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Final Screening Assessment of Bacillus megaterium strain ATCC 14581

Environment and Climate Change Canada

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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 Bacillus megaterium strain ATCC 14581.

B. megaterium strain ATCC 14581 is a Gram positive bacterium that has characteristics in common with other strains of this species. B. megaterium can be found in both aquatic and terrestrial environments, in association with plants, animals and humans, as a contaminant of foods and in man-made environments. Like other Bacillus species, B. megaterium is able to form thick-walled spores, which can withstand harsh conditions and nutrient depletion. It is also able to form biofilms, allowing it to persist and survive in suboptimal conditions. Various characteristics make B. megaterium suitable for applications in wastewater treatment, bioremediation and biodegradation, cleaning and deodorizing, drain and septic treatment as well as enzyme and chemical production.

B. megaterium can have both beneficial and adverse effects in terrestrial plants. In Canada, B. megaterium strain ATCC 14581 is not recognized as a plant pest and has been reported to act as a plant growth promoting rhizobacterium. Although B. megaterium or its secondary metabolites can adversely affect some invertebrate species in the context of experimental investigations into their biocontrol potential, B. megaterium strain ATCC 14581 did not cause effects in a terrestrial invertebrate. No effects in aquatic plants, invertebrates or vertebrates or terrestrial vertebrates have been reported in the scientific literature.

In spite of the widespread distribution of B. megaterium in the environment, human infection with B. megaterium is very rarely reported. Adverse human health effects have not been attributed to B. megaterium strain ATCC 14581. The Domestic Substances List (DSL) strain ATCC 14581 does not carry enterotoxin genes which have occasionally been associated with other strains of B. megaterium. Antibiotic susceptibility testing performed by Health Canada scientists demonstrated that, in the unlikely event of infection, clinically relevant antibiotics are effective against this strain.

This assessment considers the aforementioned characteristics of B. megaterium strain ATCC 14581 with respect to the environment and human health effects associated with consumer and commercial product uses and in 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, the Government launched a mandatory information-gathering survey under section 71 of CEPA, as published in the Canada Gazette, Part I, on October 3, 2009 (section 71 notice). Information submitted in response to the section 71 notice indicates that 10 000 to 100 000 kg of products containing B. megaterium strain ATCC 14581 were imported into or manufactured in Canada in 2008. Reported uses include products or activities in the consumer, commercial and industrial sectors.

Based on the information available, it is concluded that B. megaterium strain ATCC 14581 does not meet the criteria under paragraph 64(a) or (b) of CEPA as it is 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. megaterium strain ATCC 14581 does not meet the criteria under paragraph 64(c) of CEPA as it is 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 the Minister of Health are required to conduct screening assessments of those living organisms added to the Domestic Substances List (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]. This strain was added to the DSL under subsection 25(1) of CEPA 1988 and the DSL under subsection 105(1) of CEPA because it was manufactured in or imported into Canada between January 1, 1984 and December 31, 1986.

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 3, 2009. Further details on the risk assessment methodology used are available in the Risk Assessment Framework document "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 the DSL-listed strain B. megaterium strain ATCC 14581 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 organism included its 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, CAB Abstracts and Google Scholar), web searches, and key search terms for the identification of human health and environmental hazards. Information identified up to June 2015 was considered for inclusion in this screening assessment 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 other domestic and international jurisdictions

Domestic

B. megaterium is not considered to be a plant pest or invasive species based on the list of Pests Regulated by Canada and is included on the Canadian Food Inspection Agency (CFIA) list of “Organisms that do not Require a Plant Protection Permit to Import” under the Plant Protection Act (CFIA 2011; CFIA 2014).

B. megaterium is currently categorized as Risk Group 1 (low individual and community risk) for both humans and animals (Public Health Agency of Canada (PHAC), personal communication).

International

Germany’s Federal Institute for Occupational Safety and Health has placed B. megaterium in “Risk Group 1” (BAuA 2010; European Commission 2010) and this is considered to also apply to strain ATCC 14581.

No other mention was found regarding decisions on B. megaterium by international bodies[4].

[4] Government agencies and organizations searched include: the United States Environmental Protection Agency (U.S. EPA); United States Food and Drug Administration (U.S. FDA); United States Animal and Plant Health Inspection Services (APHIS); American Biological Safety Association (ABSA); World Health Organization (WHO) Australian Department of Health; European Food Safety Authority (EFSA); European Agency for Safety and Health at Work; European Food Standards Agency; Joint FAO/WHO Expert Committee on Food Additives.

1. Hazard assessment

1.1 Characterization of Bacillus megaterium

1.1.1 Taxonomic identification and strain history

Binomial name: Bacillus megaterium

Taxonomic designation:

Kingdom: Bacteria
Phylum: Firmicutes
Class: Bacilli
Order: Bacillales
Family: Bacillaceae
Genus: Bacillus
Species: Bacillus megaterium de Bary 1884 (Orrell 2015; Skerman et al. 1980)
Strain: ATCC 14581 (type strain)

Synonyms: Bacillus megatherium (Buchanan et al. 1951), Bacillus fructosus (USDA 2014a) and Bacillus megaterium de Bary 1884 (Euzéby and Tindall 2004). At one time there was consideration given to “B. carotarum” as a synonym for B. megaterium but this was rejected (Logan and Berkeley 1984).

Other equivalent type strain designations: BCRC 10608, CCM 2007, CCUG 1817, CIP 66.20, DSM 32, HAMBI 2018, IAM 13418, JCM 2506, KCTC 3007, LMG 7127, NBRC 15308, NCCB 75016, NCIMB 9376, NCTC 10342, NRIC 1710, NRRL B-14308, VKM B-512, Bacillus sp. JP44SK2, Bacillus sp. OS42, CCRC 10608, and IFO 15308 (ATCC 2014, Verslyppe et al. 2014).

Strain history

B. megaterium was named by De Bary in 1884; however, the original culture became unavailable and so the strain “Ford 19” as supplied to culture collections became the new type culture, acquiring the accession numbers ATCC 14581, NCIB 9376 and NCTC 10342 (Lapage et al. 1967; Smith et al. 1964; Sneath and Skerman 1966). It passed from Ford (strain 19) to T. Gibson (strain 1060), to R.E.Gordon and then to the ATCC (DSMZ 2014). The original source from which ATCC 14581 was isolated is unknown (Allen et al. 1983).

1.1.1.1 Phenotypic identification and biochemical profile

B. megaterium strain ATCC 14581 has rod-shaped cells that are 2 to 5 µm in length and 1.2 to 1.5 µm in diameter (Willeke et al. 1996). B. megaterium spores are between 0.5 × 1.0 µm up to 1.0 × 14.8 µm and are frequently club-shaped in appearance (Drucker and Whittaker 1971).

The colony morphology of B. megaterium strain ATCC 14581 observed by Health Canada scientists was consistent with that reported by the American Type Culture Collection (Table 1-1).

Table 1-1: Colony morphologies of B. megaterium strain ATCC 14581

Characteristic	Growth on nutrient agar after 24 hours at 30°C[a]	Growth on nutrient agar after 48 hours at 30°C[a]	Growth on TSB[b] agar after 24 hours at 37°C[a]	Growth on TSB agar after 48 hours at 37°C[a]	Growth on ATCC Medium 3 (nutrient agar), 24 hours at 30°C[c]
Shape	Irregular	Irregular	Irregular	Irregular	Circular or irregular
Diameter size (mm)	2-3	2-3	4	4	Not available
Margin	Entire	Entire	Entire	Entire	Entire
Elevation	Convex	Convex	Convex	Convex	Convex
Colour, pigment	Not available	Golden-tan	Not available	Golden-tan	Not available
Texture	Smooth and glistening or mucoid	Butyrous	Smooth and glistening or mucoid	Butyrous	Smooth and glistening or mucoid
Opacity	Opaque	Opaque	Opaque	Opaque	Opaque

[a] Data generated by Environmental Health Science and Research Bureau, Health Canada
[b] TSB, tryptic soy broth
[c] Description from ATCC (ATCC 2013)

B. megaterium strain ATCC 14581 has often been grouped with members of the B. subtilis group in the scientific literature (Logan and Berkeley 1984). They are similar in terms of electrophoretic mobility of spores (White et al. 2012), position of spores, beta-galactosidase activity, lecithovitellin reaction and gelatin hydrolysis (Logan and Berkeley 1984). Additional metabolic tests were conducted by Health Canada scientists (Appendix A).

B. megaterium (including ATCC 14581) also exhibits similarities to the B. cereus group, which includes the human and animal pathogens B. cereus and B. anthracis (Beesley et al. 2010) and the insect pathogen B. thuringiensis. While most B. megaterium strains are strictly aerobic, B. megaterium strain ATCC 14581 is capable of anaerobic growth like members of the B. cereus group (Beesley et al. 2010; Xiang et al. 2011). B. megaterium, like members of the B. cereus group, have larger cells (>1.0 µm long) and can also be mistaken for B. anthracis based on antigenic similarity of the antiphagocytic capsule (Beesley et al. 2010).

When a larger number of phenotypic characteristics are compared, a pattern emerges that allows B. megaterium to be clearly differentiated from either the B. subtilis or B. cereus groups. B. megaterium is distinguishable from both species groups using the API system, which is based on biochemical methods and differences in substrate utilization (Logan and Berkeley 1984; Product sheet 2004). Based on the results of 139 observations, analysis of 600 strains of Bacillus demonstrated that B. megaterium strains formed a tight group and were distantly positioned from strains of the B. subtilis group (Logan and Berkeley 1984).

B. megaterium can be differentiated from members of the B. cereus and B. subtilis groups based on morphological, biochemical and molecular characteristics (Table 1-2). Health Canada scientists also used fatty acid methyl ester (FAME) analysis to confirm the identity of the DSL strain (Appendix B). The guanine-cytosine content (G+C%) of B. megaterium strain ATCC 14581 is greater compared to type strains of B. anthracis and B. thuringiensis and can be used to differentiate it from these species. (Logan and De Vos 2009).

Table 1-2: Differentiation of B. megaterium from B. cereus and B. subtilis[a]

Characteristic	B. megaterium strain ATCC 14581	B. cereus	B. subtilis
Length of rods >1.0 µm	+	+	-[c]
Anaerobic growth	+	+	-
Growth at 50°C	-	-	+
Voges-Proskauer reaction	-	-	+
Lecithovitellin reaction	-	+	-
Glucose fermentation test[b]	A^-G^+	A^+G^-	A^+G^-
Lactose fermentation test[b]	A^-G^+	A^-G^-	A^-G^-
Mannitol fermentation tes [b]	A^-G^+	A^-G^-	A^+G^-
Type strain G+C content (%)	37.2[c]	36.2	42.9

+ indicates a positive reaction; - indicates a negative reaction
[a] Data for table compiled from Logan and De Vos (2009); Slepecky and Hemphill (2006); Beesley et al. (2010); Radhika et al. (2011); Xiang et al. (2011) and Heath Canada scientists (personal communication)
[b] A^-G^+, acid negative, gas positive; A^+G^-, acid positive, gas negative; A^-G^-, acid negative, gas negative

B. megaterium strain ATCC 14581 can be distinguished from other strains of B. megaterium by several morphological and biochemical characteristics (Table 1-3).

Table 1-3: Morphological and biochemical characteristics to distinguish between B. megaterium strain ATCC 14581 and two phosphate solubilizing strains of B. megaterium

Characteristic	ATCC 14581	DSM 3228	QW10-11[a]
Colony pigmentation[b]	Pale	Pale	Buff
Cell morphology[b]	Ellipse	Ellipse	Line[c]
Cell arrangement[b]	Short chain	Single, pairs	Chain
Cell size (µm)[b]	1.2 – 1.5 × 2.0 - 5.0	1.8 – 2.6 × 6.0	0.8 × 30 - 100
Motility[b]	+[d]	+	-
Spore morphology[b]	Ellipse	Ellipse	Round
Anaerobic growth[b]	+	-	-
Milk peptonisation[b]	-	+	+
Casein hydrolysis[b]	+	+	-
Nitrate reduction[b]	+	+	-
NaCl tolerance[b]	7%	5%	15%

+ indicates a positive reaction; - indicates a negative reaction
[a] QW10-11 cells were isolated from a and have adapted to a hypersaline environment (10% (w/v) NaCl)
[b] Information obtained from Xiang et al. (2011)
[c] Cells extend without dividing; authors attributed this morphology to physiological changes required to adapt to the hypersaline growth environment
[d] Xiang et al. (2011) and Logan and De Vos (2009) report the type strain to be motile however, this characteristic was not observed by Heath Canada Scientists

1.1.1.2 Molecular identification

In spite of the above-noted phenotypic differences, molecular methods show B. megaterium to be closely related to both the B. cereus and B. subtilis groups. In one study, 51 species of Bacillus were arranged in five phylogenetically distinct clusters based on comparison of their 16S rRNA gene sequences. B. megaterium strain ATCC 14581, B. cereus, B. anthracis and B. subtilis are all grouped together (Ash et al. 1991). The close relationship between B. megaterium and species of the B. cereus and B. subtilis groups based on 16S rRNA phylogeny suggests that lateral gene transfer may be to a large extent responsible for the phenotypic differences between these species (Eppinger et al. 2011). Health Canada scientists used 16S rRNA gene sequences to analyze the phylogenetic relationship between B. megaterium strain ATCC 14581 and other Bacillus species (Figure 1-1). The DSL strain B. megaterium strain ATCC 14581 was not observed to group with either the B. subtilis group or the pathogenic B. cereus group.

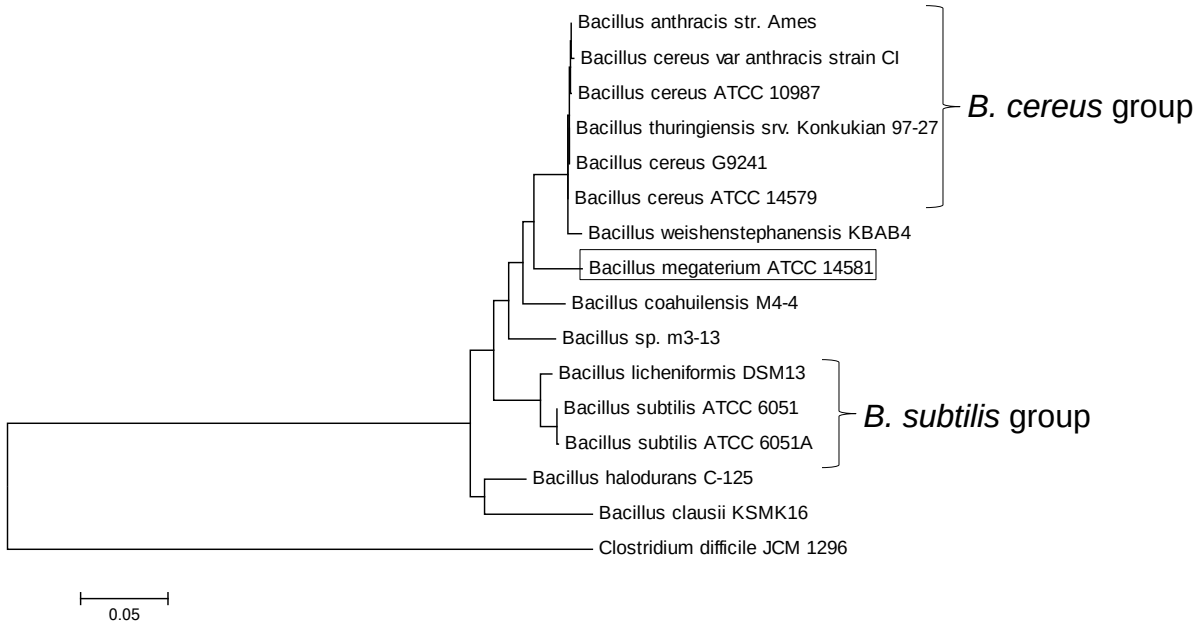

Figure 1-1: Phylogenetic tree derived in-house[5], using 16S rRNA gene sequences of B. megaterium strain ATCC 14581 and sequences identified from literature searches

Several methods can be applied to distinguish between B. megaterium and other Bacillus species, including B. cereus and B. subtilis, such as Randomly Amplified Polymorphic DNA (RAPD) (Quingming and Zongping 1997) and Ultra Violet Raman Resonance Spectroscopy (UVRR) (Lopez-Diez and Goodacre 2004). Some B. megaterium strains, including B. megaterium strain ATCC 14581, have fully

[5] Environmental Health Science and Research Bureau, Health Canada

sequenced genomes which can potentially be used to determine distinguishing genotypic features between strains (Table 1-4).

Table 1-4: Some industrial strains of B. megaterium with fully sequenced genomes

Strain	Accession number	Base pairs (Mbp)	Number of plasmids	G+C content (%)	Reference
B. megaterium strain ATCC 14581	JJMH00000000	5.7	6	37	(Arya et al. 2014; Rosso and Vary 2005; Vary et al. 2007)
B. megaterium de Bary 1884	JMQB00000000	5.6	Not available	37.7	(Daligault et al. 2014)
B. megaterium strain QM B1551	CP001983	5.1	7	38.2	(Eppinger et al. 2011)
B. megaterium strain DSM319	CP001982	5.1	0	38.2	(Eppinger et al. 2011)
B. megaterium strain WSH-002	CP003017	4.14	3	39.1	(Liu et al. 2011)

Plasmids within the genus Bacillus

Plasmids are relatively rare among Bacillus species (Yoshimura et al. 1983); however, B. megaterium strains are rich in plasmids, usually containing four or more (Slepecky and Hemphill 2006). B. megaterium strain 216 contains 10 plasmids and B. megaterium strain QM B1551 has 7 stable plasmids comprising 11% of total cellular DNA. Although the plasmids of QM B1551 carry genes encoding enzymes and proteins for heavy metal export, transport, acyl carriers, sigma factors, sterols, redox, mobilization, sporulation and germination, the plasmid-borne genes do not seem to be required for growth. In one study, a modified strain of QM B1551 with seven of its plasmids removed showed similar growth to the wild-type under laboratory conditions (Kieselburg et al. 1984; reviewed in Vary et al. 2007).

Among its plasmids, B. megaterium strain ATCC 14581 carries a 12 kb plasmid which distinguishes it from nine other strains of B. megaterium (Rosso and Vary 2005; Vary et al. 2007).

1.1.2 Biological and ecological properties

1.1.2.1 Natural occurrence

B. megaterium as a species is generally considered ubiquitous and is found in a variety of habitats, including:

- fresh and salt water (Allen et al. 1983; Rusterholtz and Mallory 1994; Kong et al. 2010) as well as soil (Smalla et al. 2001; Taupp et al. 2005; Sakurai et al. 2007);
- plant rhizosphere (Srinavasan et al. 1996; Smalla et al. 2001; Sakurai et al. 2007; Aballay et al. 2011; Xiang et al. 2011; Kavamura et al. 2013) and other plant-associated sites including ovules and seeds (Mundt and Hinkle 1976; Cottyn et al. 2001), leaves (Ercolani 1978; Purkayastha and Bhattacharyya 1982;

Araújo et al. 2001; West et al. 2010;), roots (Liu and Sinclair, 1993; McInroy and Kloepper 1995; Srinavasan et al. 1996; Surette et al. 2003; West et al. 2010) and stems (McInroy and Kloepper 1995; West et al. 2010)

- in association with animals (Osborn et al. 2002; Barbosa et al. 2005; Hillesland et al. 2008; Bulushi et al. 2010) and humans (Weber et al. 1988; Rowan et al. 2001; Dib et al. 2003; Beesley et al. 2010; Subbiya and Mahalakshmi 2012); and
- compost piles (Perestelo et al. 1989), processing waste (Srinath et al. 2002; Raj et al. 2007; Priya et al. 2014), soilless growing medium (Welbaum et al. 2009), the interior of granite rock (Fajardo-Cavazos and Nicholson 2006); air ducts (Lushniak and Mattorano 1994), chewing tobacco (Rubinstein and Pedersen 2002), wood surfaces (Austin et al. 1979), paper linerboard (Namjoshi et al. 2010) and as contaminants of food (Kurtzman et al. 1971; López and Alippi 2009; Rowan et al. 2001; Yossan et al. 2006).

1.1.2.2 Growth parameters

Strains of B. megaterium, including ATCC 14581, grow over a wide range of temperatures, with minimum growth temperatures between 3°C and 10°C, an optimal growth temperature of 37°C, and a maximum growth temperature of 40 to 45°C. Although B. megaterium strain ATCC 14581 was not observed to grow at 50°C (Table 1-2), obligate thermophilic variants are capable of growth at 55°C (Ståhl and Olsson 1977) and other variants show cardinal temperature growth maxima up to 73°C (Rilfors et al. 1978). B. megaterium is able to survive in nutrient broth for 28 days at room temperature. However, when the temperature was increased to 48°C a 1 log decrease of colony forming units (CFU) was reported (Velineni and Brahmaprakash 2011). Health Canada scientists measured the optical density of 24-hour B. megaterium strain ATCC 14581 cultures in various liquid media grown at various temperatures (Appendix C). B. megaterium strain ATCC 14581 was observed to grow well in TSB at 28°C but limited growth was observed in serum-containing mammalian culture media at this temperature. In contrast, as the temperature increased, growth was observed to increase slightly in most serum containing media but decreased in TSB.

B. megaterium strain ATCC 14581 grows poorly on minimal media and requires supplementation with L-threonine and L-valine whether grown at 30°C or 37°C with glucose or glycerol (White 1972); the most rapid growth occurred at 30°C with glycerol. B. megaterium strain ATCC 14581 grows well on ATCC Medium #3, with a pH of 6.8 ± 0.2 (ATCC 2013).

1.1.2.3 Survival and persistence

The persistence of B. megaterium strain ATCC 14581 in soil microcosms was investigated (Providenti et al. 2009). The results of the study indicated that an initial concentration of 1×10^4 CFU/g soil would decline to the detection limit of 1×10^2 CFU/g soil within 105 days. B. megaterium would also likely be able to persist at some lower concentrations as spores.

B. megaterium may undergo sporulation in response to nutrient depletion (Brown and Hodges 1974). The formation of endospores allows Bacillus species to persist for long periods under dry conditions and to resist high temperatures, ultraviolet radiation and chemical disinfectants. Spores of Bacillus species can withstand temperatures about 45°C higher than vegetative cells (Coleman et al. 2010). Spores of B. megaterium strain ATCC 14581 are also relatively resistant to ultraviolet light compared to other Bacillus species tested (Fajardo-Cavazos and Nicholson 2006; Newcombe et al. 2005).

The conditions in which B. megaterium undergoes sporulation may affect the resistance of the spores to various conditions (Soper et al. 1976; Khoury et al. 1987). For example, spores that had undergone freezing were more susceptible to chlorine and ultraviolet light inactivation treatments compared to their unfrozen counterparts (Gao et al. 2007; Soper et al. 1976). It is also important to note that the spores of different strains of B. megaterium may behave differently than ATCC 14581 (Dr. Rebekka Biedendieck, Postdoctoral fellow, Technische Universität Braunschweig, personal communication).

B. megaterium spores demonstrated better survival in sterile river water as opposed to raw (unsterilized) river water (López et al. 1995), suggesting that predation may limit persistence of spores in this environment. However, spores may survive predation better than vegetative cells. Vegetative cells of B. megaterium do not withstand passage through the digestive system of the nematode Caenorhabditis elegans, whereas spores do (Laaberki and Dworkin 2008).

1.1.2.4 Biocontrol

B. megaterium produces antimicrobial compounds, such as the bacteriocins megacin A and megacin C, that are of potential interest for biocontrol of bacterial and fungal plant pathogens (Tersch and Carlton 1983). B. megaterium strain ATCC 14581 cytochrome P-450 (specifically CYP102A1) can oxidize (i.e., degrade) the quorum signalling molecules acyl homoserine lactones produced by other micro-organisms giving it a competitive advantage (Chowdhary et al. 2007). Some experimentally observed biocontrol activities for isolates of B. megaterium are outlined in Table 1-5.

Table 1-5: Biocontrol activity of B. megaterium

Host plant species	Pathogenic organism (disease)	Reference
Tomato	Ralstonia solanacearum (bacterial wilt disease)	(Nguyen et al. 2011)
Cabbage	Plasmodiophora brassica	(Gao and Xu 2014)
Jute	Colletotrichum corchori	(Purkayastha and Bhattacharyya 1982)
Oilseed rape	Sclerotinia sclerotiorum	(Hu et al. 2013)
Wheat seed and maize seed	Smuts	(Kollmorgen 1976; reviewed in Hosford 1982)
Peanut kernels	Aspergillus flavus	(Kong et al. 2010)
Apples	Venturia inaequalis (Apple scab on leaves and fruit)	(Poleatewich et al. 2012)

1.1.2.5 Biosorption of metals and biodegradation

B. megaterium is able to increase the bioavailability of metals in contaminated soils including boron, lead and cadmium (Esringu et al. 2014). A strain of B. megaterium isolated from forest soil was able to solubilize iron, manganese and copper from phosphogypsum, a waste product of the production of fertilizer from phosphate rock (Ştefănescu et al. 2011).

Some strains of B. megaterium are capable of bioaccumulating metals, including cobalt, manganese, nickel, zinc, uranium, aluminum and cadmium (Selenska-Pobell et al. 1999; Rajkumar et al. 2013). In one study, B. megaterium was reported to bioaccumulate 32.0 mg Cr/g dry weight (Srinath et al. 2002). Moderately halotolerant strains of B. megaterium have been reported to reduce selenite (SeIV) to less toxic red elemental selenium (Mishra et al. 2011).

In addition to reactions with metals, B. megaterium can degrade organic compounds, including the herbicide atrazine (Marecik et al. 2008).

1.1.2.6 Resistance to antibiotics, metals and chemical agents

Although one strain of B. megaterium isolated from a hospital environment in Nigeria was resistant to 70% of the antibiotics with which it was challenged (Atata et al. 2013), B. megaterium is generally sensitive to a greater spectrum of antibiotics than B. cereus, though not as universally susceptible as B. subtilis (Larsen et al. 2014; Reva et al. 1995; Sadiq and Ali 2013; Appendix D).

Health Canada scientists[6] determined the minimum inhibition concentration of antibiotics against B. megaterium strain ATCC 14581 using the TREK Sensititre broth microdilution method (Thermo Scientific) conducted in liquid medium (Table 1-6 and Table 1-7). Overall, B. megaterium strain ATCC 14581 was inhibited by many of the antibiotics tested.

Table 1-6: B. megaterium strain ATCC 14581 minimal inhibitory concentration (MIC, µg/mL) using the GPN3F Sensititre panel in liquid medium

Antibiotic	Breakpoint (MIC µg/mL)[a]	Results (MIC µg/mL)[a]	MIC Interpretation[a]
Erythromycin	S ≤0.5, I 1-4, R ≥8	≤ 0.25	Susceptible
Clindamycin	S ≤0.5, I 1-2, R ≥4	>2	Intermediate
Synercid (Quinupristin/ Dalfopristin)	S ≤1, I 2, R ≥4	2	Intermediate
Daptomycin	S ≤1, R N/A	1	Susceptible
Vancomycin	S ≤2, I 4-8, R ≥16	≤ 1	Susceptible
Tetracycline	S ≤4, I 8, R ≥16	≤ 2	Susceptible
Ampicillin	S ≤0.25, R ≥0.5	>16	Resistant
Gentamicin	S ≤4, I 8, R ≥16	≤ 2	Susceptible
Rifampin	S ≤1, I 2, R ≥4	≤ 0.5	Susceptible

[6] Environmental Health Science and Research Bureau, Health Canada

Antibiotic	Breakpoint (MIC µg/mL)[a]	Results (MIC µg/mL)[a]	MIC Interpretation[a]
Levofloxacin	S ≤2, I 4, R ≥8	≤ 0.25	Susceptible
Linezolid	S ≤ 4, R N/A	2	Susceptible
Penicillin	S ≤0.12, R ≥0.25	≥ 8	Resistant
Ciprofloxacin	S ≤1, I 2, R ≥4	≤ 0.5	Susceptible
Trimethoprim/sulfamethoxazole	S ≤2/38, R ≥4/76	≤ 0.5/9.5	Susceptible
Ceftriaxone	S ≤8, I 16-32, R ≥64	≤ 8	Susceptible
Gatifloxacin	S ≤2, I 4, R ≥8	≤ 1	Susceptible
Oxacillin+2%NaCl	S ≤2, R ≥4	≤ 0.25	Susceptible

N/A indicates information not available; S indicates susceptible; I indicates intermediate; R indicates resistant
[a] Breakpoints and interpretation of MIC results according to Clinical and Laboratory Standards Institute (CLSI M45-A2, and VET01-S2) (CLSI, 2012; CLSI, 2013)

Table 1-7: B. megaterium strain ATCC 14581 minimal inhibitory concentration (MIC; µg/mL) using the BOPO6F sensititre panel in liquid medium

Antibiotic	Breakpoint (MIC µg/mL)[a]	Results (MIC µg/mL)	MIC Interpretation[a]
Ceftiofur	S ≤2, I 4, R ≥8	≤ 0.25	Susceptible
Gentamicin	S ≤4, I 8, R ≥16	≤ 1	Susceptible
Florfenicol	R ≥16[b]	4	Susceptible
Tiamulin	R ≥32	≥ 32	Resistant
Chlortetracycline	N/A	≤ 0.5	N/A
Oxytetracycline	R ≥16	≤ 0.5	Susceptible
Penicillin	S ≤0.12, R ≥0.25	≥8	Resistant
Ampicillin	S ≤0.25, R ≥0.5	>16	Resistant
Danofloxacin	N/A	≤ 0.12	N/A
Neomycin	N/A	≤ 4	N/A
Trimethoprim/sulfamethoxazole	R ≥4/76	≤ 2/38	Susceptible
Spectinomycin	N/A	32	N/A
Tylosin tartrate[c]	S ≤0.25, R ≥32	≤ 0.5	Susceptible
Tulathromycin	R ≥64	8	Susceptible
Tilmicosin	R ≥32	≤4	Susceptible
Clindamycin	S ≤0.5, I 1-2, R ≥4	≥ 16	Resistant
Sulphadimethoxine	N/A	< 256	N/A
Enrofloxacin	R ≥4	≤ 0.12	Susceptible

N/A indicates not available; S indicates susceptible; I indicates intermediate; R indicates resistant
[a] Breakpoints and interpretation of MIC results according to Clinical and Laboratory Standards Institute (CLSI M45-A2 and VET01-S2) (CLSI, 2012; CLSI 2013)
[b] (Kehrenberg and Schwarz 2006)
[c] (Scott, et al. 2010)

Strains of B. megaterium are also susceptible to a number of different disinfection methods (Table 1-8).

Table 1-8: Susceptibility of strains of B. megaterium to other disinfecting agents

Strain	Agent	Concentration/ effect	Reference
B. megaterium strain ATCC 14581	Palm kernel expeller peptides	MIC: 150 to 300 µg/mL	(Tan et al. 2011)
B. megaterium	Lysozyme	91% reduction of optical density at 50 µg/mL	(Suzuki and

Strain	Agent	Concentration/ effect	Reference
ATCC 9885 (spores)			Rode 1969)
B. megaterium strain ATCC 14581 (spores)	Lysozyme	1% reduction of optical density at 50 µg/mL	(Suzuki and Rode 1969)
B. megaterium	Ethanol	90% (v/v) ethanol caused a reduction in or absence of colony growth and reduction in spore survival	(Thomas 2012)
B. megaterium (spores)	High-pressure CO_2	58 ATM, 60°C and 30 h produced a 7 log decrease in the number of viable cells (CFUs)	(Enomoto et al. 1997)
B. megaterium (spores)	Cold atmospheric plasma[a]	> 6 log reduction in CFUs after 90 minutes of exposure	(Shimizu et al. 2014)
B. megaterium (spores)	Ozone	Lethal threshold concentration: 0.19 mg/L (exposure time: 5 minutes)	(Broadwater et al. 1973)
B. megaterium ATCC 8245	Gamma radiation	Resistance is proportional to the density of spores	(Salih 2001)
B. megaterium strain ATCC 14581 (actively dividing culture)	NO, NO_2	Unaffected by concentrations up to 1.9 ppm (NO) or ≤5.5 ppm (NO_2)	(Mancinelli and McKay 1983)

[a] Cold atmospheric plasma is an ionized gas with decontaminating potential, that can be derived from various gases such as helium, argon, or nitrogen (Hoffmann et al. 2013)

Lytic phages have been used in the control of B. cereus group species. The lytic phage vB_BceM_Bc431v3 infects all B. cereus, B. anthracis, B. licheniformis, B. thuringiensis and B. weihenstephanensis strains, as well as B. megaterium strain ATCC 14581 (El-Arabi et al. 2013).

1.1.2.7 Pathogenic and toxigenic characteristics

Some B. megaterium strains possess characteristics of pathogenic Bacillus species that are associated with virulence, such as adherence and invasion, toxin and secondary metabolite production, biofilm formation and hemolysis.

Health Canada scientists verified the whole genome sequence of B. megaterium strain ATCC 14581 (GenBank accession JJMH00000000.1; Arya et al. 2014) using a variety of search strategies (nucleotide and protein searches) and did not find any major virulence factor genes. Another complete B. megaterium strain ATCC 14581 genome sequence that appears in GenBank (CP009920.1, CP009916.1-CP009919.1 & CP009921.1) was also investigated and yielded the same results.

1.1.2.7.1 Adherence and invasion

B. megaterium isolated from honey was reported to adhere to and invade human intestinal epithelial Caco-2 cells and the supernatant from the spent cultures resulted in detachment and necrosis of the Caco-2 cells (López et al. 2013). A strain of B. megaterium isolated from infant milk formula was determined to be non-cytotoxic, yet demonstrated adherence similar to that shown by a strain of Listeria monocytogenes as well as some invasion capability (Rowan et al. 2001). There is no evidence to indicate that the DSL strain is able to adhere to and invade mammalian cells.

1.1.2.7.2 Toxins

A clinical B. megaterium strain, associated with sepsis and pyrexia, was demonstrated to produce hemolysin BL (HBL) enterotoxin (Rowan et al. 2001). Enterotoxigenic genes cytK, bceT, those related to the HBL complex and the NHE complex have been reported in B. megaterium strains isolated from honey (López and Alippi 2010; López et al. 2013; López and Alippi 2009). Searches of the annotated genome did not identify known toxin genes or operons associated with cytK, bceT, HBL or NHE in B. megaterium strain ATCC 14581.

1.1.2.7.3 Other metabolites

In plants inoculated with pathogenic strains of B. megaterium pectolytic and cellulolytic enzymes were present, causing disintegration and collapse of invaded tissues (Abdel-Monaim et al. 2012). Relative to other Bacillus species, the activity of xylanase and cellulase in two strains of B. megaterium isolated from African soil was reported to be low (Larsen et al. 2014). There is no evidence to suggest that the DSL strain is capable of producing these enzymes.

1.2.7.4 Biofilm formation

Although cells of B. megaterium produce a glucose-based polysaccharide polymer that can contribute to biofilm formation (Gandhi et al. 1997; Welbaum et al. 2009), two soil isolates of B. megaterium did not form biofilms (Larsen et al. 2014). It is not known whether the DSL strain produces a biofilm.

1.2.7.5 Hemolysis

Hemolytic activity was demonstrated in 77% of 53 B. megaterium strains isolated from Argentinean honey (primarily of the β-hemolysis type) and 10% of these produced a discontinuous hemolytic pattern typically associated with enterotoxin activity. In addition, coagulase activity was found in 74% of the strains (López and Alippi 2009). No hemolytic activity was observed in B. megaterium strain ATCC 14581 by Health Canada scientists.

1.1.3 Effects

1.1.3.1 Environment

1.1.3.1.1 Plants

Some strains of B. megaterium are reported to be pathogenic to terrestrial plants (Shark et al. 1991) causing bacterial blight or white blotch on foliage and heads of wheat (Australian Government Department of Health and Ageing 2005; Hosford 1982; USDA 2014b). B. megaterium adversely affects the subtropical ornamental tree (Radermachera sinica) (Li et al. 2014), in which its pathogenic role was confirmed by experimental reinfection of healthy plants. B. megaterium has been implicated as one of several causative agents of wetwood wilt and dieback of certain tree species, especially elms and poplars (University of Illinois 1999). Six strains of B. megaterium were isolated from diseased lupines and re-inoculated into healthy plants, which subsequently

became soft and grayish-brown, eventually collapsing (Abdel-Monaim et al. 2012). Interestingly, barley, wheat, sunflower, cocklebur, spinach and 13 other legume species were challenged with the two most pathogenic strains and no effects were reported with the exception of fava bean leaves which exhibited small necrotic spots (Abdel-Monaim et al. 2012).

Environment and Climate Change Canada scientists investigated effects in red fescue (Festuca rubra) after exposure to B. megaterium strain ATCC 14581 (Environment Canada 2005). Replicates were conducted in 1 L polypropylene vessels containing 500 ± 0.5 g wet weight of soil which were inoculated with 3.48×10^8 CFU of B. megaterium strain ATCC 14581 at the start of the study. No statistically significant difference was observed between the exposed plants and the control plants as measured by mean emergence, shoot and root length, and mass after 32 days following the initial inoculation event.

B. megaterium also has beneficial effects on terrestrial plants as a plant-growth promoting rhizobacterium (Table 1-9). B. megaterium solubilizes phosphorus (Sandeep et al. 2011; Sadiq and Ali 2013; Xiang et al. 2011), and mineralizes organic nitrogen (Sakurai et al. 2007), thereby making soil nutrients available to plants (Armada et al. 2014; Hu et al. 2013; Kieselburg et al. 1984). It also produces plant growth hormones (auxins), including indole acetic acid, and enzymes that promote growth (Armada et al. 2014; Sadiq and Ali 2013; Shaharoona et al. 2006).

Table 1-9: Growth promotion activity of B. megaterium in terrestrial plants

Strain	Species treated or point of isolation	Activity and mechanism of growth promotion	Reference
B. megaterium strain ATCC 14581	Withania somnifera (medicinal plant)	Mechanism not reported	(Saikia et al. 2013)
B. megaterium A6	Oilseed rape	Phosphate solubilization	(Hu et al. 2013)
B. megaterium-GC subgroup A	Tomato and cucumber	Higher average fruit weight and potential to increase growth, yield and mineral content (mechanism not reported)	(Dursun et al. 2010)
B. megaterium in combination with Arthrobacter chorophenolicus and Enterobacter	Wheat	Increased height, grain yield, straw yield and test (mechanism not reported)	(Kumar et al. 2014)
B. megaterium	Tomato	Mechanism not reported	(Porcel et al. 2014)
B. megaterium	Ruta (medicinal plant)	Mechanism not reported	(Patil et al. 2013)
B. megaterium	Trifolium repens	Production of stimulating hormones such as indole acetic acid	(Armada et al. 2014)
B. megaterium	Isolated from the rhizosphere and inside the root of Phaseolus vulgaris	Production of indole acetic acid; potential role in root nodulation	(Srinavasan et al. 1996)
Two strains of B. megaterium	Isolated from the rhizosphere of maize	Auxin production and 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity	(Sadiq and Ali, 2013; Shaharoona et al. 2006)

An in-depth review of the scientific literature did not identify adverse effects of B. megaterium or B. megaterium strain ATCC 14581 in aquatic plant species.

1.1.3.1.2 Invertebrates

Adverse effects of B. megaterium in terrestrial invertebrates have been observed in experiments exploring its potential as a biocontrol agent. Several examples are listed below:

- 10% mortality in fifth instar moth larvae (Hylesia metabus) inoculated with 3-4 × 10^7 CFU of B. megaterium isolated from fourth instar moth larvae (Osborn et al. 2002).
- Antagonistic effects in parasitic nematodes including Xiphinema index (Aballay et al. 2011); Meloidogyne graminicola (Padgham and Sikora 2007) and Meloidogyne incognita (Saikia et al. 2013): in M. graminicola, B. megaterium reduced nematode penetration and gall formation in rice roots and reduced migration of M. graminicola to the root zone by nearly 60% compared to non-treated roots; secondary metabolites of B. megaterium reduced nematode egg hatching by 60% compared to eggs not exposed to the bacteria.
- Antagonistic effects in weevils (Rhynchophorus ferrugineus) and moths (Dendrolimus pini (L)) (Bertone et al. 2011; Hardin and Suazo 2012).
- Secondary metabolites of B. megaterium killed mosquito larvae (Aedes aegypti) but were non-toxic to humans (Radhika et al. 2011).
- B. megaterium isolates killed aphids (Aphis pomi De Geer) (Askoy and Ozman-Sullivan 2008)

Environment and Climate Change Canada scientists investigated effects in springtails (Folsomia candida) after exposure to B. megaterium strain ATCC 14581 (Environment and Climate Change Canada 2005). Test jars were filled with 30 g wet weight of soil and inoculated with 9.28 × 10^7 CFU of B. megaterium strain ATCC 14581 24 hours prior to the start of the study. No statistically significant difference between treatments was observed in mean adult survival or mean juvenile production after 28 days following the initial inoculation event.

An in-depth search of the scientific literature identified no reports of B. megaterium adversely affecting aquatic invertebrates with the exception of the mosquito larvae identified above; however, it has been reported to act as a growth promotant when added to shrimp feed (Olmos et al. 2011; Yuniarti et al. 2013).

1.1.3.1.3 Vertebrates

An in-depth review of the scientific literature identified no reports of B. megaterium adversely affecting aquatic or terrestrial vertebrates. However, it has been added to fish feed as a growth promotant (Parthasarathy and Ravi2011). In addition, B. megaterium strain ATCC 14581 was included with other bacteria in a patent to reduce vertebral compression syndrome in salmonid fish (Aubin et al. 2006).

1.1.3.2 Human health

In spite of its widespread presence in the environment, B. megaterium has rarely been implicated in human infections. When it has been associated with infection, it was not always clear if the clinical isolates were opportunistic pathogens or contaminants (Beesley et al. 2010). No information was identified implicating B. megaterium strain ATCC 14581 in adverse human health effects.

A strain of B. megaterium was associated with pyrexia and sepsis in the patient from which it was isolated (Rowan et al. 2001). In a study of 89 Bacillus species isolated from blood cultures associated with significant bacteremia, 13 were B. megaterium (Weber et al. 1988). B. megaterium was isolated from the infected eye in one case of lamellar keratitis 2 weeks after eye surgery (Ramos-Esteban et al. 2006). B. megaterium was also implicated in the infection of a dental crown (Subbiya and Mahalakshmi, 2012) that was thought to be related to its ability to secrete collagenase.

In clinical isolates, B. megaterium has been mistaken for B. anthracis. In one case, a non-hemolytic, non-motile Bacillus isolated from a blood culture, suspected to be B. anthracis, was in fact B. megaterium (Dib et al. 2003). In another case a B. megaterium skin infection was mistaken for cutaneous anthrax, in a young, immunocompetent patient (Duncan and Smith 2011). The infection was treated successfully with ciprofloxacin. B. megaterium cultures can be distinguished from B. anthracis based on characteristics discussed previously (1.1.1.1 Phenotypic identification and biochemical profile and 1.1.1.2 Molecular identification); however, standard clinical microbiological methods used for preliminary diagnosis may not differentiate between them (Beesley et al. 2010; Dib et al. 2003).

Bacillus species, including strains of B. megaterium, isolated from tobacco products have been implicated in infections, pulmonary inflammation and allergic sensitivities, and plasma exudation and tissue dysfunction in the mouth (Rooney et al. 2005; Rubinstein and Pedersen 2002).

1.2 Hazard severity

1.2.1 Environment

The environmental hazard potential of B. megaterium strain ATCC 14581 is assessed to be low because in spite of the ubiquity of B. megaterium, no adverse effects have been reported at the population level in the environment. B. megaterium can have both beneficial and adverse effects in terrestrial plants. In Canada, B. megaterium strain ATCC 14581, is not recognized as a plant pest and has been reported to act as a plant growth promoting rhizobacterium. No negative effects were reported in plants exposed to B. megaterium strain ATCC 14581 as observed in studies conducted by Environment and Climate Change Canada scientists. Although B. megaterium or its secondary metabolites can adversely affect some invertebrate species under biocontrol conditions, the DSL strain, B. megaterium strain ATCC 14581 did not cause effects when tested on the terrestrial invertebrate Folsomia candida. No adverse effects in aquatic plants,

invertebrates or vertebrates or terrestrial vertebrates have been reported. Growth promotion was observed in aquatic invertebrates (shrimp) and in aquatic vertebrates (fish) fed B. megaterium as a feed supplement. Although B. megaterium strain ATCC 14581 has been used in Canada for several decades, there are no reports in the literature implicating it in adverse environmental effects.

1.2.2 Human health

The human hazard potential of B. megaterium strain ATCC 14581 is assessed to be low because in spite of its widespread distribution in the environment, human infection with B. megaterium is very rarely reported. Adverse human health effects have not been attributed to B. megaterium strain ATCC 14581 and it has not been shown to carry enterotoxin genes which have occasionally been associated with other strains of B. megaterium. Antibiotic susceptibility testing performed by Health Canada scientists demonstrated that, in the unlikely event of infection, clinically relevant antibiotics are effective against this strain.

Hazards related to micro-organisms used in the workplace should be classified under the Workplace Hazardous Materials Information System (WHMIS)[7].

2. Exposure assessment

2.1 Sources of exposure

This assessment considers exposure to B. megaterium strain ATCC 14581 resulting from its deliberate addition to consumer or commercial products and its use in industrial processes in Canada.

B. megaterium strain ATCC 14581 was nominated to the DSL for use in consumer or commercial products.

Responses to a voluntary questionnaire sent in 2007 to a subset of biotechnology companies, combined with information obtained from other federal government regulatory and non-regulatory programs, indicate that B. megaterium strain ATCC 14581 was in commercial use in 2006.

The Government conducted a mandatory information-gathering survey under section 71 of CEPA, as published in the Canada Gazette, Part I, on October 3, 2009 (section 71 notice). The section 71 notice applied to any persons who, during the 2008 calendar year, manufactured or imported B. megaterium strain ATCC 14581, whether alone, in a mixture or in a product. Between 10,000 and 100,000 kg of products containing B.

[7] 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.

megaterium strain ATCC 14581 were imported into or manufactured in Canada in 2008 for a variety of uses in consumer, commercial and industrial applications.

B. megaterium strain ATCC 14581 is available for purchase from the ATCC. As it is on the DSL, and can be used in Canada without prior notification under CEPA, it could be an attractive choice for further commercialization. A search of the public domain (internet, patent databases, MSDS, etc.) revealed the following consumer, commercial and industrial applications of other strains of B. megaterium. These represent possible uses of the DSL strain, as B. megaterium strain ATCC 14581 is likely to share characteristics (modes of action) with other commercialized B. megaterium strains. Growth in the market for “greener” microbial-based products for both commercial and consumer applications may increase direct human exposure to B. megaterium strain ATCC 14581, which has potential applications in these products (Spök and Klade 2009).

- as a production or modifying organism for a variety of biochemicals and biopolymers (Kieselburg et al. 1984; Honorat et al. 1990; Shark et al. 1991; reviewed in Vary 1994; Xu et al. 1997; Schallmey et al. 2004; Biedendieck et al. 2007; Shimizu et al. 2007; Obruca et al. 2009; Muffler et al. 2011; Hastings et al. 2013; Mohammed et al. 2014);
- in biocontrol (Branly and Atkins 2001; Product sheet 2004; Chowdhary et al. 2007; Drahos and Petersen 2010);
- in biodegradation (Saxena et al. 1987; Quinn et al. 1989; Bianchi et al. 2008; Marecik et al. 2008; Singh et al. 2013);
- in bioremediation (Esringu et al. 2014);
- in wastewater treatment (Bianchi et al. 2007);
- in commercial cleaning and deodorizing products for use in drain and septic maintenance (Calfarme 2010; Vandini et al. 2014);
- in calcite precipitation to provide improved strength of fly ash-amended concrete (Achal and Pan 2011; Dhami et al. 2013; Soon et al. 2014);
- in bioleaching or “biohydrometallurgy” (Vasanthakumar et al. 2013);
- in the rapid biosynthesis of silver nanoparticles (Saravanan et al. 2011);
- as a bioindicator (Lillehoj and Ciegler 1970; Garvey et al. 2013; Verma et al. 2013);
- as an aquaculture feed additive (Aubin et al. 2006) and probiotic (Barbosa et al. 2005; Olmos et al. 2011; Parthasarathy and Ravi 2011; Yuniarti et al. 2013);
- in the production of food flavouring agents (Taupp et al. 2005); and
- as an adjuvant modulator of the immune system in mice (Ruiz-Bravo et al. 1981).

2.2 Exposure characterization

2.2.1 Environment

The environmental exposure to B. megaterium strain ATCC 14581 is expected to be medium based on the wide range of uses reported in response to the section 71 notice.

The magnitude of environmental exposure to B. megaterium strain ATCC 14581 will depend on the nature, quantity, duration and frequency of use and on its persistence and survival in the environment to which it is released. The concentration of vegetative cells of B. megaterium strain ATCC 14581 introduced into soil was demonstrated to decrease by two orders of magnitude within 105 days (Providenti et al. 2009). High numbers of vegetative cells are unlikely to be maintained in water or soil due to competition for nutrients (Leung et al. 1995) and microbiostasis, which is an inhibitory effect of soil that results in the rapid decline of populations of introduced bacteria (van Veen et al. 1997). Spores of B. megaterium strain ATCC 14581 are more resistant to harsh conditions compared to their vegetative counterparts. It is unlikely that populations of B. megaterium strain ATCC 14581 will be maintained as vegetative cells in soil and water since they have little competitive advantage over naturally-occurring populations and would be subject to predation and competition for nutrients with indigenous flora. Spores of B. megaterium strain ATCC 14581 are likely to persist and could accumulate in the environment.

Aquatic exposure to B. megaterium strain ATCC 14581 is expected to be greatest for organisms in the vicinity of direct application to aquatic ecosystems for water treatment. Aquatic species may also be exposed to B. megaterium strain ATCC 14581, from its introduction into the wastewater through use in consumer or commercial products and as a result of runoff from terrestrial applications.

Terrestrial exposure to B. megaterium strain ATCC 14581 is expected through agricultural applications, biodegradation and bioremediation and bioleaching, in the vicinity of treated sites.

Aquatic or terrestrial exposure to B. megaterium strain ATCC 14581 as a result of its release from facilities manufacturing enzymes or biochemicals is expected to be limited by the application of good manufacturing processes (for example, to be in conformity with municipal and provincial waste water regulations).

2.2.2 Human health

Human exposure to B. megaterium strain ATCC 14581 is expected to be medium based on the wide range of uses reported in the section 71 survey. Human exposure to B. megaterium strain ATCC 14581 is expected to be greatest through the direct use of consumer products containing spores or viable cells. Handling and application of such products would be expected to result in direct exposure of the skin and inhalation of aerosolized droplets or lofted spores. Inadvertent ingestion following use on or near food preparation surfaces and contact with the eyes are possible secondary routes of exposure. Growth in the market for “greener” microbial-based products may increase direct human exposure to B. megaterium strain ATCC 14581, which has potential applications in these products (Spök and Klade 2009).

Humans may also be exposed to B. megaterium strain ATCC 14581 as bystanders during the commercial application of cleaning, water treatment, agricultural or

biodegradation products. The extent of bystander exposure will depend on the mode of application, the volume applied and the proximity of bystanders to the site of application. In general, exposure is expected to be low for these applications.

Indirect human exposure to B. megaterium strain ATCC 14581 released into the environment subsequent to its use in water treatment, agricultural applications or biodegradation is also expected to occur in the vicinity of treated sites, but is expected to be less than direct exposure from the use of these organisms in consumer products. Human exposure to bodies of water treated with B. megaterium strain ATCC 14581 (e.g., through recreational activities), could result in exposure of the skin and eyes, as well as inadvertent ingestion; however, dilution of these products is expected to significantly reduce exposure relative to the use of consumer products. Human activity on soils recently treated with B. megaterium strain ATCC 14581 could loft spores, which could then be inhaled and could expose the skin and eyes, but this exposure is also expected to be low relative to direct use of consumer products.

In the event that spores of B. megaterium strain ATCC 14581 enter the source waters of municipal drinking water treatment systems through release from intended and potential uses, drinking water treatment processes (e.g., coagulation, flocculation, ozonation, filtration and chlorination) are expected to effectively eliminate these micro-organisms and so limit their ingestion.

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. megaterium strain ATCC 14581 to be low for the environment and low for human health. Environmental and human exposure to B. megaterium strain ATCC 14581 is estimated to be medium based on the wide range of uses reported for this strain, 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).

B. megaterium strain ATCC 14581 has properties that make it suitable for use in a range of products, and there is reason to expect new uses of B. megaterium strain ATCC 14581 will emerge. In particular, there is growth in the market for “greener” microbial-based cleaning products, that may increase human exposure to B. megaterium strain ATCC 14581 because of its potential application in these products (Spök and Klade 2009; Vandini et al. 2014). Nevertheless, the risk from foreseeable future uses is also expected to be low, given the low hazard associated with B. megaterium strain ATCC 14581.

4. Conclusion

Based on the information presented in this screening assessment, it is concluded that B. megaterium strain ATCC 14581 is 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 this substance does not meet the criteria as set out in section 64 of the CEPA.

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Appendices

Appendix A: Metabolism by B. megaterium strain ATCC 14581

Table A-1: Metabolic tests for B. megaterium strain ATCC 14581

Test	Result
Catalase	Positive
Oxidase	Positive
Motility visual	Most often negative, but motility was observed[a]
Casein hydrolysis	Positive
Gelatin hydrolysis	Positive
Nitrate reduction test	Gas (negative), nitrate to nitrite (negative)
Starch hydrolysis	Positive
Urea hydrolysis	Positive (48 hours)
Egg yolk reaction	Negative
Acid from mannitol and maltose	Weak at 48 hours, positive after 7 days

Data generated by Environmental Health Science and Research Bureau, Health Canada

[a] Cells were motile in fetal bovine serum at 37°C; however, the bacterium was non-motile within minutes of removal from the incubator

Appendix B: Characteristics of B. megaterium – Fatty acid methyl ester (FAME) analysis

Table B-1: MIDI identification of B. megaterium strain ATCC 14581

Context	Frequency	Similarity index	First Choice
Environment context	16/16	0.816	Bacillus-megaterium-GC subgroup A
Clinical context	10/10	0.884	Bacillus-megaterium

Data generated by Environmental Health Science and Research Bureau, Health Canada shows the best match between the sample and the environmental and clinical MIDI databases and the fatty acid profile similarity index (average of all matches) along with the number of matches (number of matches/total number of tests, parentheses). For methods and additional details, see MIDI labs.

Appendix C: Growth characteristics of B. megaterium strain ATCC 14581

Table C-1: Optical density (500 nm) of B. megaterium strain ATCC 14581 after growth in liquid media for 24 hours at varying temperatures

Liquid media	28°C	32°C	37°C	42°C
TSB[a]	0.26	0.20	0.23	0.12[b]
10% FBS[c]	0.16	0.14[d]	0.14[d]	0.09[d]
100% FBS	0.03[b]	0.03	0.07[e]	0.10
10% sheep serum	0.01[f]	0.02	0.02	0.10
100% sheep serum	0.02	0.01	0.03	0.11[g]
DMEM[h] w FBS and glutamine	0.00	0.00	0.04[e]	0.07[i]

Data generated by Environmental Health Science and Research Bureau, Health Canada

[a] TSB, tryptic soy broth

[b] Long chains observed by microscopy

[c] FBS, fetal bovine serum

[d] Substantial bacilli observed by microscopy

[e] Spores/debris observed by microscopy

[f] Spores observed by microscopy

[g] Lysed bacilli observed by microscopy

[h] DMEM, Dulbecco's Modified Eagle's Medium

[i] Appears to be fewer than time=0 min (by microscopy)

Appendix D: Antibiotic susceptibility profiles of B. megaterium strains reported in the literature

Table D-1: Antibiotic susceptibility profile of B. megaterium, B. cereus and B. subtilis determined by mean zone of inhibition (mm)

Antibiotic[a]	Amount of antibiotic per disk	B. megaterium[b] (mm)	B. cereus[b] (mm)	B. subtilis[b] (mm)
Oleandomycin	15 µg	15 ± 3	17 ± 2	20 ± 1
Oxacillin	10 µg	22 ± 2	7 ± 1	27 ± 2
Chloramphenicol	30 µg	22 ± 3	21 ± 3	25 ± 2
Ampicillin	10 µg	21 ± 3	7 ± 1	21 ± 2
Carbenicillin	25 µg	24 ± 2	7 ± 1	23 ± 2
Ristomycin	30 µg	16 ± 2	16 ± 1	18 ± 2
Tetracycline	30 µg	23 ± 3	17 ± 4	17 ± 3
Benzylopenicillin	10 units	23 ± 2	7 ± 1	19 ± 3

[a] Breakpoints for susceptibility cannot be determined using the disk diffusion method because limited data currently exist for this genus (CLSI 2013)
[b] Results of 10 strains of B. megaterium, 10 strains of B. cereus and 30 strains of B. subtilis (Reva et al. 1995)

Table D-2: Antibiotic susceptibility profile of strains of B. megaterium (Bm), B. cereus (Bc) and B. subtilis (Bs) determined by zone of inhibition (mm)[a]

Strain	Erythromycin 15 µg/disc (mm)	Ampicillin 10 µg/disc (mm)	Oxytetracyclin 30 µg/disc (mm)	Carbenicillin 100 µg/disc (mm)
Bm McR-8	24 (S)	16 (S)	27 (S)	16 (S)
Bm ZmR-3	25 (S)	17 (S)	28 (S)	17 (S)
Bm ZmR-4	25 (S)	21 (S)	29 (S)	19 (S)
Bm ZmR-6	23 (S)	17 (S)	26 (S)	16 (S)
Bm OsR-3	25 (S)	20 (S)	28 (S)	19 (S)
Bc McR-3	25 (S)	0 (R)	19 (S)	0 (R)
Bs McR07	22 (I)	25 (S)	11 (R)	20 (S)

S indicates susceptible; I indicates intermediate; R indicates resistant
[a] Data and interpretation of antibiotic susceptibility from Sadiq and Ali (2013)

Table D-3: Antibiotic susceptibility profile of B. megaterium (Bm) and B. subtilis (Bs) strains determined by MIC (mg/L) and interpretation of results[a]

Antibiotic	Breakpoint values[b] (<S, >R)	Bm 15538	Bm 15545	Bs 15511	Bs 15514	Bs 15541	Bs 15549
Gentamicin	4	0.5 (S)	0.5 (S)	1 (S)	0.5 (S)	0.5 (S)	0.5 (S)
Kanamycin	8	2 (S)	2 (S)	2 (S)	2 (S)	2 (S)	2 (S)
Streptomycin	8	1 (S)	1 (S)	4 (S)	16 (R)	8 (I)	64 (R)
Tetracycline	8	1 (S)	0.5 (S)	0.25 (S)	0.25 (S)	4 (S)	4 (S)
Erythromycin	4	0.25 (S)	0.25 (S)	0.25 (S)	0.25 (S)	0.25 (S)	0.25 (S)
Clindamycin	4	>16 (R)	>16 (R)	1 (S)	2 (S)	1 (S)	2 (S)
Chloramphenicol	8	4 (S)	16 (R)	2 (S)	4 (S)	4 (S)	4 (S)
Ampicillin	NR	0.25	0.25	0.03	0.03	0.03	0.03
Vancomycin	4	0.25 (S)	0.25 (S)	1 (S)	1 (S)	0.5 (S)	0.5 (S)

S indicates susceptible; I indicates intermediate susceptibility; R indicates resistant: NR indicates, not required
[a] (Larsen et al. 2014)
[b] Strains with MIC values (mg/L) higher than the breakpoint are considered to be resistant (EFSA 2008)