation procedures in the production chain such as heat treatment, high pressure processing, combined treatments, and cold plasma. The third stage is avoiding or diminishing the bacterial growth. Growth of *B. cereus* can be inhibited or reduced by increasing the generation time, increasing doubling times, or the lag phase under refrigeration storage. This can be done by storing the products at refrigeration temperatures to prevent bacterial growth, which can be combined with other methods, such as the application of antimicrobial agents (Rodrigo *et al*., 2021).

Conclusion

Bacillus cereus is widely distributed in the environment. It is considered a major foodborne pathogen which may cause diarrhoea and/or emetic syndrome. The emergence of a cold-tolerant strains belonging to the *B. cereus* group, *B. weihenstephanensis*, has become a new problem for the food industry due to its ability to grow and proliferate at refrigeration temperatures. Chilled and minimally processed products are the primary foods which can easily be contaminated by the *B. cereus* group. Therefore, the application of good hygienic practices and appropriate hygienic design of equipment as additional measures to control the contamination of products is definitely required. The informational basis related to this bacterium provided in the present review is expected to be useful to increase consumers' health awareness, as well as the local governing agencies to implement appropriate

food safety measures to minimise the associated risk factors which may lead to potentially significant problems.

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