Final Screening Assessment for

Paenibacillus polymyxa ATCC 842

Paenibacillus polymyxa ATCC 55407

Paenibacillus polymyxa 13540-4

Environment Canada

Health Canada

August 2015

Canada

Cat. No.: En14-230/2015E-PDF
ISBN: 978-0-660-02556-8

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Synopsis

Pursuant to paragraph 74*(b)* of the *Canadian Environmental Protection Act, 1999* (CEPA 1999), the Ministers of Environment and of Health have conducted a screening assessment on *Paenibacillus polymyxa* strains ATCC 842, ATCC 55407 and 13540-4.

P. polymyxa strains ATCC 842, ATCC 55407 and 13540-4 share characteristics with other *P. polymyxa* strains that occur in the environment. *P. polymyxa* is a facultatively anaerobic bacterium that is present in many environments. It has been isolated from soils, the rhizosphere and roots of plants and from marine sediments. *P. polymyxa* has a broad host range as a plant growth-promoting bacterium. It has characteristics that make it of potential use in biocontrol, plant growth promotion, in the production of enzymes and specialty chemicals, water and wastewater treatment, and cleaning and degreasing applications.

P. polymyxa is not known as an animal or plant pathogen. Release of these strains into the environment is not expected to adversely affect ecosystems.

P. polymyxa is not known as a human pathogen. Despite its ubiquity, there have been only two reported cases of *P. polymyxa* infection in humans, both involving individuals suffering from pre-existing health conditions. Of those cases, only one indicated *P. polymyxa* as the sole microorganism involved.

This assessment considers the aforementioned characteristics of *P. polymyxa* ATCC 842, ATCC 55407 and 13540-4 with respect to environmental and human health effects associated with product use and industrial processes subject to CEPA 1999, 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 1999 as published in the *Canada Gazette*, Part I, on October 3, 2009. Information submitted in response to the notice indicates that *P. polymyxa* ATCC 55407 was not imported into or manufactured in Canada in 2008, but that *P. polymyxa* ATCC 842 and 13540-4 are used in consumer and commercial products.

Considering all available lines of evidence presented in the Screening Assessment, there is a low risk of harm to organisms and the broader integrity of the environment from *P. polymyxa* ATCC 842, ATCC 55407 and 13540-4. It is concluded that *P. polymyxa* ATCC 842, ATCC 55407 and 13540-4 do not meet the criteria under paragraph 64(*a*) or (*b*) of CEPA 1999 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.

Also, based on the information presented in the Screening Assessment, it is concluded that *P. polymyxa* ATCC 842, ATCC 55407 and 13540-4 do not meet the criteria under paragraph 64(*c*) of CEPA 1999 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 1999), the Ministers of Environment and of Health are required to conduct screening assessments of living organisms listed on the *Domestic Substances List* (DSL) 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 1999).[1] *Paenibacillus polymyxa* ATCC strains 842 and 55407 and *Paenibacillus polymyxa* 13540-4 were nominated and added to the DSL under subsection 25(1) of CEPA 1988 because they were 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 unpublished research data, as well as comments from scientific peer reviewers. Exposure information was obtained from the public domain and from a mandatory CEPA 1999 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 *Framework on the Science-Based Risk Assessment of Micro-organisms* under the *Canadian Environmental Protection Act, 1999*.

Data that are specific only to the two non-masked DSL-listed strains, *P. polymyxa* ATCC 842 and ATCC 55407, are identified as such. Any information specific to the name, use and manufacture or import quantity of the masked strain has been concealed at the request of the nominator, pursuant to the *Masked Name Regulations* of CEPA 1999, and cannot be disclosed. Surrogate organisms are identified to the taxonomic level provided by the source. Literature searches were conducted using scientific literature databases (SCOPUS, CAB Abstracts, and NCBI), web searches, and key search terms for the identification of human health and environmental hazards of each of the DSL strains assessed in this report. Information identified as of January 2014 was considered for inclusion in this report.

[1] A determination of whether one or more criteria of section 64 of CEPA 1999 are met is based upon 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 1999 may not be relevant to, nor does it preclude, an assessment against the criteria specified in the *Controlled Products Regulations* or 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..

Decisions from Domestic and International Jurisdictions

Domestic

P. polymyxa is not subject to any plant or animal health requirements according to Invasive Alien Species & Domestic Programs at the Canadian Food Inspection Agency (CFIA). This organism does not require an import permit under the *Plant Protection Act.*

P. polymyxa is considered a Risk Group 1 organism for humans and terrestrial animals according to the Public Health Agency of Canada (PHAC). PHAC does not require an import permit for this micro-organism.

International

P. polymyxa is listed in the United States Toxic Substances Control Act (TSCA) Chemical Substance Inventory under CAS RN number 68038-68-6 (EPA 2011; EPA 1994).

Other *P. polymyxa* strains are used in three microbial pesticide products: Hydroguard, used to suppress and resist damping-off (bacterial) diseases, is registered in the United States; and Topseed and NH, both used to control damping-off powdery mildew in cucumber, are registered in Korea (Kabaluk and Gazdik 2005).

The U.S. Food and Drug Administration (U.S. FDA) published import alerts for cosmetics products contaminated with *P. polymyxa* (U.S. Food and Drug Administration 2013).

1. Hazard Assessment

1.1 Characterization of *Paenibacillus polymyxa*

1.1.1 Taxonomic identification and strain history

Binomial name: *Paenibacillus polymyxa* (Prazmowski 1880) (Ash et al. 1994)

Taxonomic designation:

Kingdom Bacteria
Phylum Firmicutes
Class Bacilli
Order Bacillales
Family *Paenibacillaceae*
Genus *Paenibacillus*
Species *polymyxa*
Strains

- ATCC 842
- ATCC 55407
- 13540-4

Superseded names: *Bacillus polymyxa* (Prazmowski 1880) Mace 1889 (Approved Lists 1980); *Aerobacillus polymyxa* (Prazmowski 1880) Donker 1926; *Granulobacter polymyxa* (Prazmowski 1880) Beijerinck 1893; *Clostridium polymyxa* (Prazmowski 1880)

1.1.1.1 Strain history

P. polymyxa ATCC 842 was isolated by Prazmowski in 1880 (Smith et al. 1964). It was originally deposited by A. J. Kluyver to the American Type Culture Collection (ATCC) and then, through a chain of changing custody, was eventually deposited to the Belgian Co-Ordinated Collections of Micro-Organisms (BCCM) by Nolan in 1993. There, it was given the accession number LMG 13294 (BCCM 2013). *P. polymyxa* ATCC 842 is the type strain of the species and has been deposited in a number of other culture collections, as listed in Table 1-1.

Table 1-1: Listing of current strain designations for *P. polymyxa* ATCC 842

Culture Collection	Strain Designations
DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany	DSM 36^T
Biologicky Ustav, Czech Akademie Ved, Prague, Czech Republic	BUCSAV 162
Czech Collection of Microorganisms, Masaryk University, Brno, Czech Republic	CCM 1459
Japan Collection of Microorganisms, RIKEN BioResource Center, Japan	JCM 2507
Laboratorium voor Microbiologie, Universiteit Gent, Gent, Belgium	LMG 13294
National Collection of Industrial Bacteria, Torry Research Station, Aberdeen, Scotland, UK (incorporated with NCIMB)	NCIB 8158
National Collection of Type Cultures, Central Public Laboratory Service, London, UK	NCTC 10343
Korean Collection for Type Cultures, Genetic Resources Center, Korea Research Institute of Bioscience and Biotechnology, Taejon, Republic of Korea	KCTC 3858
Agricultural Research Service Culture Collection, National Center for Agricultural Utilization Research, U.S. Department of Agriculture, Peoria, IL, USA	NRRL B-4317

P. polymyxa ATCC 55407 was isolated from a barn in Dublin, Virginia (USA), and deposited to the ATCC by Sybron Biochemical Corporation. However, it is no longer available from the ATCC (ATCC 2012). *P. polymyxa* 13540-4 was isolated from spoiled starch; the name of this strain has been masked at the request of the nominator, pursuant to the *Masked Name Regulations* of CEPA 1999, and cannot be disclosed.

1.1.1.2 Phenotypic and molecular characteristics

Because of its rod-shaped cells, ability to form endospores, and other similarities to the genus *Bacillus*, *P. polymyxa* was originally assigned to that genus by Mace in 1889 (Montefusco et al. 1993). Later, Ash et al. (1991) grouped this organism into a phylogenetically distinct cluster, referred to as “group 3,” based on comparative analysis of 16S rRNA primary sequences of 1450 and 1490 nucleotides in length, corresponding to approximately 95% of the whole 16S ribosomal subunit (Ash et al. 1991). The organism was eventually reclassified into a separate family Paenibacilliaceae and its type genus *Paenibacillus* (Priest 2009). The genus *Paenibacillus* can be differentiated from members of the Bacillaceae by polymerase chain reaction (PCR) amplification of 16S rRNA gene fragments, using either the original diagnostic probe that led to reclassification of *P. polymyxa* and several other members into the genus *Paenibacillus* (Ash et al. 1993), or a highly specific detection primer PAEN515F that also successfully differentiated *Paenibacillus* (also including *P. polymyxa* ATCC 842) from other taxa belonging to Bacillaceae (Shida et al. 1997). The presence of anteiso-C15:0 as the major cellular fatty acid (36.9–81.0%) is also diagnostic of the genus (Shida et al. 1997).

Figure 6-1 (Appendix A) describes the phylogenetic relationships of *P. polymyxa* to most validly published *Paenibacillus* species based on the alignment of a 1320 nucleotide fragment of 16S ribosomal RNA gene sequences from representative strains. The DSL strains were not included among the representative strains sequenced.

P. polymyxa is a genetically diverse species exhibiting high levels of interstrain variability (Lal and Tabacchioni 2009; Nübel et al. 1996). Currently the genomes of ten *P. polymyxa* strains have been sequenced; they show a wide spectrum of sizes, with the largest and smallest genomes differing by 2.1 megabase pairs (NCBI Genome 2014), suggesting the possibility of future taxonomic reclassification of certain members of this species. Characteristics of the type strain, ATCC 842, are reported in Tables 1-2, 1-3, 1-4 and 1-5.

Table 1-2: Growth conditions of *P. polymyxa* ATCC 842

Characteristic	*P. polymyxa* ATCC 842	Reference
Optimum temperature	30ºC	BCCM 2013
Growth at 50ºC	None	Priest 2009
Optimum pH	7.0	Shida et al. 1997
Growth at pH 5.6	Positive	Shida et al. 1997
Growth in the presence of 5% NaCl	None	Shida et al. 1997

Table 1-3: Morphological Properties of *P. polymyxa* ATCC 842

Characteristic	*P. polymyxa* ATCC 842	Reference
Gram staining	Positive: can stain variably or negative	Priest 2009; Shida et al. 1997
Cell shape	Rod	Priest 2009
Cell size	0.5–0.8 × 2–5 µm	Priest 2009
Flagella	Peritrichous	Priest 2009; Shida et al. 1997
Motility	Motile	Priest 2009
Spore shape	Oval with thick walls and star cross-section,[a] swollen sporangia	Comas-Riu and Vives-Rego 2002; Shida et al. 1997
Fatty acid profile	$C_{15:0\ anteiso}$ at 62.9% and $C_{17:0\ anteiso}$ at 16.9%	Priest 2009; Shida et al. 1997

Characteristic	*P. polymyxa* ATCC 842	Reference
Colonies	Nutrient agar: Pale and thin, often with amoeboid spreading Glucose agar: usually heaped and mucoid with a matt surface	Priest 2009

a. Available information pertains to strain CECT 155, which is a derivative of *P. polymyxa* ATCC 842

Table 1-4: Biochemical properties of *P. polymyxa* ATCC 842

Characteristic	*P. polymyxa* ATCC 842	Reference
Respiration	Facultative anaerobe	Priest 2009
Metabolism	Organoheterotrophic: ferments glucose and variety of other carbohydrates	Priest 2009; Shida et al. 1997
Catalase	Positive	Priest 2009
Oxidase	Negative	Priest 2009
Nitrate reduction (to nitrite)	Positive	Priest 2009
Nitrogen fixation	Positive	Priest 2009
Secondary metabolites	Acetylmethyl-carbinol Indole Xylanase Levan (capsule component)	Lal and Tabacchioni 2009; Lal et al. 2012; Priest 2009; Shida et al. 1997; Tong et al. 2013
Antimicrobial production	Polymyxin-Colistitin-Circulin family[a] Fusaricidin-type compounds[a]	Raza et al. 2008
Biofilm	Positive[b]	Timmusk et al. 2005

a. Available information pertains to non-DSL strains
b. Available information pertains to strains B1 and B2

Table 1-5: Molecular properties of *P. polymyxa* ATCC 842

Characteristic	*P. polymyxa* ATCC 842	Reference
G+C Content (mol%)	44.9%	Jeong et al. 2011; Tong et al. 2013
Genome size (Mb)	5.9	Jeong et al. 2011; Tong et al. 2013
GenBank Accession no. (16S rRNA)	AJ320493	NCBI Nucleotide 2001; Priest 2009
GenBank Accession no. (Whole Genome)	AFOX01000000	NCBI Nucleotide 2014

1.1.2 Biological and ecological properties

P. polymyxa is a spore-forming bacterium (Priest 2009). As a facultative anaerobe, it can be active under anaerobic conditions and thrives in semi-anaerobic environments, and has a broad host range as a plant growth–promoting bacterium. *P. polymyxa* is present in many environments: it occurs naturally in marine sediments (Lal and Tabacchioni 2009), and has also been isolated from various soils and from the rhizosphere and roots of forest trees and crop plants, including cultivated olive trees in an organic orchard farm (Blibech 2012), wheat, barley, white clover, perennial ryegrass, crested wheatgrass, green bean, garlic (Raza et al. 2008), maize, sorghum and sugar cane (Lal and Tabacchioni 2009).

A soil persistence study was conducted by Providenti et al. (2009) in which closely related strains of *P. polymyxa* could be discriminated using quantitative PCR targeting strain-specific non-coding regions in the genome. The study showed that if released into sandy loam soil (pH 5.0, 22°C and 80% relative humidity) at initial densities of ~1 × 10^6 CFU/g soil, *P. polymyxa* strains NRRL B-4317 (ATCC 842), ATCC 55407 and 13540-4 would, within one to six months, decline to concentrations near or below the detection limit of ~1 × 10^2 CFU/g soil (Figure 1). Nine soil cores were prepared for each strain where only strains ATCC 55407 and 13540-4 were detected in at least one soil core beyond day 105. In a second experiment, strain NRRL B-4317 fell below the detection limit by day 14 post-inoculation (data not shown).

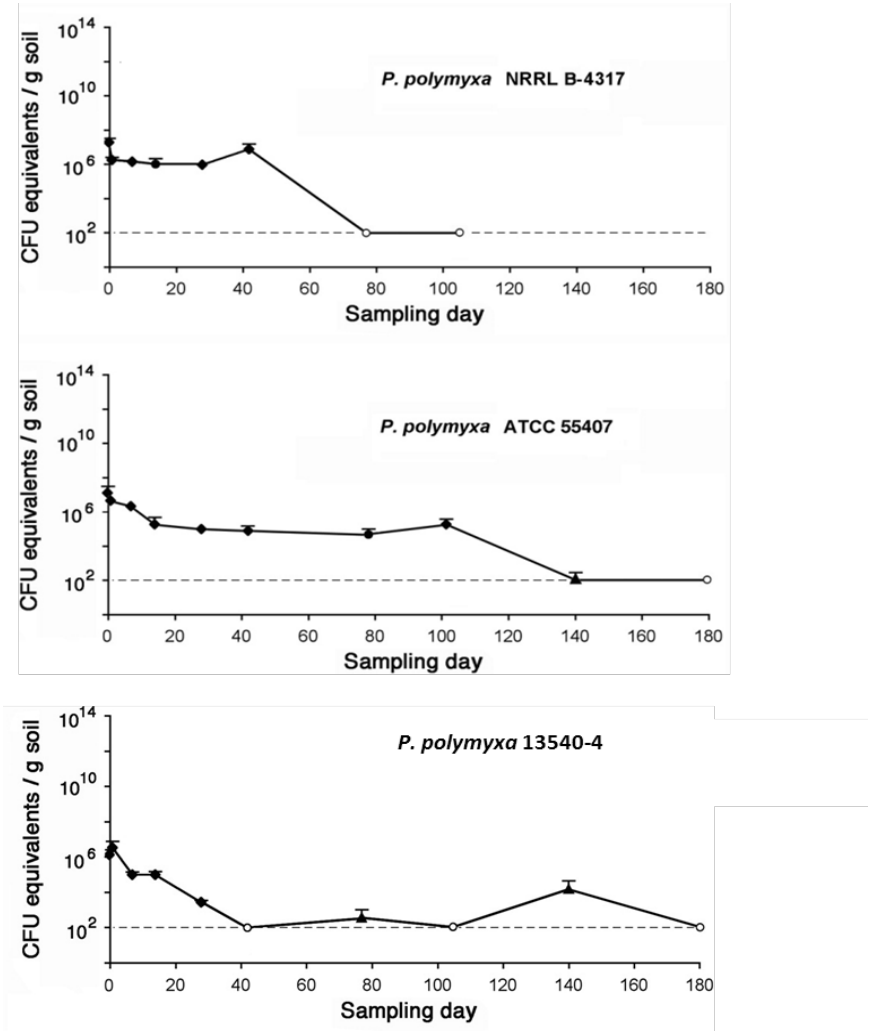

Figure 1-1: Persistence of DSL strains of *P. polymyxa* in soil, based on qPCR analyses of extractable soil DNA (taken from Can. J. Microbiol. 55, 1166-1175, with permission)

1.1.2.1 Plant growth promotion

P. polymyxa promotes plant growth by increasing nutrient availability (fixing nitrogen and solubilizing phosphorous), improving soil porosity, and producing a number of phytohormones that promote growth. *P. polymyxa* can fix atmospheric nitrogen under anaerobic conditions (Gaby and Buckley 2012). Soluble phosphorus is a limiting plant nutrient in soil (Hesham, A. E. and Hashem, M. 2011; Malboobi et al. 2009), and a high proportion of available phosphorus in chemical fertilizers becomes rapidly insoluble, and so unavailable to plants (Malboobi et al. 2009). *P. polymyxa* efficiently solubilizes inorganic phosphorus through the excretion of organic acids. Greenhouse experiments show that inoculating corn with *P. polymyxa* under different levels of phosphorus chemical fertilizer in calcareous soil caused significant increases in the shoot and root dry weights (28.53 g vs. 26.91 g [6.02% increase] and 2.29 g vs. 1.74 g [31.61% increase], respectively) as well as in phosphorus uptake in the shoots and roots of the plant (81.04 mg vs. 75.98 mg [6.67% increase] and 3.84 mg vs. 2.78 mg [38.13% increase], respectively) as compared to the uninoculated controls (Hesham, A. E. and Hashem, M. 2011). *P. polymyxa* can increase the mass of soil adhering to wheat roots by 57%. This mass increase is accompanied by a change to a more porous soil structure near the roots, which could improve water retention and nutrient transfer in the rhizosphere (Raza et al. 2008). The soil aggregation effect of *P. polymyxa* may be mediated by levan synthesis (Raza et al. 2008), as the ratio of root-adhering soil dry mass to root tissue dry mass was significantly higher for plants inoculated with a levan-producing wild-type strain CF43 than for plants inoculated with a levan-defective mutant strain SB03 (Bezzate et al. 2000).

Levan may also play a role in biofilm formation. *P. polymyxa* can form biofilms, which are microbial colonies encased in an adhesive material, usually polysaccharides, attached to a surface. Levan is the polysaccharide produced by *P. polymyxa* when grown on sucrose (Timmusk 2003). Following colonization of thale cress (*Arabidopsis thaliana*) roots with *P. polymyxa* B1::pCM20, the bacteria formed an intensive biofilm around the root tips of the plant. Several purposes have been suggested for *P. polymyxa*'s biofilm in relation to its role as a plant growth–promoting bacterium: (1) the possibility that the *P. polymyxa* biofilm formation on the roots coincides with the potential colonization sites of pathogens and thereby functions as a protective layer to prevent their access through competitive exclusion; (2) the protective layer might also contribute to the plant-enhanced drought tolerance; and (3) the damage caused by the biofilm at the root tip could in turn cause activation of defense genes against pathogens (Timmusk et al. 2005).

P. polymyxa produces a wide variety of phytohormones that help regulate plant growth and development. These include auxins, especially indole-3-acetic acid (IAA), and cytokinins, including *iso*-pentenyladenine (iP). Auxin production was evaluated in a study involving 68 strains of *P. polymyxa*, including the DSL strain ATCC 842 (DSM 36). All could produce IAA up to 17 µg/mL in the culture supernatant. The DSL strain ATCC 842 (DSM 36) was among those producing the lowest level of IAA at 1 µg/mL culture

supernatant (Da Mota et al. 2008). In a study involving stationary growth phase of *P. polymyxa* strain B2 isolated from wheat rhizosphere, *iso*-pentenyladenine and another unknown cytokinin-like compound were extracted from the growth medium at a concentration of 1.5 nM. Cytokinins may be active in very low quantities because of the very small volume of the micro-environments into which they are excreted and the co-presence of auxins in the rhizosphere that could act synergistically with the cytokinin (Timmusk et al. 1999). Volatile organic compounds, including acetoin and 2,3-butanediol produced by *P. polymyxa*, also promote plant growth (Ryu et al. 2003). *P. polymyxa* produces acetoin in the presence of oxygen, and accumulates 2,3-butanediol in significant quantities as a product of fermentation under intermediate oxygen concentrations (De Mas et al. 1988; Mankad and Nauman 1992).

P. polymyxa has the potential to be used in biosorption of certain heavy metals. *P. polymyxa* strain P13 has been described as an exopolysaccharide (EPS) producer: it was shown that 100 mL of a stationary phase P13 culture formed 27±4 mg and 15±4 mg EPS in Brain-Heart Infusion (BHI) medium containing 1 M NaCl and in control BHI medium without NaCl, respectively. Strain P13 exhibited significant biosorption capacity of industry-originated Cu(II). EPS production was associated with hyperosmotic stress by high salt concentrations of 1 M NaCl, leading to a significant increase in the biosorption capacity of whole cells (Acosta et al. 2005). The absorption of *P. polymyxa* cells or EPS production by these micro-organisms on the surface of several minerals has been reported as a method to selectively separate certain metal ions from binary mixtures. Bioflocculation of high-ash Indian coals using *P. polymyxa* showed a 60% decrease in ash, suggesting that selective flocculation of coal was possible (Lal and Tabacchioni 2009).

1.1.2.2 Biocontrol

P. polymyxa has biocontrol potential against insects and nematodes, as well as bacterial and fungal plant diseases.

It is thought that *P. polymyxa* can prevent the proliferation of the nematode *Meloidogyne javanica* by producing siderophores that bind most of the Fe (III) in the area around the plant root. The resulting lack of iron prevents the nematode from proliferating in the immediate vicinity of the roots (Siddiqui et al. 2007). Similarly, reports of adverse effects caused by two different strains of *P. polymyxa* against another nematodes (*Meloidogyne incognita*) were later reported by Siddiqui and Akhtar (2008), who studied protective effects of an unidentified strain in tomatoes, and also by Akhtar and Panwar (2013), who tested biocontrol properties of strain MTCC 122 in peas.

Exposure of the root-knot nematode *Meloidogyne incognita* to various concentrations (5–100%) of *P. polymyxa* GBR-I culture filtrate significantly reduced egg hatching of the nematode and caused substantial mortality of its juveniles. At higher concentrations of 25–100%, egg hatching was inhibited by 84–91% after 2 days of exposure as compared to sterile distilled water controls. Overall, the severity of effects was proportional to the

concentrations of the *P. polymyxa* culture filtrates and exposure durations. Those results were further confirmed by the application of a bacterial suspension of *P. polymyxa* GBR- I into the soil of potted tomato plants 3 days prior to inoculation of the nematode, which reduced nematode populations by 91.3% and root galling by 62.5%, compared with untreated controls (Khan et al. 2008).

In a greenhouse, tomato seedlings were challenged with *M. incognita* (3000 eggs). Compared with untreated controls, seedlings treated with *P. polymyxa* strain T79 (1.0×10^9 CFU per pot) showed a significant (P=0.05) reduction in per-gram root numbers of the second-stage juveniles, females, total nematodes, egg masses, and number of nematode eggs per egg mass (Liu et al. 2012).

In another greenhouse experiment involving different bacteria, inoculation of *M. incognita*–infested tomato plants (1000 fresh second-stage juveniles per plant) with *P. polymyxa* NFB7 (2×10^8 CFU per pot) after 60 days resulted in high reductions in nematode population compared with the uninoculated, nematode-infested controls, as follows: 95.8% for number of hatched juveniles/root, 85% for number of females per root, and 95.16% for number of juveniles per kilogram of soil (El-Hadad et al. 2011).

P. polymyxa also has biocontrol potential against the olive tree insect pests *Hylesinus oleiperda* and *Phloeotribus scarabaeoides*. In feeding assays, *P. polymyxa* isolates Pp10, Pp11, Pp12, Pp22 and Pp24, at an inoculation dose of 3×10^7 CFU per petri dish containing larvae, caused mortality of up to 55% in larvae of these coleopterans 7 days post-inoculation. Proteins found in *P. polymyxa* supernatants were toxic to *P. oleae* and *P. scarabaeoides* larvae, with LC50 values of 37.6 µg/mL and 12.4 µg/mL, respectively (Blibech et al. 2012). Similarly, in another study, Blibech (2012) found a different strain of *P. polymyxa* (strain I10) to be pathogenic to coleopteran insects. These results, taken together with the ability of *P. polymyxa* to survive in the olive tree roots, can be considered promising for biocontrol of these agricultural pests (Blibech 2012).

Other *Paenibacillus* species are also active against coleopteran larvae. *P. popilliae* and *P. lentimorbus* are pathogens of the Japanese beetle (*Popillia japonica*) larvae, and larvae of several related scarab beetles (white grubs). They cause Milky Spore Disease in the beetle larvae and so have potential use in biocontrol (Dingman 2008).

Antibiotic production is a frequently encountered but non-uniform characteristic of *P. polymyxa* (Beatty and Jensen 2002), which may contribute to biocontrol of bacterial and fungal plant diseases. It is known to produce two families of chromosomally encoded peptide antibiotics (Kim et al. 2010a; Raza et al. 2008). In addition, there are many reports of antimicrobial and antifungal properties in which the nature of the inhibitory agent is undefined (Raza et al. 2008), and which require the presence of living bacteria for continuous suppression (Dijksterhuis et al. 1999). The Polymyxin-Colistin-Circulin family includes polymyxins (A, B_1, B_2, C, D, E_1, E_2, M, S_1 and T_1), polypeptins (A), polyxin, jolipeptin and saltavalin. Polymyxin B and Polymyxin E (Colistin) are used

clinically for their antibacterial activities against a wide variety of Gram-negative bacteria, including *Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp., *Pseudomonas aeruginosa* and *Acinetobacter* spp., *Salmonella* spp., some *Shigella* spp., *Pasteurella* spp., and *Haemophilus* spp. (Landman et al. 2008; Park et al. 2012).

However, a wide variety of Gram-negative bacteria are resistant to polymyxins (Landman et al. 2008). Polymyxins are cationic agents that bind to the anionic bacterial outer membrane and disrupt membrane integrity and permeability. They show a high affinity for the lipid moiety of lipopolysaccharide and can inactivate endotoxins (Landman et al. 2008; Raza et al. 2008) and inhibit cellular respiration (Raza et al. 2008). Because of the disruptive effect on membrane integrity, Gram-negative bacteria may become even more susceptible to hydrophobic antimicrobials (e.g. erythromycin) following exposure to polymyxins (Landman et al. 2008).

The Fusaricidin family includes Fusaricidins A, B, C and D (produced by *P. polymyxa* strains PKB1 and SQR-21), gavaserin, gatavalin (produced by *P. polymyxa* ssp. Colistinus koyama) and a series of peptides designated as LI-F03, LI-F04, LI-F05, LI-F07 and LIF08 (Beatty and Jensen 2002). The antimicrobial activity of the fusaricidins varies depending on the amino acids present at three variable positions in the peptide moiety (Li et al. 2007), but the family is less diverse than the Polymyxin-Colistin-Circulin group (Raza et al. 2008). Fusaricidins have an excellent antifungal activity against plant pathogenic fungi such as *Fusarium oxysporum*, *Aspergillus niger*, *Aspergillus oryzae* and *Penicillium thomii*. Fusaricidin B, in particular, has antagonistic activity against *Candida albicans* and *Saccharomyces cerevisiae*. Fusaricidins also have an excellent germicidal activity against Gram-positive bacteria such as *Staphylococcus aureus* and *Micrococcus luteus*. In addition, they have antifungal activity against *Leptosphaeria maculans*, which causes black root rot of canola (Choi et al. 2008). While fusaricidins are strongly active against fungi and Gram-positive bacteria, they have no activity even at 100 µg/mL against all Gram-negative bacteria tested (Kajimura and Kaneda 1997).

Lectins are glycoconjugate-binding proteins with some enzymatic activity that are widely distributed in nature (Lakhtin et al. 2011). Lectins I and II isolated from *P. polymyxa* 1460 were able to suppress the growth of *Rhizobium leguminosarum* 252 and *Bacillus subtilis* 36 at nearly all the concentrations tested (from 1–10 µg/mL). Lectin I was inhibitory to *Azospirillum brasilense* 245 and *Erwinia carotovora* subsp. *citrulis* 603, while lectin II exerted bactericidal activity against *Xanthomonas campestris* B-610 and B-611 and *A. brasilense* 245. The inhibitory activity of lectins is thought to be mediated by their interactions with the lectin-specific receptors occurring on the bacterial membrane, leading to conformational changes in the membrane and concurrent malfunction of the metabolism of bacterial cells (Karpunina et al. 2003).

P. polymyxa strains produce many hydrolytic enzymes, making them valuable antagonists to control plant pathogens (Raza et al. 2008).

Chitinase and β-1,3-glucanase were produced when different strains of *P. polymyxa*, including the DSL strain ATCC 842 (Mavingui and Heulin 1994), were grown in the

presence of colloidal chitin as the sole carbon source. Chitinases are thought to aid in the antagonistic effects of bacteria against fungal plant pathogens such as Basidiomycetes (Mavingui and Heulin 1994; Nielsen and Sørensen 1997). *P. polymyxa* strains CM5-5 and CM5-6 can produce cellulase and mannanase (Nielsen and Sørensen 1997). Two xylanases have been isolated from *P. polymyxa* strains CECT 153 and ATCC 842 (LMG 6319) (Morales et al. 1993); these enzymes are involved in degradation of cellulose and certain glycoproteins available in the cell walls of *Oomycetes* (Nielsen and Sørensen 1997), and therefore have potential in biocontrol.

In addition, certain *P. polymyxa* strains isolated from poultry production environments (i.e. NRRL B-30507, NRRL B-30509 and NRRL B-30508) were shown to produce bacteriocins with inhibitory action against *Campylobacter jejuni* (Svetoch et al. 2005). This finding suggests a potential application for these organisms in the production of food bio-preservatives for the management of *Campylobacter* infection in poultry.

1.1.2.3 Probiotic Activity

P. polymyxa has been used in aquaculture due to its probiotic properties (reviewed in (Hong et al. 2005). Health-promoting effects were seen in fish-feeding trials involving blends of *P. polymyxa* with other bacteria. All studies showed significant positive effects in various health and growth parameters measured in larvae of Persian Sturgeon (*Acipencer percicus*) (Jafarian et al. 2009a) and Rainbow Trout (*Oncorhynchus mykiss*) (Jafarian et al. 2009b). No adverse effects were reported as a result of consumption of the probiotic formulations, in any of the studies discussed.

1.1.2.4 Spore formation

P. polymyxa forms ellipsoidal spores that distinctly swell the sporangium or mother cell (Lal and Tabacchioni 2009) and that have thick walls with a star-shaped cross-section (Comas-Riu and Vives-Rego 2002). Spores of *P. polymyxa* can resist harsh environmental conditions, including extremes of temperature, pH, high pressure, aridity, UV irradiation and chemical infiltration (reviewed in Huo et al. 2012). The spores remain in a dormant state for long periods of time and germinate when conditions are suitable for vegetative growth (Setlow 2003). In *P. polymyxa* SQR-21 (a well-recognized biocontrol agent), the sporulation temperature has been found to affect the resistance and germination properties of its spores. For example, spores prepared at a higher temperature of 37°C showed more heat resistance than those prepared at 25°C and 30°C. However, the germination rate was negatively correlated with the sporulation temperature (Huo et al. 2012).

1.1.2.5 Horizontal gene transfer

No plasmids have been identified in the DSL strains of *P. polymyxa*, and it is not known whether they possess other mobile genetic elements. However, two other strains carry large plasmids (Table 1-6) encoding a number of vital genes essential for metabolism.

The 510 115 base pair plasmid of strain SC2 contains essential genes involved in the metabolism of purines, pyrimidines and lipids, and genes for ribosomal proteins, translation elongation factors and DNA methyl transferases (Ma et al. 2011). The 366 576 base pair plasmid in strain M-1 contains essential genes encoding ribosomal proteins and genes involved in replication, DNA repair and methylation, transcription, translation initiation, metabolism of amino acids and carbohydrates, transport, and drug resistance (Niu et al. 2011).

Table 1-6: Sequenced genomes and plasmids of *P. polymyxa* strains

Strain/plasmid	Type	Size (Mb)	Guanine-cytosine content (%)	No. proteins encoded	GenBank Accession No.
SC2	Chr	5.73	45.2	5406	CP002213
pSC	Plsm	0.51	37.6	626	CP002214
M1	Chr	5.86	45.2	5061	HE577054
pPPM1a	Plsm	0.37	38.4	295	HE577055

In an experimental setting, the use of a previously described soil-isolated *P. polymyxa* bacteriophage IPy1 and *P. polymyxa* strain Loutit, which is a naturally compatible host for IPy1 (Seldin et al. 1984), was investigated as a host-vector system for molecular biology. The phage successfully mediated transduction of plasmid DNA into the bacterium. Transductions of that strain containing the antibiotic resistance-marked plasmids pC194, pBC16 and pRJ45 were detected at frequencies of 5×10^{-7} to 1.2×10^{-6} per plaque-forming unit (PFU). Rearrangements or deletions were not detected in these plasmids as a consequence of transduction. Plasmid pC194 was also transferred to different *P. polymyxa* strains by the IPy1 phage. Different fragments of phage IPy1 DNA cloned into plasmid pRJ45 resulted in an increase of frequency of transduction of the new plasmids (pRJI13, pRJI21 and pRJI41) into *P. polymyxa* strain Loutit at a frequency of 1.2 to 6.5×10^{-4} per PFU (Ferreira et al. 1999).

1.1.2.6 Pathogenic and toxigenic properties and antibiotic susceptibility

Literature searches did not identify virulence determinants or toxins associated with *P. polymyxa* that would allow it to infect aquatic or terrestrial plants or animals, or humans.

The only information available on antimicrobial susceptibility testing of *P. polymyxa* pertains to screening of the organism isolated from the blood culture of one of the two reported bacteremia cases, which indicated that *P. polymyxa* was resistant to penicillin but sensitive to cefazolin, vancomycin, chloramphenicol, tobramycin, gentamicin, ciprofloxacin and clindamycin (Galanos et al. 2003).

Although *P. polymyxa* could theoretically acquire virulence genes (including antibiotic resistance determinants) through horizontal transfer from other bacteria, the potential for this is no greater for the DSL strains than for other *P. polymyxa* strains that are naturally present in the environment.

1.1.3 Effects

1.1.3.1 Effects on the environment

Terrestrial plants

In spite of the well-accepted role of *P. polymyxa* as a plant growth–promoting bacterium, a few studies have shown that it can also act as a deleterious rhizobacterium under certain circumstances. Inoculation of thale cress (*Arabidopsis thaliana*) with *P. polymyxa* (in the absence of biotic or abiotic stress) resulted in a 30% reduction in plant growth, as well as a stunted root system, compared to non-inoculated plants (Timmusk and Wagner 1999; Timmusk 2003).

In another study involving colonization of roots of *Arabidopsis thaliana* ecotype C24 by *P. polymyxa* strain B1 (isolated from rhizosphere of wheat), the bacterium predominantly colonized the root tips, where it formed biofilms and behaved as a root-invading organism. This was evident by heavy accumulation of bacterial cells at 10^6 cells/g root weight and formation of a semi-transparent material suggestive of an extracellular matrix within only 2 hours of inoculation. Due to the tight cover that the abundant colonizing cells formed, initially it was difficult to assess the damage caused by the bacteria. Nevertheless, indications of damage at the epidermal root cap during 5 hours of incubation were evident in the images provided. During the longer inoculation period, a second biofilm zone formed in the root differentiation region, and the root became progressively more affected. At 24 hours, the root was even more heavily populated with the organism at 10^9 CFU/g root weight and was severely damaged (Timmusk et al. 2005). The authors, attempting to reconcile the deleterious and beneficial activities of *P. polymyxa* observed in the same plant host system, suggested (1) the possibility that the *P. polymyxa* biofilm formation on the roots coincides with the potential colonization sites of pathogens and thereby functions as a protective layer to prevent their access through competitive exclusion; (2) the protection layer might also contribute to the plant-enhanced drought tolerance; and (3) the damage caused by the biofilm at the root tip could in turn cause activation of defense genes against other pathogens (Timmusk et al. 2005).

P. polymyxa caused blight in tomato seedlings grown from seeds inoculated with a suspension of 1×10^6 CFU/mL *P. polymyxa* isolated from naturally diseased seedlings. Numerous necrotic flecks were observed on the stems, cotyledons, and, occasionally, the roots of seedlings. Similar results were observed following artificial inoculation of germ-free tomato seeds with *P. polymyxa* ATCC 842 and 8523 (Caruso et al. 1984).

Aquatic plants and algae

An unidentified *Paenibacillus* species was isolated from brown Irish edible seaweeds (*Himanthalia elongata*, *Laminaria sachharina* and *Laminaria digitata*), apparently as part

of the normal microflora. No associated adverse effects were reported (Gupta et al. 2010). A comprehensive scientific literature search did not reveal any further reports of *P. polymyxa* or other *Paenibacillus* species in association with aquatic plants.

Terrestrial invertebrates

P. polymyxa has been investigated for use as a biocontrol agent for its ability to adversely affect pest nematodes and insects. Outside of experimental challenge settings, adverse effects in invertebrates have not been reported in the literature. However, given data obtained from the challenge experiments, it is possible that such effects could be observed in some invertebrates at high *P. polymyxa* concentrations.

The genus *Paenibacillus* contains a number of entomopathogenic species, such as *P. larvae*, *P. alvei*, *P. popilliae* and *P. lentimorbus.*

P. larvae is the causative agent of American foulbrood, which is one of the most deleterious bacterial honey bee diseases, affecting the larval stages of bees. This disease kills the infected larvae and has the potential to destroy infected colonies (reviewed in Genersch 2008). Whole genome analysis of *P. larvae* revealed open reading frames very similar to those of (a) synthetases of the antibiotic plipastatin (that inhibits phospholipase A2); (b) a surfactin (with hemolytic activity); (c) putative type I polyketide synthetases whose products are secondary metabolites with potential antibiotic, immunosuppressant or toxic effects; and (d) homologues of a putative iron-siderophore ABC transporter, which enables iron uptake from the medium (Chan et al. 2011). In addition, *P. larvae* secretes highly active extracellular proteases during the process of infection, which facilitates invasion of the haemocoel and killing of the larva (Genersch 2008). In the phylogenetic analysis of the genus *Paenibacillus* (Appendix A), *P. polymyxa* clusters distantly from *P. larvae*.

P. alvei, an organism that can occur as a secondary invader of honey bees (*Apis mellifera*) during outbreaks of European foulbrood, is capable of producing signs in larvae that are similar to the signs produced by *P. larvae* (Djordjevic et al. 2000). Analysis of the *P. alvei* genome sequence revealed that this organism harbours genes to produce a variety of toxins, such as a putative mosquitocidal toxin as well as an insecticidal toxin complex that produces orally active toxins located on the surface of the organism. In addition, *P. alvei* has genes encoding chitinases (discussed in section 1.1.2.3) and hyaluronate lyases that are known virulence factors which damage eukaryotic connective tissues (Djukic et al. 2012). *P. polymyxa*, in its role as a plant growth–promoting bacterium, is known to stimulate plants to produce chitinase, which can protect them against some fungal pathogens. Only one study (Mavingui and Heulin 1994) has demonstrated chitinase activity in *P. polymyxa*. Nevertheless, despite *P. polymyxa*'s ability to produce chitinase, this organism has never been reported as a pathogen of honey bees. A comprehensive literature search did not find any reports on

P. polymyxa-specific hyaluronate lyases. In addition, the phylogenetic analysis of the genus *Paenibacillus* (Appendix A) clearly shows that *P. polymyxa* clusters distantly from *P. alvae*.

Aquatic invertebrates

As indicated earlier, different strains of *P. polymyxa* (including the DSL strain ATCC 842) are capable of producing chitinase (Mavingui and Heulin 1994), an enzyme that can degrade chitin, which is a major component of the exoskeleton of aquatic crustaceans. Accordingly, different chitinolytic prokaryotic and eukaryotic microbes are thought to be involved in the generation of shell disease and associated shell lesions in crustaceans (reviewed in Quinn et al. 2013). However, surveys of bacterial communities involved with shell lesions/disease in the American Lobster *(Homarus americanus)* or Edible Crab (*Cancer pagurus*) affected by that disease did not find *P. polymyxa* or other members of the *Paenibacillus* genus among the micro-organisms involved in those cases (Quinn et al. 2013; Vogan et al. 2002, respectively).

In addition, a comprehensive scientific literature search did not reveal any further reports involving *P. polymyxa* or other members of the *Paenibacillus* genus as a pathogen of aquatic invertebrates.

Aquatic vertebrates

There is evidence implicating *P. thiaminolyticus* as a source of thiaminase in aquatic and terrestrial animals. Under laboratory conditions, *P. thiaminolyticus*, which is found in viscera of certain prey fish, caused mortality when it was fed to Lake Trout. Thiaminase I, a vitamin B_1–degrading enzyme produced by *P. thiaminolyticus*, has been blamed for thiamine deficiency and related mortality in fish. However, further investigations showed no relationship between thiaminase activity and either the amount of *P. thiaminolyticus* thiaminase I protein or the abundance of *P. thiaminolyticus* cells measured in multiple trophic levels of Great Lakes food webs (fish, zooplankton, and dreissenid mussels). Therefore, it was concluded that *P. thiaminolyticus* was not the primary source of thiaminase activity affecting Great Lakes salmonides (Richter et al. 2012). Phylogenetically, *P. thiaminolyticus* is most closely related to *P. popillae*, which causes disease in the Japanese Beetle (Iiyama et al. 2013), and which clusters distantly from *P. polymyxa*.

In addition, there are feeding studies where *P. polymyxa* (alone or blended with other organisms) was investigated for its probiotic potential in different species of fish, as discussed in Section 1.1.2.3 (Jafarian et al. 2009a, Jafarian et al. 2009b). No associated adverse effects were reported as a result of consumption of the probiotic formulations, in any of the studies reviewed.

Terrestrial vertebrates

A *Paenibacillus* species was cultured from the blood of a Friesian dairy cow showing signs consistent with bacterial endocarditis. The source of infection was suggested as accidental ingestion of a contaminated metal object and its penetration into the wall of the reticulum (Watts and Tulley 2013). The isolated *Paenibacillus* species was not adequately identified in the report to determine the extent of its relatedness to the DSL strains assessed here.

1.1.3.2 Effects on human health

Thus far, there are only two reports of *P. polymyxa* associated with human infection, and in only one case was *P. polymyxa* the only isolated organism. One case of bacteraemia due to *P. polymyxa* involved a 93-year-old woman hospitalized for a cerebral infarction. The authors speculated that *P. polymyxa* might have entered her circulation through broken skin on her hands while gardening (Nasu et al. 2003). Another case of recurrent bacteremia involving an unspecified strain of *P. polymyxa* and two other bacillus species were reported in an 18-year-old immune-competent female patient with an underlying mental illness (Munchausen syndrome) involving at least one prior episode of self-injection of soil. The source of the organisms was postulated to be self-injection of a polymicrobial product containing *Bacillus* spores, given the patient's past history of psychiatric illness and self-destructive behavior (Galanos et al. 2003).

Other *Paenibacillus* species occasionally cause disease in humans or are isolated from clinical samples. *P. alvei* is the species most commonly reported in association with human infection. It has been isolated from samples of cerebrospinal fluid, blood and pleural fluid, and on a foreign body extracted from the eye (Ouyang et al. 2008). In additional cases of infection attributed to *P. alvei* and *P. macerans*, the taxonomic identity of the causative agent was not definitive. These include cases of endophthalmitis, meningitis, prosthetic hip infections, wound infections and catheter-associated infections (Kim et al. 2010).

Table 1-7: Human case reports involving other *Paenibacillus* species

Case Description	Associated Bacteria	Clinical Details/Outcome	References
Bacteremia in a patient with multiple medical problems and an indwelling catheter	*P. thiaminolyticus*	Intravenous antibiotic therapy (pipericillin and an aminoglycoside) effectively controlled the infection.	Ouyang et al. 2008

Case Description	Associated Bacteria	Clinical Details/Outcome	References
Bacteremia involving five injection drug users	*P. larvae*	All but one recovered either with no treatment or treated with β-lactam antibiotics.	Rieg et al. 2010
Brain abscess following a periorbital injury	*P. macerans* and a *Clostridium* species	Antimicrobial therapy was ineffective and patient died.	Bert et al. 1995
Endocarditis with recent history of tympanoplasty	*P. popilliae*	The infection was controlled by intravenous penicillin G therapy.	Wu et al. 1999
Cardiac device associated endocarditis	*P. glucanolyticus*	The infection was controlled with trimethoprim-sulfamethoxazole and erythromycin.	Ferrand et al. 2013
The organism was isolated from sputum of a patient with pulmonary disease	*P. sputi*	Signs or symptoms of a serious infection were absent. The organism was susceptible to ampicillin, chloramphenicol, erythromycin, neomycin, penicillin G, rifampicin, streptomycin, tetracycline, trimethoprim/sulfamethoxazole and vancomycin, and resistant to kanamycin.	Kim et al. 2010
Secondary mediastinitis	*P. pasadenensis*	The patient presented signs of infection, e.g., malaise, fever and elevated inflammation markers as well as discharge of clear liquid from the sternal wound. Treatment with vancomycin, clindamycin and ciprofloxacin controlled the infection.	Anikpeh et al. 2010

The phylogenetic analysis of the genus *Paenibacillus* (Appendix A) clearly demonstrates that *P. polymyxa* clusters distantly from the other *Paenibacillus* species associated with human infection. All reported cases of *Paenibacillus* infection (including *P. polymyxa*) involved patients with pre-existing health conditions or broken skin, or who had undergone invasive medical procedures.

1.2 Hazard severity

There is little information in the literature to suggest that *P. polymyxa* is pathogenic to aquatic or terrestrial plants, vertebrates or invertebrates, in spite of its widespread distribution in the environment. No reports of animal or plant diseases are attributed to *P. polymyxa* strains ATCC 842, ATCC 55407 or 13540-4. As discussed previously in this document, *P. polymyxa* has promising plant growth–promoting/biocontrol properties, and appears to have a history of safe use based on the range of products known to be in commerce and on an absence of reports of adverse effects. A few experimental challenge studies have suggested some potential for adverse effects in plants when directly inoculated with high concentrations of *P. polymyxa* (~1 × 10^6 bacteria per mL) (Timmusk et al. 2005). Adverse effects in certain pest nematodes and insects were also observed at inocula of 10^7–10^9 CFU (Blibech et al. 2012; El-Hadad et al. 2011; Liu et al. 2012). This suggests that the effective dose for deleterious effects is likely to be high, and therefore the hazard posed to these target organisms can be considered low. In addition, *P. polymyxa* has been used as a probiotic in Persian Sturgeon and Rainbow Trout without negative effects reported.

As discussed earlier, certain other *Paenibacillus* species are implicated in disease among insects under natural conditions, but these are phylogenetically distinct from *P. polymyxa*. The environmental hazard severity for *P. polymyxa* strains ATCC 842, ATCC 55407 and 13540-4 is therefore estimated to be low.

To date, there are only two reports of human infection involving *P. polymyxa*, one involving self-injection of a polymicrobial product containing *P. polymyxa* and the other involving a serious comorbidity (Galanos et al. 2003; Nasu et al. 2003). Taken together with the ubiquity of this organism, the significance of these two cases appears to be negligible. Case reports of other *Paenibacillus* species isolated from blood or bodily fluids involved species thought not to be closely related to *P. polymyxa*. These cases were also associated with pre-existing health conditions, invasive medical procedures (Ouyang et al. 2008), or significant breaches in normal barriers to infection (Anikpeh et al. 2010; Galanos et al. 2003; Nasu et al. 2003). Furthermore, the implicated species are phylogenetically distinct from *P. polymyxa*. In the unlikely event of *P. polymyxa* infection, there are effective antibiotic treatments available (Galanos et al. 2003). The human hazard severity for *P. polymyxa* strains ATCC 842, ATCC 55407 and 13540-4 is estimated to be low.

Hazards related to micro-organisms used in the workplace should be classified under the Workplace Hazardous Materials Information System (WHMIS).[2]

2. Exposure Assessment

2.1 Sources of exposure

This assessment focuses on exposure to *P. polymyxa* ATCC 842, ATCC 55407 and 13540-4 from their deliberate addition to consumer or commercial products or their use in industrial processes.

P. polymyxa ATCC 842, ATCC 55407 and 13540-4 were nominated to the DSL in 1997 and 1998 because they were manufactured in or imported into Canada between January 1, 1984 and December 31, 1986. Strain ATCC 55407 was nominated for use in industrial applications. Uses of strains ATCC 842 and 13540-4 are kept confidential at the request of the nominators.

In 2007, a voluntary questionnaire was sent to a subset of key biotechnology companies in Canada. The responses, combined with information obtained from other federal government regulatory and non-regulatory programs, indicated that *P. polymyxa* 13540-4 was not used. However, survey responses indicated that up to 9400 kg of products potentially containing *P. polymyxa* ATCC 842 and up to a total of 6×10^{12} CFU *P. polymyxa* ATCC 55407 (the formulation and concentration were unknown in both cases), as well as small quantities of strain ATCC 55407 for research and development, were imported into or manufactured in Canada in 2006.

In 2009, the Government conducted a mandatory information-gathering Notice under section 71 of CEPA 1999 as published in the *Canada Gazette*, Part I, on October 3, 2009 (hereafter referred to as the s.71 Notice). The s.71 Notice applied to any persons who, during the 2008 calendar year, manufactured or imported *P. polymyxa* ATCC 842, ATCC 55407 or 13540-4, whether alone, in a mixture, or in a product. Multiple responses were received relating to the DSL-listed *P. polymyxa* strains, indicating that approximately 2000 kg of products containing ATCC 842, ATCC 55407 or 13540-4 were imported into or manufactured in Canada in 2008 for consumer and commercial uses.

[2] A determination of whether one or more criteria of section 64 of CEPA 1999 are met is based upon an assessment of potential risks to the environment and/or 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 1999, on *P. polymyxa* ATCC 842, ATCC 55407 and 13540-4, is not relevant to, nor does it preclude, an assessment against the hazard criteria for WHMIS that are specified in the *Controlled Products Regulations* or the *Hazardous Products Regulations* for products intended for workplace use.

In a follow-up to the s.71 Notice, the nominator confirmed that they no longer import *P. polymyxa* ATCC 55407. Furthermore, this strain is no longer available for purchase from the ATCC, reducing the likelihood of its use in future applications.

The DSL *P. polymyxa* strains have properties that make them of commercial interest in a variety of industries, particularly in bioremediation, water treatment and consumer products. The recent increase in the number of publications related to this organism may also be reflective of a growing commercial interest in *P. polymyxa*.

With the exception of biocontrol and plant growth promotion, *P. polymyxa* is primarily used as a production organism for a variety of enzymes and specialty chemicals:

- Production of the optically active (R,R) isomer of 2,3-butanediol (Ji et al. 2011; Yu et al. 2011), which is used as an antifreeze (Häßler et al. 2012).
- Production of acetoin, which is used as a food flavour (Zhang et al. 2012).
- Production of enzymes (e.g. 1,3-glucanases, cellulases, chitinases, proteases and xylanase) with applications in pre-treatment of feedstocks for biofuel production, bleaching of paper pulp (Kumar et al. 2012; Lan Pham et al. 1998; Morales et al. 1993), detergent formulation, the food industry, leather processing, chemical synthesis, waste management (Alvarez et al. 2006), and drain opener formulations (Griffin et al. 1995 [U.S. Patent Application Number 870057]).

In other applications, *P. polymyxa* could be used alone or with other micro-organisms to colonize a particular environment and produce enzymes and other molecules in situ to perform a specific function. A search of the public domain identified the following ongoing consumer, commercial and industrial applications of other naturally occurring *P. polymyxa* strains:

- Wastewater treatment: industrial and municipal (Product Sheet A 2014)
- Control of grease build-up in sewer lines (Product Sheet B 2007)
- Water restoration, including aquariums, aquacultures and garden ponds (Material Safety Data Sheet B 2010)
- Odour eliminator for use in hospitals, nursing homes, schools, prisons, apartment houses, funeral homes, kennels, medical clinics (for grease traps), drains, downpipes, portable toilets, septic tanks, RVs, marinas, sump pumps, wet wells, garbage cans, waste receptacles, locker rooms and carpets (Material Safety Data Sheet A 2003)
- Drain cleaner (Material Safety Data Sheet C 2004)
- For use in performance testing of media, stains, reagents and identification kits, and for the evaluation of bacteriological procedures (Product Sheet C 2014)
- For use as a feed probiotic in aquaculture (Product Sheet D 2014)

2.2 Exposure characterization

Human or environmental exposure to *P. polymyxa* ATCC 55407 is not currently expected, based on responses to the s. 71 Notice (which indicates that the strain was not used in consumer or commercial products or for industrial processes in Canada in 2008), on confirmation from the nominator that they no longer import this strain into Canada, and on its being unavailable for purchase from the ATCC. However, human and environmental exposure to *P. polymyxa* strains ATCC 842 and 13540-4 is estimated to be medium, because imported products were reported in the s.71 survey.

Given the range and scale of known and potential applications of the species, human and environmental exposure scenarios arising from potential future uses of the DSL *P. polymyxa* strains, which could increase exposure, have been considered along with persistence and survival properties of these micro-organisms.

2.2.1 Environment

Environmental exposure scenarios based on known uses of DSL-listed and other *P. polymyxa* strains and on probable future uses described in Section 2.1 indicate that sources of exposure are likely to introduce *P. polymyxa* ATCC 842 and 13540-4 into aquatic and terrestrial ecosystems.

The magnitude of plant and animal exposure to *P. polymyxa* ATCC 842 and 13540-4 will depend on their persistence and survival in the environment. *P. polymyxa* is an endospore-forming facultative anaerobe, capable of thriving in environments with limited oxygen availability. This explains its ubiquity in soils and in the rhizosphere of various plants as well as in deeper sub-surface zones in marine sediments. Its spores can remain in a dormant state for long periods, being resistant to many environmental stressors. Under favourable conditions the spores germinate and produce vegetative cells. As discussed earlier, a soil persistence study (Providenti et al. 2009) showed that, within one to six months in terrestrial systems, *P. polymyxa* strains NRRL B-4317 (ATCC 842) and 13540-4 decline from initial densities of ~1×10^6 CFU/g soil to concentrations near or below the detection limit of ~1×10^2 CFU/g soil. This rapid decline of *P. polymyxa's* population size, following its introduction into the soil, could be due to the common phenomenon of soil microbiostasis, which is defined as the growth or survival-inhibitory effect of soil that is attributed to the scarcity of available nutrient sources for microbes in soil, and to the hostility of the soil environment to incoming microbes due to a myriad of adverse abiotic and biotic factors (van Veen et al. 1997). Although this evidence suggests that *P. polymyxa* strains introduced to soil and water will likely decrease to background levels over time, under sub-optimal conditions the spores of *P. polymyxa* strains are likely to persist and could accumulate in the environment. Should *P. polymyxa* ATCC 55407 become available in Canada, its exposure scenarios are expected to be similar to *P. polymyxa* ATCC 842 and 13540-4.

2.2.2 Human

Human exposure is expected primarily through direct contact with consumer products containing *P. polymyxa* ATCC 842 or 13540-4. Skin and eye contact, and inhalation of aerosolized droplets or particles containing the strains, are likely routes of direct exposure.

Secondary to product application, residual *P. polymyxa* ATCC 842 or 13540-4 on surfaces and in reservoirs such as treated drains could result in dermal exposure, as well as exposure through inadvertent ingestion where the organism persists on food preparation surfaces and through inhalation where aerosols are generated (e.g. from kitchen garbage disposal units). Since *P. polymyxa* is expected to persist in certain sites (such as drains) following application, such exposures may be temporally distant from the time of application.

Should commercial products containing *P. polymyxa* ATCC 55407 become available in Canada, the general population could be exposed as bystanders during commercial product application. 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, but in general is expected to be low.

Indirect exposure to *P. polymyxa* ATCC 842 and 13540-4 in the environment subsequent to its use in water and wastewater treatment, water restoration of aquacultures and garden ponds, degreasing of sewer lines, lawn maintenance, or disposal of waste from its use in the production of enzymes is also likely to occur in the vicinity of application or disposal sites, but is expected to be no greater than direct exposure from the use of the organism in consumer products.

In the event that the organisms enter municipal drinking water treatment systems through release from potential uses, the water treatment process, which includes coagulation, flocculation, ozonation, filtration and chlorination, is expected to effectively eliminate these micro-organisms from drinking water.

Growth in the market for “greener” microbial-based products may increase human exposure to the DSL *P. polymyxa* strains, which have potential applications in these products. In the event that the potential consumer, commercial or industrial uses of *P. polymyxa* ATCC 842, ATCC 55407 and 13540-4 are realized, the human exposure scenarios described above can be expected, and could include direct and possibly repeated exposure to concentrated preparations of *P. polymyxa* ATCC 842, ATCC 55407 or 13540-4.

3. Risk Characterization

In this assessment, risk is characterized according to a paradigm embedded in section 64 of CEPA 1999 that 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 *P. polymyxa* ATCC 842, ATCC 55407 and 13540-4 to be low for the environment and low for human health. Environmental and human exposure to *P. polymyxa* ATCC 55407 from its 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. Human and environmental exposure to *P. polymyxa* strains ATCC 842 and 13540-4 is estimated to be medium because imported products containing these strains were reported in the s.71 survey, but, based on the low estimation of hazard, the risk associated from these two strains from current levels of exposure is estimated to be low.

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

P. polymyxa ATCC 842, ATCC 55407 and 13540-4 have useful properties that make them of interest for use in additional industrial processes or consumer or commercial products. In the event that these potential consumer, commercial or industrial uses of *P. polymyxa* ATCC 842, ATCC 55407 and 13540-4 are realized, the level of environmental and human exposure to these strains could increase. Nevertheless, the risk from foreseeable potential uses of *P. polymyxa* ATCC 842, ATCC 55407 and 13540-4 remains low, given that there is no evidence of effects on human health or adverse ecological effects at the population level for environmental species, in spite of widespread distribution of *P. polymyxa* in the environment and the history of industrial, environmental and commercial uses of strains ATCC 842, ATCC 55407 and 13540-4.

4. Conclusion

Based on the information presented in the Screening Assessment, it is concluded that *P. polymyxa* strains ATCC 842, ATCC 55407 and 13540-4 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;
- 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 these substances do not meet the criteria as set out in section 64 of CEPA 1999.

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6. Appendix: Phylogeny of the genus *Paenibacillus*

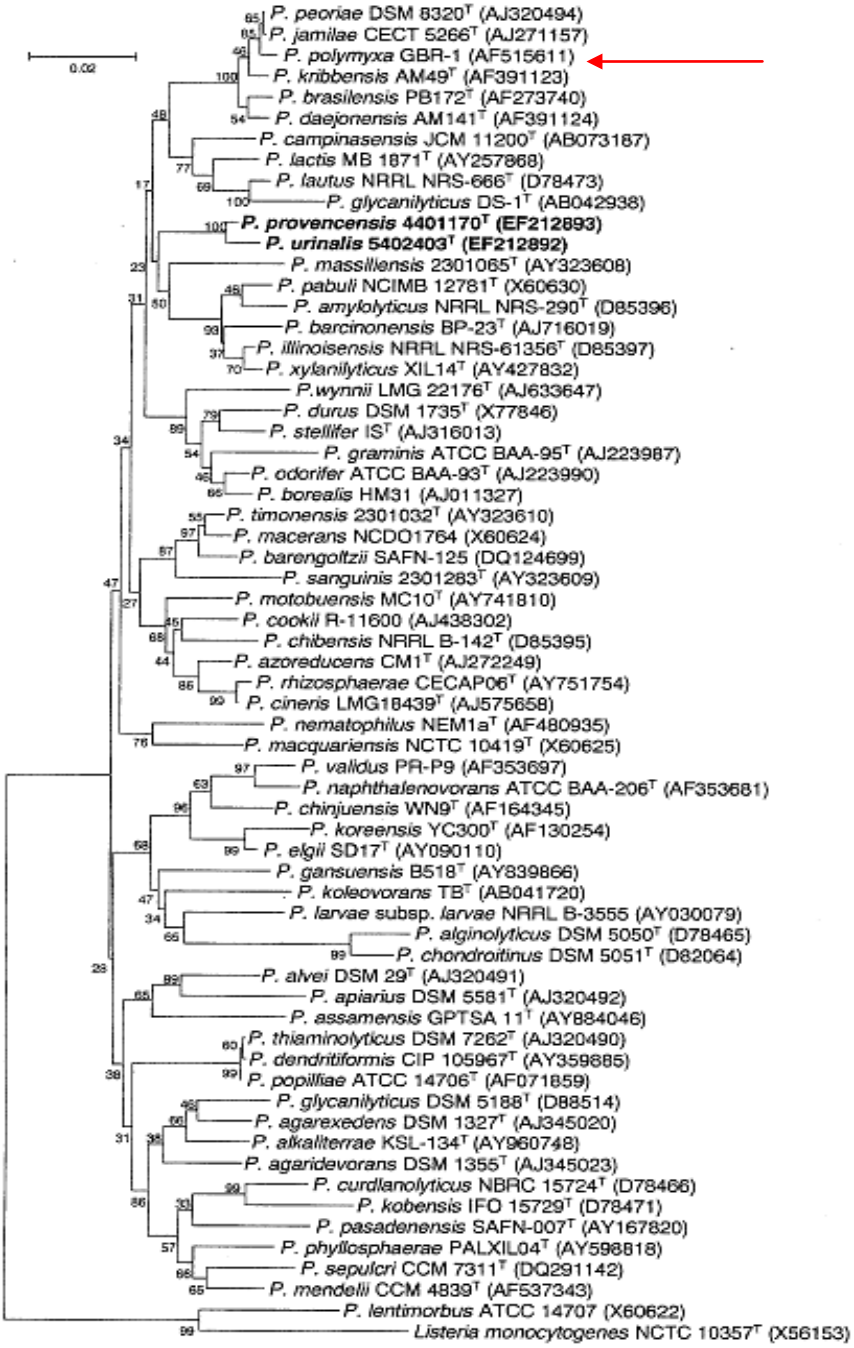

Figure 6-1: Extended phylogenetic tree of the genus *Paenibacillus* inferred from 16S rRNA gene sequence comparison - 1320 nt fragment (taken from Int. J. Syst. Evol. Microbiol. 58, 682-687, with permission)