Government of Canada / Gouvernement du Canada

Final Screening Assessment for

Pseudomonas putida ATCC 12633

Pseudomonas putida ATCC 31483

Pseudomonas putida ATCC 31800

Pseudomonas putida ATCC 700369

Environment Canada

Health Canada

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Canada

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 on four strains of *Pseudomonas putida (P. putida)* (ATCC 12633, ATCC 31483, ATCC 31800, ATCC 700369).

P. putida strains ATCC[1] 12633 (= type strain), ATCC 31483, ATCC 31800 and ATCC 700369 have characteristics in common with other strains of the same species. *P. putida* is a bacterium generally considered to have ubiquitous distribution in the environment that can adapt to varying conditions, and that thrives in soil, water and the rhizosphere of many plants. *P. putida* can also thrive in extreme and contaminated environments with low nutrient availability. Some members of the species *P. putida* are known to metabolize chemical compounds such as hydrocarbons and solvents. Furthermore, some strains can sequester and reduce heavy metals. These properties allow for potential uses of *P. putida* in bioremediation and biodegradation, waste water treatment, cleaning and degreasing products, and in production of enzymes and biochemicals used for industrial biocatalysis and in pharmaceuticals.

Despite its widespread presence in soil, water and rhizosphere ecosystems, *P*. *putida* has rarely been reported to cause adverse effects in aquatic or terrestrial plants or animals. Some *P*. *putida* infections have been reported in captive-bred fish, but rarely in wild fish populations. Overall, there is no evidence to suggest that *P. putida* has adverse ecological effects at the population level for vertebrates, invertebrates or plants. *P. putida* is considered a plant growth-promoting rhizobacterium, and some strains have anti-bacterial and anti-fungal properties, making the species of commercial interest in agriculture, and as a biocontrol agent against pest micro-organisms. Overall, there is no evidence specifically implicating the *Domestic Substances List* (DSL) strains of *P. putida* (ATCC 12633, ATCC 31483, ATCC 31800, and ATCC 700369) in adverse effects in the environment.

P. putida infrequently causes infection in healthy humans, but can act as an opportunistic pathogen in individuals predisposed to infection because of compromised immunity or debilitating disease. *P. putida* colonizes moist surfaces in hospitals, including medical devices and solutions, and can grow at temperatures typical of refrigerated storage. This characteristic has enabled it to proliferate in stored blood products and, in rare cases, cause sepsis in transfused patients. *P. putida* is resistant to some clinical antibiotics; however, a number of

[1] American Type Culture Collection

antibiotics are effective in treating *P. putida* infections. There have been no reported human infections attributed specifically to the DSL strains *P. putida* ATCC 12633, ATCC 31483, ATCC 31800, and ATCC 700369.

This assessment considers the aforementioned characteristics of *P. putida* strains ATCC 12633, ATCC 31483, ATCC 31800, and ATCC 700369 with respect to human health and environmental effects associated with product use and industrial processes subject to CEPA, including releases to the environment through waste streams and incidental human exposure through environmental media. To update information about current uses, 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 *P. putida* ATCC 12633, ATCC 31483, ATCC 31800 and ATCC 700369 were imported into or manufactured in Canada in 2008 for uses including wastewater treatment and bioremediation.

Based on the information available, it is concluded that *P. putida* ATCC 12633, ATCC 31483, ATCC 31800 and ATCC 700369 do not meet the criteria under paragraph 64(*a*) or (*b*) of CEPA as they are not entering the environment in a quantity or concentration or under conditions that have or may have an immediate or long-term harmful effect on the environment or its biological diversity or that constitute or may constitute a danger to the environment on which life depends. It is also concluded that *P. putida* ATCC 12633, ATCC 31483, ATCC 31800 and ATCC 700369 do not meet the criteria under paragraph 64(*c*) of CEPA as they are not entering the environment in a quantity or concentration or under conditions that constitute or may constitute a danger in Canada to human life or health.

Table of Contents

List of Tables

List of Figures

Introduction

Pursuant to paragraph 74*(b)* of the *Canadian Environmental Protection Act, 1999* (CEPA), the Minister of the Environment and 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).[2] These strains were added to the DSL under subsection 25(1) of CEPA 1988 and the DSL under subsection 105(1) of CEPA 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 from unpublished research data generated by Health Canada[3] and Environment Canada[4] 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 entitled "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 *P. putida* ATCC 12633, 31483, 31800 or 700369 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, Google Scholar and NCBI PubMed), web searches, and key search terms for the

[2] 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.

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

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

identification of human health and environmental hazards. Information identified up to April 2014 was considered for inclusion in this screening assessment report.

Decisions from Domestic and International Jurisdictions

Domestic

The Public Health Agency of Canada (PHAC) assigned the species *P. putida* to 'Risk Group 1' (low individual risk, low community risk) for both humans and terrestrial animals (personal communication, PHAC 2014). *P. putida* is not considered to be a plant pest in Canada by the Canadian Food Inspection Agency (CFIA), but it is considered an aquatic pathogen (AQC-2 in vitro) (personal communication, CFIA 2014).

International

The United States Environmental Protection Agency (U.S. EPA) assessed 6 genetically modified strains of *P. putida* for environmental release under the *Toxic Substances Control Act.* Its determination that the proposed small scale field trials would not present an unreasonable risk of injury to health or the environment was supported in part by a finding that *P. putida,* as a species, is a "common, wide-spread, soil bacterium that is not pathogenic to plants or animals" (U.S. EPA, 2012 a, b, c, d).

No other international regulatory decisions were found regarding *P. putida*.[5]

[5]Government agencies and organizations searched include: the United States Environmental Protection Agency; United States Food and Drug Administration; United States Animal and Plant Health Inspection ServicesUnited States Department of Agriculture; American Biological Safety Association; World Health Organization; United States Centers for Disease Control; Biosecurity NZ; Australian Department of Health; European Food Safety Authority; European Centre for Disease Prevention and Control; and the Invasive Species Specialist Group

1. Hazard Assessment

1.1 Characterization of *Pseudomonas putida*

1.1.1 Taxonomic identification and strain history

Binomial name: *Pseudomonas putida*

Taxonomic designation:

Kingdom:	Bacteria
Phylum:	Proteobacteria
Class:	Gammaproteobacteria
Order:	Pseudomonadales
Family:	*Pseudomonadaceae*
Genus:	*Pseudomonas*
Species:	*putida* (Trevisan 1889) Migula 1895
DSL strains:	ATCC 12633 (type strain) ATCC 31483 ATCC 31800 ATCC 700369

Synonyms, common and superseded names:

The most common synonyms associated with the species *P. putida* include: *Pseudomonas ovalis* (Palleroni 2005); *Bacillus putidus* (Palleroni 2005); *Arthrobacter siderocapsulatus* (Chun et al. 2001); and *Pseudomonas barkeri* (DSMZ 2014).

Strain history

P. putida ATCC 12633, the type strain for the species, was originally isolated from soil by enrichment methods (Stanier 1947). The strain was deposited to ATCC as *Pseudomonas fluorescens* Migula by A.B. Pardee, who had obtained the strain from R.Y. Stanier (ATCC 2014). Other noted designations for this strain include: A.3.12, ATCC 23467, NCIB 9494, NCTC 10936, R.Y. Stanier 90, HUT 8100, NCIB 9494, CCUG 12690, CFBP 2066, CIP 52.191, DSM 291, DSM 50202 (historical number), HAMBI 7, ICPB 2963, LMG 2257, NCAIM B.01634, NCIB 9494, NCCB 68020, NCCB 72006, NCTC 10936 , and WDCM 00117 (ATCC 2014; NBRC 2013; StrainInfo 2014).

P. putida strain ATCC 31483 was isolated from a wastewater lagoon in South Carolina and deposited to the ATCC by Sybron Biochemical Corp. as *Pseudomonas fluorescens* Migula. Other designations for this strain include: 3P (ATCC 2014), BCRC 14347, and CCRC 14347 (StrainInfo 2014).

P. putida strain ATCC 31800 was isolated from wastewater from a textile chemical plant in Welford, South Carolina based on its ability to utilize phenol and deposited to the ATCC by Sybron Biochemical Corp. as *Pseudomonas putida* (Trevisan) Migula. Other designations for this strain include: CB 173 (ATCC 2014), BCRC 14365, and CCRC 14365 (StrainInfo 2014).

P. putida strain ATCC 700369 was isolated from soil and deposited to the ATCC by Sybron Chemicals, Inc. as *Pseudomonas putida* (Trevisan) Migula. This strain is also designated as HC 7219 (ATCC 2014).

1.1.2 Phenotypic and molecular characteristics

Members of the *Pseudomonas* genus are Gram negative, diverse, widely distributed and dominated by non-pathogenic, saprophytic colonizers of soil, water, and rhizosphere ecosystems and non-pathogenic commensal colonizers of healthy human skin (Cogen et al. 2008; Li et al. 2013). The genus *Pseudomonas* (*sensu lato*) historically included members from the alpha, beta, gamma-beta and gamma proteobacteria, many of which have been or are likely to be reclassified based on modern taxonomic methods. A subgroup of *Pseudomona*s species within the gamma proteobacteria are considered to represent the genus (*sensu stricto*). These include the *P. aeruginosa*, *P. chloroaphis*, *P. fluorescens*, *P. putida*, *P. stutzeri* and *P. syringae* species groups.

P. putida is Gram-negative, obligately aerobic, rod-shaped, non-spore forming and motile by one or several polar flagella, with a cell size of 0.5-1.0 × 1.5-5.0 µm, characteristic of the type strain ATCC 12633 (Palleroni 2005).

P. putida is distinguished from *P. aeruginosa* by its inability to liquefy gelatin, produce phenazine pigments, denitrify or give an egg-yolk reaction, or to grow at 41°C. In addition, optimal growth of *P. putida* occurs within a temperature range of 25-30°C, while the optimum growth temperature of *P. aeruginosa* is 37°C (Palleroni 2005; Stanier et al. 1966).

P. putida is distinguished from *P. fluorescens* (Bossis et al. 2000; Chapalain et al. 2007; Palleroni 2005; Stanier et al. 1966) by its inability to liquefy gelatin, metabolize L-arabinose, denitrify or give an egg-yolk reaction.

Other phenotypic methods such as the API 20NE commercial kit (BioMerieux, Marcy l'Etoile, France fluorescence spectroscopy (Belal et al. 2010; Tourkya et al. 2009), and Biolog GN microplates (Regenhardt et al. 2002) can be used for the rapid identification of *P. putida*, but these techniques do not differentiate between *P. putida* strains.

In addition to the selected characteristics shown in Table 1-1, Health Canada also independently characterized the four DSL strains for their growth kinetics in liquid

culture at different temperatures on different media (Appendix 1) and taxonomic classification by fatty acid methyl-ester (FAME) analysis (Appendix 2).

Table 1-1: Characteristics of the DSL strains of *P. putida*

Characteristic	ATCC 12633	ATCC 31483	ATCC 31800	ATCC 700369
Optimum temperature (°C)	25 °C to 30 °C : Grows in TSB at 28 °C and 32 °C, not at 37 °C or 42 °C	Grows in TSB at 28 °C with low level growth at 32 °C, but not at 37 °C or 42 °C	30 °C : Grows in TSB at 28 C and 32 °C, not at 37 °C or 42 °C	Grows in TSB at 28°C and 32 °C, low level growth at 37 °C
Optimum pH	4 to 8	Unspecified	7	Unspecified
Colonies	Two morphologies observed: 1) entire, rough, circular, gray and flat on nutrient agar 2) entire, smooth, circular, and convex on nutrient agar	Entire, smooth, circular, glistening, convex, translucent on nutrient agar	Entire, smooth, undulated edges, diffusible green pigment on nutrient agar	Entire, smooth, circular, cream and opaque on trypticase soy agar
Morphology on trypticase soy agar after 7 days at room temperature	Two morphologies were observed: 1) beige, semi-translucent, glossy butyrous, entire-undulated, raised, circular colony with an average size 9mm 2) Light-beige/ off white, semi-translucent, moist, entire, flat, circular-irregular colony with size between 5-7 mm	Beige, semi-translucent, moist, entire-undulate, raised, circular colony with an average size of 11 mm	Two morphologies were observed: 1) beige off white/ cream/ beige, semi-translucent, moist, entire-undulated, raised, circular colony with an average size 4 mm 2) beige, semi-translucent, glossy-butyrous, entire, flat, circular colony with size between 3-5 mm	Two morphologies were observed: 1) beige, semi-translucent, glossy-butyrous, entire-undulated, raised, circular colony with an average size 9mm 2) beige-tan, opaque, moist, undulate, raised/flat, circular-irregular colony with size between 4-8 mm

Information presented in the table is derived from the following references: ATCC 2014; Stanier 1947; Palleroni 2005; Annadurai et al 2008; Moore et al 2006; Data generated by Health Canada's Healthy Environments and Consumer Safety Branch.

FAME analysis of *P. putida* ATCC 12633, 31483, 31800 and 700369 by Health Canada Scientists indicates that the DSL strains are not closely related to pathogenic micro-organisms, such as *P. aeruginosa* and *P. syringae*. Comparison using the environmental and clinical MIDI databases matched the *P. putida* DSL strains to the species *P. putida* and *P. fluorescens* (Appendix 2).

The high phenotypic heterogeneity between strains of *P. putida* has led to the subdivision of the species into two main biovars (or biotypes), A and B (Table 1-2) (Palleroni 2005). However, recent studies using ribotyping suggest that the internal subdivision of this species should be revised again (reviewed in Palleroni 2005). Mulet et al. (2013) concluded that biovar differentiation is an obsolete taxonomic classification but that associated phenotypic characteristics may still be useful for strain differentiation.

Most *P. putida* strains have been assigned to biovar A (including the DSL strain ATCC 12633). Yamamoto and Harayama (1998) indicate that *P. putida* strains within biovar B show a greater phylogenetic similarity to *P. fluorescens* than to *P. putida* biovar A. It is not known which biovar better fits the phenotypic characteristics of the other three DSL strains, ATCC 31483, 31800, and 700369.

Table 1-2: Phenotypic differences between biovar A and B[a]

Characteristics	Biovar A	Biovar B
Utilization of anthranilate	-	+
Utilization of D-galactose	-	d
Utilization of L-kynurenine	-	+
Utilization of nicotinate	d	-
Utilization of L-tryptophan	-	+
Growth at 4 °C	d	+
Mol % G+C of DNA	62.5	60.7

[a]Information presented in the table is based on Palleroni 2005
d = 11 to 89% of the strains are positive; - = 90% or more of the strains are negative; + = 90% or more of the strains are positive.

Phenotypic identification of *P. putida* using culture-based methods is complemented by genotypic identification using methods based on nucleic acid sequence, composition, structure, and protein expression. MALDI-TOF MS analysis of the total bacterial proteome can be used for rapid identification of fluorescent *Pseudomonas* (including *P. putida*) (Anderson et al. 2012). Phylogenetic analysis of fluorescent *Pseudomonas* (including *P. putida*) can be also achieved by using the outer membrane protein OprD as reference for comparison (Chevalier et al. 2007). *P. putida* can be differentiated from other members of the *Pseudomonas* genus by a number of genotypic methods,

including 16S rRNA gene sequence analysis, which can distinguish *P. putida* from other pseudomonads such as *P. aeruginosa, P. fluorescens, P. mosselii,* and *P. monteilii* (Andreote et al. 2009). Additional methods useful for the identification and classification of *Pseudomonas* species include: phylogenetic analysis using *gyrB* and *rpoD* genes sequences (Yamamoto et al. 2000); and high density oligonucleotide microarrays, which are discriminatory of pseudomonads including *P. putida* (Ballerstedt et al. 2007; Elomari et al. 1997). Furthermore, the following bacterial repetitive elements can be used to differentiate *P. putida* from other pseudomonads:

- Repetitive extragenic palindromic (REP) sequences, REP-PCR (Regenhardt et al. 2002);
- BOX-A1R-based repetitive extragenic palindromic PCR, BOX-PCR (Andreote et al. 2009; Louws et al. 1994; Mehri et al. 2011; Regenhardt et al. 2002);
- Enterobacterial repetitive intergenic consensus (ERIC) sequences (Louws et al. 1994; Regenhardt et al. 2002); and
- Amplified rDNA restriction analysis, ARDRA (Mehri et al. 2011).

At the strain level, effective taxonomic classification can be achieved using multilocus sequence analysis of *Pseudomonas* 16S rRNA, *gyrB* and *rpoD* genes (Mulet et al. 2010). This method might be used to differentiate between DSL strains, however, this has not yet been tested. Siderotyping of genes encoding siderophores can also differentiate pseudomonads at the strain level, and can distinguish the *P. putida* type strain from other strains (Mehri et al. 2011; Meyer et al. 2007; Molina et al. 2006; Sutra et al. 2000; Tripathi et al. 2005; Ye et al. 2013).

Identification of the DSL *P. putida* strains was confirmed by Health Canada scientists using 16S ribosomal RNA gene sequence analysis along with match searches of Microseq and Ribosomal Database Project libraries (Appendix 3). A phylogenetic tree was generated to model the relationships of the DSL strains to representatives of the two biovars and to the type strains of different species (Figure 1-1). The figure shows that ATCC 700369 and 31483 are grouped together, close to the biovar A DSL strain ATCC 12633, suggesting they could also be part of *P. putida* biovar A. The last strain, ATCC 31800, appears to be closely related to *P. fluorescens* and appears closest to a biovar B strain, which could suggest that the strain is part of biovar B. An alternative interpretation is that ATCC 31800 has been misidentified and is in fact *P*. *fluorescens*. An assessment by the Government of Canada of a strain of *P*. *fluorescens* (ATCC 13525) reached a similar conclusion to the current *P*. *putida* assessment (Final Screening Assessment - *Pseudomonas fluorescens* ATCC 13525). None of the DSL strains appear directly related to the species *P. aeruginosa,* including a highly virulent representative, strain PAO1.

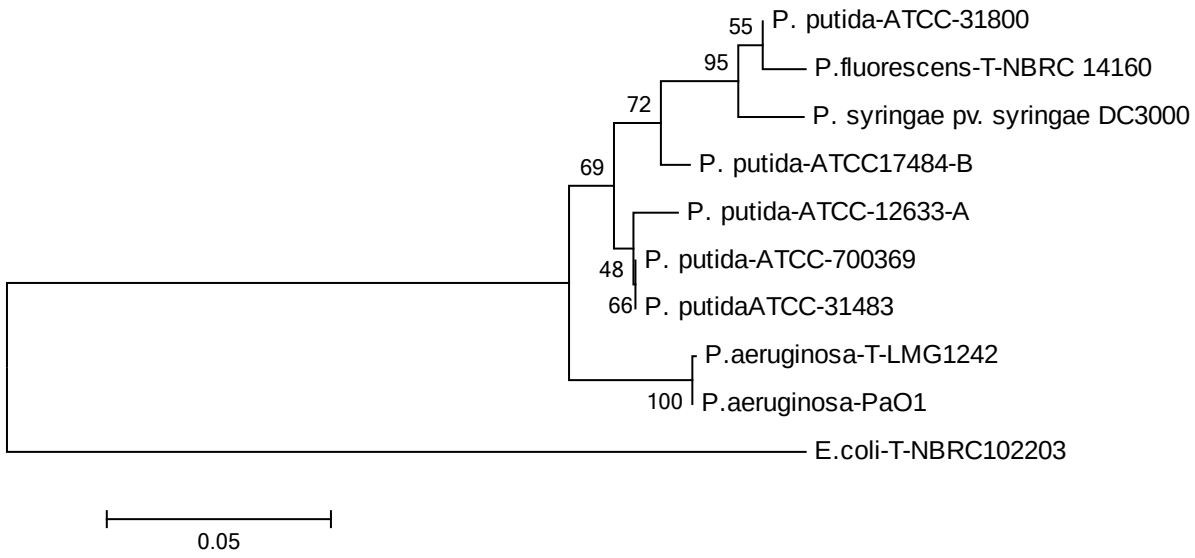

Figure 1-1: Phylogenetic tree of DSL *P. putida* strains using 16S rRNA gene sequences. The tree was generated by the Environmental Health Science and Research Bureau using 16S rRNA gene sequences of *P. putida* strains obtained in house and from the literature. The phylogenetic tree was constructed using the methodology from Tamura et al. (2011), first by alignment of the sequences by the MUSCLE method and then analyzed with the Hasegawa-Kishino-Yano distance model within the MEGA version 5.2 platform.

The genome sequence is available for one of the DSL strains of *P. putida* (ATCC 12633), as reported in the NCBI Entrez Genome Project Database (NCBI 2014).

The genome of the DSL strain ATCC 12633 contains:

- 6.16 Mb;
- 62.3% guanidine-cytosine (G+C) content;
- 5,449 coding sequences (Ohji et al. 2014); and
- 3,031 coding sequences which are shared with the nine other *P. putida* strains that have been sequenced.

Although approximately 85% of the genomes of *P. putida* and *P. aeruginosa* are shared (presumed essential or core gene sequences for housekeeping functions), *P. putida* genomes differ significantly and are distinguishable taxonomically from *P. aeruginosa* and other pseudomonads. Higher functional diversity was reported for *P. putida* compared to *P. aeruginosa* as estimated by higher numbers of paralogous gene families (894 versus 809) (Moore et al. 2006). For non-housekeeping genes, 105 islands of *P. putida* genes were assigned to only 14 of the 25 functional categories established for *P. aeruginosa*, many associated with the metabolic proficiency of *P. putida* as a saprophytic omnivore (Weinel et al. 2002).

Many pseudomonads initially identified by morphology or other phenotypic characteristics have been re-classified as a result of genotypic studies. Two of the DSL *P. putida* strains, ATCC 12633 and 31483, were initially misidentified as *P. fluorescens* and are now identified as *P. putida.*

1.2 Biological and ecological properties

1.2.1 Natural occurrence

P. putida is found in most soil and water habitats where there is oxygen available (Danhorn and Fuqua 2007; Olapade et al. 2005; Palleroni 2005), but it may be best suited to live in terrestrial environments (Olapade et al. 2005). It has been isolated from various sources including but not limited to:

Soils and rhizospheres:

- Rhizospheres of rice in Brazil, bananas in French West Indies, sugarcane (geographical location not specified), and peas in Sweden (de Castro et al. 2010; Silva et al. 2009; Sutra et al. 2000; Hassan et al. 2011; Berggren et al. 2005);
- Soybean root and soil water samples (location not specified), and tomato and wheat roots (location not specified) (Kloepper et al. 1985; Danhorn and Fuqua 2007);
- Maize plants (which recruit *P. putida* through the secretion of allelochemicals) (location not specified) (Mendes et al. 2013);
- Soil samples in Japan, Antarctica, Sub-Alpine Himalaya, and yard soil from a house in a Louisville, KY (USA) metropolitan area (Nakazawa 2002; Pandey et al. 2006; Shivaji et al. 1989; Remold et al. 2011); and
- Polluted soils (location not specified) (Timmis 2002).

Animals:

- Frog skin in Columbia (Flechas et al. 2012);
- Footpads, mouth, and ears of cats in Louisville, KY USA (Remold et al. 2011);
- Kidney samples collected from ornamental fish of various species, from Columbia, Florida USA, and Singapore, sampled for the presence of bacteria (Rose et al. 2013).

Aquatic environments:

- Within and around lakes in Antarctica (Shivaji et al. 1989);
- Marine environments with oil contamination in Malaysia (Jalal et al. 2012);
- Streams in the USA, at concentrations of 6.9×10^2 cells/mL to 7.3×10^3 cells/mL (Olapade et al. 2005); in sediment, the *P. putida* concentration ranged from 5.7×10^4 cells/g dry weight to 1.2×10^7 cells/g dry weight; and

- Freshwater and wastewater mixed liquor samples of the Kat River in Fort Beaufort and the Tyume River in Alice, South Africa (Igbinosa et al. 2012).

Humans:

- Homemakers' hands were sampled in Manhattan, USA, and *P. fluorescens*/*putida* was the most prevalent bacterium (from 59 of 204 hands) (Aiello et al. 2003).

Built environments

- Security swipe cards in hospitals in United Kingdom (Sultan et al. 2009);
- Water in public distribution water tanks in Massachusetts, USA (Penna et al. 2002);
- Household surfaces, water, drains, and garbage in Louisville, KY USA (Remold et al. 2011);
- Dental chair units in Finland (O'Donnell et al 2005); and
- Moist surfaces in hospitals, including drains and sinks, plastics, tubing, medical devices, and solutions in France and Massachusetts, USA (Aumeran et al. 2007; Penna et al. 2002).

Food:

- Spoiled refrigerated meat, and fish in Italy, poultry, and dairy products in Australia (Barrett et al. 1986; Eller 1969; Neumeyer et al. 1997; Pin and Baranyi 1998; Pin et al. 1999; Speranza et al. 2010); and
- Refrigerated raw cow and goat milk samples in France and Turkey (Callon et al. 2007; Uraz and Çitak 1998).

Other:

- Found in metal working fluid samples that contained biocides in France (Chazal 1995);and
- Isolated in air sample from dusts from polluted harbor air in Rouen, France (Duclairoir Poc et al. 2007).

1.2.2 Survival, persistence and dispersal in the environment

P. putida has the ability to proliferate and to be competitive in a variety of environments (reviewed in Cray et al. 2013), and can also persist in environments with low nutrient availability (Palleroni 2005). Factors known to affect the persistence of *P. putida* in terrestrial environments include both the soil type and the water content of the substrate (Iwasaki et al. 1994; Mirleau et al. 2005). Additionally, the characteristic flagellar motility

of the species is known to contribute to its dispersal in hydrated soil and aquatic environments (reviewed in Dechesne et al. 2010).

Terrestrial:

P. putida is able to colonize and survive in the rhizosphere soil and rhizoplane of wheat, barley, maize, and apple seedlings (Vancura 1988). An unspecified strain of *P. putida* coated onto soybean seeds was reported to establish a population density of 10^6 to 10^8 colony forming units (CFU)/seed (depending on the inoculation level) within 48 hours of inoculation (Kloepper et al. 1985). In another study, soils were inoculated with ~5x10^7 CFU/g of *P. putida* strain BIRD-1, and were shown to adhere to and colonize corn seeds with a population density of 10^4 to 10^5 CFU/seed; *P. putida* strain BIRD-1 was also able to colonize tomato, pepper, zucchini, and strawberry roots (Roca et al. 2013).

Aquatic:

In a study to assess the survival of *P. putida* in lake water related to organic load, *P. putida* strain DSM 3931 was released into a number of lake mesocosms in high concentrations with or without growth medium for up to 10 weeks. *P. putida* survived for 70 days in these lake water mesocosms, with a decrease in population by several orders of magnitude throughout the duration of the experiment. Population losses were attributed to grazing by invertebrates, sedimentation, and partially through attachment to particles and aggregation with other cells in the lake water, including algae (Brettar et al. 1994). *P. putida* was also reported to survive in distilled water for a year with no external nutrient input, possibly by reusing nutrients from dead cells (Lynch 1990).

P. putida was able to spread inside drinking water pipes at a mean rate of 4.3 cm/day (Gagniere et al. 2006). The presence of water in the pipes appeared to favour faster motility rates with cell densities reaching up to 2.5×10^3 CFU/cm^2.

1.2.3 Growth parameters

P. putida grows in extreme and contaminated environments. *Pseudomonas* species are generally considered psychrotrophic and halotolerant. *P. putida* grows at neutral pH, and has an optimal growth temperature between 25 to 30°C. However, temperature ranges vary by strain with some strains able to grow at temperatures as low as 4°C, others at temperatures as high as 50°C (Fonseca et al. 2011; Moreno and Rojo 2013; Palleroni 2005).

The species can be grown in iron deficient media, due to the presence of siderophores that allow the high affinity binding and transport of iron (Cray et al. 2013; Kloepper et al. 1988; Palleroni 2005). *P. putida* is also able to grow on media containing various concentrations of glucose (Banitz et al. 2012).

1.2.4 Role in nutrient cycling

P. putida plays an important role in decomposition, is involved in element cycling, and the recycling of organic compounds in aerobic compartments of the environment (Timmis 2002). *P. putida* strain AF7 was inoculated into 3 different soil types at two concentrations (3.5×10^4 CFU/ g dry weight soil; 3.5×10^5 CFU/g dry weight soil) and soil enzyme activities were measured. *P. putida* rapidly reduced organic carbon availability and β-glucosidase levels (de Castro et al. 2010; Silva et al. 2009), suggesting that it could affect the carbon cycle. Various properties of the species in a soil microcosm were investigated (Jones et al. 1991), and *P. putida* was reported to have no significant effect on ammonification, nitrification or denitrification, or on the population dynamics of the micro-organisms involved.

As a species, *P. putida* is known for a high level of solvent tolerance (Couillerot et al. 2009; Schweizer 2003). Some strains are tolerant to high concentrations of toluene (90%), tolerant of acetate, styrene, p- and m-xylene, cyclohexane, heptanol, ethyl benzene, octanol, and dimethyl phthalate (Sardessai and Bhosle 2002). *P. putida* is able to metabolize a wide variety of substrates (depending on the specific strain) such as: aromatics (including phenols) and aliphatic compounds (Moreno and Rojo 2013); chlorinated compounds including BTEX (TSCA); and 17β-estradiol, estrone, estriol, naphthalene, phenanthrene and fluorine (Liang et al. 2012). However, in the environment, *P. putida* growth is potentially limited by tannins produced from leaf detritus (Olapade et al. 2005).

The ability of *P. putida* to metabolize hydrocarbon compounds may be transferable to other bacteria in the environment, as conjugation can occur with *P. putida* in the environment and in contaminated industrial sites. *P. putida* intra-species transfer of the TOL plasmid pWWO coding for alkylbenzoate metabolism and toluene and xylene degradation has been reported to occur in soils (Greated et al. 2002; OECD 1997). Furthermore, transfer of the pHCL catabolic plasmid coding for hydrocarbon degradation from the terrestrial *P. putida* HC1 strain to marine bacteria *Micrococcus luteus* and *M. varians* was seen successfully in in situ and in vitro conjugation testing, allowing for possible routes of bioremediation by natural marine species (Latha and Lalithakumari 2001).

1.2.5 Antibiotic and Heavy Metal Resistance

Veterinary and clinical antibiotics have been used to successfully treat *P. putida* infections, including aminoglycosides, carbapenems, fluoroquinolones, piperacillin, ceftazadime, levofloxacin and ciprofloxacin (Anaissie et al. 1987; Chen et al. 2005; Chiu et al. 1998; Dervisoglu et al. 2008; Lombardi et al. 2002); however, resistance to carbapenem (Kim et al. 2012), cephalothin, ampicillin, chloramphenicol and carbenicillin (Moody et al.,1972) was observed in some clinical isolates of *P. putida.* Environmental isolates have been resistant to penicillin, ampicillin, oxacillin, cephalothin, erythromycin, vancomycin and trimethoprim (Igbinosa et al. 2012).

Multi-drug-resistant isolates of *P. putida* have been found in the urine of intensive care patients with nosocomial infections (Lombardi et al. 2002). These isolates contained conjugative and non-conjugative R-plasmids encoding IMP- and VIM-type metallo-β-lactamases, which confer high-level resistance to carbapenems and other β-lactams. Moreover, Novel Class 1 integrons coding for multi-drug resistance were isolated from 12 *P. putida* strains in southern China (Wu et al. 2012). Multi-drug-resistant clinical *P. putida* strains could be a nosocomial reservoir of transferable resistance determinants (Gilarranz et al. 2013). Resistance to the antimicrobial agent triclosan has been observed in *P. putida* strain TriRY, which also has the ability to utilize it as a carbon source (Meade et al. 2001).

The DSL *P. putida* strains were tested against antibiotics from a number of classes by Health Canada scientists (Table 1-3). The susceptibility pattern is similar to that reported for resistant strains in the literature. Overall, the most effective antibiotic was ciprofloxacin, while amoxicillin, amphotericin B, cefotaxime, erythromycin, nalidixic acid, trimethroprim, and vancomycin were inactive against all DSL strains.

Table 1-3: Antibiotic susceptibility profiles of the DSL *P. putida* strains

Antibiotic	ATCC 12633	ATCC 31483	ATCC 31800	ATCC 700369	S[a]≤	R[a]>
Amoxycillin	>24	>24	>24	>24	-	-
Aztreonam[b]	18.0±8.5	>24	>24	>24	1	16
Ceftazidime[c]	7.2±2.7	4.7±4.3	4.0±2.3	9.9±10.3	8	8
Cefotaxime	>24	>24	>24	>24	-	-
Ciprofloaxcin	0.4	0.4±0.2	0.4±0.2	0.4	0.5	1
Colistin	0.5±0.2	1.7±1.4	0.5±0.2	2.2±4.3	4	4
Doxycycline	1.0±0.4	4.7±5.0	10.4±22.1	3.1±2.3	-	-
Erythromycin	>24	>24	>24	>24	-	-
Gentamicin	0.4	6.8±11.5	1.4±0.3	1.1±1.0	4	4
Meropenem	7.2±2.7	1.5±1.2	0.5±0.2	9.4±7.4	2	8
Nalidixic acid	>24	>24	22.6±17.7	>24	NA	NA
Trimethoprim	>24	>24	>24	>24	-	-
Vancomycin	>24	>24	>24	>24	-	-

Tests were conducted using a TSB-MTT liquid assay method (Seligy and Rancourt 1999). Values correspond to the minimal inhibitory concentration (µg/mL ± standard deviation) for *P. putida* strains (1 582×10^6cfu/ mL) grown in the presence of antibiotic for 24 hours at 37°C.
S, susceptible; R, resistant; - indicates that susceptibility testing is not recommended as the species is a poor target for therapy with the drug (isolates may be reported as R without prior testing); NA, not applicable
[a] Interpretive criteria (MIC µg/mL; Eucast 2014)
[b] For this antibiotic, the resistant breakpoint relates to high dose therapy. The susceptible breakpoint is set to ensure that wild type isolates are reported intermediate.
[c] For this antibiotic, breakpoints relate to high dose therapy.

P. putida can persist and grow in heavy metal contaminated water and sequester heavy metals via biosorption and bioaccumulation (Kamika and Momba 2013).

Resistances/tolerances to the following heavy metals have been observed: mercury (Zhang et al. 2012); as well as methylmercury, copper, lead, nickel, chromate, zinc, cobalt, manganese, and barium over a wide range of pH and temperature (Cabral et al. 2013).

1.2.6 Pathogenic and toxigenic characteristics

P. putida ATCC 12633 contains 111 out of the 453 known virulence factor genes for *Pseudomonas*, including genes responsible for the synthesis of flagella, type IV pili, alginate and pyoverdine. However, *P. putida* ATCC 12633 does not contain complete gene sets for a type III secretion system and associated proteins (Ohji et al. 2014). As the genomes of the other DSL strains have not been sequenced, it is unclear whether they carry a similar gene complement. Health Canada scientists evaluated the cytotoxic potential of the DSL *P. putida* strains, ATCC 12633, 31483, 31800 and 700369. The DSL strains were not cytotoxic towards human colonic epithelial cells (HT29) at 4 hours of exposure, and minimal cytotoxic effects were seen at 24 hours of exposure. No hemolytic activity was observed when the DSL strains were grown on sheep blood agar for 24h or 48h at 37°C.

P. putida is known to produce a variety of enzymes, toxins, and other metabolites (Appendix 4, Table A-9). Some of these genes are associated with virulence in the related species *P. aeruginosa*, such as those coding for pili; flagellum; siderophores; pyocyanin; elastase; proteases; rhamnolipid; alginate; other polysaccharides; lipopolysaccharide. Many of these are core genes of pseudomonads that contribute to their survival in the environment by enhancing their ability to colonize surfaces and form biofilms, but may also allow them to behave as opportunistic pathogens under certain conditions (Nelson et al. 2002; Palleroni 2005).

Virulence traits have been investigated in other *P. putida* strains. The complete genome of strain KT2240 was analyzed by Nelson et al. (2002). The only genes in *P. putida* KT2440 related to virulence-associated traits encode adhesion proteins PP0168, PP1449 and PP0806. Exotoxin A is absent, as is the transcriptional regulatory gene *algM*/*mucC*, which may account for its non-mucoid phenotype, as loss of this gene in *P. aeruginosa* stops the overproduction of alginate that is the hallmark of lung infections in cystic fibrosis patients. It also lacks the genes conferring known plant-related virulence traits, such as type III secretion systems, corresponding secreted substances and plant cell wall-degrading enzymes (Nelson et al. 2002). Similarly, in a review by Wu et al. (2011), which compares the genomes of four *P. putida* strains (KT2440, W619, F1 and GB-1), there is no evidence of putative functions required for the biosynthesis of exotoxin A, phospholipase C or pectin lyase which are usually present in animal and plant pathogens (Nelson et al. 2002).

Nevertheless, other isolates of *P. putida* can produce phytotoxins, such as phenazine-1-carbonic acid (PCA) (Vancura 1988). Phytotoxins can interfere with regulatory mechanisms in plants, and can lead to wilting, growth abnormalities, watersoaking, and

others (Durbin 1991). PCA has been shown to be an acyl-CoA synthetase inhibitor (Kim 2000).

Biofilm production by *P. putida* is conditional, and dependent on the amount and the type of the carbon source available (Klausen et al. 2006). No information was found about the ability of the DSL strains to form biofilms. Flagella play a role when *P. putida* planktonic cells initially attach, colonize and begin to form biofilms on fungal hyphae and on the outer surface of plant roots (Klausen et al. 2006). In water-limiting conditions, transient alginate expression has been found in *P. putida* cells within biofilms (Li et al. 2010). *P. putida* can develop biofilms on charged and uncharged surfaces and on hydrophobic and hydrophilic surfaces (Shrove et al. 1991). Charged surfaces support the maximum biofilm accumulation for *P. putida*.

The *P. putida* strain KT2440 is known to have genes coding for homoserine lactone production; however, it does not appear to produce sufficient quantities of this signalling molecule to be of concern for virulence as it is in quorum-sensing *P. aeruginosa* strains (Nelson et al. 2002). No information was found about the ability of the DSL strains to produce homoserine lactone.

1.3 Effects

No cases of pathogenicity or toxicity related to the DSL strains were found in the literature. An in depth scientific literature search on *P. putida* yielded only a few cases of pathogenicity towards plants or animals, despite the widespread presence of this species in the environment.

1.3.1 Environment

Microbiota

P. putida is known to antagonize plant pathogenic bacteria and fungi (Haas and Defago 2005; Mazzola 1999; Moore et al. 2006; Naik and Sakthivel 2006; Palleroni 2005; Scherlach et al. 2013; Sutra et al. 2000). One such mode of antagonism by *P. putida* involves its ability to secrete antimicrobial substances, including volatile organic compounds, biosurfactants, and karalicin (an antibiotic with some inhibitory action on yeasts) (Cray et al. 2013). In addition, siderophore formation by *P. putida* can lead to iron limitation inhibition of other species in the surrounding environment (Haas and Defago 2005; Kloepper et al. 1988). *P. putida* can inhibit the following microbial species:

- *Glomerella tucumensis* (Hassan et al. 2011);
- *Batrachochytrium dendrobatidis* (Flechas et al. 2012);
- *Pythium ultimum* (Paulitz 1991);
- *Phytophthora parasitica* (Lee and Cooksey 2000);
- *Fusarium oxysporum* (Lopez-Berges et al. 2013; Srinivasan et al. 2009);

- *Rhizobium leguminosarum* (Berggren et al. 2005); and
- *Phytophthora nicotianae*, *Peronophythora litchi*, *Erwina caeatovora*, *Phytophota capsici*, *Collectotrichium gloeosporioides*, *C. higginsianum* and *Alternaria tenuis* (Shi et al. 2010).

Plants

P. putida is not considered a plant pathogen, although some strains can aid in root rot or other plant diseases. For instance, *P. putida* in the rhizosphere of paddy rice plants has been implicated in "suffocation disease" (Bradbury 1986), and *P. putida* strain GR12-2 was reported to inhibit root elongation of wheat (Hall et al. 1996, as cited in Barazani and Friedman 2001). Additionally, *P. putida* produces antimicrobial substances that were shown to interfere with symbiotic processes in pea plants (Berggren et al. 2005).

Conversely, *P. putida* is also considered a plant growth promoting rhizobacterium (PGPR) (Kloepper et al. 1985); it colonizes the rhizosphere of plants in a mutualistic relationship, resulting in increased plant growth and the selective inhibition of other bacteria and fungi (Cray et al. 2013; Kloepper et al. 1985). *P. putida* can produce allelopathic compounds such as plant growth hormone indole-3-acetic acid (IAA) (Barazani and Friedman 2001) that can help or hinder plant growth depending on the compound, plant species, and concentration (Cray et al. 2013).

P. putida also has the ability to make essential elements available to plants in the soil. *P. putida* can fix atmospheric nitrogen (Shabayev 2010), and strain BIRD-1 can solubilize various sources of insoluble phosphate (Roca et al. 2013). Some examples of the positive effects *P. putida* exhibits towards plants include:

- Enhancement of maize root growth from corn seeds (Roca et al. 2013);
- Promotion of root growth in lettuce, tomato, and canola (Barazani and Friedman 2001);
- Increase of strawberry plant dry mass (Vancura 1988);
- Stimulation of fructification in button mushrooms (*Agaricus bisporus*) (Scherlach et al. 2013); and
- The ability to induce systemic resistance in bean plants (Ongena et al. 2004).

Pathogenicity and toxicity studies were performed by Environment Canada scientists using *Festuca rubra* (red fescue) exposed to *P. putida* ATCC 12633, 31800, or 700369 in artificial soil. The addition of these strains had no effect on the growth of red fescue

compared to controls, and no significant differences between the three strains were detected.[6]

No data were identified on adverse effects or experimental challenge in aquatic plants.

Vertebrates:

P. putida was found on the endotracheal tube of a male cynomolgous macaque that died post-anesthesia (Matchett et al. 2003). After an environmental analysis of the laboratory found no traces of *P. putida*, the authors concluded that the *P. putida* was a commensal bacterium and that possible immunosuppression from anesthesia allowed for infection leading to death. Post-mortem analysis revealed multifocal areas of mild pulmonary edema and neutrophil infiltration in the lungs. In another report, a wild female koala captured for routine survey that died shortly after release was found to have melioidosis associated with *P. pseudomallei* infection and *P. putida* was isolated from the lungs (Ladds et al. 1990).

Members of the genus *Pseudomonas* are reported to be opportunistic fish pathogens. *P. putida* is known to cause disease in fish under stress conditions, more commonly in captive fish, and rarely in wild fish (reviewed in Smolowitz et al. 1998). Opportunistic infections reported in farm-raised, ornamental, and experimental fish populations, such as yellowtail, European Eel, Rainbow Trout, and the Large Yellow Croaker, have involved *P. putida* and other pseudomonads (Mao et al. 2013; Nishimori et al. 2000; Rose et al. 2013; Smolowitz et al. 1998). *P. putida* infection in yellowtails was identified after the fish developed large abscesses on their skin surface (Kusuda and Toyoshima 1976). *P. putida* was isolated from the kidney and spleen of the moribund yellowtails. In Rainbow Trout, a disease outbreak presenting dorsal fin discoloration followed by epithelial necrosis and ulcers was found to be caused by *P. putida* (Altinok et al. 2006). The authors did not attribute the outbreak to stress in the fish and suggest the source of bacteria to have been the water bed where soil was present.

P. putida infections in two experimental tanks holding 50 toadfish (wild collected, and held in flow through tanks) caused multifocal mucus beads on dorsal and lateral surfaces externally (Smolowitz et al. 1998). Internally, peritonitis was observed, with colonization in the spleen, sex glands, swim gland, and possibly the liver and kidney parenchyma. It was not clear if the tanks were colonized or if the fish were stressed, allowing colonization. In a study about the development of a vaccine against *P. putida* in the Large Yellow Croaker, it was observed that most of the fish used as a control died after 3 to 9 days of being challenged by intraperitoneal infection with 10^6 cells/mL of *P.*

[6] Unpublished data generated by Environment Canada's Biological Methods Division

putida (Mao et al. 2013). Even the vaccinated fish had a high mortality after being challenged (from 60% up to 100%). *P. putida* injected into the fish caused white nodules on the spleens and kidneys and in some cases, fish developed signs of congestion within a few days post-injection.

Zhang et al. (2012) report that the LD_{50} for the *P. putida* SP1 strain in flounder and turbot exposed via intraperitoneal injection and monitored for 14 days was 1.5×10^9 CFU per fish. Juvenile Rainbow Trout exposed to 5×10^6 CFU/mL for one hour before resuming flow through in tanks were observed twice daily for 60 days (Altinok et al. 2006). Cumulative mortality of 45% was reported and effects included skin ulcers and epithelial necrosis. *P. putida* was isolated from skin lesions, liver, spleen, and kidneys of infected fish.

Invertebrates:

Cupulated female spider mites (*Tetranychus urticae*) were exposed to a suspension (10^8 to10^9 CFU/mL) of *P. putida* biovar B to determine if it had potential as a biocontrol agent. Total egg numbers and egg hatching were reduced, and there was overall high mortality seen in the test species, but the mechanism of this effect was not clear (Aksoy and Kutluk Yilmaz 2008). *P. putida* induced a high mortality rate in the female Olive fly (*Dacus oleae*) when it was exposed to the bacteria via a diet containing 10% of the inoculum (unknown concentration) (Haniotakis and Avtzis 1977). However, *P. putida* has also been suggested as a probiotic in the rearing of olive flies for biocontrol through the sterile insect technique (Sacchetti et al. 2014).

Pathogenicity and toxicity studies were performed by Environment Canada scientists using adult *Folsomia candida* (collembolan or springtail) exposed to *P. putida* ATCC 12633, 31800, and 700369 in artificial soil. The addition of these strains had no effect on growth of adult *Folsomia candida*, or survival or reproduction of the species. No significant differences between the three strains were detected.[7]

Septicemia caused by *P. putida* and *P. fluorescens* has been reported in crayfish (Boemare and Vey 1977); however, *P. putida* has been reported to be a component of the crayfish microflora (Devesa et al. 2005). In a study that investigated bacterial populations associated with moribund snails (*Biomphalaria glabrata*), *P. putida* was found to be present in 13% of the 100 organisms tested (Cheng 1986). Death of the snails was not specifically associated with the presence of *P. putida*. In *Daphnia similis* exposed for 21 days to 10^6 active units of *P. putida* per mL of culture water, there was a

[7] Unpublished data generated by Environment Canada's Biological Methods Division

12% decrease in number of neonates produced per day, but no significant difference in survival rate for the exposed organism was observed (de Castro et al. 2010).

1.3.2 Human health

P. putida is considered a rare opportunistic pathogen and is infrequently isolated from clinical samples. *P. putida* has been implicated in infections in patients with debilitating health conditions and/or immunosuppression, or whose normal barriers to infection had been breached by surgical procedures, exposure to medical devices or physical trauma. *P. putida* has been reported to cause infection at several sites including the bloodstream (Anaissie et al. 1987; Chiu et al. 1998; Erol et al 2014; Korcova et al. 2005; Ladhani and Bhutta 1998; Martino et al. 1996; Rolston et al. 2005; Shah et al. 2007); traumatic wounds (Carpenter et al. 2008; Yang et al. 1996); soft tissue (Chen et al. 2005; Ladhani and Bhutta 1998; Taylor et al. 1984; Thomas et al. 2013; Yoshino et al. 2011) and cornea (Ying-Cheng et al. 2006). *P. putida* has been implicated in infections including peritonitis (Dervisoglu et al. 2008; Lew and Gruia 2005) and septic arthritis (Macfarlane et al. 1991; Madhavan et al. 1973). One recent case report refers to a *P. putida* infection in an otherwise healthy individual with an eye (conjunctival) infection (Zuberbuhler and Carifi 2012). In addition, multidrug-resistant and carbapenem-resistant *P. putida* infections are associated with a reported mortality rate of 39% (Kim et al. 2012). *P. putida* contamination of blood products has been associated with post-transfusional sepsis (Tabor and Gerety 1984; Taylor et al. 1984; Zavizion et al. 2003), and mortalities due to the presence of *P. putida* in the bloodstream have been reported (Tabor and Gerety 1984; Taylor et al. 1984) but are rare.

No cases of allergic reactions have been reported specifically related to *P. putida*.

In in vivo testing at Health Canada using the DSL strains ATCC 12633, 31483, 31800 and 700369, four replicate BALB/c mice exposed to 1×10^6 CFU/25µL by endotracheal instillation showed no changes in behavior or physical appearance aside from some ruffled fur that resolved within the monitoring period; a transient increase in levels of proinflammatory cytokines in the lungs early after exposure, and a transient increase in levels of pulmonary granulocytes between 24 and 48 hours post-exposure were observed. These results are indicative of transient local inflammation resolving within two days. The mice were able to clear ATCC 12633, 31483, 31800 and 700369 from the lungs within one week post-exposure.

The effects of experimental challenge towards murine species using *P. putida* have also been reported in the literature. In one study, no clinical alterations were identified on Wistar rats exposed to a single dose of 10^8 units of *P. putida* per gavage (de Castro et al. 2010). The animals were sacrificed at 3, 16, and 24 hours post-exposure. *P. putida* was identified in lung homogenates at the 16 hour post-dose time point only, at a concentration of 3.31×10^4 CFU/g tissue. Bacteria were cleared from the lungs by 24 hours. In another study by George et al. (2000), CD-1 male mice were exposed to *P. putida* ATCC 12633 orally with 1.86×10^8 CFU. The micro-organism was cleared from

the pulmonary system within 1 day, and no mortality was reported up to a dose of 1.0×10^9 CFU. *P. putida* was observed in the mesenteric lymph nodes and liver 3 hours post-exposure indicating translocation; however, no adverse effects were identified in any of these organs.

1.4 Hazard severity

1.4.1 Environment

The environmental hazard potential of *P. putida* DSL strains ATCC 12633, ATCC 31483, ATCC 31800 and ATCC 700369 is assessed to be medium for fish, and low for other aquatic and terrestrial plants and animals based on the following considerations:

- A combination of morphological, biochemical, and physiological traits allow *P. putida* to be reliably discriminated from other pathogenic *Pseudomonas* species, especially *P. aeruginosa*;
- There are no reports in the literature implicating the DSL *P. putida* strains in adverse effects in the environment; and
- At the species level, reports of adverse effects associated with *P. putida* are limited, despite its widespread presence in soil, water, and rhizosphere ecosystems. Some adverse effects have been reported in aquatic vertebrates. Cases of *P. putida* infection have been reported in farm-raised, ornamental, and experimental fish populations but rarely in wild fish populations. Overall, despite its prevalence and association with various environmental species and habitats, there is no evidence in the scientific literature to suggest that *P. putida* has adverse ecological effects at the population level for plants, vertebrates or invertebrates.

1.4.2 Human Health

The human health hazard potential of *P. putida* DSL strains ATCC 12633, ATCC 31483, ATCC 31800 and ATCC 700369 is assessed to be low for healthy individuals and medium for immunocompromised individuals based on the following considerations:

- A combination of morphological, biochemical, and physiological traits allow *P. putida* to be reliably discriminated from other pathogenic *Pseudomonas* species, especially *P. aeruginosa*;
- The DSL *P. putida* strains have no reported history of pathogenicity in humans, and these strains did not induce adverse effects upon endotracheal administration in a mouse model;
- *P. putida* human infections have been reported in the literature, although almost exclusively in individuals with pre-existing conditions or a history of medical or surgical procedures, or physical trauma. Reports of mortality associated with *P. putida* infections are rare;

- Although *P*. *putida* infections are rare, when they do occur, antibiotic resistance could limit the effectiveness of treatment. In most reported cases, *P*. *putida* infections were effectively treated with antibiotics; however susceptibility profiles vary significantly, and multi-drug-resistant *P. putida* clinical infections have been reported. All of the DSL strains of *P. putida* are susceptible to Ciprofloxacin, and individually other treatment options are available; and
- Contaminated medical devices and blood products have been implicated in nosocomial infections and sepsis and represent a hazard to individuals undergoing medical treatment.

Hazards related to micro-organisms used in the workplace should be classified accordingly under the Workplace Hazardous Materials Information System (WHMIS).[8]

2. Exposure Assessment

2.1 Sources of exposure

The focus of this assessment is to characterize exposure to *P. putida* ATCC 12633, 31483, 31800 and 700369 from their deliberate addition to consumer or commercial products or their use in industrial processes in Canada. *P. putida* ATCC 12633, 31483, 31800 and 700369 were nominated to the DSL for industrial, commercial and consumer uses.

In 2007, a voluntary questionnaire was sent to a subset of key biotechnology companies. Responses to this survey indicate that 10000 to 100 000 kg of products containing the DSL strains were imported into Canada for a variety of applications in 2006.

[8] 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.

To update information on current uses, the Government conducted a mandatory information-gathering survey (Notice) under section 71 of CEPA (hereafter referred to as the section 71 Notice), as published in the *Canada Gazette*, Part I, on October 3 2009. The section 71 Notice applied to any persons who, during the 2008 calendar year, manufactured or imported, *P. putida* ATCC 12633, 31483, 31800 or 700369 whether alone, in a mixture, or in a product. A variety of environmental, industrial and household applications using *P. putida* ATCC 12633, 31483, 31800 or 700369 such as in wastewater and bioremediation were reported in response to the s. 71 Notice (for quantities see Table 2-1).

Table 2-1: Quantities of products containing DSL *P. putida* strains reported to be imported or manufactured in Canada in 2009

Strain	Total Amount Range[a] (kg)
ATCC 12633	10,000-100,000
ATCC 31483	10,000-100,000
ATCC 31800	1,000-10,000
ATCC 700369	1,000-10,000

a Combined amount of all products containing the micro-organisms manufactured in or imported to Canada.

P. putida is metabolically diverse, which could make it of commercial interest in a variety of industries, particularly in the degradation of xenobiotic compounds. A search of the public domain and the Canadian Intellectual Property Office Patent Database (CIPO 2014), yielded the following potential uses of other naturally occurring strains of *P. putida*:

- Activated sludge in wastewater treatment processes (Pramanik et al. 2011);
- Wastewater treatment of pharmaceutical drugs containing a mix of antibiotic drugs including amoxicillin and cefadroxil (Krifa et al. 2013);
- Commercial production of bulk and fine chemicals and industrial biocatalysis (Cray et al. 2013; Hassan et al. 2011; Poblete-Castro et al. 2012; Puchalka et al. 2008);
- Production and preparation of chemical compounds
  - Microbiological process for the oxidation of methyl groups (Hoeks 1992, Patent CA 2046430);
  - Process for producing optically active beta-amino alcohols (Sakamoto et al. 2001, Patent CA 2404668).
- Pharmaceutical applications with uses in antimicrobial treatments, chemotherapy, and vaccine development;
  - Sustained release of anti-infectives (Boni et al. 2014, Patent CA 2614764);

        - Cellular and viral inactivation (Raviv et al. 2005, Patent CA 2557800).
    - Removal of volatile organic compound in industrial gas effluxes (Zhao et al. 2014);and
    - Microbial biodegradation of chemicals and environmental contaminants, used for bioremediation activities and in commercially-available cleaning compositions.
        - Analog enrichment decontamination process (Focht 1990, Patent CA 1265082);and
        - Drain foam composition and method of using the same (Ipser and Tilyou 2009, Patent CA 2718523).

2.2 Exposure characterization

2.2.1 Environment

The environmental exposure to *P. putida* ATCC 12633, 31483, 31800 and 700369, originating from its presence in commercial products, is estimated to be medium based on responses to the Section 71 Notice.

The magnitude of plant and animal exposure to these strains will depend on the nature of the use, on the proximity of environmental species to the sites of application or disposal, the mass or volume released in the environment, and their persistence and survival in the environments to which they are released. Uses, such as bioremediation, wastewater treatment are likely to introduce these DSL strains to terrestrial and aquatic ecosystems. Organisms at the site of application or disposal are likely to be the most directly exposed. Vertebrates could ingest *P. putida* ATCC 12633, 31483, 31800 and 700369 while feeding on plants or invertebrates growing in treated or contaminated soils or water. Aquatic species may come into contact with the DSL strains from runoff subsequent to terrestrial application or disposal of wastewater from facilities that use the organism for production of enzymes and biochemicals. Subsequent rainfall events would also introduce these micro-organisms in treated soils into waterways. Growth in the market for “greener” microbial-based products may increase such exposures (Spök and Klade 2009).

As mentioned in Section 1.2.1, *P. putida* has a ubiquitous distribution which reflects the ability of the species to adapt to, persist and thrive under a variety of environmental conditions. Nevertheless, introduced populations are not expected to persist above background levels (Van Veen et al. 1997).

2.2.2 Human

Based on commercial activity in Canada according to responses to the Section 71 Notice, the overall human exposure estimation for *P. putida* ATCC 12633, 31483, 31800 or 700369 is medium.

Human exposure is expected to be greatest through the direct use of consumer products containing 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 dusts containing the *P. putida* ATCC 12633, 31483, 31800 or 700369.

Inadvertent ingestion following use on or near food preparation surfaces and contact with the eyes, are possible secondary routes of exposure. Humans may also be exposed as bystanders during application of commercial 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 the DSL *P. putida* strains could occur subsequent to their use in wastewater and waste treatment, bioremediation, and as a production organism. Human exposure to bodies of water and soils treated with the DSL *P. putida* strains (e.g., through recreational activities), could result in exposure of the skin and eyes, as well as inadvertent ingestion; however, such exposures may be temporally distant from the time of release and are expected to be significantly lower relative to exposure to *P. putida* ATCC 12633, 31483, 31800 or 700369 in cleaning products. Furthermore, uses for enzyme and biochemical production in manufacturing facilities that do not release wastes into the environment should not result in human exposure.

In the event that the organism enters municipal drinking water treatment systems through release from intended and 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.

As mentioned above in section 2.2.1, growth in the market for “greener” microbial-based products may increase human exposure to the DSL *P. putida* strains which have potential applications in these products (Spök and Klade 2009).

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.

For the environment, the hazard of DSL *P. putida* strains ATCC 12633, 31483, 31800 and 700369 to captive-raised fish is estimated to be medium and the hazard to other terrestrial and aquatic plants and animals is estimated to be low. Exposure, as assessed through the s. 71 Notice for the 2008 calendar year, from its deliberate use in industrial processes or consumer or commercial products in Canada is expected to be medium for environmental species. Fish could be exposed to elevated concentrations of *P. putida* through its use in wastewater treatment, or from runoff subsequent to

terrestrial applications near rivers or lakes for bioremediation, or for disposal of treated wastewater or biosolids. The overall risk from these uses to fish is nevertheless expected to be low.

Based on the low level of human health hazard of *P. putida* strains ATCC 12633, 31483, 31800 and 700369 to the general population and the medium potential for exposure as assessed through the s. 71 Notice for the 2008 calendar year, the risk is estimated to be low with respect to the general population. Although individuals undergoing medical treatment could be at greater risk than the general population, current use patterns do not suggest a risk that medical devices or blood products could become contaminated from deliberate uses of *P. putida* strains ATCC 12633, 31483, 31800 and 700369.

It is therefore proposed to conclude that *P. putida* strains ATCC 12633, 31483, 31800 and 700369 do not meet any of the criteria set out in section 64 of CEPA.

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

The risk to the environment from foreseeable future uses is expected to be low as no new exposure scenario is expected from those uses.

The risk to human health from foreseeable future uses is expected to be low, but could increase to medium for individuals undergoing medical treatment, if they occur in healthcare settings.

P. putida strains ATCC 12633, 31483, 31800 and 700369 have properties that make them suitable for use in a range of products, and there is reason to expect new uses of *P. putida* strains ATCC 12633, 31483, 31800 and 700369 in health care settings could emerge. In particular, there is growth in the market for “greener” microbial-based cleaning products, (Spök and Klade 2009). As these products have potential uses in health care settings, there is some potential for harm.

Therefore, although effects in the general population are not expected, it is possible that new activities not considered in this assessment could increase the risk of nosocomial infections or sepsis resulting from contamination of medical devices or blood products.

4. Conclusion

Based on the information presented in this screening assessment, it is concluded that *P. putida* ATCC 12633, 31483, 31800 and 700369 are not entering the environment in a quantity or concentration or under conditions that:

- have or may have an immediate or long-term harmful effect in the environment or its biological diversity;

- constitute or may constitute a danger to the environment on which life depends; or
- constitute or may constitute a danger in Canada to human life or health.

Therefore, it is concluded that *P. putida* ATCC 12633, 31483, 31800 and 700369 do not meet the criteria set out in section 64 of CEPA.

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A. Appendices

Appendix A: Growth of *P. putida* ATCC 12633, 31800, 31483 and 700369 in various media

Table A-1: Growth of *P. putida* ATCC 12633 in liquid media at various temperatures

Medium	28°C	32°C	37°C	42°C
Trypticase Soy Broth	+	+	–	–
100% Defibrinated Sheep Blood	–	–	–	–
100% Fetal Bovine Serum	+	+	–	–
Dulbecco's Modified Eagles Medium (with FBS, glucoses, glutamine)	~	+	–	–

Data generated by Health Canada's Environmental Health Science and Research Bureau
– no growth, + growth, ~ low level growth

Table A-2: Growth of *P. putida* ATCC 31483 in liquid media at various temperatures

Medium	28°C	32°C	37°C	42°C
Trypticase Soy Broth	+	~	–	–
100% Defibrinated Sheep Blood	–	–	–	–
100% Fetal Bovine Serum	+	+	–	–
Dulbecco's Modified Eagles Medium (with FBS, glucoses, glutamine)	~	–	–	–

Data generated by Health Canada's Environmental Health Science and Research Bureau
– no growth, + growth, ~ low level growth

Table A-3: Growth of *P. putida* ATCC 31800 in liquid media at various temperatures [a]

Medium	28°C	32°C	37°C	42°C
Trypticase Soy Broth	+	+	+	–
100% Defibrinated Sheep Blood	–	–	–	–
100% Fetal Bovine Serum	+	+	–	–
Dulbecco's Modified Eagles Medium (with FBS, glucoses, glutamine)	~	~	–	–

[a] Data generated by Health Canada's Environmental Health Science and Research Bureau
– no growth, + growth, ~ low level growth

Table A-4: Growth of *P. putida* ATCC 700369 in liquid media at various temperatures [a]

Medium	28°C	32°C	37°C	42°C
Trypticase Soy Broth	+	+	~	–
100% Defibrinated Sheep Blood	–	–	–	–
100% Fetal Bovine Serum	+	+	~	–
Dulbecco's Modified Eagles Medium (with FBS, glucoses, glutamine)	~	~	–	–

[a] Data generated by Health Canada's Environmental Health Science and Research Bureau
– no growth, + growth, ~ low level growth

Appendix B: Fatty acid methyl ester (FAME) analysis of *P. putida* ATCC 12633, 31483, 31800, 700369

Data generated by Health Canada's Healthy Environments and Consumer Safety Branch 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 www.midilabs.com/fatty-acid-analysis. As a general rule of thumb, samples that cluster within a Euclidian distance of 2.5, 6 and 10 represent samples derived from the same strain, subspecies and species, respectively.

Table B-1: FAME analysis of *P. putida* ATCC 12633

Environmental Frequency - Best Match (Similarity Index)	**Clinical** Frequency - Best Match (Similarity Index)
8/21 *Pseudomonas putida* biotype A (0.861)	7/16 *Pseudomonas putida* biotype A (0.467)
6/21 No match	3/16 *Pseudomonas putida* biotype B (0.719)
4/21 *Pseudomonas fluorescens* biotype A (0.700)	2/16 *Pseudomonas fluorescens* biotype G & C (0.875)
1/21 *Pseudomonas fluorescens* biotype B (0.908)	2/16 *Aeromonas veronii* GC subgroup B (biogroup sobria) (0.128)
1/21 *Pseudomonas vancouverensis* (0.765)	2/16 No match
1/21 *Pseudomonas putida* biotype B (0.023)	Not applicable
1/21 *Pseudomonas vancouverensis* (0.765)	Not applicable
1/21 *Pseudomonas putida* biotype B (0.023)	Not applicable

Table B-2: FAME analysis of *P. putida* ATCC 31483

Environmental Frequency - Best Match (Similarity Index)	**Clinical** Frequency - Best Match (Similarity Index)
20/24 *Pseudomonas putida* biotype A (0.577)	9/17 *Pseudomonas putida* biotype A (0.698)
2/24 No match	6/17 *Pseudomonas fluorescens* biotype G & C (0.737)
1/24 *Variovorax paradoxus* GC subgroup A (*Alcaligenes paradoxus*) (0.700)	1/17 *Pseudomonas fluorescens* biotype A
1/24 *Corynebacterium diphtheria* gravis &	1/17 No Match

Environmental Frequency - Best Match (Similarity Index)	**Clinical** Frequency - Best Match (Similarity Index)
mitis (0.205)	

Table B-3: FAME analysis of *P. putida* ATCC 31800

Environmental Frequency - Best Match (Similarity Index)	**Clinical** Frequency - Best Match (Similarity Index)
10/21 *Pseudomonas fluorescens* biotype C/P. mandelii (0.832)	5/5 *Chromobacterium violaceum* (0.605)
7/21 *Pseudomonas putida* biotype B (0.703)	Not Applicable
2/21 *Pseudomonas savastanoi fraxinus* (0.814)	Not Applicable
2/21 *Pseudomonas syringaetabac*i (0.870)	Not Applicable

Table B-4: FAME analysis of *P. putida* ATCC 700369

Environmental Frequency - Best Match (Similarity Index)	**Clinical** Frequency - Best Match (Similarity Index)
10/17 *Pseudomonas putida* biotype A (0.557)	6/7 *Pseudomonas putida* biotype A (0.801)
5/17 No Match	1/7 *Pseudomonas fluorescens* biotype G & C (0.725)
1/17 *Paucimonas lemoignei* (0.317)	Not applicable
1/17 *Pseudomonas chlororaphis /aureofaciens/aurantiaca* (0.389)	Not applicable

Appendix C: 16S ribosomal RNA gene sequence analysis of *P. putida* ATCC 12633, 31800, 31483 and 700369

16S ribosomal RNA gene sequence data generated by Health Canada's Healthy Environments and Consumer Safety Branch. The 16S ribosomal RNA gene sequences of the four *P. putida* strains on the DSL were compared to the Microseq database and the Ribosomal Database project release 11 (Cole et al. 2014) and top 10 matches are shown. The match hit format is: identification code, similarity score (if available), S ab score, unique common oligomers and sequence full name.

Table C-1: Results of 16S Ribosomal RNA Gene Sequence Analysis of *P. putida* ATCC 12633

Identification Code	Seqmatch Score	Unique Common Oligomers	Sequence Full name
S000127497	0.999	1391	*Pseudomonas putida; KF715; AB109776*
S000407787	0.996	1389	*Pseudomonas sp. WSCIII; AY344806*
S000428777	0.997	1388	*Pseudomonas putida; ATCC 12633; AF094736*
S000428786	0.996	1379	*Pseudomonas putida; ATCC 11172; AF094745*
S000558712	1.000	1400	*uncultured bacterium; MP104-0916-b40; DQ088809*
S000649419	0.999	1380	*uncultured Pseudomonas sp.; SQ9_Pitesti; DQ366089*
S000774932	0.999	1395	*Pseudomonas sp. OCR2; AB240201*
S000976786	0.999	1397	*uncultured Pseudomonas sp.; AV_5N-G03; EU341207*
S001004690	0.995	1229	*uncultured bacterium; Ana200UA-10; EU499717*
S001153798	0.993	1361	*Pseudomonas sp. WMQ-7; EU807744*

The Seqmatch results support the identification of this strain as *P. putida*

Table C-2: Results of 16S Ribosomal RNA Gene Sequence Analysis of *P. putida* ATCC 31843

Identification Code	Seqmatch Score	Unique Common Oligomers	Sequence Full name
S000006483	0.997	*1392*	*Pseudomonas plecoglossicida (T); FPC951; AB009457*
S000434662	0.997	*1418*	*Pseudomonas sp. HR 13; AY032725*
S000752253	0.997	*1393*	*Pseudomonas sp. BJS-X-1; EF068265*
S000892479	0.997	*1384*	*Pseudomonas putida; ISSDS-590; EF620456*
S001099019	0.997	*1364*	*Pseudomonas putida; AS01; EU661866*
S001175076	0.997	*1392*	*uncultured bacterium; E33; EU556988*
S001794722	0.997	*1424*	*Pseudomonas monteilii; SB 3067; GU191931*
S002199718	0.997	*1394*	*uncultured Pseudomonas sp.; CapF3B.10; HM152577*
S002199755	0.997	*1364*	*uncultured Pseudomonas sp.; Filt.26; HM152614*
S002199766	0.997	*1364*	*uncultured Pseudomonas sp.; Filt.37; HM152625*

The Seqmatch results show matches with *P. putida*, *P. plecoglossicida*, *P. monteilii*, and other species.

Table C-3: Results of 16S Ribosomal RNA Gene Sequence Analysis of *P. putida* ATCC 31800

Identification Code	Seqmatch Score	Unique Common Oligomers	Sequence Full name
S000426303	0.970	1196	*Pseudomonas sp. E3; AY745742*
S000536080	0.970	1399	*uncultured bacterium; rRNA003; AY958776*
S000536117	0.974	1414	*uncultured bacterium; rRNA040; AY958813*
S000536122	0.969	1415	*uncultured bacterium; rRNA045; AY958818*
S000769445	0.971	1228	*Pseudomonas sp. RBE1CD-131; EF111137*
S000901674	0.969	1405	*Pseudomonas veronii; MPU43; AB334768*
S000980300	0.971	1411	*Pseudomonas sp. Hg4-06; EU304252*
S001351336	0.970	1296	*Pseudomonas sp. IMER-B4-19; FJ796439*
S001416054	0.968	1440	*Pseudomonas fluorescens SBW25; AM181176*
S001575909	0.976	1319	*Pseudomonas sp. N54.43.896; GQ214545*

The Seqmatch results show matches with *P. veronii* and *P. fluorescens*.

Table C-4: Results of 16S Ribosomal RNA Gene Sequence Analysis of *P. putida* ATCC 700369

Identification Code	Seqmatch Score	Unique Common Oligomers	Sequence Full name
S000537373	0.998	1404	*Pseudomonas putida; DQ060242*
S000557499	0.998	1402	*Pseudomonas sp. ONBA-17; DQ079062*
S000558711	0.998	1396	*uncultured bacterium; MP104-0927-b22; DQ088808*
S000626304	0.998	1402	*Pseudomonas sp. PHD-8; DQ301785*
S000711306	0.998	1365	*Pseudomonas putida; WAB1889; AM184230*
S001095516	0.998	1374	*Pseudomonas taiwanensis (T); BCRC 17751; EU103629*
S001265294	0.998	1404	*Pseudomonas sp. BJQ-D4; FJ600361*
S001577029	0.998	1405	*Pseudomonas sp. TSWCW20; GQ284465*
S001577035	0.998	1408	*Pseudomonas sp. PCWCW2; GQ284471*
S001794716	0.998	1428	*Pseudomonas monteilii; SB 3091; GU191925*

The Seqmatch results show matches with *P. putida*, *P. taiwanensis*, *P. monteilii* and other species.

Appendix D: List of toxins and secondary metabolites produced by *P. putida*

Table D-1: List of toxins and secondary metabolites produced by *P. putida*

Toxins	Actions	References
Lipopolysaccharide (LPS), endotoxin	• Endotoxin associated with Gram-negative bacteria. • A complex amphiphilic molecule essential for outer membrane functions, particularly during host-pathogen interactions. • Major virulence factor in the infectious process responsible for membrane depolarisation in cerebellar granule neurons. Causes the reduction of two of the major voltage-dependant potassium currents. • In sepsis, the lipid A component stimulates the innate immune response by binding to the phagocyte LPS receptor. This activates the release of the inflammatory cytokines TNF, IL-1, IL-6, IL-8 and IL-12, which in the bloodstream can cause septic shock.	(Balaji et al. 2004; Berndt et al. 2003; Hernandez Duquino and Rosenberg 1987; Yildiz 1998)
Hydrogen cyanide	• Produced by clinical isolates of *P. aeruginosa* from cystic fibrosis patients at low oxygen tension and high cell densities during the transition from exponential to stationary growth phase. • Inhibitor of plant roots and a broad-spectrum of compounds. • A potent inhibitor of cellular respiration that is produced under microaerophilic growth conditions at high cell densities. • Cyanide levels are associated with impaired lung function.	(Blumer and Haas 2000; Castric 1983; Flores-Vargas and O'Hara 2006; Pessi and Haas 2000; Ryall et al. 2008)
Pyoverdine	• High-affinity strain specific, yellow-green fluorescent siderophore. • In iron-limiting conditions, pyoverdine enables the acquisition of iron from the environment by chelating with iron when	(Gross and Loper 2009; Moon et al. 2008)

Toxins	Actions	References
	secreted in the extracellular environment and resulting in a ferri-pyoverdine complex that will be transported back into the bacteria by a cell surface receptor protein.	
Pyochelin	• Iron-scavenging metabolites.	(reviewed in Gross and Loper 2009)
Pseudomonine	• Secondary siderophore with similar function.Consists of salicylic acid and two heterocyclic amino acids.	(reviewed in Gross and Loper 2009)
Pyocins	• Antibacterial agents (active against closely related species or strains) usually associated with *P. aeruginosa* which exist in three types (R, F and S). • R- and F- type pyocins resemble tails of bacteriophage. The R- type has a non-flexible and contractile rod-like structure and the F- type has a flexible and non-contractile rod-like structure • R-type pyocin arrests the synthesis of macro molecules and releases intracellular material, which is followed by cell death caused by depolarisation of the cytoplasmic membrane. • Production starts when adverse conditions provoke DNA damage and at optimal temperatures (37°C).	(Iwalokun et al. 2006; Mavrodi et al. 2009; Michel-Briand and Baysse 2002)
Cyclic Lipopeptides (such as viscosin, massetolide and orfamide)	• Class of compounds with diverse structures containing fatty acyl residues ranging from C_5-C_{16} in length and chains of 7-25 amino acids of which 4-14 form a lactone ring. • Divided in 6 groups: Viscosin, Syringomycin, Amphisin, Putisolvin, Tolaasin and Syringopeptin. • Lowers surface tension and alters cellular membrane integrity by interaction due to their amphiphilic properties. • Increases the bioavailability of water-	(Reviewed in Gross and Loper 2009)

Toxins	Actions	References
	insoluble substrates, promotes cellular swarming and enhances virulence or antagonism against other micro-organisms.	
Pyrrolnitrin	• Strong antifungal activity that inhibits the fungal respiratory chain. • Compound used as a topical antimycotic in humans.	(Reviewed in Gross and Loper 2009)
Phenazines	• Over 50 compounds in this large family of colorful nitrogen-containing tricyclic molecules. • Antibiotic, antitumor and antiparasitic activities due to interaction with polynucleotides, topoisomerase inhibition and the generation of free radicals • Intracellular signals have an influence on transcriptional regulation and broad effects on bacterial physiology and fitness.	(Reviewed in Gross and Loper 2009)
Pyoluteorin	• Substance composed of a bichlorinated pyrrole linked to a resorcinol moiety • Shows antifungal properties. • Moderates plant disease caused by other pathogens such as oomycetes fungi.	(Hammer et al. 1997; Nowak-Thompson et al. 1999)