Final Screening Assessment of *Bacillus cereus* strain ATCC 14579 and *Bacillus subtilis* strain 11685-3 (*B. cereus*)

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Synopsis

Pursuant to paragraph 74(b) of the Canadian Environmental Protection Act, 1999 (CEPA), the Minister of the Environment and the Minister of Health have conducted a screening assessment of two strains of Bacillus cereus (B. cereus strain ATCC 14579 and B. subtilis strain 11685-3). B. subtilis strain 11685-3 was listed on the Domestic Substances List (DSL) as a strain of B. subtilis; however, in testing by Health Canada scientists, it was discovered to be in fact a strain of B. cereus. For the purposes of the screening assessment, it will be referred to as B. subtilis strain 11685-3 (B. cereus).

B. cereus strain ATCC 14579 and B. subtilis strain 11685-3 (B. cereus) are bacteria that have characteristics in common with other strains of the species. B. cereus is generally considered ubiquitous, and has the ability to adapt to and thrive in many aquatic and terrestrial niches. It is resistant to a range of antibiotics and heavy metals. B. cereus forms endospores that permit survival under sub-optimal environmental conditions. Various characteristics of B. cereus make it suitable for use as an active ingredient in commercial and consumer products, including detergents, degreasers, additives for biodegradation and bioremediation, and in various industrial processes.

B. cereus can infect some animals and causes a range of debilitating symptoms, and even death, but under normal circumstances it is unlikely to be a serious hazard to healthy livestock or other organisms in the environment. B. cereus can cause mastitis in cows, but affected animals recover rapidly upon treatment with veterinary antibiotics. There are no cases where B. cereus has been shown in the scientific literature to cause adverse effects in organisms in the Canadian environment. B. cereus strain ATCC 14579 reduced the rate of reproduction in springtails (a soil invertebrate), and decreased shoot and root length in red fescue (a terrestrial plant). However, these effects were observed under specific laboratory conditions, which are not a concern under the current known exposure scenarios.

In humans, B. cereus has pathogenic potential in both the otherwise-healthy general population and in individuals who are susceptible because of compromised immunity, debilitating disease or extremes of age. B. cereus is a gastrointestinal pathogen that can also cause other types of infection, including endophthalmitis and skin infections. B. cereus is resistant to several clinical antibiotics, which could make infections harder to treat. Laboratory data show that B. cereus strain ATCC 14579 and B. subtilis strain 11685-3 (B. cereus) produce extracellular enzymes and toxins that are known factors for pathogenicity in humans.

This assessment considers the aforementioned characteristics of B. cereus with respect to environmental and human health effects associated with consumer and commercial product use and industrial processes subject to CEPA, including

releases to the environment through waste streams and incidental human exposure through environmental media. To update information about current uses of these living organisms, the Government launched a mandatory information-gathering survey (section 71 notice) under section 71 of CEPA as published in the Canada Gazette, Part I, on September 23, 2017. Information submitted in response to the notice indicates that neither B. cereus strain ATCC 14579 nor B. subtilis strain 11685-3 (B. cereus) was imported into or manufactured in Canada, except (in the case of B. cereus strain ATCC 14579) for limited quantities for academic research, teaching, and research and development activities. The likelihood of exposure to this living organism in Canada resulting from commercial and consumer activity is low.

Based on the information available, it is concluded that B. cereus strain ATCC 14579 and B. subtilis strain 11685-3 (B. cereus) do not meet the criteria under paragraph 64(a) or (b) of CEPA as they are not entering the environment in a quantity or concentration or under conditions that have or may have an immediate or long-term harmful effect on the environment or its biological diversity or that constitute or may constitute a danger to the environment on which life depends. It is also concluded that B. cereus strain ATCC 14579 and B. subtilis strain 11685-3 (B. cereus) do not meet the criteria under paragraph 64(c) of CEPA as they are not entering the environment in a quantity or concentration or under conditions that constitute or may constitute a danger in Canada to human life or health.

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Introduction

Pursuant to paragraph 74*(b)* of the *Canadian Environmental Protection Act, 1999* (CEPA), the Minister of the Environment and Climate Change and the Minister of Health are required to conduct screening assessments of those living organisms added to the (DSL) by virtue of section 105 of the Act to determine whether they present or may present a risk to the environment or human health (according to criteria as set out in section 64 of CEPA)[1]. These strains were added to the DSL under subsection 25(1) of CEPA 1988 and the DSL under subsection 105(1) of CEPA 1999 because they were manufactured in or imported into Canada between January 1, 1984 and December 31, 1986. *B. subtilis* strain 11685-3 was nominated to the DSL as a strain of *B. subtilis*; however in testing by Health Canada scientists it was discovered to be in fact a strain of *B. cereus*. For the purposes of the screening assessment report, it will be referred to as *B. subtilis* strain 11685-3 (*B*. *cereus*).

This screening assessment considers hazard information obtained from the public domain and from unpublished research data generated by Health Canada[2] and Environment and Climate Change Canada[3] research scientists, as well as comments from scientific peer reviewers. Exposure information was obtained from the public domain and from a mandatory CEPA section 71 notice published in the *Canada Gazette,* Part I, on October 23, 2017. Further details on the risk assessment methodology used are available in the "Framework on the Science-Based Risk Assessment of Micro-organisms under the *Canadian Environmental Protection Act, 1999*" (Environment Canada and Health Canada, 2011).

In this report, data that are specific to DSL-listed strains *B. cereus* strain ATCC 14579 and *B. subtilis* strain 11685-3 (*B*. *cereus*) are identified as such. Where strain-specific data were not available, surrogate information from literature searches was used. When applicable, literature searches conducted on the organisms included their synonyms, and common and superseded names. Surrogate organisms are identified in each case to the taxonomic level provided by the source. Literature searches were conducted using scientific literature databases (SCOPUS, PubMed, CAB abstracts), web searches and key search terms for the identification of human health and environmental hazards in this report. Information identified as of April 2017 was considered for inclusion in this report.

[1] A determination of whether one or more criteria of section 64 of CEPA are met is based on an assessment of potential risks to the environment and/or to human health associated with exposure in the general environment. For humans, this includes, but is not limited to, exposure from air, water and the use of products containing the substances. A conclusion under CEPA may not be relevant to, nor does it preclude, an assessment against the criteria specified in the *Hazardous Products Regulations*, which is part of the regulatory framework for the Workplace Hazardous Materials Information System (WHMIS) for products intended for workplace use.

[2] Testing conducted by Health Canada's Environmental Health Science and Research Bureau

[3] Testing conducted by Environment and Climate Change Canada's Ecotoxicology and Wildlife Health Division

Decisions from Domestic and International Jurisdictions

Domestic

B. cereus is a Risk Group 2 human and animal pathogen and it is regulated by the Public Health Agency of Canada and by the Canadian Food Inspection Angency. They are regulated under the *Human Pathogens and Toxins Act* and their use in research and teaching laboratories should be in compliance with the Canadian Biosafety Standard Second Edition, 2015 (CBS 2015). A license under the *Human Pathogens and Toxins Regulations* is required for controlled activities with Risk Group 2 human pathogens.

B. cereus is listed in the *Transportation of Dangerous Goods Regulations* (TDGR) as a category B infectious substance. Arrangements for shipping of *B. cereus* must also meet requirements under Canada's *Transportation of Dangerous Goods Act and Regulations*. These measures are designed to prevent any human or environmental exposure to the organism in the laboratory setting. Human and environmental exposure to *B. cereus* through R&D and teaching uses reported under the section 71 notice is therefore expected to be low.

International

Outbreaks caused by *B. cereus* have been published by the United States Centers for Disease Control. *B. cereus* has been included in the Bad Bug Book published by the United States Food and Drug Administration. Another strain of *B. cereus* has been assessed by the United States Environmental Protection Agency for its use in a pesticide product.

In the European Community (EC) *B. cereus* is considered to be a Risk Group 2 pathogen. Regulation (EC) No 1831/2003 requires that an application be submitted for authorisation of feed additives for use in animal nutrition, including additives that may contain micro-organisms such as *B. cereus*. Two scientific opinions regarding two strains of *B. cereus* concluded that given the presence of genes coding for enterotoxin in the genome of those strains, there is a risk to individuals exposed to the organisms or the product containing them. The European Food Safety Authority has several publications on the risks to public health related to *Bacillus* species including *B. cereus* in foods.

The Australian Department of Health reported on foodborne diseases across Australia which included *B. cereus* often in association with rice. Biosecurity New Zealand recently published a risk profile on *B. cereus* in dairy products.

112 No other international decisions regarding *Bacillus cereus* were found[4].

[4] Government agencies and organizations searched include: United States Animal and Plant Health Inspection Services United States Department of Agriculture; American Biological Safety Association; World Health Organization; and European Centre for Disease Prevention and Control

1. Hazard Assessment

1.1 Characterization of *Bacillus cereus*

1.1.1 Taxonomic identification and strain history

Binomial name: *Bacillus cereus*

Taxonomic designation:

Kingdom:	Bacteria
Phylum:	Firmicutes
Class:	Bacilli
Order:	Bacillales
Family:	*Bacillaceae*
Genus:	*Bacillus*
Species:	*Bacillus cereus*
DSL strains:	ATCC 14579 and 11685-3

Strain history:

B. cereus strain ATCC 14579 was first isolated from the air in a cow shed in the United Kingdom (Frankland and Frankland 1887). *B. cereus* strain ATCC 14579 is the type strain and has several accession numbers in other culture collections including DSM 31 (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH) and NCCB 75008 (Netherlands Culture Collection of Bacteria).

Although it was nominated to the DSL as a strain of B. subtilis, and is listed in culture collections under that species name, B. subtilis strain 11685-3 was discovered by Health Canada scientists to be a strain of *B. cereus*. *B. subtilis* strain 11685-3 (*B*. *cereus*) was nominated to the DSL under a masked strain designation. Although it is housed in a recognized culture collection, no information on its source of isolation is available from the culture collection. There are very few publications in the scientific literature referring to this strain, and phenotypic data are not available.

1.1.1.1 Phenotypic characteristics

Bacillus cereus is a Gram-positive, facultatively anaerobic, spore-producing, motile, rod-shaped bacterium. *B. cereus* spores are ellipsoidal, subterminal and do not swell the sporangium. *B. cereus* cells tend to occur in chains and the stability of these chains determines the form of the colony, which may vary from strain to strain (Logan and De Vos 2009).

Table 1-1 compares colony morphologies of *B. cereus* from various sources including both DSL strains.The phenotypic characteristics summarized in Table 1-2 provide an overview of the metabolic capabilities of *B. cereus* strain ATCC 14579 compared to other members of the *B. cereus* group. The discrepancies between data from Health Canada, American Type Culture Collection (ATCC), and Bergey's manual are within the range of acceptability for *B. cereus*, and may be due to variable culture conditions. Results of phenotypic testing (other than colony morphology) for *B. subtilis* strain 11685-3 (*B*. *cereus*) are not available.

Table 1-1: Colony morphology of *B. cereus* strain ATCC 14579 and *B. cereus sensu stricto*

Characteristic	ATCC 14579	ATCC 14579	Strain 11685-3	*B. cereus* sensu stricto
Shape	Circular, irregular	Irregular	Circular	Circular to irregular
Size (mm)	5-8	N/A	2	2-7
Margin	Undulate	Erose	Entire	Entire to undulate, cremate or fimbriate
Elevation	Flat	Flat	N/A	N/A
Colour	Cream	N/A	Off-white	Whitish to cream
Texture (surface)	Moist	Dull	Smooth	Matte or granular (smooth and moist)
Opacity	Opaque	Opaque	Opaque	Opaque
Pigment	None	N/A	N/A	Pinkish-brown, yellow diffusible or yellow-green fluorescent possible
Data source	Heath Canada[a]	American Type Culture Collection[b]	Health Canada	Bergey's manual[c]

N/A indicates data not available
[a] appearance on TSB agar after 7 days of growth at room temperature
[b] appearance on nutrient agar at 30°C
[c] appearance on blood agar after 24-36 hours at 37°C

Table 1-2: Characteristics of closely related *B. cereus* group species

Characteristics	*B. cereus* strain ATCC 14579[a]	*Bacillus cereus*[b]	*Bacillus cereus* Emetic biovar[b]	*Bacillus anthracis*	*Bacillus thuringiensis*[b]
Motility	+	+	+	-	+
Catalase	+	+	+	+	+

Characteristics	*B. cereus* strain ATCC 14579[a]	*Bacillus cereus*[b]	*Bacillus cereus* Emetic biovar[b]	*Bacillus anthracis*	*Bacillus thuringiensis*[b]
Oxidase	+	-	-	N/A	-
Egg-yolk reaction	N/A	+	+	+	+
Hydrolysis of Casein	+	+	+	+	+
Hydrolysis of Esculin	+	+	+	+	+
Hydrolysis of Gelatin	+	+	+	+	+
Acid from Glycogen	N/A	+	-	+	+
Acid from Starch	N/A	+	-	+	+
Degradation of Tyrosine	+	+	N/A	-	+
Utilization of Citrate	+	+	+	d	+
Utilization of Propionate	+	N/A	N/A	N/A	N/A
Parasporal Crystal	-	-	-	-	+
Reduction of Nitrate	+	d	+	+	+
Voges-Proskauer	+	+	+	+	+
Deamination of Phenylalanine	N/A	-	-	N/A	-

+ indicates greater than > 85% positive; - indicates 0-15% positive; N/A indicates data not available; d indicates different strains give different reactions

[a] nominator data

[b] based on information summarizing phenotype of several strains form various publications available in Bergey's manual (Logan and De Vos 2009)

Unpublished data generated by Health Canada[5], including growth in liquid media at different temperatures, growth on solid media at 28°C and 37°C, biochemical testing and fatty acid methyl-ester (FAME) analysis are presented in Appendix A for *B. cereus* strain ATCC 14579, but are not available for *B. subtilis* strain 11685-3 (*B. cereus*). These techniques cannot be used to differentiate *B. cereus* strain ATCC 14579 from other *B. cereus* strains. The FAME analysis of *B. cereus* strain ATCC 14579 showed high similarity with *B. thuringiensis,* which is expected, given the genetic similarity among the *B. cereus* group members.

1.1.1.2 Molecular characteristics

Genotypic methods, such as full genomic sequencing (Ivanova et al. 2003), amplified fragment length polymorphism (AFLP) (Ticknor et al. 2001), rep-PCR fingerprinting (Cherif et al. 2003),16S rRNA and 23S rRNA gene sequence analysis (Ash et al. 1991), multi-locus enzyme electrophoresis (MLEE)(Ash and Collins 1992;

[5] Environmental Health Science and Research Bureau

Helgason et al. 2000b), multi-locus sequence typing (MLST) (Helgason et al. 2004; Priest et al. 2004; Tourasse et al. 2006) and suppression subtractive hybridization (SSH) (Radnedge et al. 2003), have been extensively used to demonstrate phylogenetic relationships and to understand the few genomic variations among the *B. cereus* group species. The genetic relatedness between members of the *B. cereus* group is so close that from a strictly phylogenetic point of view they can be seen as a single species.

The *B. cereus* group members are usually divided into three main phylogenetic clades (Appendix B); Clade I comprises *B. anthracis* and some *B. cereus* and *B. thuringiensis*, mostly from clinical sources; Clade II contains *B. cereus* strain ATCC 14579 and several other *B. cereus* strains, but is mostly composed of *B. thuringiensis* strains, few from clinical sources; and Clade III contains the non-pathogenic *B. mycoides* and *B. weihenstephanensis* (Didelot et al. 2009; Helgason et al. 2000b; Kolsto et al. 2009;Priest et al. 2004; Vassileva et al. 2006). Different lineages based on MLST have also emerged from Clades I and II. *B. cereus* strain ATCC 14579 belongs more specifically to the Tolworthi lineage (Barker et al. 2005; Priest et al. 2004; Vassileva et al. 2006).

16S rRNA gene sequences of *B.cereus* ATCC 14579, prepared by Health Canada scientists from stock obtained from ATCC, show 100% homology with *B. cereus* strain ATCC 14579 on the proprietary MicroSeq ® ID library and more than 99% homology with other members of the *B. cereus* group included on the database (*B. thuringiensis* strain ATCC 33679 and ATCC 10792, *B. anthracis* Ames and *B. mycoides* strain ATCC 6462). This confirmed that the 16S rRNA gene sequence from *B. cereus* strain ATCC 14579 obtained from the ATCC matched the published 16S rRNA gene sequence from *B. cereus* strain ATCC 14579. *B. cereus* strain ATCC 14579 16S rRNA gene sequences also showed the same high similarity when compared to published *B. cereus* sequences in NCBI.

16S rRNA gene sequences prepared from a stock of *B. subtilis* strain 11685-3 obtained directly from the culture collection, were discovered by Health Canada scientists to have more than 99% homology with members of the *B*. *cereus* group on the proprietary MicroSeq ® ID library, including *B*. *cereus* strain ATCC 14579. The possibility that the stock had become contaminated in the Health Canada laboratory was ruled out, as similar results were observed with stocks obtained from Carleton University, and with new stocks ordered from the culture collection. To confirm this finding, the genome was sequenced. Comparison of marker genes via the *B*. *cereus* Multi Locus Sequence Typing website (Jolley 2014) showed a close but non-exact match to an existing strain (Table 1-3).

Table 1-3: Comparison of allele profiles in the *B. cereus* multi locus sequence typing database (adapted from Jolley 2014)

MLST allele	***B. cereus* strain ATCC 14579**	***B. subtilis* strain 11685-3 (*B*.**	**Closest match *(B. cereus* strain**

		***cereus*)**	**ST204)**
glp	13	15	15
gmk	8	6	6
ilv	11	29	29
pta	11	8	8
pur	12	4	4
pyc	7	8	8
tpi	4	39	14

In order to determine its relatedness to other sequenced *B*. *cereus* genomes, a whole-genome phylogenetic tree was constructed using 16S rRNA gene sequences from both DSL strains (Figure 1-1). Both strains group within the *B. cereus* group along with the other pathogens of the *Bacillus* genus. This method confirmed that strain *B. subtilis* strain 11685-3 is in fact a strain of *B*. *cereus*.

Central to the identification of members of the *B. cereus* group is analysis of pathogenicity traits, and of extra-chromosomal elements, which reflect the species' virulence spectra. The extra-chromosomal elements that differ between members of the *B. cereus* group are presented in Appendix C. The plasmids determining pathogenicity patterns in the *B. cereus* group include pXO1 and pXO2 of *B. anthracis,* which contain the anthrax pathogenicity island, pBtoxis of *B. thuringiensis,* coding for the insecticidal protein, and pCER270 of *B. cereus*, encoding an emetic toxin. Extrachromosomal elements can also differ between strains of the same species. While pXO1 has been found in some *B. cereus* strains, such as G9241, and others carry pCER270, these are not features of *B. cereus* strain ATCC 14579, which only contains one extrachromosomal element, pBClin15 (Ivanova *et al.* 2003). The plasmid pBClin15 does not contain genes associated with any known pathogenicity traits. Searches of annotated genome data did not identify known toxin genes or operons associated with pXO1 (cya/edema factor, lef/lethal factor, pagA/protective antigen repressor), PXO2 (potentially positive for CapA, but no other capsule genes), pCER270 (emetic toxin gene cluster) in *B. subtilis* strain 11685-3 (*B. cereus*). By gel electrophoresis, very faint bands suggest plasmids may be present at low copy number. These putative plasmids are small (3 kb and 5 kb), whereas pXO1 is 181 kb in size; pXO2 is 94.8 kb; pCER270 is 270.1 kb; and pBtoxis is 127.9 kb, and are unlikely to carry considerable gene content. To rule out the possibility that *B. subtilis* strain 11685-3 (*B*. *cereus*) is *B*. *thuringiensis*, the genome was searched and PCR tested for vegetative insecticidal protein (vip3) and crystal protein (cry) genes. Neither was detected.

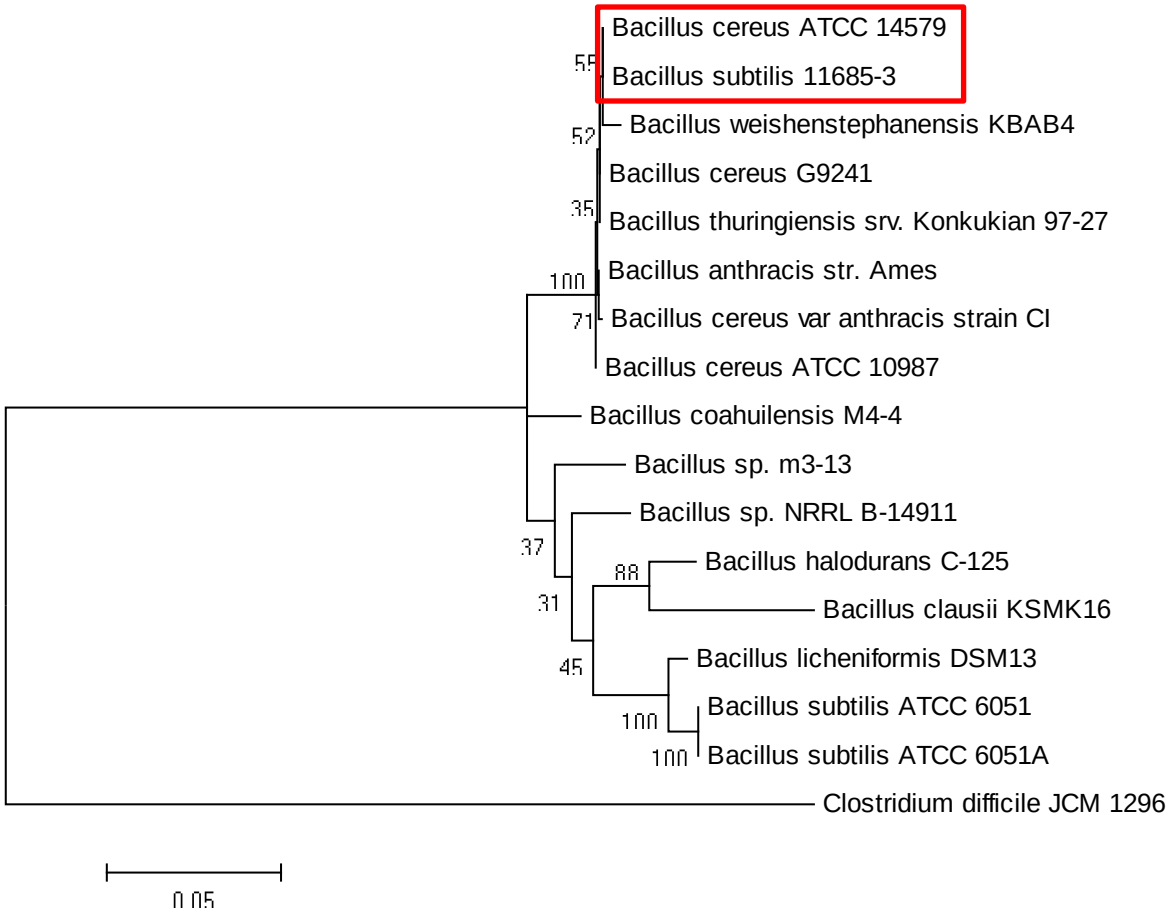

Figure 1-1: Phylogenetic tree generated by the Environmental Health Science and Research Bureau using the 16S rDNA sequences of *Bacillus* species, identified from literature searches. The phylogenetic tree was constructed first by alignment of the sequences by the MUSCLE method and then analyzed with the Kimura 2-parameter distance model within the MEGA version 5.2 platform (Tamura et al., 2011)

1.1.2 Biological and ecological properties

1.1.2.1 Growth parameters

B. cereus has minimal nutritional requirements and grows over a range of temperatures and pH. The minimum temperature for growth is generally around 10 to 20°C and the maximum is 40 to 45°C with an optimal growth temperature of about 37°C (Logan and De Vos 2009). Sme physchotolerant strains have been isllated at 6°C (Logan and De Vos 2009). Additional information on the growth requirements specific to the DSL strains is provided in Appendix A.

1.1.2.2 Survival, persistence and dispersal in the environment

B. cereus has the ability to form spores; therefore, its vegetative cells have the capacity to colonize a variety of niches and its spores to persist indefinitely in many environments (Kotiranta et al. 2000). *B. cereus* forms endospores that permit survival under sub-optimal environmental conditions. These have a tough outer keratin-like layer which is heat-, chemical-, radiation-, disinfectant- and desiccation-resistant, often withstanding temperatures of 126°C for more than 90 minutes (Pillai et al. 2006). The spores are found in many types of soil and in sediments, dust and plant material, are described as having a ubiquitous presence in nature (Stenfors Arnesen et al. 2008) and may passively spread in the environment. The spores are not easily destroyed by means that eliminate vegetative cells and may germinate when in contact with organic matter, or once inside insect or animal hosts (Stenfors Arnesen *et al.* 2008).

Nevertheless, conditions required for growth and survival vary with the strain (Bassen et al. 1989; Gibriel and Abd-el Al 1973; Jaquette and Beuchat 1998; Rizk and Ebeid 1989; Rossland et al. 2003). The optimal growth temperature for most strains is between 30ºC and 37ºC, normally with no growth above 55ºC or below 5ºC. The optimal pH depends on the growth medium used (Andersson 1995), with no growth seen in media of pH lower than 4.3 or higher than 10.5.

Persistence test data were obtained by Environment and Climate Change Canada on *B. cereus* strain ATCC 14579 in agricultural soil. After inoculation of soil with live cells, samples from various time points were collected and the presence of *B. cereus* strain ATCC 14579 DNA was tested by specific AFLP PCR. DNA from this strain was detected for 127 days post-inoculation (Xiang *et al*. 2010). No persistence data are available for *B. subtilis* strain 11685-3 (*B*. *cereus*). Another study (West et al. 1985) artificially inoculated natural (un-autoclaved) dry soil with 10^4 spores of *B. cereus* and the population level increased to 10^5 over the span of the experiment (64 days). This is consistent with the finding that spores can be maintained in the environment and are resistant to some of the factors that cause vegetative cell numbers to decline after artificial inoculation. Therefore, it is reasonable to believe that repeated releases of *B. cereus* spores into the natural environment could lead to increased numbers of spores being maintained in those environments.

B. cereus is a persistent micro-organism that is frequently isolated from natural environments. However, studies in the scientific literature that contain data on population levels of *B. cereus* in the natural environment are very limited. One study (Tucker and McHugh 1991) showed that, in various soils containing varying floral populations, the concentration of *B. cereus* reached 6×10^4 CFU/g of soil. As well, no relevant reports concerning environmental persistence of toxins produced by *B. cereus* have been found. While large inputs of *B. cereus* strain ATCC 14579 or *B. subtilis* strain 11685-3 (*B*. *cereus*) into the environment could result in concentrations greater than background levels of *B. cereus*, high numbers of vegetative cells are unlikely to be maintained in water and in soil due to competition

(Leung et al. 1995) and microbiostasis (van Veen et al. 1997), which is an inhibitory effect of soil, resulting in the rapid decline of populations of introduced bacteria. Nevertheless, *B. cereus* spores are likely to persist and accumulate in the environment, as indicated by the information presented above. No reports documenting elimination of *B. cereus* spores following environmental contamination were found in the literature.

It has been shown that *B. cereus* strain ATCC 14579 is able to produce a bacteriocin-like inhibitory substance (BLIS) that is highly active against closely related *Bacillus* species (Risoen et al. 2004). However, there are currently no published reports or research articles indicating that *B. cereus* strain ATCC 14579 is harmful to microbiota in the environment at the population level (for the purpose of this assessment, this indicates a significant number of organisms of the same species inhabiting a given area). It is not known if *B. subtilis* strain 11685-3 (*B. cereus*) produces BLIS or similar compounds.

1.1.2.2 Involvement in biogeochemical cycling

B. cereus is most likely involved in biogeochemical nutrient cycling, as it produces a wide range of extracellular enzymes and can grow on decaying organic matter (Borsodi et al. 2005).Therefore, it is capable of playing a role in the decomposition and recycling of soil organic matter, when it appears in a vegetative state, however its widespread occurrence in soils is notably as spores. *B. cereus* is also capable of reducing nitrate to nitrite and ammonium and can thereby play a role in the nitrogen cycle (Andersson 1995).

1.1.2.3 Genetic transfer

Various plasmids are found in different strains of *B. cereus* (Appendix C) and some harbour genes linked to pathogenicity or environmental adaptation (Helgason et al. 2000a; Hoffmaster et al. 2004; Rasko et al. 2005; Rasko et al. 2007). Generally these plasmids are present in low copy number, and are not self-transmissible, but they can be mobilized with the help of other plasmids carrying homologs of key components of a conjugative secretion system (Van der Auwera and Mahillon 2005). Transduction (phage-mediated horizontal gene transfer) is a potentially important mechanism of gene transfer in natural environments. Bacteriophage CP-51, a generalized transducing phage for *B. cereus*, *B. anthracis* and *B. thuringiensis*, mediates transduction of plasmid DNA (Ruhfel et al. 1984). The only plasmid found in *B. cereus* ATCC 14579 is linear plasmid pBClin15 (Ivanova 2003), closely resembling the Bam35 phage, a common bacterial virus (Stromsten et al. 2003) but no transduction events have been associated with pBClin15 in the scientific literature. Putative plasmids in *B. subtilis* 11685-3 (*B. cereus*) are small (3 kb and 5 kb) and are unlikely to carry considerable gene content.

Insertion sequences (IS) are another type of mobile element that can be involved in horizontal gene transfer. IS elements are composed of inverted repeats flanking a

transposase gene (De Palmenaer et al. 2004) and have been found in various members of the *B. cereus* group. IS*231* has been implicated in the translocation of mobile insertion cassettes which may contain genes involved in antibiotic resistance or adaptation to the environment (Chen et al. 1999; De Palmenaer et al. 2004). An IS*231* variant was identified in *B. cereus* strain ATCC 14579 and is composed of two putative genes; one is 60% identical to a haloacid dehalogenase and the other is 55% identical to an acetyltransferase (De Palmenaer et al. 2004).

Group II introns were also identified in the genome of *B. cereus* group members (chromosome and plasmids), including one copy in *B. cereus* ATCC 14579. Even though these do not contain any pathogenicity genes, they are self-splicing, mobile retro-elements implicated in genetic transfer (Tourasse and Kolsto 2008). Other elements that can facilitate gene transfer may also be present in *B. cereus*. Økstad et al. (2004) identified a DNA repeated element specific to the *B. cereus* group, *bcr1*. This element is present in *B. cereus* ATCC 14579 in 54 copies and possesses the characteristics of a mobile element. Therefore, *bcr1* could be implicated in horizontal gene transfer within the *B. cereus* group. Furthermore, the full genome analysis of the sequence of *B. cereus* ATCC 14579 (Ivanova et al. 2003) revealed the presence of 28 transposase genes, which could be involved in horizontal gene transfer (Kolsto et al*.* 2009).

Gene transfer is possible and could increase the hazard potential of *B. cereus*, as occurred when strain G9241 acquired the *B. anthracis* pXO1 plasmid carrying the anthrax pathogenicity island. However, the presence of vegetative cells seems to be essential for conjugation (Santos et al. 2010). Since *B. anthracis* exists in the natural environment mainly as dormant spores, and its vegetative cells survive poorly outside the host, the acquisition of *B. anthracis* plasmids by other members of the *B. cereus* group is extremely rare, and may be restricted to, or at least be more common in, areas where anthrax is endemic (Hoffmaster et al. 2006). Moreover, growth of *B. anthracis* outside a host usually leads to loss of virulence (reviewed in Dragon and Rennie 1995). In a laboratory setting, the conjugal transfer of an insecticidal plasmid of *B. thuringiensis* to *B. anthracis* was observed at a ratio ranging from 6.9×10^{-4} to 1.9×10^{-7}, but no naturally occurring insecticidal *B. anthracis* isolates have yet been reported (Yuan et al. 2010). Conjugation of *B. thuringiensis* plasmid pAW63 and pXO16 to one strain of *B. cereus* and between *B. thuringiensis* strains has been reported in food matrices under laboratory conditions (Van der Auwera et al. 2007). Conjugal transfer of plasmid pHT73- EM^R from *B. thuringiensis* var*. kurstaki* to *B. cereus* strain ATCC 14579 had frequencies of $1.1 \pm 0.90 \times 10^{-9}$ on nitrocellulose filter and was not detected on LB broth or on *Bombyx mori* larvae (Santos et al. 2010).

While it is possible that *B. cereus* strain ATCC 14579 and *B. subtilis* strain 11685-3 (*B. cereus*) could acquire virulence plasmids from pathogenic relatives, the probability of such an occurrence is no higher than for other naturally occurring strains of *B. cereus*. The DSL strains do not contain plasmids bearing virulence

factors, so they cannot be implicated in the conjugal transfer of virulence factors to other bacteria in the environment.

1.1.2.4 Resistance to antibiotics, metals and chemical agents

Resistance of *B. cereus* to different antibiotics is widely variable between strains (Bernhard et al. 1978; Weber et al. 1988). Most strains of *B. cereus* produce β-lactamase and are therefore considered to be resistant to β-lactam antimicrobial agents (Coonrod et al. 1971). Most *B. cereus* strains have been found to be resistant to penicillin, semisynthetic penicillin, cephalosporin (Stretton and Bulman 1975; Weber et al. 1988), ampicillin, colistin, polymyxin, kanamycin, tetracycline, bacitracin and cephaloridine (Bernhard et al. 1978; Wong et al. 1988). Mols et al. 2007 reported that *B. cereus* strain ATCC 14579 is resistant to antibiotics targeting cell wall components such as cefazolin, ketoprofen and moxalactam. Even with appropriate antibiotic regimens, there are reports in the literature presenting refractory *B. cereus* infection leading to fatal outcomes (Musa 1999; Tuladhar 2000). Antibiotic susceptibility tests conducted by Health Canada on 10 classes of antibiotics have shown that *B. cereus* strain ATCC 14579 is highly resistant to amoxycillin, aztreonam and trimethoprim, that it had intermediate sensitivity to cephotaxim and nalidixic acid but that it is sensitive to doxycyline, erythromycin, gentamicin and vancomycin (Table 1-4). The antibiotic susceptibility profile of *B. subtilis* strain 11685-3 (*B*. *cereus*) is not available.

Table 1-4: Minimal Inhibitory Concentrations (MIC, µg/mL) for *B. cereus* strain ATCC 14579

Antibiotic	MIC breakpoints[a] (µg/mL)	*B. cereus* strain ATCC 14579[b]	Interpretation of results
Amoxycillin	N/A	>24	N/A
Aztreonam	N/A	>24	N/A
Cephotaxime	S≤8; I 16-32; R≥64	>12	I
Doxycycline	N/A	<0.37	N/A
Erythromycin	S≤0.5; I 1-4; R≥64	<0.37	S
Gentamicin	S≤4; I 8; R≥16	1.5	S
Nalidixic acid	N/A	6	N/A
Trimethoprim	R≥4	>24	R
Vancomycin	S≤4	1.5	S

S indicates susceptible; I indicates intermediate susceptibility; R indicates resistant; N/A indicates not available

[a] Breakpoints to determine the susceptibility of the strain were taken from Clinical and Laboratory Standard Institute's Methods for Antimicrobial Dilution and Disk Susceptibility Test of Infrequently Isolated or Fastidious Bacteria; Approved Guideline – Second Edition (CLSI 2012)

[b] Work conducted using TSB-MTT liquid assay method (Seligy *et al.* 1997). The reported values are based on a minimum of three independent experiments. Values correspond to the minimal inhibitory concentration (µg/ml) for *B. cereus* strain ATCC 14579 (20, 000 CFU/well) grown in the presence of antibiotic for 72 hrs at 37°C

1.1.2.5 Pathogenic and toxigenic characteristics

Toxins

B. cereus can cause two types of food poisoning: an emesis syndrome, resulting in vomiting through the action of the emetic toxin cereulide and a diarrheal syndrome produced through the action of various enterotoxins (Granum 2001; Kotiranta et al. 2000; Stenfors Arnesen et al. 2008). Cereulide is a peptide toxin, that must be present in the food at the time of ingestion to cause vomiting, but live cells are not required for emesis (Agata et al. 2002). For the diarrheal syndrome, it is unclear whether enterotoxins present in food or produced in the small intestine by live bacteria cause the effect. However, enterotoxins are unstable at pH <4 and can be degraded by pepsin, trypsin and chymotrypsin (Granum 1994), so it is likely that they are produced in the small intestine. Five toxins have been proposed as potential causes for the diarrheal syndrome: HBL, NHE, BceT, EntFM and CytK, but only three (HBL, NHE and CytK) have been related to food borne outbreaks (Agata et al. 1995a; Lund et al. 2000; Lund and Granum 1997; Stenfors Arnesen et al. 2008; Schoeni and Lee Wong 2005).

Both *B. cereus* strain ATCC 14579 and *B. subtilis* strain 11685-3 (*B*. *cereus*) produce the enterotoxins hemolysin BL [HBL], nonhemolytic enterotoxin [Nhe], hemolysins (hemolysin II [HlyII] and III [HlyIII]) and phospholipase C (PLC) of which three variants are recognized: phosphotidylinositol hydrolase (PIH), phosphotidylcholine hydrolase (PC-PLC) and sphingomyelinase (SMase) (see Appendix D) (Ivanova et al. 2003). Unpublished PCR-analyses by Health Canada Scientists confirmed the presence of enterotoxins in the chromosome of *B. cereus* strain ATCC 14579 and *B. subtilis* strain 11685-3 (*B. cereus*) (Table 1-5). The emetic toxin-encoding gene is located on a plasmid, pCER270, which is not carried by *B. cereus* strain ATCC 14579 or *B. subtilis* strain 11685-3 (*B*. *cereus*), making these strains unlikely to produce cereulide (Haggblom *et al.* 2002). Phospholipase C and hemolysins produced by *B. cereus* are necrotic toxins that mimic the effects of some staphylococcal or clostridial toxins, resulting in invasive, non-gastrointestinal infections (Turnbull and Kramer 1983).

Table 1-5: Toxin genes identified by PCR in *B. cereus* strain ATCC 14579 and *B. subtilis* strain 11685-3 (*B*. *cereus*)

Toxin gene	***B. cereus* strain ATCC 14579**	***B. subtilis* strain 11685-3 (*B*. *cereus*)**
Hemolytic enterotoxin BL (*hblA*, *hblC*, *hblD*)	+	+
Non-hemolytic enterotoxin *Nhe*	+	+
Enterotoxin FM1	+	+
Cytotoxin K	+	+
Phosphatidylinositol specific phospholipase C	+	+
Cereolysin A, B	+	+
Cereolysin O	+	+
Hemolysin II	+	-
Hemolysin III	+	+

Toxin gene	*B. cereus* strain ATCC 14579	*B. subtilis* strain 11685-3 (*B. cereus*)
Vegetative insecticidal protein	-	-
Crystal protein	-	-

Adhesion and biolfim formation

Adherence of enteropathogens to the intestinal epithelium is an essential first step required for colonization. Attachment of the bacterium is linked to the presence of fimbriae, which recognize a specific site on the enterocytes. A crystalline cell surface protein (S-layer) has also been implicated in attachment, but was not detected on the cell surface of *B. cereus* strain ATCC 14579 (Kotiranta et al. 1998). The enterotoxin components are either expressed on the bacterial cell membrane or secreted into the intestinal lumen. There, the toxins cause diarrhea by perturbing the exchange of water and electrolytes (Belaiche 2000). It has been suggested that *B. cereus* HBL, Nhe and CytK enterotoxins form pores in the membrane of intestinal epithelial cells, which causes osmotic lysis (Beecher and Wong 1997; Hardy et al. 2001; Haug et al. 2010).

In a recent study, pili on the surface were suggested to protect *B. cereus* from early intraocular clearance (Callegan et al 2017). This could potentially contribute to its virulence during the early onset of endophthalmitis. However, both the piliated wild-type (strain ATCC 14579) as well as the nonpiliated mutant resulted in significant vision loss when infections were left untreated.

The exosporium layers are an external, loosely fitting, hydrophobic, glycosylated and balloon-like (Abhyankar et al. 2013). In addition to increasing the resistance of the spores, these layers provide the spores with the ability to adhere to surfaces (Abhyankar et al. 2013). One hundred spore coat and exosporium proteins were identified in strain ATCC 14579, 11 of which are hypothesized as being likely to be specifically involved in the attachment of spores to surfaces (Abhyankar et al. 2013). The exosporium looks like a hair-like nap in which BclA is the major glycoprotein on top of a paracrystalline basal layer (reviewed in Lequette et al. 2011b). BlcA has been demonstrated to be a key factor in the adhesion of *B. cereus* strain ATCC 14579 kspores to stainless steel surfaces (lequette et al. 2011a). The BclA sequences are well conserved within the *B. cereus* group (Lequette et al. 2011b).

Biofilm formation is in general associated with pathogenicity and increased resistance to antimicrobial agents. The species *B. cereus* is reported to have the ability to form biofilms; however, no biofilm formation was observed for *B. cereus* strain ATCC 14579 after incubation of an exponential phase culture at an OD (600 nm) of 0.01 into LB medium in a 96-well polyvinylchloride microliter plate during 72 h at 30˚C, whereas biofilm formation was observed under the same conditions in *B. cereus* strain ATCC 10987 (Auger et al. 2006). In another study, *B. cereus* strain ATCC 14579 formed biofilms on Y1 medium after 24 h at 20°C and 30°C, but after 48h the biofilms dispersed (Wijman et al. 2007). The conclusion of this study was

that bioflm production was found to be strongly dependent on incubation time, temperature, and medium, as well as the strain used. In another study, free iron availability was observed to enhance strain ATCC 14579 biofilm formation (Hayrapetyan et al. 2015). It is not known if *B. subtilis* strain 11685-3 (*B. cereus*) forms biofilms. The ability of strains of *B. cereus* to form biofilms has a big impact on the food industry as a possible source of contamination (Ribeiro et al. 2017).

Secondary metabolites

Other virulence factors specific to *B. cereus* include proteases, notably metalloproteases (Cadot et al. 2010; Guillemet et al. 2010), and other degradative enzymes play a role in the establishment and development of infection, and in circumventing the host immune system (Appendix E). Some of these have been implicated in both human and non-human target infections (see Appendix F). The transcription factor PlcR is seen as a virulence factor as it is involved in the expression of most known virulence factors in *B. cereus*, including HBL, Nhe, CytK, PLCs and several proteases in *B. cereus* on the DSLand may be in part responsible for the variability of virulence amoung *B. cereus* stains (Gohar et al. 2008). The ability of the *B. cereus* strain ATCC 14579 strain to grow at 37°C, as shown in Appendix A, is another concern from a human health standpoint.

Cytotoxicity testing

Unpublished data generated by Health Canada with *B. cereus* strain ATCC 14579 and *B. subtilis* strain 11685-3 (*B. cereus*) (cells and/or culture filtrates) showed cytotoxic activity towards a human colon cancer cell line and a mouse macrophage cell line at 37°C that is consistent with findings from other laboratories. The toxicity of *B.subtilis* 11685-3 (*B. cereus*) was significant, but markedly lower than that of *B. cereus* strain ATCC14579. Also, *B. cereus* strain ATCC 14579 showed high cytotoxicity on Vero cells when grown at 37°C and 15°C in BHIG (L. P. Stenfors Arnesen, personal communication[6]). Linbäck et al. (1999) demonstrated the cytopathogenic effect of *B. cereus* strain ATCC 14579 (supernatant) on Vero cells and strong hemolytic activity against sheep erythrocytes, both at 37°C. Although cytotoxicity is evident in these studies, the results vary depending on the growth temperatures.

Virulence genes

Due to the high genetic similarity among *B. cereus* group members, clinical isolates sharing the toxins known to be present in *B. cereus* strain ATCC 14579 and *B. subtilis* strain 11685-3 (*B*. *cereus*) are considered good surrogates for characterizing the potential human health hazards of *B. cereus* strain ATCC 14579.

[6] Lotte Pia Stenfors Arnesen, Associate professor and head of the food microbiology laboratory at the Norwegian School of Veterinary Science (NVH), Dep. of Food Safety and Infection, Oslo, Norway

However, it should be recognized that *B. cereus* strain ATCC 14579 and *B. subtilis* strain 11685-3 (*B*. *cereus*) differ from the highly pathogenic strains of the *B. cereus* group in that they do not carry the virulence plasmids that are associated with the *B*. *cereus* emetic syndrome or anthrax *(*Didelot *et al.* 2009*;* Helgason *et al.* 2000b; Kolsto *et al.* 2009; Rasko *et al.* 2005; Vassileva *et al.* 2006). *B. cereus* strain ATCC 14579 and *B. subtilis* strain 11685-3 (*B. cereus*) can also be distinguished from the highly pathogenic strains of the *B. cereus* group based on their genome sequences and phylogenetic position in Clade II of the *B. cereus* group. Clade II comprises the majority of *B. thuringiensis* isolates (Priest et al. 2004), which also include clinical isolates (Barker et al. 2005; Hoffmaster et al. 2008), whereas the highly pathogenic strains (*B. anthracis* and *B. cereus* emetic strains) are grouped in Clade I. Guinebretière et al. (2008) proposed a new classification of the *B. cereus* group based on AFLP. This new classification includes seven groups, each of which is associated with a particular growth temperature range and potential for pathogenicity. Under this scheme, *B. cereus* strain ATCC 14579 belongs to group IV, which includes those *B. cereus* and *B. thuringiensis* strains that grow at 37ºC and are implicated in food poisoning (Guinebretière et al. 2008).

1.1.3 Effects

1.1.3.1 Environment

B. cereus can have a range of effects on non-human species, depending on the host and method of exposure (Appendix F). Some examples include diarrhea (monkeys), mastitis (cattle), inflammation (rabbits) and death (range of organisms).

Vertebrates

Four cases involved mastitis in cattle, which were fatal in some cases (Appendix F). However, it is known that with the appropriate treatment, animals can survive such infections (Schiefer et al. 1976). In vertebrates, effects reported from various sources included necrotic inflammation at the site of subcutaneous injection, fluid accumulation in a rabbit ileal loop model, increased vascular permeability, presence of abscesses and/or nodules following intradermal injection, calcification and necrotic skin ulcers following intramuscular injection in rabbits, diarrhea following ingestion in monkeys, abortion in cattle and sheep injected intravenously with high doses, and mortality in mice. Specific details of these experiments are provided in Appendix F. Based on the available information, it is worth noting that the pathogenic effects noted in Appendix F are not expected to occur to biota in the environment given that the route of administration bypassed natural physical barriers to infection and/or the concentrations of bacteria used were higher than would be expected in the natural environment.

A number of experimental studies challenged a variety of target organisms with *B. cereus* (with *B. cereus* strain ATCC 14579 where indicated by an asterix (*)). These included guinea pigs, rabbits, mice*, cattle, monkeys and cats). Some of the

methods of exposure included free ingestion or gavage, injection (intravenous, intradermal, intravitreal, intraperitoneal and subcutaneous), nasal instillation or dermal exposure to cultures or cell-free supernatants. The objectives of the studies varied and included some of the following: characterization of the role of specific genes in virulence, investigation of the opportunistic properties of *B. cereus* and models for human *B. cereus* pathogenicity.

B. cereus was implicated as the causative agent in an outbreak causing the sudden death of 12 parrots belonging to several species at a zoo (Godoy et al. 2012). *B. cereus* was isolated from blood as well as sterile organs. Extensive areas of lung hemorrhage, hepatic congestion, hemorrhagic enteristis and cardia congestion were observed during necropsy.

No data or specific information on effects in vertebrates of *B. subtilis* strain 11685-3 (*B*. *cereus*) are available.

Invertebrates

With respect to invertebrates, two studies reported that *B. cereus* isolate WGPSB-2 has potential as a biocontrol agent against white grubs (Selvakumar *et al.* 2007; Sushil *et al.* 2008). Another study that invesgated the effects of several bacterial species on white grubs (known potato pest) reported that *B. cereus* strain CRPI14 induced the highest mortality (51.85% in seven days) (Sharma et al. 2013). Mortality increased throughout the duration of the study to 100% thirty days after treatment.

B. cereus was implicated in a case of hepatopancreas necrosis syndrome of farmed *Litopenaeus vannamei* (shrimp) along with several other bacterial species (Huang et al. 2016). *B. cereus* has been previously reported as the causative agent in white patch disease which can cause severe disease outbreak in shrimp aquaculture with a mortality of more than 70% within three to five days of disease outbreak (Velmurugan et al. 2015). Symptoms of white patch disease include changes in colouration in the carapace and muscles, necrosis and loss of appetite.

A number of experimental studies challenged a variety of target organisms with *B. cereus* (with *B. cereus* strain ATCC 14579 where indicated by an asterix (*)) (Appendix F). These included Lepidopteran*, Blattarian*, Galleria and Coleopteran insects and crustaceans). Some of the methods of exposure included free ingestion and injection (intrahemocoelic and intracoelomic). The objectives of the studies varied and included some of the following: characterization of the role of specific genes in virulence, investigation of the opportunistic properties of *B. cereus*, the suitability of specific organisms as an oral infection model for entomobacterial pathogens, investigation of natural pathogens for different pests, pathogenicity testing to characterize cause of larval death, purification and identification of a soil bacterial exotoxin.

The results of the studies referred to above also varied, depending on the target organisms, the strains of *B. cereus* used and the method of exposure. In many of the studies on lepidopteran invertebrates, *B. thuringiensis* insecticidal crystal toxin (Cry1C) was co-administered with spores of *B. cereus* strain ATCC 14579. *B. cereus* spores contributed synergistically to mortality in these studies, and mortality in the absence of Cry1C was low. Nevertheless, a specific strain of *B. cereus* was identified as a lepidopteran pathogen by Koch's postulates in *Trichoplusia ni* and *B. cereus* strain ATCC 14579 sphingomyelinase was demonstrated to be toxic to silkworms and cockroaches. Elm bark beetle larvae suspended in *B. cereus* cultures showed 63.6% mortality. *B. cereus* was also pathogenic toward orally inoculated Southern pine beetle larvae and showed varying degrees of toxicity and mortality when freely ingested by Boll weevil and Black Bean aphids (but not by Egyptian cotton leafworm). Water fleas exposed to *B. cereus* cultures died within 8 to 16 days. Pathogenicity and toxicity testing on terrestrial organisms were also performed at Environment and Climcate Change Canada laboratories[7]. Results of chronic testing with *B. cereus* strain ATCC 14579 using the invertebrate species *Folsomia candida* (springtail; a soil invertebrate) demonstrated no effect on adult mortality, but a depression in juvenile reproduction at 10^8 cfu/g soil (Environment Canada 2010). No data on effects in springtails of *B. subtilis* strain 11685-3 (*B*. *cereus*) are available.

Plants

Pathogenicity and toxicity tests of *B. cereus* strain ATCC 14579 on plants were performed at Environment Canada laboratories. Plant testing using *Festuca rubra* (red fescue) demonstrated a significant decrease in shoot length (21% reduction relative to control response), root length (28% reduction) and root dry mass (42% reduction), but no effect on shoot dry mass in comparison to control growth in conducted tests (Environment Canada 2010). Despite the observed adverse effect on red fescue at the concentration tested, this strain is not suspected to be a frank plant pathogen nor is it expected to be used at this concentration in any industrial or consumer application to plants. No data on effects in red fescue of *B. subtilis* strain 11685-3 (*B*. *cereus*) are available.

1.1.3.2 Human health

Gastrointestinal illnesses are the most common infections associated with *B. cereus.* In healthy individuals the symptoms are generally mild, but complications can lead to more serious disease, or even death (Ginsburg 2003; Girish et al. 2003; Lund et al. 2000; Shiota 2010; Dierick et al. 2005). *B. cereus* gastrointestinal outbreaks have been reported around the world (Appendix G). It is implicated in 1 to 33% of cases of food poisoning (Anonymous, 2005) with varying degrees of severity. The true number of cases is likely underestimated since most foodborne diseases are

[7] Tests were conducted at the Biological Assessment and Standardization Section, Soil Biotechnology Lab according to "*Guidance Document for Testing the Pathogenicity and Toxicity of New Microbial Substances to Aquatic and Terrestrial Organisms* (EPS 1/RM/44, March 2004).

underreported. In Canada, *B. cereus*-related diseases are not notifiable and outbreaks are investigated at the local Health Unit level (J. Greig, personal communication). There have been foodborne outbreaks reported in Canada (Todd et al. 1974; McIntyre et al. 2008; Gaulin et al. 2009), but no reported laboratory-acquired infections to date (J. Greig, personal communication).

B. cereus also causes non-gastrointestinal illness (reviewed in Bottone 2010; Drowbnieski 1993). Endophthalmitis is a severe infection caused by the introduction of bacteria into the eye following trauma or surgery. Case reports of *B. cereus* endophthalmitis or panophthalmitis have been reported in the literature. Among the organisms that infect the eye, *B. cereus* has one of the most rapidly evolving courses of infection (Brinton et al. 1984) and is one of the most destructive (Levin and D'Amico 1991; Parke 1986; Pflugfelder and Flynn 1992). An experiment conducted on rabbits by Callegan et al. 2003 showed reproducible endophthalmitis caused by *B. cereus* strain ATCC 14579. Despite aggressive drug and/or surgical intervention, *B. cereus* endophthalmitis typically results in migration of bacteria throughout the eye and a remarkably rapid progression to a severe intraocular inflammatory response, resulting in loss of functional vision within 24 to 48 hours (Davey and Tauber 1987; Vahey and Flynn 1991).

B. cereus can produce a variety of skin and soft tissue infections, including cellulitis (Dancer et al. 2002; Latsios et al. 2003) and necrotizing cellulitis (Darbar et al. 2005;Hutchens et al. 2010; Sada et al. 2009). Wound infections, mostly in otherwise-healthy persons, have been reported following trauma, surgery and burns (Crane and Crane 2007; Dubouix et al. 2005; Moulder et al. 2008; Pillai et al. 2006; Ribeiro *et al.* 2010; Shimoni et al. 2008; Stansfield and Caudle 1997). Catheter use was often associated with *B. cereus* infection (Crane and Crane 2007;Flavelle *et al.* 2007; Koch and Arvand 2005; Monteverde *et al.* 2006; Ruiz *et al.* 2006; Srivaths *et al.* 2004).

B. cereus endocarditis is a rare condition that is associated with intravenous devices or recreational drug injections (Abusin et al. 2008). Morbidity and mortality associated with *B. cereus* endocarditis are high among patients with valvular heart disease (Cone et al. 2005; Steen et al. 1992).

Some cases of *B. cereus* meningoencephalitis (Evreux et al. 2007; Lebessi et al. 2009; Lequin et al. 2005; Manickam et al. 2008; Puvabanditsin et al. 2007) and bacteremia (Girisch et al. 2003; Hilliard et al. 2003; John et al. 2007; Tuladhar et al. 2000; Van Der Zwet et al. 2000) have been reported in neonates. Neonatal meningoencephalitis caused by *B. cereus* is rare, but it carries a high mortality. Cases of infection are often associated with medical devices. Bacteremia caused by *B. cereus* has been reported in intravenous drug users (Benusic et al. 2015).

Some cases of *B. cereus* pneumonia have been reported. Pulmonary infections due to *B. cereus* are rare compared to those attributed to *B. anthracis,* but can be just as life threatening in immunocompromised persons. The majority of cases were in

metalworkers and immunocompromised patients who have greater susceptibility to infection. Avashia et al. (2007) reported the cases of two healthy metalworkers who died from *B. cereus* pneumonia. Another fatal case of a metal worker occurred in an area where anthrax occurs naturally in herbivores (Hoffmaster et al. 2006). In each of these cases, plasmid pX01 (but not pX02) was found in all *B. cereus* samples and the route of exposure was suspected to be inhalation. Cases of *B. cereus* pneumonia in cancer patients were reported by Frankard et al. (2004), Fredrick et al. (2006), Katsuya et al. (2009), and Sotto et al. (1995). In most cases, the route of infection was unknown but linked to existing *B. cereus* infections in the patients. In all but one case, the infection resulted in death. One survey conducted in the USA reported that a variety of *B. cereus* subgroup species are commonly present in urban aerosols across all seasons in 11 major American cities, but the reported incidence of respiratory infection due to *B. cereus* is extremely low in the USA (Merrill et al. 2006).

Non-gastrointestinal *B. cereus* outbreaks (Appendix G) are less frequent, and most are identified as nosocomial in origin. Season and temperature (e.g. summer months) have implicated in the acquisition on *B. cereus*-bloodstream infections in patients with indwelling devices in hospital settings (Kato et al. 2014). In addition, laundered linen and construction work has been implicated as sources of nosocomial *B. cereus* colonisation and infections (Dohmae et al. 2008; Balm et al. 2012; Hosein et al. 2013).

One study in BALB/c mice showed that inhalation of either spores or vegetative cells of *B. cereus* strain ATCC 14579 had adverse effects. Salimatou et al. (2000) reported that ninety percent of mice died after 24h after nasal instillation of 10^7 spores, while all died after administration of 6×10^6 vegetative cells. The cause of death was not determined but did not seem to depend on the growth of bacteria in the mice. Flaws in the study make its results questionable. The experiment was done only once, and the instillation of a large dose volume could have been the cause death by asphyxiation and pulmonary hemorrhage.

Tayabali *et al.* (2010) reported no toxicological effects in BALB/c mice exposed to 10^7 spores of *B. cereus* strain ATCC 14579 one week after endotracheal instillation. However, severe shock-like signs (lethargy, hunched appearance, ruffled fur, and respiratory distress) occurred 4 hours after exposure to 10^5 or 10^6 vegetative cells. An increase of inflammatory cytokine levels was observed in the blood and lungs, but not in all mice, resulting in a high standard deviation. Post-testing revealed an intermediate cytokine response after exposure to 10^4 and no response to lower vegetative cell exposure (10^2 and 10^3) (A. Tayabali[8], personal communication).

In unpublished studies by Health Canada scientists, BALB/c mice were endotracheally exposed to 10^6 spores or vegetative cells of *B. subtilis* strain 11685-3 (*B. cereus*). Clearance of vegetative cells and spores was rapid and almost

[8] Azam Tayabali, Environmental Health Science and Research Bureau, Health Canada

complete within one week. Animals did not demonstrate shock-like symptoms or elevated pulmonary or plasma cytokines.They did not show elevated serum amyloid A, which is indicative of a systemic acute phase response. These results demonstrate that the virulence of this strain in a mouse model was not as potent as that observed with *B. cereus* strain ATCC 14579.

In comparison to the Salimatou study, the Tayabali and Health Canada studies were better controlled and better standardized the production of spores and vegetative cells. Pre-study work on methodology limited the effect of the instillation procedure in the final results.

The range of reported non-gastrointestinal infections is wider in immunocompromised individuals. Necrotizing meningitis, subarachnoid hemorrhage and brain abscesses have been reported with systemic *B. cereus* infections in patients with leukemia (Gaur et al. 2001; Nishikawa et al. 2009). Other local and systemic *B. cereus* infections have also been reported in patients with compromised immunity (Akiyama et al. 1997; El Saleeby et al. 2004; Hernaiz et al. 2003; Kiyomizu et al. 2008; Kobayashi et al. 2005; Le Scanff et al. 2006; Musa et al. 1999; Nishikawa et al. 2009).

Clinical reports demonstrate that the severity of *B. cereus* infection significantly correlates with its ability to synthesize toxins (Beecher et al. 2000; Ghelardi et al. 2002) and depends on the immune competence of the host and the virulence of the microbe. As mentioned in section 1.1.3, genes encoding for hemolysin BL, nonhemolytic enterotoxin (Nhe), hemolysins (hemolysin II and III), and phospholipase C (phosphotidylinositol hydrolase, phosphotidylcholine hydrolase and sphingomyelinase) are present in *B. cereus* strain ATCC 14579 and *B. subtilis* strain 11685-3 (*B*. *cereus*), and both have been shown to express diarrheagenic enterotoxin in testing at Health Canada using a reversed passive latex agglutination (RPLA) test kit (Denka-Seiken, Campbell CA, U.S.A) and a Duopath Cereus test kit for NHE and HBL (Millipore, Etobicoke ON, Canada). Hemolysin II and metalloproteases InHA1 and NprA can also serve as indicators of pathogenicity (Cadot et al. 2010), however it is impossible to predict which *B. cereus* strains are able to cause gastrointestinal disease based solely on the presence of these virulence factors (Anonymous 2005) since not all strains containing these factors cause adverse effects.

1.2 Hazard Severity

1.2.1 Environment

The environmental hazard potential for *B. cereus* strain ATCC 14579 and *B. subtilis* strain 11685-3 (*B*. *cereus*) is assessed to be medium. Considerations that may result in a finding of medium hazard include that the *B. cereus* species is known as an opportunistic pathogen, has some adverse but reversible effects, in the intermediate term, and effective treatments or mitigation measures are available. *B. cereus* can

infect some animals and cause a range of effects that can debilitate the host and even kill it, but under normal circumstances it is unlikely to be a serious hazard to healthy livestock or other organisms in the environment. *B. cereus* can cause mastitis in cows, but affected animals recover rapidly upon treatment with veterinary antibiotics. There are no cases where *B. cereus* has been shown to cause adverse effects to organisms in the Canadian environment in the scientific literature. Unpublished Environment Canada data show that *B. cereus* strain ATCC 14579 causes a reduced rate of reproduction in springtails, and decreased shoot and root length in red fescue.

1.2.2 Human health

The human hazard potential for *B. cereus* strain ATCC 14579 and *B. subtilis* strain 11685-3 (*B*. *cereus*) is assessed to be medium. Both DSL strains carry genes encoding hemolysin BL, nonhemolytic enterotoxin (Nhe), hemolysins (hemolysin II and III), and phospholipase C (phosphotidylinositol hydrolase, phosphotidylcholine hydrolase and sphingomyelinase), which are recognized as important factors for pathogenicity in susceptible and in healthy individuals. *B. cereus* is primarily a gastrointestinal pathogen and gastrointestinal infections in healthy humans are mild, self-resolving and usually treatable, even so, a few fatalitities have been reported in children. Non-gastrointestinal *B. cereus* diseases are less frequent, and are generally associated with invasive medical procedures. The range of reported non-gastrointestinal infections, e.g., pulmonary infections, endocarditis, meningoencephalitis, is wider in susceptible individuals (immunocompromised, neonate, cancer patient, etc) and these infections have a higher mortality rate. Wound infections have also been documented for *B. cereus* in otherwise-healthy individuals; however, these are rare and there is no indication that *B. cereus* strain ATCC 14579 or *B. subtilis* strain 11685-3 (*B*. *cereus*) could penetrate intact skin to cause dermal infection. Since skin is a natural barrier to microbial invasion of the human body, infections are likely to occur only if the skin has been damaged through abrasions or burns (Dubouix et al. 2005). Similarly, although *B. cereus* is highly virulent in the eye, infection is likely only in cases of prior injury to the eye. Antibiotics effective against *B. cereus* infections are available; however, the treatment of *B. cereus* strain ATCC 14579 or *B. subtilis* strain 11685-3 (*B*. *cereus*) infections could be hampered by existing resistance to several antimicrobial drugs.

Hazards related to micro-organisms used in the workplace should be classified under the Workplace Hazardous Materials Information System (WHMIS)[9].

[9] A determination of whether one or more criteria of section 64 of CEPA are met is based on an assessment of potential risks to the environment and/or to human health associated with exposure in the general environment. For humans, this includes, but is not limited to, exposure from air, water and the use of products containing the substances. A conclusion under CEPA may not be relevant to, nor does it preclude, an assessment against the criteria specified inthe *Hazardous Products Regulations*, which is part of the regulatory framework for the Workplace Hazardous Materials Information System (WHMIS) for products intended for workplace use.

2. Exposure Assessment

2.1 Sources of Exposure

This assessment considers exposure to *B. cereus* strain ATCC 14579 and *B. subtilis* strain 11685-3 (*B*. *cereus*) resulting from their deliberate addition to consumer or commercial products and their use in industrial processes in Canada.

B. cereus strain ATCC 14579 and *B. subtilis* strain 11685-3 (*B*. *cereus*) were nominated to the DSL based on past use in consumer and commercial products. *B. cereus* as a species has properties that make it of commercial interest in a variety of industries.

Responses to a voluntary questionnaire sent in 2007 to a subset of key biotechnology companies, combined with information obtained from other federal regulatory and non- regulatory programs, indicated that 10,000 to 100,000 kg of products potentially containing *B. cereus* strain ATCC 14579 (formulation and concentration unknown) were imported into or manufactured in Canada in 2006-2007 for use in consumer and commercial products. However, survey responses indicated that *B. subtilis* strain 11685-3 (*B*. *cereus*) was not used.

The Government conducted a mandatory information-gathering survey under section 71 of CEPA, as published in the *Canada Gazette*, Part I, on September 23, 2017 (section 71 notice). The section 71 notice applied to any persons who, during the 2016 calendar year, manufactured or imported *B. cereus* strain ATCC 14579 or *B. subtilis* strain 11685-3 (*B*. *cereus*), whether alone, in a mixture, or in a product. No commercial or consumer activities using *B. cereus* strain ATCC 14579 or *B. subtilis* strain 11685-3 (*B*. *cereus*) were reported in response to the section 71 notice. *B. cereus* strain ATCC 14579 was reported to be used in very small quantities for research and development (R&D) and teaching activities.

The 2007 and 2017 surveys differed significantly in target and scope. In this assessment, results from the 2009 survey were used to estimate exposure from current uses because it requested information on uses of the micro-organism strain that is listed on the DSL, whereas the 2007 survey asked about uses of the products that had been associated with the micro-organism at the time it was nominated to the DSL. Because product formulations may have changed, information from the 2009 survey may more accurately represent current uses. Uses reported in the 2007 voluntary survey were also considered in the assessment of potential uses.

Although no consumer, commercial or industrial uses were reported for *B. cereus* strain ATCC 14579 or *B. subtilis* strain 11685-3 (*B*. *cereus*) during the mandatory survey, strain ATCC 14579 is available for purchase from the ATCC. As it is on the DSL, and so can be used in Canada without prior notification, it could be an attractive choice for commercialization. A search of the public domain (internet, literature and patent databases) revealed the following consumer, commercial and

industrial applications of other strains of *B. cereus.* These represent possible uses of the DSL strains, as strains ATCC 14579 and 11685-3 are likely to share the characteristics (modes of action) with other commercialized *B. cereus* strains:

- food processing;
- pharmaceuticals;
- pulp and paper and textile processing;
- biochemical and enzyme production;
- bioremediation and biodegradation;
- bioleaching and biomining;
- municipal and industrial wastewater treatment; and
- agricultural applications including as livestock probiotics and as microbial pest control agents.

2.2 Exposure Characterization

The exposure characterization is based on activities reported in the section 71 notice (R&D and teaching). *B. cereus* is a Risk Group 2 human and animal pathogen and it is regulated by the Public Health Agency of Canada and by the Canadian Food Inspection Angency. They are regulated under the *Human Pathogens and Toxins Act*. A license under the *Human Pathogens and Toxins Regulations* is required for controlled activities with Risk Group 2 human pathogens. Measures to reduce human and environmental exposure to Risk Group 2 pathogens are set out in Canadian Biosafety Standard Second Edition, 2015 (CBS 2015). These include specific laboratory design, operational practices and physical requirements. For example, all material must be contained and is decontaminated prior to disposal or reuse in such a way as to prevent the release of an infectious agent, and equipment for emergency and decontamination response must be readily available and maintained for immediate and effective use.

2.2.1 Environment

Based on the absence of consumer or commercial activity in Canada according to the section 71 notice, the overall environmental exposure estimation for *B. cereus* strain ATCC 14579 and *B. subtilis* strain 11685-3 (*B*. *cereus*) is low. Nevertheless, given the range and scale of known and potential applications of the species *B*. *cereus* listed in Section 2.1, there is potential for an increase in environmental exposure to products containing *B. cereus* strain ATCC 14579 or *B. subtilis* strain 11685-3 (*B*. *cereus*), and exposure scenarios arising from these products have been considered.

Should potential uses identified in Section 2.1 be realized in Canada they are likely to introduce *B. cereus* strain ATCC 14579 and *B. subtilis* strain 11685-3 (*B*. *cereus*) to both aquatic and terrestrial ecosystems. For example, use of *B. cereus* strain ATCC 14579 or *B. subtilis* strain 11685-3 (*B*. *cereus*) in wastewater treatment or its discharge in wastes from industrial applications, such as pulp and paper processing,

textile manufacturing and biochemical production, could introduce *B. cereus* strain ATCC 14579 into aquatic ecosystems. Similarly, its use in bioremediation and biodegradation as well as in livestock probiotics and pest control agents could introduce *B. cereus* strain ATCC 14579 into terrestrial ecosystems.

The magnitude of non-human species exposure to this micro-organism will depend on the persistence and survival of *B. cereus* strain ATCC 14579 and *B. subtilis* strain 11685-3 (*B*. *cereus*) in the environment as described in Section 1.1.2.2.

In the event that consumer, commercial or industrial activities resume the environmental exposure to *B. cereus* strain ATCC 14579 could change based on the exposure scenarios described above.

2.2.2 Human

Based on the absence of consumer or commercial activity in Canada according to the section 71 notice, the overall human exposure estimation for *B. cereus* strain ATCC 14579 or *B. subtilis* strain 11685-3 (*B*. *cereus*) is low. Nevertheless, given the range and scale of known and potential applications of the species *B*. *cereus* listed in Section 2.1, there is potential for an increase in human exposure to products containing *B. cereus* strain ATCC 14579 or *B. subtilis* strain 11685-3 (*B*. *cereus*), and exposure scenarios arising from these products have been considered.

Should potential uses identified in Section 2.1 be realized in Canada human exposure would be expected during the direct use and application of consumer or commercial products containing *B. cereus* strain ATCC14579 or *B. subtilis* strain 11685-3 (*B*. *cereus*). Skin and eye contact, inadvertent ingestion and inhalation of aerosolized droplets or particles are likely routes of direct user and bystander exposure. The use of such products in food preparation areas could result in the contamination of surfaces and foods at the time of product application. Subsequent lapses in proper food handling practices could allow *B. cereus* strain ATCC 14579 or *B. subtilis* strain 11685-3 (*B*. *cereus*) to proliferate in foods, possibly resulting in the ingestion of large numbers of cells.

Human exposure may also be temporally distant from the time of application. Subsequent to application, *B. cereus* strain ATCC 14579 and *B. subtilis* strain 11685-3 (*B*. *cereus*) is expected to remain viable and establish communities where organic matter accumulates (for example: countertops, drains, sinks, grease traps and kitchen garbage disposals). From such reservoirs, aerosols containing *B. cereus* strain ATCC 14579 or *B. subtilis* strain 11685-3 (*B*. *cereus*) could inoculate surfaces and foods. As above, subsequent lapses in proper food handling practices could allow the organism to proliferate in foods and result in the ingestion of large numbers of cells and lead to adverse effects.

Certain uses may introduce *B. cereus* strain ATCC 14579 or *B. subtilis* strain 11685-3 (*B*. *cereus*) into bodies of water, as described in section 2.2.1. Nevertheless,

human exposure to the strain through the environment is expected to be low. Moreover, drinking water treatment processes are expected to effectively eliminate these micro-organisms and so limit their ingestion through drinking water.

In the event that consumer, commercial or industrial activities resume, the human exposure to *B. cereus* strain ATCC 14579 or *B. subtilis* strain 11685-3 (*B*. *cereus*) could change based on the exposure scenarios described above.

3. Risk Characterization

In this assessment, risk is characterized according to a paradigm whereby a hazard and exposure to that hazard are both required for there to be a risk. The risk assessment conclusion is based on the hazard, and on what is known about exposure from current uses.

Hazard has been estimated for *B. cereus* strain ATCC 14579 and *B. subtilis* strain 11685-3 (*B. cereus*) to be medium for the environment and medium for human health. Environmental and human exposure to *B. cereus* strain ATCC 14579 and *B. subtilis* strain 11685-3 (*B. cereus*) from their deliberate use in industrial processes or consumer or commercial products in Canada is not currently expected (low exposure), so the risk associated with current uses is estimated to be low for both the environment and human health.

The determination of risk from current uses is followed by consideration of the estimated hazard in relation to foreseeable future exposures (from new uses).

The potential use of *B. cereus* strain ATCC 14579 or *B. subtilis* strain 11685-3 (*B. cereus*) in consumer or commercial products could result in an increased level of human and environmental exposure, as described in Section 2.2, and this would increase the estimation of risk.

Risks to the environment from foreseeable future uses:

Non-human species may be exposed to *B. cereus* strain ATCC 14579 primarily through water and soil mainly through its release from industrial or manufacturing activities. Uses involving introduction into terrestrial environments could become problematic, as it has been shown that high (10^7-10^8 CFU per gram of dry soil) concentrations of *B. cereus* strain ATCC 14579 can cause adverse effects in springtails and red fescue (Environment Canada 2010) and there is a lack of information on the potential adverse effects of *B. cereus* on aquatic species.

Risks to human health from foreseeable future uses:

The risk to human health will depend on the route of exposure. Of all routes identified, exposure through ingestion is of primary concern since *B. cereus* is primarily a gastrointestinal pathogen. *B. cereus* strain ATCC 14579 and *B. subtilis*

strain 11685-3 (*B. cereus*) are known to produce important pathogenic factors (e.g., extracellular enzymes and toxins) implicated in gastrointestinal disease. The infectious dose of *B. cereus* is reported to range from 10^2 to 10^8 CFU per gram of food or water and it is generally believed that any food containing concentrations of *B. cereus* exceeding 10^3 to 10^5 cells or spores per gram is not safe for consumption (Anonymous 2005; Haggblom *et al.* 2002; Stenfors Arnesen *et al.* 2008). The use of products containing *B. cereus* strain ATCC 14579 or *B. subtilis* strain 11685-3 (*B. cereus*) in food preparation areas could result in the inoculation of foods and subsequent lapses in proper food handling practices could allow bacteria to proliferate. Cycles of reheating and inadequate refrigeration are particularly problematic for spore-forming bacteria like *B. cereus*, because spores are not inactivated during heating, and vegetative cells can germinate, multiply and re-sporulate between heating cycles. In this way, the number of viable cells in food increases in exponential fashion, eventually reaching a level that can lead to human gastrointestinal infection.

Skin and eye contact have been identified as potential routes of exposure, but these are less likely to result in adverse health effects. Wound infections have been documented for *B. cereus* in otherwise-healthy individuals; however, these are rare and there is no indication that *B. cereus* strain ATCC 14579 or *B. subtilis* strain 11685-3 (*B. cereus*) could penetrate intact skin to cause dermal infection. Since skin is a natural barrier to microbial invasion of the human body, infections are likely to occur only if the skin was damaged through abrasions or burns (Dubouix et al. 2005). Similarly, although *B. cereus* is highly virulent in the eye, infection is likely only in cases of prior injury to the eye.

Inhalation of *B. cereus* strain ATCC 14579 or *B. subtilis* strain 11685-3 (*B. cereus*) cells or spores aerosolized through mechanical or air disturbances, either during or subsequent to product application, could lead to pulmonary exposure to spores or vegetative cells, but the number of inhaled spores or cells is unlikely to reach an infectious dose in healthy individuals.

4. Conclusion

Based the information presented in this screening assessment, it is concluded that *B. cereus* strain ATCC 14579 and *B. subtilis* strain 11685-3 (*B. cereus*) are not entering the environment in a quantity or concentration or under conditions that:

- have or may have an immediate or long-term harmful effect in the environment or its biological diversity;
- constitute or may constitute a danger to the environment on which life depends; or
- constitute or may constitute a danger in Canada to human life or health.

Therefore, it is concluded that *B. cereus* strain ATCC 14579 and *B. subtilis* strain 11685-3 (*B. cereus*) do not meet any of the criteria as set out in section 64 of CEPA.

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Appendices

Appendix A: Characterisation of *B. cereus* strain ATCC 14579

Table A-1: Growth of *B. cereus* strain ATCC 14579 in liquid media at various temperatures

Medium	28°C	32°C	37°C	42°C
Trypticase Soy Broth (TSB)	+	+	+	+
Sheep Serum	-	-	(+)	~
Fetal Bovine Serum	+	+	+	-
Dulbecco's Modified Eagles Medium	(+)	~	-	-

+ indicates growth; – indicates no growth; (+) indicates low and delayed growth (after 15h); ~ indicates low level growth

Data generated by Health Canada's Environmental Health Science and Research Bureau. Growth of *B. cereus* strain ATCC 14579 in broth culture was measured by increase in absorbance at 500 nm, in four different growth media and over a range of temperatures. Concentration of bacteria at time zero was 1×10^6 CFU/mL. Measurements were taken every 15 minutes over a 24-hour period with a multi-well spectrophotometer.

Table A-2: Growth characteristics of *B. cereus* strain ATCC 14579 on solid media at various temperatures

Medium	28°C	37°C
Nutrient agar	+	+
Citrate[a]	-	-
Lysine Iron[b]	+	+
Growth on MacConkey Agar[c]	-	-
Mannitol Salt Agar[d]	-	-
MYP supplements[e]	+	+
Growth on Starch agar[f]	N/A	+
Starch Hydrolysis[f]	N/A	+
Triple Sugar Iron - with phenol red[g]	+	-
Hydrolysis of Urea[h]	+	+
Catalase activity on TSB[i]	-	+
Catalase activity on Sheep Blood agar[i]	+	+
Hemolysis[j]	+	+

+ indicates positive for growth or test; - indicates negative for growth or test; N/A indicate data not available

Data generated by Health Canada's Environmental Health Science and Research Bureau

[a] Citrate utilization test, ability to use citrate as the sole carbon source

[b] Simultaneous detection of lysine decarboxylase and formation of hydrogen sulfide

[c] Detection of coliform organisms; tests for ability of organism to ferment lactose

[d] Isolation and differentiation of *Staphylococci*

[e] *B. cereus* selective agar

[f] Differential medium that tests the ability of an organism to produce extracellular enzymes that hydrolyze starch

[g] Gram-negative enteric bacilli based on glucose, lactose, and sucrose fermentation and hydrogen sulfide production

[h] Screening of enteric pathogens from stool specimens - Urea metabolism

[i] Catalase enzyme assay measures by enzymatic detoxification of hydrogen peroxide

[j] Hemolysis of sheep blood. Bacteria (5000 CFU, 20 µl) were spotted onto the blood-agar and incubated for 24h

Table A-3: Fatty Acid Methyl Ester (FAME) analysis of *B. cereus* strain ATCC 14579

Environmental Database		Clinical Database	
B. cereus group A	39/46 (0.889)	*B. thuringiensis* group B	24/35 (0.751)
B. megaterium subgroup A	1/46 (0.045)	*B. cereus* group A	8/35 (0.751)
No Match	6/46	No match	3/35

Data generated by Health Canada's Environmental Health Science and Research Bureau. Data presented show the best match between the sample and different MIDI databases (clinical and environmental), along with the number of matches (fraction of total number of tests) and the fatty acid profile similarity index (in parentheses; average of all matches). MIDI is a commercial identification system that is based on the gas chromatographic analysis of cellular fatty acid methyl esters.

Appendix B: Relationships within the *B. cereus* group

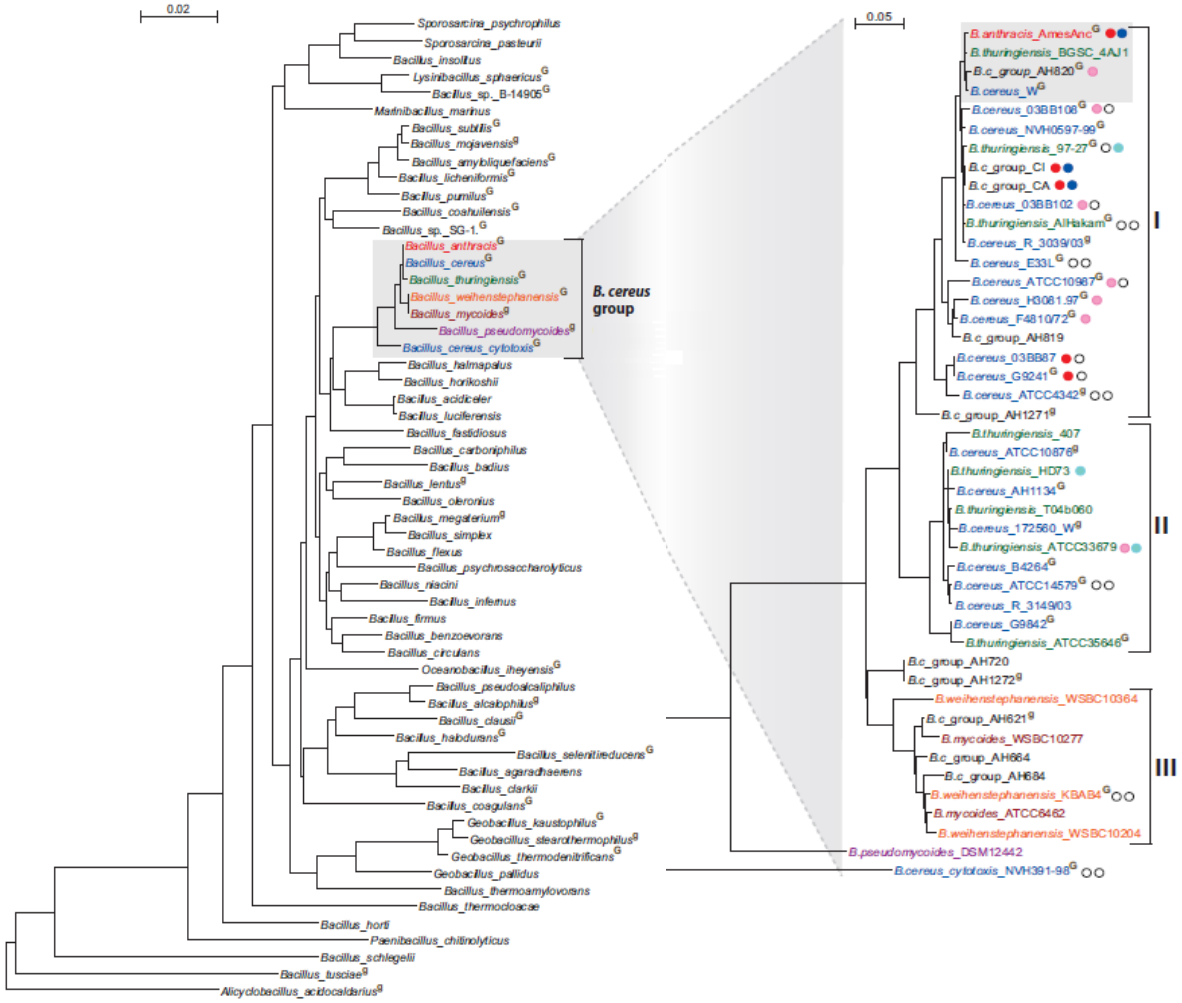

Figure B-1: Phylogenetic tree based on the neighbor-joining method applied to a matrix of pair-wise distances shows 16S ribosomal RNA (rRNA) gene sequences relationship between 57 Bacillus species. Taken from Figure 1 from Kolsto et al. 2009

Appendix C: *B. cereus* group mobile genetic elements

Table C-1: List of some plasmids found in the *B. cereus* group and related traits

Name	*Bc*[a]	*Ba*[b]	*Bt*[c]	Associated traits	References
pAW63[d]	N/A	N/A	subsp. *kurstaki*	- No known homology to *cry* and *cyt,* - Contains mobile elements and putative proteins	(Schnepf et al. 1998; Van der Auwera and Mahillon 2005)
pBc10987[e]	10987	N/A	N/A	- Tn*554*, AbrB (regulator hom.) - Bc1A (spore coat determinate)	(Rasko et al. 2004)
pBC218	G9241	N/A	N/A	Polysaccharide capsule	(Hoffmaster et al. 2004)
pBClin15[f]	14579	N/A	N/A	Prophage feature, similar to Bam35	(Stromsten et al. 2003; Verheust et al. 2005)
pBClin29	G9241	N/A	N/A	Prophage feature	(Hoffmaster et al. 2004)
pBCOX1[g]	G9241	N/A	N/A	Lethal toxin complex *pagA, lef, cya*	(Hoffmaster et al. 2004)
pBT9727[d]	N/A	N/A	97-27[h]	- No known homology to *cry* and *cyt,* - Contains mobile elements and putative proteins	(Rasko et al. 2005)
pBToxis	N/A	N/A	+	insecticidal protein toxin (*cry, cyt*)	(Berry et al. 2002)
pCER270	AH1134 AH187	N/A	N/A	Emetic toxin (cereulide)	(Ehling-Schulz et al. 2006; El Emmawie et al. 2008; Rasko et al. 2007)
pE33L[i] (series)	E33L[j]	N/A	N/A	Possesses a number of transposable genes and mobile elements	(Rasko et al. 2005)
pPER272	AH820 AH818	N/A	N/A	Associated with periodontal isolates	(Rasko et al. 2007)
pXO1	N/A	+	NA	Lethal toxin complex, *pag*, *lef* and *cya* genes	(Okinaka et al. 1999)
pXO2	N/A	+	N/A	- D-glutamic acid caspsule, - Operon *cap* BCADE	(Drysdale et al. 2005)
pXO16	N/A	N/A	subsp. *israelensis*	Aggregation phenotype	(Jensen et al. 1995)
pCI-XO1[g]	CI	N/A	N/A	Lethal toxin complex, *pag*, *lef* and *cya* genes	(Klee et al. 2010)
pCI-XO2[k]	CI	N/A	N/A	- D-glutamic acid caspsule, - Operon *cap* BCADE	(Klee et al. 2010)
CI-14	CI	N/A	N/A	- Unknown function - Cryptic plasmid	(Klee et al. 2010)

N/A indicates data not available; + indicates multiple strains;
[a] *Bacillus cereus* strains known to carry mobile genetic elements
[b] *Bacillus anthracis* strains known to carry mobile genetic elements

[c] *Bacillus thuringiensis* strains known to carry mobile genetic elements
[d] Shares conserved backbone with *B. anthracis* pX02
[e] Shares conserved backbone with *B. anthracis* pX01
[f] Linear plasmid
[g] Shares 99% and greater genetic identity with pX01
[h] *B. thuringiensis* subsp. *konkukian* 97-27 isolated from a case of severe human necrosis
[i] Similar to pXO2 and pBC218
[j] Isolate from a dead zebra suspected of having died of anthrax, (phylogenetically close to *B. anthracis*)
[k] Shares 100% genetic identity with pX02

Table C-2: List of phages found in the *B. cereus* group

Name	Bc[a]	Ba[b]	Bt[c]	References
Bam35	N/A[d]	N/A	+	(Ackermann et al. 1978)
CP-51	+	N/A	N/A	(Ruhfel et al. 1984)
GIL01	N/A	N/A	+	(Verheust et al. 2005)

N/A indicates data not available; + indicates multiple strains
[a] *Bacillus cereus* strains known to carry mobile genetic element
[b] *Bacillus anthracis* strains known to carry mobile genetic element
[c] *Bacillus thuringiensis* strains known to carry mobile genetic element

Table C-3: List of mobile genetic elements found in the genome of the *B. cereus* group, and related traits

Type	Name	*Bc*[a]	*Ba*[b]	*Bt*[c]	Associated traits	References
Transposon	Tn5084	RC607 VKM684	+	+	- Resistance to mercury	(Huang et al. 1999;Narita et al. 2004)
DNA repeated element	*bcr1*	+ (incl. 14579)	+	+	- exhibits characteristics of a mobile element	(Okstad et al. 2004)
Insertion Sequence	*IS*231	+ (incl. 14579)	+	+	- transposase	(De Palmenaer et al. 2004)
Group I intron	*recA*	+ (incl. 10987 E33L)	+	+	- Ribozyme (catalytic RNA)	(Tourasse et al. 2006)
Group I intron	*nrdE*	+ E33L G9241 10987	+	+	- Ribozyme (catalytic RNA)	(Tourasse et al. 2006)
Group II intron	*B.c.*I1	10987 14579	N/A	N/A	N/A	(Tourasse et al. 2006)
Group I IStron	Bc*ISt1*	10987 E33L G9241 (not 14579)	+	+	- Self-splicing group I introns associated with IS element	(Tourasse et al. 2006)

+ indicates multiple strains; N/A indicates data not available
[a] *Bacillus cereus* strains known to carry mobile genetic elements
[b] *Bacillus anthracis* strains known to carry mobile genetic elements
[c] *Bacillus thuringiensis* strains known to carry mobile genetic elements

Appendix D: Toxin genes present in the *B. cereus* strain ATCC 14579 genome (NC 004721)

Table D-1: Chromosomal genes coding for toxins in *B. cereus* strain ATCC 14579

CDSs in *B. cereus*[a]	Function
BC3103, BC3102, BC3102	Hemolytic enterotoxin BL
BC1809, BC1810, BC0560	Non-hemolytic enterotoxin Nhe
BC2081	Enterotoxin T, BceT
BC1953	Enterotoxin FM1
BC1110	Cytotoxin K
BC3761	Phosphatidylinositol-specific phospholipase C
BC0670	Phosphatidylcholine-specific phospholipase C
BC0671	Sphingomyelinase
BC5101	Cereolysin O
BC3523	Hemolysin II
BC2196	Hemolysin III

[a] Adapted from Ivanova et al. 2003

Appendix E: Virulence factors produced by *B. cereus*

Table E-1: List of toxins produced by *B. cereus*

Toxin	Structural Characteristics	Toxic Dose and Effects	References
Cereulide	- Lipophilic peptide, heat-stable, emetic toxin - Cyclic dodecadepsipeptide resembling valinomycin (OLeu-Ala-OVal-Val-)$_3$ - K+-specific ionophore similar to valinomycin	- Amount of cereulide found in food samples implicated in emetic food poisoning cases ranges from 0.01 to 1.28 µg/g of food - Toxic dose in human is 8 µg kg^{-1} body weight (human emesis-causing dose) in Rhesus monkey 10 µg/kg and in *Suncus murinus* is 8 µg/kg - The ED50 in *Suncus murinus* is 12.9 µg kg^{-1} by oral administration - Cytotoxic and mitochondriotoxic to primary cells and cell lines of human and other mammalian origins - In an assay for detection of cereulide production in *B. cereus* strains, boar sperm exposed *in vitro* to 2 µg/L of cereulide showed observable mitochondrial damage - Toxic towards porcine fetal Langerhans islets and beta cells - Inhibits hepatic mitochondrial fatty-acid oxidation which can cause liver failure - Inhibit natural killer cells at concentration 20-30 µg/L	(Agata et al. 1994; Agata et al. 1995b; Agata et al. 2002; Haggblom et al. 2002; Jaaskelainen et al. 2003; Mahler et al. 1997; Mikkola et al. 1999; Paananen et al. 2002; Shinagawa et al. 1995; Virtanen et al. 2008)
Cytotoxin K (CytK)	- Two variants of the protein: CytK-1 and CytK-2 - Sequence comparisons suggest that the protein may belong to the family of β-barrel pore-forming toxins	- CytK-1 and CytK-2 are able to form pores in lipid bilayers but the distribution of channel conductance is lower in CytK-2 - CytK-1 is associated with more severe forms of gastrointestinal disease - Highly cytotoxic, necrotic & haemolytic effects produced by CytK-1 or CytK-2 - This is the cytotoxin that may cause necrotic enteritis - Preliminary tests in guinea pigs using intracutaneous injections suggest that CytK is dermonecrotic - CytK-1 is highly toxic toward human intestinal Caco2 cells and Vero cells compare to CytK-2 - Cyt-K-2 proteins are toxic to Caco-2 and bovine erythrocytes but not to the same extent as the original CytK-1 - No information available on the effective dose of the toxin	(Brillard and Lereclus 2004; Fagerlund et al. 2004; Guinebretiere et al. 2006; Hardy et al. 2001; Lund et al. 2000)
Hemolysin BL (HBL)	Three-component (B, L_1,L_2) pore-forming toxin	- The major virulence factor associated with diarrheal syndrome. HBL responsible for the major enterotoxigenic activity and the main cytopathogenic activity of *B. cereus* strain ATCC 14579	(Agata et al. 1995a; Beecher and Macmillan 1991; Beecher et al. 2000;

Toxin	Structural Characteristics	Toxic Dose and Effects	References
		- Enterotoxin responsible for the diarrheal food poisoning syndrome - Toxic activities when three HBL components are combined: hemolysis, cytotoxicity, vascular permeability, dermonecrosis, enterotoxicity and ocular toxicity - Lysis caused by formation of a membrane attack complex on the cell surface - Enterotoxigenic; damages membranes of a variety of different cell types - Exhibits Vero cell, Chinese hamster ovary (CHO) cell and retinal cell cytoxicity and is lethal to mice upon injection - Causes necrosis of intestinal tissue, fluid accumulation in a ligated mouse ileal loop, and vascular permeability and necrosis in rabbit skin - HBL toxin does not contribute significantly to *B. cereus* haemolytic activity against human erythrocytes; HBL is most active against sheep and calf erythrocytes - Necrotic to rabbit retinal tissue with maximal activity in dose between 50 to 150 µg/L - Induces apoptosis in macrophages	Beecher et al. 2000; Beecher et al. 1995b; Beecher and Wong 1994a; Beecher and Wong 1994b; Beecher and Wong 1994c; Beecher and Wong 1997; Beecher and Wong 2000; Lindback et al. 1999; Tran et al. 2010a)
Non Hemolytic enterotoxin (Nhe)	- Three-component complex (Nhe A, Nhe B and Nhe C). A binding factor (Nhe B), a complex formation factor (Nhe C) and a lysis factor (Nhe A) - Nhe is fundamentally a two-component toxin (NheA and NheB) but a third component (NheC) is necessary for the full cytotoxicity in some cells - Optimal cytotoxic effect with ratio NheA:NheB:NheC of 10:10:1 . Concentration of	- Enterotoxin with no detectable haemolytic effects - Cytotoxic/enterotoxic properties	(Fagerlund et al. 2008; Granum et al. 1999; Haug et al. 2010; Lindback et al. 2004; Linback et al. 2010; Lund and Granum 1996; Wijnands et al. 2001)

Toxin	Structural Characteristics	Toxic Dose and Effects	References
	NheC higher than 10% of that of NheA and NheB inhibited the toxic activity - Mechanism of cytotoxicity is osmotic lysis following pore formation in the plasma membrane		
Enterotoxin T (BceT or bc-D-Ent)	Unknown	- Unknown type of enterotoxic action - Proposed as an *B. cereus* enterotoxin but the proposition was disproved after the cloned *bceT* construct was suggested to be a cloning artifact	(Agata et al. 1995a; Choma and Granum 2002; Guinebretiere et al. 2006; Hansen et al. 2003; Lindbäck and Granum 2006)
Enterotoxin FM (entFM)	Unknown	- Mechanism of action and role unknown - Increases vascular permeability in rabbit, and causes fluid accumulation in mouse ligated intestinal loops - Cytotoxic to Vero cells and lethal to mice - Sequence analysis revealed that EntFM is related to cell wall peptidases (CwpS) and has homology to *B. subtilis* cell wall hydrolase, suggesting that the protein might not be a toxin - However, EntFm might still have a role in *B. cereus* virulence	(Asano et al. 1997; Lindbäck and Granum 2006; Tran et al. 2010b; Shinagawa et al. 1991a; Shinagawa et al. 1991b)

Table E-2: List of membrane-damaging virulence factors produced by *B. cereus*

Factor	Structural Characteristics	Toxic Dose and Effects	References
Hemolysin II (HlyII)	- Member of the β-barrel pore-forming toxin family - HlyII is a structural and functional homolg of staphylococcal α-hemolysin	- Hemolytic protein - Hemolysin II is able to lyse different kinds of eukaryotic cells. Hemolytic activity in rabbit erythrocytes have a HC_{50} value of 1.64 µg/L (HC_{50}: Concentration of hemolysin to reach 50% of erythrocyte lyse) - Exhibit cytolytic activity on erythrocytes of human and rabbit. Bovine and mouse erythrocytes are least sensitive to HlyII	(Andreeva et al. 2006; Andreeva et al. 2007; Miles et al. 2002)

Factor	Structural Characteristics	Toxic Dose and Effects	References
	- Binds to surface of cells and assemble into oligomeric transmembrane pores leading to cell permeation and lysis		
Hemolysin III (HLy-III)	Pore-forming hemolysis with functional diameter of pores about 3-3.5 nm	- Hemolytic protein - Three steps of hemolysis: i) the temperature-dependent binding of the Hly-III monomers to the erythrocyte membrane; ii) the temperature-dependent formation of a transmembrane pore by multiple molecules of the hemolysin; iii) temperature-independent erythrocyte lysis	(Baida and Kuzmin 1995; Baida and Kuzmin 1996)
Cereolysin O (CLO)	- Pore-forming toxin from the cholesterol-binding cytolysin (CBC) family - Cross reacts with streptolysin-O	- Hemolytic protein - Causes disorganization of the cytoplasmic membrane and intracellular organelles - Is thiol activated, heat labile and poorly susceptible to proteolysis - Pathogenic role in extraintestinal infection - CBCs are lethal to animals and highly lytic toward eukaryotic cells, including erythrocytes	(Alouf 2000; Granum 1994)
Phosphatidylinosol hydrolase (PIH)	Phospholipase C that hydrolyzes phosphatidylinositol (PI) and PI-glycan-containing membrane anchors, which are important structural components of one class of membrane proteins	No hemolytic activity	(Granum 1994; Beecher and Wong 2000)
Sphingomyelinase (SMase)	Highly specific phospholypase C that hydrolyzes sphingomyelin (SM) to produce ceramide and phosphocholine	- SMase lysed ruminant erthrocytes (46-53% of SM) - Exhibits hemolytic action against mammalian erythrocytes and hemolyzes sheep erythrocytes with and without cold shock - Data for lysis is 222 HD^{50}/unit for Sheep erythrocytes and 27.8 HD^{50}/unit for human erythrocytes (HD^{50}: Higher enzyme dilution that cause 50% lysis of erythrocytes)	(Beecher and Wong 2000; Fujii et al. 2004; Ikezawa et al. 1980)
Phosphatidylcholine(PC) preferring phospholipase C	- Phospholipase C that hydrolyzes phosphatidylcholine, phosphatidyleth	- Cooperative action with SMase is needed to lyse swine and human erythrocytes (22-31% PC and 28-25% SM) - Inhibits HBL lysis of sheep erythrocytes and enhances the discontinuous hemolysis pattern	(Beecher et al. 2000; Beecher and Wong 2000; Granum 1994)

Factor	Structural Characteristics	Toxic Dose and Effects	References
(PC-PLC)	anolamine and phosphatidylcholine - The enzyme might be capable of binding to a membrane interface with little or no specific substrate present - Little published information regarding the binding of PL-PLC	- Second major contributor to retinal toxicity - PC-PLC is expressed by the great majority of isolates	

Table E-3: List of damaging enzymes produced by *B. cereus*

Enzyme	Structural Characteristics	Toxic Dose and Effects	References
ADP-ribosylating toxin (ADP-ribosyltransferase)	Unknown	- Exoenzyme - Member of C3-like transferase which selectively ribosylates the small GTP-binding protein Rho - Produced by *Bacillus cereus* strain 2339, a clinical isolate	(Just et al. 1992)
Vip (vegetative insecticidal protein)	- Composed of VIP1, a cell-binding component, and VIP2, an ADP-ribosyltransferase that targets actin - Belongs to the family of binary bacterial toxins resembling mammalian clostridial toxins of the C2 and iota-like family	- VIP2 exerts its intracellular poisoning effect by modifying actin and preventing actin polymerization - Insect-killing properties on Northern and Western corn rootworms	(Barth et al. 2004; Jucovic et al. 2008)

Appendix F: Pathogenicity of *B. cereus* to invertebrates and vertebrates

Details of experiments mentioned in Section 1.1.3.2. The following tables provide information specific to invertebrates and vertebrates.

Table F-1: Laboratory pathogenicity testing of *B. cereus* in insects

Organisms	Experimental Conditions	*B. cereus* strains used	Results	Reference
Tobacco hornworm *(Manduca sexta*) 5th instar larvae) Sex not specified **Purpose:** Insect infection model to characterize the role of the iron-responsive regulator *fur* gene in the virulence of *B. cereus*	- Single Injection of vegetative cells (compartment not specified) 20 larvae per dose group	569 WT 569 *Δfur*	- Wild-type LD_{50} value = 1859 cfu (1142-2774) 95% CI - Mutant LD_{50} value = 4932 cfu (3609-6912) 95% CI - Reduced virulence for the *B. cereus* 569 *Δfur* mutant - The *Δfur* mutant constitutively expresses siderophores and accumulates iron intracellularly to a level threefold greater than the WT	(Harvie et al. 2005)
Wax moth *(Galleria mellonella)* Last instar larvae Sex not specified **Purpose:** Investigation of the opportunistic properties of acrystalliferous *B. thuringiensis* (Bt) and *B. cereus* strain and the role of the *plcR* gene, a pleiotropic regulator of extracellular factors	- Force-feeding co-ingestion - Intrahaemocoelic injection - 10 µl of spore suspension per larvae for both methods - For the force-feedings, spores were in association with crystal toxins (Cry1C) - 30 larvae used for each dose and for each method	ATCC 14579	- Mortality observed after 2 days - Very low (>10%) with crystals or spores alone - ≈70% mortality caused by co-ingestion of 10^6 spores with a sublethal (1µg) quantity of Cry1C toxin Clear pattern of synergism between the spores of *B. cereus* and the toxin of *B. thuringiensis*	(Salamitou et al. 2000)
Wax moth *(Galleria mellonella)* 2^{nd} and 5^{th} instar larvae **Purpose:** To evaluate whether *Galleria mellonella* can function as an oral infection model for human and entomo-bacterial pathogens	- Oral infection - Free ingestions**:** on 2^{nd} instar larvae of mixtures of 50% pollen with 50% water containing 10^8 spores/mL alone or along with 2 µg of Cry1C - Force feedings: on 5^{th} instar larvae using a micro injector 5×10^5 - 1×10^6 spores or vegetative cells per larva, with (2 – 3 µg) and without Cry1C	ATCC 14579 diarrheal strains: D6 (F4370/ 75) D23 (F284/78) D17 (1651-00) D19 (NvH391/ 98) D24 (F352/90)	- Mortality observed for free ingestion: 2 ± 2% for ATCC 14579 spores alone; 5 ± 5% for Cry1C toxin alone; ranging from 12 ± 7% (D24) to 57 ± 20% (D23) for co-ingestion of Cry1C toxin - Mortality observed for force feeding: 0% (D19) to 8 ± 6% (D23) without toxin; 10 ± 8% (D19) to 50 ± 13% (D23) in co-ingestion - These results demonstrate synergy - The low virulence of D19 (10%) was unexpected since it	(Fedhila et al. 2010)

Organisms	Experimental Conditions	*B. cereus* strains used	Results	Reference
	toxin		is a highly virulent human pathogen Insect mortality values did not correlate with the pathogenic potential of the bacterial strains	
Cabbage looper *(Trichoplusia ni*) 1 to 8-day old healthy larvae from a stock culture **Purpose:** Pathogenicity test to characterize the non-viral cause of larvae death in a study on NPV	- Free ingestion of contaminated diet pathogenicity test - Suspension pipetted onto the surface of freshly prepared artificial diet in a 1-oz plastic cup - bacteria, virus or combination used (3 test groups) - 50 larvae per dosage	Isolate from dead or moribund larvae	- The cause of death and symptoms was identified as *B. cereus* on the basis of criteria of A. Krieg's key, 1970 - The highest level, 7.2×10^8 cells caused 100% mortality in 11 days - 69 and 50% mortality occurred among larvae exposed to 3.6 and 1.8×10^8 cells - 70 to 100% died within 10 days - Symptoms were identical to those observed in larvae from which original isolations were found: larvae ceased to feed, showed paralysis, darkening of integument and ultimately died - 1-day-old larvae appeared more susceptible than 2 to 8-day-old larvae - 1-day-old cultures of *B. cereus* caused greater and more rapid mortality than did 2, 3 or 20-day-old cultures - Combinations of the two pathogens resulted in slightly higher mortality than either pathogen alone, no synergistic effects - Pathogenicity to *T. ni* was not associated with any demonstrable toxin	(To et al. 1975)
Silkworm 5^{th} instar larvae **Purpose:** Purification and identification of a soil bacteria exotoxin, sphingomyelinase C	- Injection into the hemolymph through the dorsal surface - 0.05 ml of an overnight culture or culture supernatant - Two-fold dilutions of purified sphingomyelinase - 2 silkworms for each dose of culture or culture supernatant 5 silkworms for each dose of the toxin	ATCC 14579 25 distinct colonies of which 16 killed silkworm 9 undesignated strains of *Bacillus* sp isolated from soil	- Of 25 distinct isolates, 16 killed silkworms - 5 out of 16 culture supernatants had a killing activity against silkworms; these 5 strains were identified as *Bacillus* species (16S rRNA sequences) - The toxin purified from one isolate was identified as sphingomyelinase C from *B. cereus* - Sphingomyelinase C from *B. cereus* strain ATCC 14579 LD_{50} of 0.7 µg	(Usui et al. 2009)
German cockroaches	- Injection into the abdomen	ATCC 14579 Isolates from the	- Symptoms observed 10 minutes after injection	(Nishiwaki et al. 2004)

Organisms	Experimental Conditions	*B. cereus* strains used	Results	Reference
(Blattela germanica) Adult males **Purpose:** Purification and characterization of insect toxicity of sphingomyelinase C produced by *B. cereus*.	- 2 µl of cell-free supernatant or solution of protein sample - 5 cockroaches used for each dose	mandibles of last instars of antlions (*Myrmeleon bore)* producing insecticidal factors when cultured aerobically	- Minimum paralysis dose (MPD) at which at least four or five insects were paralysed - MPD of 262 ± 29 ng protein/insect - The insecticidal activity was abolished by heating at 100°C and by proteinase K treatment - Sphingomyelinase C produced by *B. cereus* is able to kill insects rapidly at low doses - The insecticidal factors produced by *B. cereus* may aid the prey-capturing action of the antlions - The insecticidal effect of sphingomyelinase C is due to its action on the nervous system	
Cockroaches *Leucophaea maderae*	Intrahemocoelic challenge	4 strains comprising: B1 and NCIB 3329	- B1 was the most pathogenic - NCIB 3329 was the least pathogenic	(Rahmet-Alla and Rowley 1989)
Elm bark beetles (*Scolytus scolytus)* 5th instar larvae Collected from infested elm logs **Purpose:** Biological control for the vector of Dutch elm disease	- Larvae suspended in a solution of 8×10^5 cells/ml cell for 1 hour	11796	- Observation over 21 days - Corrected for natural mortality: 63.6% of 40 larvae were killed - Control gave 17.5% mortality (corrected to 0%) in 40 larvae	(Jassim et al. 1990)
Southern pine beetle *(Dendroctonus frontalis)* larvae	Oral inoculation	Not specified	Strains isolated from diseased beetle were pathogenic	(Moore 1972)
Boll weevil *(Anthonomus grandis)* Egyptian cotton leafworm *(Spodoptera littoralis*) Black bean aphid *(Aphis fabae)*	Free ingestion method of supernatant	Not specified	- 4 of the 575 strains were toxic for *A. grandis* (85 to 100% mortality) - 5 of the 270 strains resulted in 41 to 97% mortality in *A. fabae* - No effect on *S. littoralis*	(Perchat et al. 2005)

Organisms	Experimental Conditions	*B. cereus* strains used	Results	Reference
Moth larvae (*Galleria mellonella*) – last instar	- 30 larvae were divided into groups of 10 at 15°C for each treatment - 5µL suspensions of vegetative bacteria (1.2 to 2.2 × 10^5 CFU) were injected intrahemocoelically into the base of the last left proleg of each larvae	- B. cereus NVH 0075-95 WT - ΔnheBC, Δsph, ΔnheBCΔsph - complementation mutant ΔnheBCΔsph comPplc	- Survival was determined over daily over 7 days - The percentage of alive larvae decreased most rapidly in the WT group - Mortality was significantly reduced in the *sph* deletion mutant and additional inactivation of the *nheB/nheC* reduced larvae mortality futher	(Doll et al. 2013)

Table F-2: Laboratory pathogenicity testing of *B. cereus* in aquatic crustaceans

Organism	Experimental Conditions	*B. cereus* strains used	Results	Reference
Water flea – newborn (*Daphnia magna*)	Culture dilutions 10^4 to 10^6 CFU/mL to jars containing individual neonates (24-hours old)	BD170 EH2 *B. subtilis* expressing *B. cereus* hemolysin II gene, *hlyII* *B. cereus* VKM B-771.	- Animal death within 8 to 16 days - Decreased fecundity	(Sineva et al. 2009)
Litopenaeis vannamaei (shrimp) and *Artemia* (shrimp)	Challenged with 10^4 to 10^8 CFU/mL	*B. cereus* WPD	Hemolytic activity, lipase activity and high mortality	(Velmurugan et al. 2015)

Table F-3: Reported *B. cereus* infection in insects in natural settings

Organism	Conditions	Strain	Symptoms	Reference
Pectinophora gossypiella larvae	- Throughout 2 resting seasons, the rate of sick larvae carrying dermal brown lesions were 4.1 and 1.7%. - The rates of dead larvae carrying dermal brown lesions were 2 and 0.4%.	Not specified	- When these larvae were kept in the laboratory, many of them died within 8-45 days - *B. thuringiensis* var. *finitimus* and *B. cereus* were isolated from these larvae, but not from the healthy larvae or dead larvae not presenting the lesions - Decreasing virulence with the advance of the resting period may indicate that the larvae catching the disease late may be or may become more resistant to its effect	(Abul Nasr et al. 1978)
White grubs *Anomala dimidiata*	Atrophied pupa	WGPSB-2 (MTCC 7182)	The strain was able to infect and cause 92 and 67% mortality in second instar larvae of *Anomala dimidiata* and *Holotrichia seticolis*, respectively	(Selvakumar et al. 2007)

White grubs *Anomala dimidiata* and *Holotrichia seticollis*	Up to one-fifth of the population was found to exhibit symptoms of bacterial infection	WGPSB-2	The most highly toxic strain, of 27 bacterial isolates tested against *A. dimidiate*, was identified as *B. cereus*	(Sushil et al. 2008)

9 Table F-4: Laboratory pathogenicity testing of *B. cereus* in mammal species

Organisms	Experimental Conditions	*B. cereus* strains used	Results	Reference
Guinea pigs *Cavia porcellus*	Injection (compartment not specified)	- ATCC 21 - N. R. Smith No. 156	Guinea pigs killed only when strains were subcultured	(Clark 1937)
Guinea pigs *Cavia porcellus*	Injection of culture filtrates (0.05 mL) intradermally	- B-4ac used for the dermal assay - 24 other *B. cereus* strains	B-4ac and 21 strains gave necrotic reactions surrounded by inflammation at the site of injection	(Glatz and Goepfert 1973)
New Zealand white rabbits *Oryctolagus cuniculus* Ligated ileal loop (Food poisoning experimental model)	6 test loops per rabbit	22 different strains designated	- Rapid accumulation of 3 to 20 mL of straw-colored, often bloody fluid - Positive responses for 19 of the 22 strains - Consistently positive responses for younger rabbits - Most of the rabbits with at least one positive loop died within 10 hours following the surgery	(Spira and Goepfert 1972)
New Zealand white rabbits *Oryctolagus cuniculus*	0.05 mL of cell-free culture filtrate injected intradermally	11 strains of *B. cereus* (including B-4ac, positive in ileal loop and guinea pig dermal assays)	- Increase in vascular permeability ranged from 4 to over 100 mm^2 for strain B-4ac - 9 of the 10 other strains produced a positive vascular reaction	(Glatz et al. 1974)
Dutch rabbits *Oryctolagus cuniculus* (Males)	- 0.1 or 0.3 mL injected intramuscularly into the flank - 0.15 mL injected subcutaneously - Concentration 10^2 cells/mL	SV1 lecithinase negative variant	- Presence of abscesses showing inflammatory response - Presence of nodules under the skin with necrotic fibres and fibrosis around its periphery - Calcification observed in 80% of the animals after 7 days	(Stretton and Bulman 1975)
Rabbit	Injected intradermally	- 50 of 136 strains isolated from dairy products - 102 positive strains for extracellular toxins	All 102 strains caused vascular permeability in rabbit skin	(Christiansson et al. 1989)

Organisms	Experimental Conditions	*B. cereus* strains used	Results	Reference
Rabbits *Oryctolagus cuniculus* Ligated ileal loop (Food poisoning experimental model)	3 enterotoxins in concentrated cell-free culture filtrate	- Isolates form diarrhea in monkeys - Isolates from raw rice, no symptoms in fed monkey - Isolates from a brain abscess (2141/74, serotype 11) - B-4ac	- 2 of the 11 exhibited a >50% probability of being positive on repeated testing - Fluid accumulation in rabbit ileal loop for two strains - On strain caused severe disruption of the mucosa in the ileal	(Turnbull 1976)
New Zealand adult white rabbits *Oryctolagus cuniculus*	- In vitro retinal toxicity assay measuring the cytolytic release of lactate dehydrogenase (LDH) treated with *B. cereus* HBL_{eq} and CET (600 ng/mL) - In vivo sterile endophtalmitis model: intravitreal injection of pure or crude exotoxin	MGBC 145	- Retinal buttons treated with either CET or HBL became completely disaggregated into cells and cell debris and collapsed upon removal - Within 4 hours, all eyes receiving ≥ 0.8 µg crude exotoxin exhibited marked exudate, conjunctival edema and hyperemia - When receiving 1-4 µg, no or little red reflex, vitreal hemorrhage, hemorrhagic chemosis of the conjunctiva, and corneal haze - Milder responses to low doses	(Beecher et al. 1995a)
Rabbit *Oryctolagus cuniculus* Ligated ileal loop (Food poisoning experimental model)	Purified 3 components of HBL.	F837/76	Caused fluid accumulation and 3 components were required together to cause maximal activity	(Beecher et al. 1995b)
New Zealand white rabbits *Oryctolagus cuniculus* (2 to 3 kg)	Eyes injected intravitreally with viable *B. cereus* (log 2.06 CFU) or cell-free supernatant	MGBC145	- Intraocular inflammation and reduction in retinal responses after 3 hours - Retinal detachment and photoreceptor layer folding and disrupting observed after 9 hours - At 18 hours, eyes demonstrated maximal inflammation, including in peri-ocular tissues. - supernatant produces similar results	(Callegan et al. 1999)
Mice *Mus musculus* Albino Namru strain (6- to 9-week old)	- Intraperitoneal and subcutaneous injections - 4 dilutions injected - Vegetative forms and	- NRS 201 - NRS 232 - NRS 1256	- 10 to 100 times more spores were required to kill mice - Death occurred upon intraperitoneal injection but not subcutaneous	(Lamanna and Jones 1963)

Organisms	Experimental Conditions	*B. cereus* strains used	Results	Reference
	spores tested		- Subcutaneous injections resulted in an open necrotic lesion	
Mice *Mus musculus*	Subcutaneous or intraperitoneal injections (0.25 mL) of a suspension (500 x10^6 cfu/mL)	No strain designation provided	- Acute lethal illness at high doses, almost all within 6 hours - The severity of the disease was dose-dependant - The minimal dose causing 84 to 100% mortality was approx. 22 × 10^7 bacilli - Low doses resulted in mild illness and sometimes by necrotic skin ulcers at the injection site	(Burdon et al. 1967)
Mice *Mus musculus* ICR mice (adult)	Intravenous injection of culture filtrate	183 strains isolated from dairy products	3/11 isolates with strong hemolysin activity killed mice	(Wong et al. 1988)
Mice *Mus musculus*	Intravenous injection of 8 µg of purified hemolysin II	FS-1	Death within 2 minutes	(Shinagawa et al. 1991a)
Mice *Mus musculus*	Vascular permeability test, intestinal necrosis reaction and mouse lethal test.	116 strains	Good correlation between production of necrosis in the skin and intestinal tests and the fluid accumulation test	(Turnbull et al. 1979)
Mice *Mus musculus* BALB/c strain 5-week-old females **Purpose:** Investigation of the opportunistic properties of a *B. thuringiensis* mutant and *B. cereus,* and the role of the *plcR* gene.	- Nasal instillation, mouse inhalation of the inoculum by breathing - 50 µl of the suspension (spores or vegetative cells). - Mortality observed after 24 hours.	- ATCC 14579 - ATCC 14579 Δ*plcR*	- 10^8 spores per mouse resulted in 100% mortality for both strains - 5 × 10^7 spores per mouse resulted in 90% and 22% mortality, respectively. - 10^7 spores per mouse resulted in 90% and 0% mortality, respectively - 6 × 10^6 vegetative cells per mouse resulted in 100% and 0% mortality, respectively - ATCC 14579 possesses additional factors, not regulated by PlcR, which may potentiate its opportunistic properties - Rapid death of the host if large doses of vegetative or sporulated cells are used	(Salamitou et al. 2000)
Mice *Mus musculus* BALB/c strain	Endotrachea	ATCC 14579	- Exposure to spores results in negligible effects - Exposure to vegetative cells experiments terminated at 4h due to severity of symptoms - elevated pyrogenic cytokines - pulmonary granulocyte infiltration	(Tayabali et al. 2010)

Organisms	Experimental Conditions	*B. cereus* strains used	Results	Reference
			- acute phase response markers	
Monkeys *Macaca mulatta* Rhesus strain **Purpose:** Determine the usefulness of Rhesus monkeys model for enteropathogenicity of *B. cereus*	- Force-feeding using stomach tubes - 3 types of test material fed: whole cultures, sterile culture filtrates or purified precipitated toxin - Fluid accumulation in rabbit ileal loops and skin capillary permeability tests also performed.	- B-4ac (food poisoning isolate) - 6 other strains (isolated from the rice-associated outbreaks)	- Diarrhea elicited by the three test materials 35-150 minutes after administration - Considerable variation in sensitivity among test monkeys - Approx. 50% of the monkeys showed positive responses - Vomiting never observed - 4 of the 6 undesignated strains were positive diarrheal - When grown on rice, B-4ac induced diarrhea in 3 of 6 monkeys but not vomiting - Direct correlation between ability to cause fluid accumulation in rabbit ileal loops, alteration of skin capillary permeability and ability to induce diarrhea in monkeys - Diarrhea is due to synthesis and excretion of a toxin by logarithmically growing cells	(Goepfert 1974)
Monkeys *Macaca mulatta* Sex not specified Young Rhesus strain of approximately 3 kg. **Purpose:** Attempt to confirm that food-associated outbreaks were caused by *B. cereus* and to determine the involvement of a new enterotoxigenic material.	- Force-feed with homogenized contaminated rice with feeding tube - In food, about 10^{10} viable organisms - In broth, about 10^{11} organisms - Also, ileal fluid accumulation tested with 12-15 fold concentrated filtrates.	- 4810/73 (isolated from vomitus) - 4433/73 (isolated from meat loaf, implicated in outbreak) - 2532B/74 isolated from rice	- Emetic activity: vomiting within 5 hours - Diarrhea: presence of watery or loose stools within 24 hours - Only cultures grown on rice could cause vomiting - 10 of 24 monkeys showed positive vomiting for strain 4810/73 - Bacteriological picture accurately reflected the quantities in the material fed - A clear distinction between the strains causing vomiting and diarrhea - The difference between the activities of the 2 first strains is reinforced by the rabbit loop test	(Melling et al. 1976)
Monkeys *Macaca mulatta* Rhesus strain 6-8 kg	- Intragastric administration - Purified cereulide - Partially purified vacuolation factor	- *B. cereus* No. 35, produces enterotoxin, but no vacuole factor - *B. cereus* No. 55, isolated from outbreak	- For cereulide at 14 000 units, all 3 monkeys showed emesis within 2-4 hours. - For partially purified factor at 30 000 units, 1 of 2 monkeys showed emesis after 6 hours. - For partially purified factor at	(Shinagawa et al. 1995)

Organisms	Experimental Conditions	*B. cereus* strains used	Results	Reference
		produces vacuolation factor but no enterotoxin	36 000 units, the 2 monkeys showed emesis after 2 and 4 hours - HEp-2 vacuolation factor is an emetic toxin like cereulide - These toxins can produce emesis in monkeys	
Mice *Mus musculus* strain CR 20-24 g.	- Intravenous injection - Purified cereulide - Partially purified vacuolation factor	- *B. cereus* No. 35 produces enterotoxin but no vacuole factor. - *B. cereus* No. 55, isolated from outbreak produces vacuolation factor but no enterotoxin.	- Lethality not observed for 100-500 units for both substances - Lethality found for more than 1000 units of toxin	(Shinagawa et al. 1995)
Sheep and cow (Young females)	- Intravenous injection. - 5.1×10^5 organisms in ewes - Heifers: - Group 1: 8×10^6 organisms. - Group 2: 8×10^5 organisms. - Group 3: 8×10^3 organisms	Isolates from an aborted bovine fetus	- 4 aborted dead lambs between 3 to 8 days postinoculation - Groups 1 and 2 aborted dead calves between 7 to 12 days postinoculation - Group 3 had normal calves at term - Lambs and calves: Varying degrees of autolytic change, blood-tinged ascites, hydrothorax, hydropericardium and subcutaneous edema - The foetal membranes were hyperemic and edematous - *B. cereus* isolated in pure cultures from tissues of the dead ewe, lambs and calves	(Wohlgemuth et al. 1972b)
Rabbits and mice	Purified enterotoxin	FM-1	- Vascular permeability in rabbits. - Lethal to mice. - Caused fluid accumulation in mouse ligated intestinal loop.	(Shinagawa et al. 1991b)
Mice and cats	Intravenous injection of purified enterotoxin	96	- Minimum lethal dose of 300 µg per mouse - 70 to 80 µg/ kg caused vomiting in cats	(Gorina et al. 1975)

Table F-5: *B. cereus* infections in vertebrates in natural settings

Organism	Conditions	*B.cereus* strain used	Symptoms	Reference
Dairy cattle *Bos taurus* **Purpose:** Describe the pathology of bovine *B. cereus* mastitis	- Injection into quarters of contaminated commercial antibiotic product - 8 dairy herds and otal 80 cows affected	None specified	- Some of the affected cows developed acute mastitis within 24 hours, most of them shortly after calving. - Watery blood that had failed to clot - Marked subcutaneous edema over the udder - Numerous dark red, well demarcated areas were scattered throughout the affected quarters - Enlarged supramammary lymph nodes - Edematous and emphysematous lungs - Enlarged, dark red and turgid spleens - Mammary glands: interstitial septa were found to be edematous, acute thrombosis of veins and lymph vessels was noted - Erythrocytes found in the interstitial tissue. - Acute lymphadenitis in sections of supramammary lymph nodes with focal areas of necrosis and large numbers of inflammatory cells. - Liver showed presence of centrolobular hypoxic necrosis - Renal tissue revealed hemoglobinemic casts in the tubules - Hyaline thrombi were evident in capillaries of glomerular tufts and in the corticomedullary junction. - Lungs revealed thickened alveolar septa due to edema, alveolar capillaries engorged with blood and hyaline thrombi	(Schiefer et al. 1976)
Cattle Various sexes and ages	3 case reports of abortions	Not provided	- Necropsy, microbiologic and histopathologic examinations conducted for each fetus - Necropsy findings: atelectatic, firm and dark red lungs; fibrinous	(Wohlgemuth et al. 1972a)

Organism	Conditions	*B.cereus* strain used	Symptoms	Reference
			pleuritis, pericarditis and peritonitis; yellow liver, twice the normal size; enlarged and congested lymph nodes - Microbiological findings: *B. cereus* was the only microorganism isolated from gastric contents and tissues - Histopathologic findings: aasculitis, edema, inflammation and necrosis in the intercotyledonary space; hyperplasia in spleen; congested liver	
Dairy cattle *Bos taurus* (Adult females)	Quarters inoculated with *B. cereus*.	Not provided	- Acute mastitis developed, followed by atrophy and cessation of milk secretion.	(Horvath et al. 1986)
Dairy cattle *Bos taurus* (Adult females)	- Accidental occurrence of *B. cereus* mastitis in several herds involved in efficacy trials of a proposed “dry-cow” therapy product - Injection into quarters of experimental product containing 500 mg of cloxacillin in peanut oil and 3% monostearate base - Deliberate injection in 151 non-lactating cows - Inadvertent injection in 33 lactating cows	Not provided (isolated from the experimental product and from the quarters)	- Gangrenous mastitis developed in 5 cows at calving - Clinical mastitis developed in 15 other infected quarters, chiefly at calving or during lactation - Only 26 of 184 cows and 37 of 735 quarters exposed were infected - The numbers of organisms in infected quarters vary widely, often being low - The number of organisms in each product tube was low and not all tubes were contaminated	(Jasper et al. 1972)
Dairy cattle *Bos taurus* Adult females	11 cows with acute mastitis between 1963 and 1973	Not provided	*B. cereus* was isolated from 1 cow	(Inui et al. 1979)
Holstein dairy cattle *Bos taurus* (Adult females) **Purpose:** Antibiotic therapy using cloxacillin as part of a herd health program	- Antibiotic program initiated in 67 cows; infusions of the antibiotic during the dry period or the lactating period, or both	Not provided (isolate from the milk of infected cows)	- Acute severe mastitis occurred in 62 of the 67 cows infused with cloxacillin - Post mortem examination of one cows revealed scarlet-colored mammary glands surrounded by	(Perrin et al. 1976)

Organism	Conditions	*B.cereus* strain used	Symptoms	Reference
	- 129 out of a 140 cow herd		gelatinous material and filled with serosanguineous fluid; mammary lymph nodes were wet in appearance and surrounded by gelatinous material - Lactating cows: all of 33 cows infused developed mastitis 1 to 30 days later - The disease occurs as the result of injection of *B. cereus* into the teat cistern when treating mastitis of other causes - Gangrenous inflammation and acute mastitis with systemic involvement have been reported - Very low numbers of *B. cereus* can produce profound pathogenic effects	
Dairy cattle *Bos taurus* Goat *Capra hircus* Adult females	- Trimmed tissues from one affected animal were fixed for sectioning. - Toxins tests with the rabbit skin vascular permeability and necrosis reaction - 28 cows and 1 goat distributed on 4 farms	Not provided	**Farm 1** - 3 cases of very acute mastitis in one week - One cow died within 24 hours - No response to antibiotic therapy - Second animal had subnormal temperature and a swollen and cold udder; animal died within 24 hours - Deep red kidney and udder, blood in the pelvis, congested liver and large white clots and blood stained fluid in the teat cistern - The third cow was newly calved and developed mastitis 2 days later and recovered from antibiotic therapy. **Farm 2:** - Symptoms were mild and response to therapy was poor **Farm 3:** - One cow recumbent after milk fever suddenly developed peracute mastitis and died - Second case occurred in	(Jones and Turnbull 1981)

Organism	Conditions	*B.cereus* strain used	Symptoms	Reference
			newly-calved, *B. cereus* was recovered from the udder **Farm 4:** - One cow died of acute mastitis the morning following a cut in the teat **Bacteriology:** - Organisms present in faeces of affected and non-affected cows at levels of 10^5-10^6 CFU/g - 10^2-10^3 CFU/g recovered from well preserved brewer's grains and 10^4-10^5 CFU/g when spoiled - *B. cereus* has been isolated on 17 other occasions in pure culture from mastitic bovine milk **Histopathology:** - Lesions, interstitial septa oedematous and containing erythrocytes - Thrombi in veins - Necrosis of alveolar cells **Permeability test:** - Only one of 19 mastitic and environmental isolates showed strong toxic activity in rabbit skin vascular permeability reation	
Dairy cattle *Bos taurus* Adult females	Bovine mastitis	- 1820/77 - 1419/77 - 1414/77 - 1589/77 - 624/76	- 1820/77: Death - 1419/77, 1414/77 and 1589/77: 2 deaths - 624/76: not available.	(Turnbull et al. 1979)
Parrot *A. hyacinthinus* (1 individual), *Diopsittaca nobilis* (1 individual), *Ara severa* (1 individual) and *A. ararauna* (9 individuals)	Acute, overwhelming bacterial septicemia resulting in sudden death	Specific strain(s) not available (isolates were lost and could not be submitted for molecular characterization)	- No clinical symptoms of disease prior to death. - Necorpsy revealed extensive areas of lung hemorrhage, hepatic congestion, hemorrhagic enteristis and cardia congestion	(Godoy et al. 2012)

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Appendix G: Outbreaks caused by *B. cereus*

Table G-1: Selected non-gastrointestinal outbreaks caused by *B. cereus* reported in the literature

Year	Place	Type of infection	Reference
2010	National Univeristy Hospotal (Singapore)	During the peak of the outbreak, 171 patients were implicated. Bacteremia was reported in 146 cases (51 of which were in immunocompromised patients, 57 in patients with indwelling devices and 39 who were categorised as both). Deep tissue involvement was identified in 20 patients.	(Balm et al. 2012)
2010	Tertiary care children's hospital (Aurora, Colorado)	Three patients had blood cultures positive for *B. cereus*. Non-sterile alcohol prep pads were determined to be the source of infection.	(Dolan et al. 2012)
2006	Jichi Medical University Hospital (Japan)	Eleven patients developed *B. cereus* bacteremia between January and August 2006 (Sasahara et al. 2011). The washing machine and hospital linens were highly contaminated by *B. cereus* and it was also isolated from intravenous lines.	(Sasahara et al. 2011)
2005	Kyushu University Hospital (Japan) Neonatal Intensive Care Unit	Bacteremia was detected in three neonates due to ineffective cleaning methods; the bacterial load in the environment increased and was spread through the facility via the airflow of the ventilation system (Shimono et al. 2012).	(Shimono et al. 2012)
2004	Georgia (United States), University Military Program	94/660 cadets with non-puritic, impetigo-like lesions on their scalps caused by *Bacillus cereus*. Infections are linked to the following potential factors: haircut, poor hygiene, sunscreen, exposure to soil and water.	(CDC 2005)
1998	Amsterdam (Netherlands) Neonatal Intensive Care Unit	Three neonates developed a series of invasive blood infections with *B. cereus* between January and August 1998. One died and the two recovered. Thirty-five neonates were found to be colonized with *B. cereus*. The source of infection was contaminated balloons used for manual ventilation.	(Van Der Zwet et al. 2000)

Table G-2: Reported *B. cereus* food-related outbreaks[a]

Year	Country	Etiology (additional information)	Cases
2002	Australia	Rice	37
2004	Australia	Potato and gravy (national franchised fast food restaurant)	6
2006	Australia	Chicken (cooked)	14
2007	Australia	Asparagus cream sauce (81-year-old male died 12 hours after consuming)	3
2003	Belgium	Pasta salad (stored at 14°C. Severe illness and death of 1 child)	5
2004	Belgium	Pasta	50
2005	Belgium	Rice	6
2006	Belgium	Milk products	70

Year	Country	Etiology (additional information)	Cases
1999	Canada	Potato salad (meal prepared by a restaurateur inexperienced in catering services & temperature control)	25
2005	Denmark	Chicken	4
2005	Denmark	Pizza	16
2004	Finland	Sauce (confirmed in left-overs; inadequate cooling and reheating and improper storage; mushroom sauce)	5
2004	Finland	Cake (confirmed in left-overs; layer cake)	10
2005	Finland	Eggs (egg-butter)	2
2005	Finland	Ham casserole (mixed dishes)	20
2005	Finland	Berries (imported from Poland)	15
2005	Finland	Macaroni and Cheese	18
2005	Finland	Meat soup	9
2007	France	Herbs and spices (school/kindergarten)	146
2006	India	Rice	140
2000	Japan	Milk, pasteurized (four tons of dairy products were recalled because investigators found *B. cereus* in bottles of milk)	3
2001	Japan	Bean jam filled rice cakes (kindergarten –kept longer than usual at room temperature)	335
2007	Jordan	Milk products (distributed under the government's School Nutrition Programme)	51
2004	Norway	Chicken (confirmed in left-overs	19
2005	Norway	Chili (workplace canteen)	6
2005	Norway	Stew	22
2005	Norway	Rice	3
2005	Norway	Pizza	3
2005	United Kingdom	Infant Cereal	2
1995	United States	Rice	21
1996	United States	Marinara sauce	22
1997	United States	Stuffing	400
1997	United States	Chicken, BBQ	3
1997	United States	Seafood corn chowder	2
1997	United States	Rice, fried[b]	4
1997	United States	Rice, fried[b]	4
1997	United States	Rice, fried	19
1997	United States	Pork, BBQ	33
1998	United States	Shrimp	118
1998	United States	Rice, fried	6
1998	United States	Turkey, roast beef	19
1998	United States	Rice, fried	7
1998	United States	Sandwich, submarine	25
1998	United States	Meat	19
1998	United States	Rice, fried	11
1998	United States	Rice, fried	4

Year	Country	Etiology (additional information)	Cases
1999	United States	Coleslaw	8
1999	United States	Rice, fried	4
1999	United States	Potato, mashed, with gravy	4
1999	United States	Rice	32
1999	United States	Rice	4
1999	United States	Sandwich, beef	2
2000	United States	Rice Milk	2
2000	United States	Rice, fried	18
2000	United States	Rice	15
2000	United States	Rice, fried	10
2000	United States	Salmon	3
2000	United States	Taco	4
2000	United States	Salad	3
2001	United States	Buttermilk peppercorns dip	10
2001	United States	Rice, fried	5
2001	United States	Rice, fried	17
2001	United States	Vegetable-based salad, lettuce-based salad	3
2002	United States	Chicken	11
2002	United States	Chicken	3
2002	United States	Rice, fried	8
2002	United States	Rice, egg-fried	2
2002	United States	Meat pizza	6
2002	United States	Chicken, fried	4
2002	United States	Chicken, mixed dish	8
2003	United States	Potato, fried	42
2003	United States	Chicken, mixed dish	8
2004	United States	Chicken chow mein	3
2004	United States	Chicken	11
2004	United States	Cheese, meat and vegetable pizza	4
2004	United States	Chicken and pasta (mixed dish)	2
2004	United States	Rice, fried	26
2004	United States	Chinese food	2
2005	United States	Taco (meat)	27
2005	United States	Tzatziki sauce	4
2006	United States	Grains	2
2006	United States	Pasta (lo mein)	2
2006	United States	Pancakes	2
2006	United States	Pork fried rice	5
2006	United States	Roasted pork	20
2006	United States	Chicken, baked	5
2006	United States	Prime rib steak	3
2006	United States	Spanish rice	4
2007	United States	Vegetable fried rice	16
2007	United States	Rice, fried	3

[a] Information courtesy of Judy Greig, food Safety Microbiologist/Epidemiologist, Laboratory for Foodborne Zoonoses, Public Health Agency of Canada

[b] Separate outbreaks