Final Screening Assessment Report for *Pseudomonas stutzeri* ATCC 17587

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Synopsis

Pursuant to paragraph 74*(b)* of the *Canadian Environmental Protection Act, 1999* (CEPA 1999), the Minister of the Environment and the Minister of Health have conducted a screening assessment of *P. stutzeri* ATCC 17587.

P. stutzeri ATCC 17587 is a bacterium that has characteristics in common with other strains of the species *P. stutzeri* that occur in nature. *P. stutzeri* has the ability to adapt to and thrive in soil, sediments and water. Multiple potential uses of *P. stutzeri* in household, industrial, commercial and agricultural sectors exist. These include treatment of ponds and aquariums (to breakdown wastes and control odours), waste management, wastewater treatment, septic tank cleaning and deodorizing, drain cleaning and degreasing, bioremediation, recovery of oil and precious metals, as well as in enzyme production for the manufacture of foods, detergents, textiles and bioethanol.

Despite the widespread presence of *P. stutzeri* in soil and water and in close association with plant roots, only one case of infection has been reported in terrestrial vertebrates. This case was in chickens, and the infection was successfully treated with antibiotics. Certain strains of *P. stutzeri* have antialgal, antibacterial and antifungal properties. Experimental challenge with *P. stutzeri* ATCC 17587 on a soil springtail, a terrestrial invertebrate, revealed a significant decrease in adult survival and juvenile production at concentrations which can be reached during bioremediation uses. However, there is no evidence that occasional applications of *P. stutzeri* ATCC 17587 to soil will adversely affect terrestrial invertebrates at the population level.

There have been no human infections attributed to the DSL strain *P. stutzeri* ATCC 17587. Some strains of *P. stutzeri* can act as an opportunistic pathogen in susceptible humans. Compared with other closely-related opportunistic *Pseudomonas* pathogens, the incidence of nosocomial or secondary infection due to *P. stutzeri* in individuals with compromised immunity and underlying medical conditions is low.

This assessment considers the aforementioned characteristics of *P. stutzeri* ATCC 17587 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 (section 71 notice) as published in the *Canada Gazette*, Part I, on October 3, 2009. Information submitted in response to the notice indicates that *P. stutzeri* ATCC 17587 was not imported into or manufactured in Canada in 2008.

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. stutzeri* ATCC 17587. It is concluded that *P. stutzeri* ATCC 17587 does not meet the criteria under paragraph 64*(a)* or *(b)* of CEPA 1999 as it is not entering the environment in a quantity or concentration or under conditions that have or may have an immediate or long-term harmful effect on the environment or its biological diversity or that constitute or may constitute a danger to the environment on which life depends.

Also, based on the information presented in the Screening Assessment, it is concluded that *P. stutzeri* ATCC 17587 does not meet the criteria under paragraph 64*(c)* of CEPA 1999 as it is not entering the environment in a quantity or concentration or under conditions that constitute or may constitute a danger in Canada to human life or health.

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Introduction

Pursuant to paragraph 74*(b)* of the *Canadian Environmental Protection Act, 1999* (CEPA 1999), the ministers of Environment and of Health are required to conduct Screening Assessments of those living organisms listed on the *Domestic Substances List* (DSL) which were in commerce between 1984 and 1986, 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] *Pseudomonas stutzeri* ATCC 17587 was added to the DSL under subsection 105(1) of CEPA 1999 because it was manufactured in or imported into Canada between January 1, 1984, and December 31, 1986, and entered or was released into the environment without being subject to conditions under CEPA 1999 or any other federal or provincial legislation.

This Screening Assessment considers hazard information obtained from the public domain and from 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 1 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 DSL-listed *P. stutzeri* ATCC 17587 are identified as such. Where strain-specific data were not available, surrogate information from literature searches was used. When applicable, literature searches conducted on the organism included its synonyms, and common and superseded names. Surrogate organisms are identified in each case to the taxonomic level provided by the source. Literature searches were conducted using scientific literature databases (SCOPUS, CAB Abstracts, and NCBI), web searches, and key search terms for the identification of human health and environmental hazards. Information identified up to August 2013 was considered for inclusion in this Screening Assessment Report.

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

The Public Health Agency of Canada (PHAC) assigned *P. stutzeri* (as a species) to Risk Group 1 for both humans and terrestrial animals (personal communication, PHAC 2013).

The Canadian Food Inspection Agency (CFIA) does not consider *P. stutzeri* as a regulated plant pest in Canada (personal communication, CFIA 2013).

International

No information found.

1. Hazard Assessment

1.1 Characterization of *Pseudomonas stutzeri*

1.1.1 Taxonomic identification and strain history

Binomial name *Pseudomonas stutzeri* (*P. stutzeri*)
Taxonomic designation
Kingdom Bacteria
Phylum Proteobacteria
Class Gammaproteobacteria
Order *Pseudomonadales*
Family *Pseudomonadaceae*
Genus *Pseudomonas*
Species *stutzeri* (Lehmann and Neumann, 1896) Sijderius 1946 (validated 1980)
Strain ATCC 17587 (equivalent to LMG 5838, NCPPB 1972, Lautrop AB180, Stanier 220)

Superseded names: "*Bacillus denitrificans* II" (Burri and Stutzer, 1895); "*Bacterium stutzeri*" (Lehmann and Neumann, 1896); "*Bacillus nitrogenes*" (Migula, 1900); "*Bacillus stutzeri*" (Chester, 1901); "*Achromobacter sewerinii*" (Bergey et al., 1923); "*Achromobacter stutzeri*" (Bergey et al., 1930); "*Pseudomonas stanieri*" (Mandel, 1966).

Strain history

P. stutzeri ATCC 17587 was originally isolated from a clinical specimen (bile) in 1956 at Statens Seruminstitut, Copenhagen, Denmark, by Dr. H. Lautrop and designated as Lautrop strain AB 180 (reviewed in NCPPB, 2013; Rainey et al., 1994; Stanier et al., 1966). The strain was received by Dr. R.Y. Stanier and designated as Stanier 220 (Stanier et al., 1966) and deposited to the American Type Culture Collection as ATCC accession number 17587. The strain was also deposited to the National Collection of Plant Pathogenic Bacteria as NCPPB 1972 (NCPPB, 2013).

1.1.2 Phenotypic and molecular characteristics

P. stutzeri is markedly heterogeneous, making each strain difficult to differentiate from another on the basis of morphology, biochemical and nutritional properties alone (reviewed in Cladera et al., 2004; Lalucat et al., 2006). Species diversity, however, has been established with the subdivision of *P. stutzeri* into distinct genomic groups, commonly referred to as genomovars (Rosselló-Mora et al., 1991). There are currently 22 genomovars assigned to the species (Scotta et al., 2013).

This grouping allows for clustering of *P. stutzeri* strains that cannot be distinguished phenotypically from others (Rosselló-Mora et al., 1991; Cladera et al., 2005). Based on the taxonomic standard for species delineation (Roselló-Mora and Amann, 2001; Stackebrandt et al., 2002), members of the same *P. stutzeri* genomovar are grouped based on DNA-DNA hybridization values greater than 70% (Rosselló-Mora et al., 1991; reviewed in Cladera et al., 2004; Lalucat et al., 2006; Sikorski et al., 2005). These hybridization values are usually less than 50% when strains from different genomovars are compared (Rosselló-Mora et al., 1991; Garcia-Valdes et al., 2010).

Comparisons of morphological, biochemical and molecular properties between *P. stutzeri* ATCC 17587, *P. stutzeri* ATCC 17591 (genomovar 2 reference strain clinical isolate) and *P. stutzeri* ATCC 17588 (genomovar 1 type strain clinical isolate) are summarized in Tables 1-1, 1-2, 1-3 and 1-4. Characteristics that differentiate *P. stutzeri* from its closest pathogenic relatives, *P. aeruginosa* and *P. fluorescens*, are also noted in the following tables.

Table 1-1 Morphological properties of *P. stutzeri*

Characteristic	***P. stutzeri* ATCC 17587 (DSL strain) genomovar 2**	***P. stutzeri* ATCC 17591 (reference strain) genomovar 2**	***P. stutzeri* ATCC 17588 (type strain) genomovar 1**
Gram staining	Negative[a]	Negative[b]	Negative[b]
Fluorescent	No[a,c]	No[b]	No[a,c]
Colonies	Colony morphologies vary with different nutrient media and culture conditions[a]	Translucent colonies with entire margin[b]	Freshly isolated: wrinkled, resemble craters with elevated ridges (coral structure), reddish brown, hard, dry, tenacious Older colonies: smooth, butyraceous, pale (Lalucat et al., 2006)
Motility	Yes[a]	Yes (Rosselló-Mora et al., 1991)	Yes[b]

[a] Unpublished data generated by Health Canada's Healthy Environments and Consumer Safety Branch. Refer to Appendix A Tables A-1 and A-2 for more morphology data on ATCC 17587
[b] Unpublished data generated through the International *Pseudomonas* Double-Blind Study funded by Health Canada
[c] Characteristic that distinguishes *P. stutzeri* from *P. aeruginosa* or *P. fluorescens*

Table 1-2 Growth and biochemical properties of *P. stutzeri*

Characteristic	*P. stutzeri* ATCC 17587 (DSL strain) genomovar 2	P. stutzeri ATCC 17591 (reference strain) genomovar 2	*P. stutzeri* ATCC 17588 (type strain) genomovar 1
Growth at 42°C	No[a]	No[a]	Yes[b]
Catalase	Positive[c]	Positive[b]	Positive[b]
Oxidase	Positive[c]	Positive[b]	Positive[b]
Starch hydrolysis	Positive[c,d]	Positive[b]	Positive[b,d]
Gelatin hydrolysis	Negative[c,d]	Negative[b]	Negative[b,d]
Denitrification	Positive[c]	Positive (Grüntzig et al., 2001)	Positive[b]

[a] Data from Rosselló-Mora et al. (1991)
[b] Unpublished data generated through the International *Pseudomonas* Double-Blind Study funded by Health Canada
[c] Unpublished data generated by Health Canada's Healthy Environments and Consumer Safety Branch
[d] Characteristic that distinguishes *P. stutzeri* from *P. aeruginosa* or *P. fluorescens*

Table 1-3 *P. stutzeri* assimilation of select substrates

Substrate	*P. stutzeri* ATCC 17587 (DSL strain) genomovar 2	P. stutzeri ATCC 17591 (reference strain) genomovar 2	*P. stutzeri* ATCC 17588 (type strain) genomovar 1
D-Fructose	Positive[a]	Positive[a]	Positive[b]
Ethanolamine	Positive[b]	Positive[b]	Negative[b]
Mannose	Negative[b]	Negative[b]	Negative[b]
Mannitol	Positive[a]	Positive[b]	Positive[b]
n-Propanol	Positive[b]	Positive[b]	Negative[b]
Propionate	Negative[b]	Negative[b]	Positive[b]
Propylene glycol	Positive[b]	Positive[b]	Negative[b]
Valerate	Negative[b]	Positive[b]	Positive[b]
Glycine	Positive[a]	Positive[a]	Positive[a]
Ethylene glycol	Positive[b]	Positive[b]	Negative[b]

[a] Data from Rosselló-Mora et al. (1991)
[b] Data from Stanier et al. (1961)

Table 1-4 Molecular properties of *P. stutzeri*

Characteristic	*P. stutzeri* ATCC 17587 (DSL strain) genomovar 2	P. stutzeri ATCC 17591 (reference strain) genomovar 2	*P. stutzeri* ATCC 17588 (type strain) genomovar 1
G+C content (%)	~60.6% (Xiang et al., 2010)	61.4% (Rossello et al., 1991)	63.9% (Chen et al., 2011)
16S rRNA (GenBank Accession #)	U25431	U26261	U26262

In addition to the properties summarized in the tables above, Health Canada also independently examined the growth on liquid media at different temperatures

(Appendix A Table A-3), growth on different solid media at 37°C (Appendix A Table A-4) and fatty acid methyl-ester (FAME) profile (Appendix A Figures 3 and 4) of *P. stutzeri* ATCC 17587.

Analyses of the sequences of 16S rDNA or housekeeping genes complement phenotypic methods used to distinguish *P. stutzeri* from closely-related *Pseudomonas* species. A 16S rDNA consensus sequence was derived at Health Canada for *P. stutzeri* ATCC 17587 and was used as a query sequence against the DNA databases of the National Center for Biotechnology Information–Basic Local Alignment Search Tool (NCBI BLAST). Sequence alignment shows 99.86% rRNA homology to *P. stutzeri* type strain ATCC 17588 and 98% similarity to other *Pseudomonas* species, such as *P. otitidis, P. pseudoalcaligenes, P. xanthomarina* and *P. putida.*

Sequence differences in other markers and housekeeping genes have also been used to differentiate *P. stutzeri* from other *Pseudomonas* species. As shown in Figure 1, the relatedness of *P. stutzeri* to other validly described *Pseudomonas* species has been examined by comparing the DNA gyrase B (*gyrB*) and the RNA polymerase sigma factor (*rpoD*) gene sequences (Yamamoto et al., 2000).

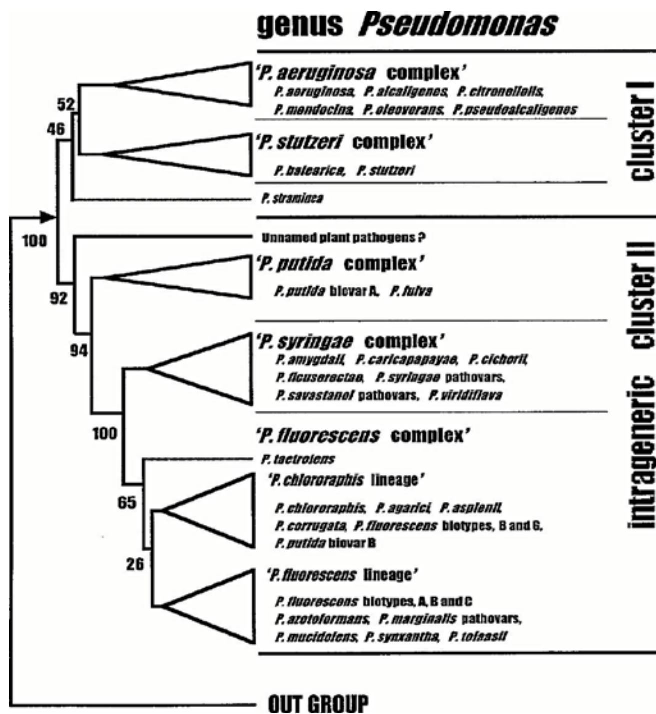

Figure 1-1: Schematic dendrogram summarizing the intrageneric structure of the genus *Pseudomonas* based on analysis of *gyrB* and *rpoD* sequences of 31 validly published species (Adapted from Yamamoto et al., 2000, with permission)

The resulting dendrogram (Figure 1-1) demonstrates the divergence of the genus *Pseudomonas* into two major intragenic clusters in which *P. stutzeri* belongs to the same cluster as *P. aeruginosa*. The PseudoMLSA Database is also used for multigenic sequence analysis of *Pseudomonas* species based on housekeeping genes, such as *gyrB*, *rpoB* and *rpoD* for *P. stutzeri* (Bennasar et al., 2010).

Housekeeping genes specifically targeting the denitrification pathway have been used to further differentiate *P. stutzeri* from closely-related species. These include the *nosZ* gene for nitrous oxide reductase (Zumft et al., 1990), the *nirS* gene for nitrite reductase, which is responsible for the reduction of nitrite (NO_2^-) to nitric oxide (NO) (Grüntzig et al., 2001), and the *nifH* gene for the nitrogenase enzyme (Chan et al., 1994).

Other phenotypic and molecular methods, such as the API 20NE commercial kit and Restriction Fragment Length Polymorphism (Bennasar et al., 1998), respectively, can also be used with other previously described techniques for the identification of *P. stutzeri*. Given the phenotypic and genotypic diversity of the *P. stutzeri* group, a polyphasic approach is important in generating a robust identification that allows for clear differentiation of *P. stutzeri* from closely-related pathogenic *Pseudomonas* species. None of the described techniques, however, can unequivocally differentiate the DSL-listed strain from other *P. stutzeri* strains.

1.2 Biological and ecological properties

The diversity of *P. stutzeri* is demonstrated by its adaptive ability to occupy a variety of ecological niches. *P. stutzeri* has been isolated from marine sediments and water column, deep-sea hydrothermal vents, wastewaters, sludge, garden soils, contaminated soils, plant roots and rhizosphere (reviewed in Lalucat et al., 2006; Bolognese at al., 1999), and as an intra-cellular symbiont with the dinoflagellate alga *Alexandrium lusitanicum* (Plumley et al., 1999). In a global study of denitrifying bacteria conducted by Gamble et al. (1997), *P. stutzeri* or *stutzeri*-like strains dominated the denitrifier population of approximately 10^6 CFU/g in soil and freshwater lake sediments at specific sites in Michigan, USA.

Long-term persistence of *P. stutzeri* ATCC 17587 in agricultural soil was investigated by Xiang et al. (2010). Using amplified fragment length polymorphism, quantitative PCR of the recovered *P. stutzeri* ATCC 17587 DNA from extracted soil was performed to estimate the cell concentration in the soil (Figure 1-2).

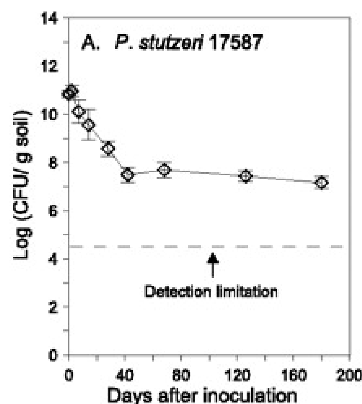

Figure 1-2: Persistence of *P. stutzeri* ATCC 17587 in soil based on qPCR analyses of extractable soil DNA (Adapted from Xiang et al., 2010, with permission)

As shown in Figure 1-2, DNA from *P. stutzeri* ATCC 17587 could be amplified from laboratory microcosms for at least 180 days after 10^8 to 10^{10} CFU/g were introduced (Xiang et al., 2010). Following an initial decline in recovered DNA over 40 days, the cell concentration appears to reach equilibrium at a concentration of approximately 10^7 CFU/g dry soil. This indicates that *P. stutzeri* ATCC 17587 is able to establish a niche and persist directly within the microcosm.

1.2.1 Growth conditions

Given the diversity of habitats where it is found, *P. stutzeri* is adaptable to a wide range of environmental conditions. Different strains of the species have different growth temperature ranges, overall spanning 4°C to 40°C (Palleroni, 2005), but growth at extremes of the temperature range is limited to selected strains (reviewed

in Lalucat et al., 2006). *P. stutzeri* ATCC 17587 does not grow at 4°C and grows well at 40°C (Palleroni et al., 1970). Most strains of the species are unable to grow at pH 4.5 (reviewed in Lalucat et al., 2006). Growth kinetics (conducted at Health Canada) in various growth media at different temperatures are shown in Appendix A Tables A-3 and A-4.

Ude et al. (2006) observed that *P. stutzeri* ATCC 17587 formed biofilms *in vitro* within 15 days of inoculation on King's B medium at 20–22°C. Other *P. stutzeri* strains have been isolated from biofilms growing on copper and galvanized steel, which are commonly used in water-based heating and cooling systems (Dogruöz et al., 2009) and on dental unit water systems (Singh et al., 2003).

1.2.2 Nutrient cycling

P. stutzeri is active in nutrient cycling in the environment. It is well known for its simultaneous capacity for denitrification and nitrogen fixation, which are of relevance to the overall nitrogen cycling in several ecosystems (reviewed in Lalucat et al., 2006; Krotzky and Werner, 1987). Denitrification is a stable trait for *P. stutzeri*; it is one of the most active denitrifying, heterotrophic bacteria. The bacterial process of denitrification is normally a facultative trait. It provides bacteria with a respiratory pathway for anaerobic life (reviewed in Lalucat et al., 2006). Under anaerobic conditions, *P. stutzeri* denitrification is highly dependent on the presence of nitrate and nitrous oxide as electron acceptors (Korner and Zumft, 1989). Aerobic denitrification can only take place in *P. stutzeri* at low dissolved oxygen concentration (Körner and Zumft, 1989).

P. stutzeri may also play an important role in the turnover of thiosulfate in marine environments (Sorokin et al., 1999). *P. stutzeri* isolated from Baltic Sea sediments at the shoreline and in anoxic water column was found to oxidize thiosulfate to tetrathionate (40–50% of the thiosulfate sulfur) as the major product and to elemental sulfur (15–30%) (Podgorsek and Imhoff, 1999).

A *P. stutzeri* strain that is related to *P. stutzeri* strain DSM 50227 (a clinical isolate) contains the *htx* gene, which encodes for hypophosphite-2-oxoglutarate dioxygenase (White and Metcalf, 2004). This enzyme allows the organism to utilize phosphonates as an alternative source of phosphorus in low phosphate environments (Dyhrman et al., 2006).

Finally, some *P. stutzeri* strains, such as JM300 and CMT.9.A, have hydrogenase activity which supplies additional energy for metabolism (reviewed in Lalucat et al., 2006; Barraquio et al., 1988).

1.2.3 Plant growth promotion and biocontrol

P. stutzeri is present in the plant rhizosphere. For instance, *P. stutzeri* A15 and CMT.9.A have been isolated from the rhizosphere of rice in China (Rediers et al.,

2003) and from the roots of a cultivar of *Sorghum nutans* (Yellow Indian Grass) in Germany (Krotzy and Werner, 1987). *P. stutzeri* strain A1501 has properties that facilitate its colonization and interaction with plants such as: motility, presence of global regulators, and nutritional and stress adaptation (reviewed in Hardoim et al., 2008). Genes promoting plant growth and preventing ethylene formation, which is an inhibitor of root elongation, were also identified in *P. stutzeri* A1501 (reviewed in García-Valdés et al., 2010). Also, *P. stutzeri* strain RP1, isolated from sunflower endorhizosphere, showed multiple plant growth-promoting attributes such as phosphate solubilization, auxin (indole-3-acetic acid), NH_3 and HCN production, and antibacterial activity (Pandey et al., 2013). A *P. stutzeri* strain isolated from cyanobacteria-deprived lichens was able to release amino acids and 3-indoleacetic acid through the production of phytohormones, directly contributing to the nutrition of those lichens by releasing amino acids available to them, but was not able to solubilize phosphates (Liba et al., 2006).

Certain strains have been reported to have anti-algal, antibacterial and antifungal properties. *P. stutzeri* YPL-1 produces lytic extracellular enzymes which inhibit mycelial growth rather than spore germination and also causes lysis of *Fusarium solani* mycelia and germ tubes (Lim et al., 1991). Pandey et al. (2013) indicated that certain *P. stutzeri* strains showed antibacterial activity against *Escherichia coli, Xanthomonas sp, Serratia marcescens* and *Bacillus cereus.* Some *P. stutzeri* strains can also produce nocardamine (Meyer and Abdallah, 1980), which is an antibiotic covalently linked to a siderophore (Braun et al., 2009). This compound has antibacterial activity against certain mycobacteria (Wencewicz et al., 2013).

Three *P. stutzeri* strains, A41, B47 and MM4, that were isolated from sea sediment and aeration tanks of a sewage plant showed lethal activity against the red tide phytoplankton *Chattonella antiqua*, which lost its mobility and lysed. Moreover, the three strains did not show any toxicity on killifish (*Cyprinodontidae* family), which implies that they could be applied for the protection of cultivated fishes from the damage caused by red tide plankton (Hayashida et al., 1991).

1.2.4 Biosorption of metals and degradation of organic compounds

P. stutzeri has the potential to be used in biosorption of certain metals. For example, *P. stutzeri* strain KCCM 34719 demonstrated sorptive capacities (q_{max}) of 47.86 and 33.16 for cadmium(II) and copper(II), respectively. The optimum pH for biosorption rates is pH 5; above this value, the metals have been found to precipitate (Hassan et al., 2009).

P. stutzeri is metabolically diverse and is capable of degrading a range of organic substrates. Some *P. stutzeri* strains are able to metabolize aromatic hydrocarbons including mono- and di-halogen benzoates, benzenesulfonate, carbazole, cresol, dibenzothiophene, fluoranthene, fluorene, indan, naphthalene (Velayutham et al., 2012), polychlorinated biphenyls, phenanthrene, phenols, pyrene, quinoline, salicylate, tetralin, toluate, toluene, and xylene (reviewed in Lalucat et al., 2006).

P. stutzeri is also capable of degrading aliphatic hydrocarbons. For example, *P. stutzeri* strain KC, isolated from groundwater aquifer solids, is capable of transforming carbon tetrachloride to carbon dioxide under anoxic iron-limited condition (45–55% of carbon as determined by ^{14}C labelling), to a non-volatile fraction (45–55% of carbon), and a cell-associated fraction (5% of carbon) (Criddle et al., 1990; Dybas et al., 1995). Strain JJ, isolated from soil contaminated with1,2-dichloroethane, oxidizes 2-chloroethanol (as sole energy and carbon source) completely to CO_2 with NO_3^- or O_2 as electron acceptor (Dijk et al., 2003).

Certain *P. stutzeri* strains have been found to degrade biocides, such as tributyltin, β-cyfluthrin, cyanide and thiocyanates, all of which are used in industrial and agricultural applications (reviewed in Lalucat et al., 2006).

1.2.5 Resistance to metals and chemical agents

Several naturally-occurring strains of *P. stutzeri* are resistant to metals such as aluminum, chromium, cobalt, copper, germanium, lead, manganese, nickel, plutonium, selenium, silver, thallium, titanium, uranium, vanadium and zinc (review by Lalucat et al., 2006). *P. stutzeri* is susceptible to a variety of chemical agents or biocides. For instance, a study comparing the effect of cationic antiseptics, mercury compounds, the parabens, phenolics and EDTA on six strains of *P. stutzeri* (NCIMB 568, 10783, 11358, 11359, JM 302, JM 375) has shown that all strains were found to be highly sensitive to chlorhexidine diacetate (CHA), organomercurials and triclosan, but less sensitive to quaternary ammonium compounds (Tattawasart et al., 1999); however, certain strains of *P. stutzeri* developed stable resistance to CHA or cetylpyridinium chloride (CPC) when exposed to gradually increasing concentrations of either antibacterial agent. Alterations in the cell envelope are likely to be responsible for non-specific changes in sensitivity to several antibacterial agents (Tattawasart et al., 1999). Resistance to CHA is likely linked to changes in the binding site(s) available at the outer membrane of the bacterium (Tattawasart et al., 2000).

1.2.6 Horizontal gene transfer

P. stutzeri is noted for its ability to become naturally competent for transformation (Sikorski et al., 2002) and can take up intraspecies and foreign DNA. Competence in *P. stutzeri* is a transient physiologic state that manifests at the transition between the log phase and stationary phase (reviewed in Lalucat et al., 2006).

Intraspecific DNA transformation through homologous recombination has been reported for *P. stutzeri* ATCC 17587. Lorenz and Sikorski (2000) have shown that ATCC 17587 is highly transformable at a frequency of $2.6 \pm 0.6 \times 10^{-4}$ when grown on LB agar in the presence of DNA extracted from *P. stutzeri* rifampicin-resistant mutants. Although possible within the *P. stutzeri* population, Rius et al. (2001) reported that recombination is rare or absent between distinct *P. stutzeri* populations based on a multilocus enzyme electrophoresis analysis of 42 *P. stutzeri* strains

belonging to several genomovars and isolated from different sources. Transformation of *P. stutzeri* ATCC 17587 with DNA from relatives within the same genus, *Pseudomonas mendocina* ATCC 25411 or *P. alcaligenes* ATCC 14909, was nearly two orders of magnitude lower than in intraspecific transformation (Lorenz and Sikorski, 2000). The frequency of foreign DNA acquisition events was only 0.0003% of the value observed for fully homologous DNA transformation (Lalucat et al., 2006).

In *P. stutzeri*, natural transformation requires the formation of functional type IV pili that affects the translocation of DNA into the cytoplasm (Graupner and Wackernagel, 2000; Graupner and Wackernagel, 2001). Transformation is also tightly controlled by *comA* gene which encodes for a competence transcription factor (Graupner and Wackernagel, 2001).

Conjugation and transduction in *P. stutzeri* also exist, but have not been studied in detail (reviewed in Lalucat et al., 2006). Transposons have been linked to the evolution and construction of catabolic pathways for degradation of organic contaminants in *P. stutzeri* (reviewed in Garcia-Valdes et al., 2010), while class 1 integrons have been associated with the dissemination of antibiotic resistance (Poirel et al., 2010) and the capture of metabolic islands (Garcia-Valdes et al., 2010). The genome of *P. stutzeri* ATCC 17587 has not been fully sequenced, so its complement of mobile genetic elements remains uncharacterized; however, Tetu and Holmes (2008) reported that *P. stutzeri* strain ATCC 17587 contains four copies of insertion sequence ISPst6. Several mobile genetic elements have been detected in other strains of *P. stutzeri* (Refer to Appendix B). Of particular importance is the presence of a class 1 integron in well-studied *P. stutzeri* species (The Uniprot Consortium, 2013; Winsor et al., 2011). The integron contains the $bla_{IMP\text{-}16}$ gene which encodes imipenemase-type metallo-beta-lactamases that catalyze the hydrolysis of a broad range of beta-lactams, including carbapenem (Lee et al., 2004; Carvalho-Assef et al., 2010; Yan et al., 2001).

Transformation frequency of DNA within a *P. stutzeri* population could contribute to the genomic plasticity and diversity of the species (Ginard et al., 1997; Sikorski et al., 1999), which enable it to adapt to new ecological niches and to act as a reservoir of antibiotic resistance genes under long-term selective pressure in the hospital environment (Yan et al., 2001; Carvalho-Assef et al., 2010).

Compared to other *P. stutzeri* strains, *P. stutzeri* ATCC 17587 has a low transformation frequency (Lorenz and Sikorski, 2000). The possibility of *P. stutzeri* ATCC 17587 acquiring virulence genes from other species in the environment is considered limited.

1.3 Effects

1.3.1 Environment

An in-depth scientific literature search on *P. stutzeri* yielded only a few cases of naturally-occurring toxicity, virulence factors and pathogenicity towards plants and animals, despite the widespread presence of this species in the environment. In addition, the genomic study of *P. stutzeri* strain A1501 provided evidence of the absence of virulence genes that are found in *P. aeruginosa* PAO1, which is an opportunistic plant and animal pathogen. *P. stutzeri* does not carry genes for type III/VI secretion systems, the synthesis of both types of quorum-sensing molecules, alginate polymer synthesis, siderophores or antibiotic biosynthesis pathways (Yan et al., 2008).

Plants

A study looking at anti-algal bacteria reported that three strains of *P. stutzeri* showed lethal activity (i.e., lost motility and lysis) against a marine alga, *Chattonella antiqua*, which is known to cause red tide leading to serious damage to the commercial cultivation of yellowtails (fish) (Hayashida et al., 1991). The *P. stutzeri* anti-algal activity reaches its maximum after two to six days of cultivation with a lowest lethal concentration of 0.5% (w/v). In addition, no toxicity was observed, over the course of one week, towards co-cultured fish belonging to the *Cyprinodontidae* family (killifish) when fed a homogenized sardine meat supplemented with anti-algal bacteria *P. stutzeri* A41 (one loopful of bacteria per 5g of sardines). The selective toxicity of *P. stutzeri* implies that it could be applied for the protection of cultivated fishes from the damage caused by red tide plankton. No other pathogenicity information on aquatic plant species was found in the literature.

In terrestrial plant testing at Environment Canada[2], red clover (*Trifolium pratense*) was grown in clay loam soil inoculated at day 0 with 10^8 CFU, at day 14 with 10^9 CFU and at day 28 with 10^9 CFU of *P. stutzeri* ATCC 17587 per gram of dry soil. A 21% decrease in root length was observed at the end of the study (day 42) for both the non-infectious control and the infectious treatment when compared with the negative control, which indicates that this effect may not be directly attributable to the live *P. stutzeri* ATCC 17587. A significant increase in shoot dry weight was observed in the non-infectious control and the infectious treatment, with no significant difference in root dry weight, regardless of treatment. Given the information above and the lack of a reduction in root dry weight, it is difficult to ascertain whether exposure to the bacteria, at these test concentrations, resulted in any adverse effect to red clover.

[2] Tests done according to Environment Canada's "Guidance Document for Testing the Pathogenicity and Toxicity of New Microbial Substances to Aquatic and Terrestrial Organisms (EPS 1/RM/44, March 2004)".

Invertebrates

Environment Canada also conducted research on the pathogenicity and toxicity of *P. stutzeri* ATCC 17587 on a soil arthropod. *Folsomia candida* (springtail, a soil invertebrate) was grown for 28 days in clay loam soil inoculated at day 0 with 10^8 CFU and at day 14 with 10^9 CFU of *P. stutzeri* ATCC 17587 per gram of dry soil. There was a significant decrease (14%) in adult survival in the infectious treatment when compared with both the negative control and non-infectious control, indicating pathogenicity to the invertebrate. There was also a decrease in juvenile production (45%) which may be attributable to a component of the bacterial cells which is still active once the cells are dead, as the difference in the effect between the live and killed cells was not statistically different.

P. stutzeri was used as a control in a study involving other pathogenic bacteria towards the nematode *Caenorhabditis elegans*. No effects were observed in the nematodes after 24 h exposure to 20 µL of viable *P. stutzeri* that was cultured on Luria broth and incubated at 37°C overnight (Balaji et al., 2004).

No reports in the literature have been found regarding pathogenicity of *P. stutzeri* towards aquatic invertebrates despite having been isolated from marine sediments and water column (reviewed in Lalucat et al., 2006).

Vertebrates

Fifteen different bacterial species were isolated from the respiratory tracts of various types of sick chickens suffering from a long-lasting syndrome or from the bone marrow of dead chickens. *P. stutzeri* was identified among these bacteria. To determine the etiological agent of infection, each bacterium was inoculated intraperitoneally (1 mL per chicken of 10^{10}CFU/mL) into ten healthy four-week-old chickens. The isolate identified as *P. stutzeri* killed 20% of animals by one week post-inoculation, compared with 58% mortality observed with an isolate identified as *P. aeruginosa*. Drugs that successfully treated the *P. stutzeri* infection in chickens were apramycin, gentamicin, spectinomycin, oxytetracycline and sulfachloropyrazine (Lin et al., 1993). Another study reported the isolation of *P. stutzeri* from the conjunctiva of a captive Kori bustard (bird native to Africa) suffering from conjunctivitis. However, the occurrence of this isolation was very low (of the 537 diseased bustards examined, 32 had conjunctivitis and only one of them contained *P. stutzeri*) and the micro-organism was not confirmed to be the causative agent (Silvanose et al., 2001). No other information on pathogenicity to avian species was found in the literature.

Studies conducted at Health Canada showed no adverse effects following endotracheal administration of 10^6 CFU *P. stutzeri* ATCC 17587 in mice. No reports in the literature have been found regarding pathogenicity of *P. stutzeri* towards mammals, particularly towards grazing mammals despite the close association of *P. stutzeri* with plant roots (Rediers et al., 2003; Krotzy and Werner, 1987).

No reports in the literature have been found regarding pathogenicity of *P. stutzeri* towards fish or other aquatic vertebrates despite its isolation from marine sediments and water column (reviewed in Lalucat et al., 2006).

1.3.2 Humans

P. stutzeri is generally not considered as an etiological agent of infection in humans, but it is occasionally isolated from clinical samples. The species accounts for only 1 to 2% of all *Pseudomonas* clinical isolates (Noble and Overman, 1994; Bisharat et al., 2012). Most frequently, the clinical isolates were recovered from wounds, pus, blood, urine, tracheal aspirates and sputum (Holmes, 1986; Ergin and Mutlu, 1999; Noble and Overman, 1994; Bisharat et al., 2012). Noble and Overman (1994) reported that in most cases, *P. stutzeri* is not found as a single isolate but as part of a polymicrobial infection. The majority of *P. stutzeri* clinical isolates were reported to belong to genomovar 1 (Garcia-Valdes et al., 2010). This was supported by a recent study of 229 *P. stutzeri* strains from various origins. Scotta et al. (2013) reported that 136 of these strains were of clinical origin in which 88% belong to genomovar 1 and only 10% belong to genomovar 2 (including ATCC 17587).

P. stutzeri has been intermittently linked to secondary infections in patients with existing health conditions or who have undergone invasive medical procedures. These include pneumonia (Campos-Herrero, 1997; Carratala et al., 1992; Loyse et al., 2006; Potvliege et al., 1987), septicemia (Bello, 2007; Potvliege et al., 1987; Priestly et al., 1996), arthritis (Bishara et al., 2000; Miron et al., 2007), conjunctivitis (Singh, 2008), endocarditis (Grimaldi et al., 2009; Rosenberg et al., 1987), panophthalmitis (Lebowitz et al., 2001) and meningitis (Chang et al., 1996; Roig et al., 1996).

Hospital-acquired *P. stutzeri* infections, such as endopthalmitis (Jiraskova and Rozsival, 1998), brain abscess (Yee-Guardino et al., 2006) and peritonitis (Ceri et al., 2010) have also been reported. Rare infections in otherwise healthy individuals include an isolated case of pneumonia and empyema (Kose et al., 2004) and osteomyelitis (Reisler and Blumberg, 1999; Rowley et al., 1987) in children.

Very few outbreaks related to *P. stutzeri* were reported, all of which occurred during the 1970s and 1980s. These include bacteremia, septicaemia and peritonitis that were attributed to contamination of intravenous fluids (Felts et al., 1972), catheters (Elting et al., 1990), a water system used for hemodialysis (Goetz et al., 1983), or soap used to prepare the skin for intravenous insertions (Keys et al., 1983). No other outbreaks of *P. stutzeri* infection have since been reported in the scientific literature. *P. stutzeri* infections have rarely resulted in mortality. Deaths are often directly attributed to significant comorbidities, rather than to the presence of *P. stutzeri* in the clinical material (Bisharat et al., 2012).

While generally resistant to cephalosporins, monobactams and macrolides, *P. stutzeri* infections have been successfully treated with a wide range of antibiotics,

including aminoglycosides, fluoroquinolones, carbapenems, antipseudomonal penicillins, and trimethoprim-sulfamethoxazole (reviewed in Lalucat et al., 2006; Noble and Overman, 1994). Antibiotic susceptibility patterns of 93 *P. stutzeri* isolated from clinical specimens during a 10-year period (2000–2010) were reviewed by Bisharat et al. (2012) and are consistent with prior studies showing that *P. stutzeri* is susceptible to many antibiotics. Excluding ceftazidime, third- and fourth–generation cephalosporins are not optimal therapies for *P. stutzeri* infections with a coverage rate ranging from 50–70% (Bisharat et al., 2012). Antibiotic susceptibility testing conducted by Health Canada in 2011 indicates that *P. stutzeri* ATCC 17587 is susceptible to ciprofloxacin (fluoroquinolone), meropenem (carbapenem), gentamicin (aminoglycoside) and ceftazidime (cephalosphorin), and is tolerant to cefotaxime (cephalosporin), which are consistent with the findings in the literature for clinical isolates of *P. stutzeri*.

Resistance to monobactams could be attributed to the presence of IMP- and VIM-type metallo-beta-lactamases in the chromosome (Yan et al., 2001; Lee et al., 2004; Carvalho-Assef et al., 2010), while resistance to macrolides could be due to alterations of the outer membrane proteins and LPS (Tattawasart et al., 2000).

In vitro and *in vivo* tests conducted at Health Canada found no evidence of cytotoxic effects on human colonic epithelial cells (HT29) after 6, 12 or 24 h of exposure to *P. stutzeri* strain ATCC 17587.

P. stutzeri strain ATCC 17587, and no haemolytic activity was observed on sheep blood agar after 24 h or 48 h at 37°C. In four BALB/c mice exposed to 1.0×10^6 CFU/25 µL by endotracheal instillation, no changes in behaviour or physical appearance were observed and the animals were asymptomatic. No significant increase in lung granulocytes over the one week sampling period and no increase in lung or blood cytokine marker levels were detected one week after exposure. All bacteria were cleared by 96 h after exposure from lungs, trachea and esophagus, and the organs remained clear at 7 days.

P. stutzeri has relatively low virulence compared to the virulent species of *Pseudomonas* and *Burkholderia*, presumably due to the absence of virulence factors in *P. stutzeri* (Yan et al., 2008). A search of the literature and of the Pseudomonas Genome Database for five fully sequenced *P. stutzeri* strains (A1501, ATCC 17588, CCUG 29243, DSM 10701 and DSM 4166) confirms the absence of the following virulence factors that play an important role in the adherence, invasion, evasion of host defences, biofilm formation, and damage to host cells:

- type III secretion system effector proteins ExoS, ExoT, ExoU, and ExoY (Winsor et al., 2011)
- type VI secretion system effector proteins Hcp and VgrG (Silverman et al., 2012; Winsor et al., 2011)
- quorum-sensing molecules: 2 -heptyl-3-hydroxy-4(1*H*)-quino and 2-alkyl-4(1*H*)-quinolones (Diggle et al., 2006; Winsor et al., 2011)

- *algD* gene for alginate polymer synthesis (Fialho et al., 1990, Winsor et al., 2011)
- extracellular toxins, such as rhamnolipids, pyocyanin, pyochelin, hydrogen cyanide (Winsor et al., 2011).

P. stutzeri has been associated with a toxin produced by red tide algae that has been linked to paralytic shellfish poisoning (Plumley et al., 1999; Martins et al., 2003). However, further investigation showed that *P. stutzeri* does not produce this toxin, but under certain growth conditions, has the potential to sequester the algal toxins in its cell wall (Baker et al., 2003; Martins et al., 2003). No toxin production by *P. stutzeri* has been reported in the literature.

No cases of allergic reaction have been reported specifically on *P. stutzeri* strain ATCC 17587; however other strains of *P. stutzeri* have been isolated along with other organisms from metal-working fluids that cause hypersensitivity pneumonitis at metalworking plants (Gilbert et al, 2010; Bracker et al., 2003). Like all micro-organisms, *P. stutzeri* contains or produces components, such as lipopolysaccharides and enzymes, that may act as immune stimulants, allergens or sensitizers. Sensitization or allergic reactions to micro-organisms could occur via dermal and respiratory routes in frequently exposed or susceptible individuals (Martel et al., 2010; Ring et al., 1992).

1.4 Hazard severity

P. stutzeri is a well-defined microbial species. A combination of morphological, biochemical, and physiological traits allow it to be reliably discriminated from other *Pseudomonas* species, especially closely-related pathogens such as *P. aeruginosa*. Despite the widespread presence of *P. stutzeri* in soil and water and in close association with plant roots, only one isolate has been reported to be pathogenic towards chickens, which were successfully treated with antibiotics. Certain strains of *P. stutzeri* have anti-algal, antibacterial and antifungal properties. As previously mentioned in Section 1.3.1, experimental challenges with *P. stutzeri* ATCC 17587 on springtails revealed a significant decrease in adult survival and juvenile production at concentrations which can be reached during bioremediation uses (Dybas et al., 2002; Silva et al., 2004). However, there is no evidence that *P. stutzeri* ATCC 17587 has adversely affected terrestrial invertebrates at the population level in spite of widespread distribution of *P. stutzeri* in the environment. Thus, the environmental hazard severity for *P. stutzeri* ATCC 17587 is estimated to be low.

No human infections have been specifically attributed to the DSL strain *P. stutzeri* ATCC 17587 in the scientific literature. Nonetheless, there have been a few reports of secondary infection from other *P. stutzeri* isolates in individuals with predisposing factors such as compromised immunity, trauma, previous surgery or invasive medical procedures, or are hospital acquired. While most of the recovered *P. stutzeri* clinical isolates belong to genomovar 1, a few strains belonging to genomovar 2 have been identified (including ATCC 17587). The human hazard severity for

P. stutzeri ATCC 17587 is therefore estimated to be low-medium.

Hazards related to micro-organisms used in the workplace should be classified accordingly under the Workplace Hazardous Materials Information System (WHMIS)[3].

[3] A determination of whether one or more criteria of section 64 of CEPA 1999 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 1999 on *G. species* ATCC 700368 is not relevant to, nor does it preclude, an assessment against the hazard criteria for WHMIS that are specified in the *Controlled Products Regulations* for products intended for workplace use.

A.Exposure Assessment

An exposure assessment identifies the mechanisms by which a micro-organism is introduced into a receiving environment (Section 2.1) and qualitatively and/or quantitatively estimates the magnitude, likelihood, frequency, duration, and/or extent of human and environmental exposure (Section 2.2). Exposure to the micro-organism itself, its genetic material, toxins, metabolites and structural components is assessed in this section.

2.1 Sources of exposure

As a species, *P. stutzeri* is naturally present in the environment. The purpose of this assessment is to characterize the exposure to *P. stutzeri* ATCC 17587 from its deliberate addition to consumer or commercial products or its use in industrial processes.

P. stutzeri ATCC 17587 was nominated to the DSL in 2005 because it was manufactured in or imported into Canada between January 1, 1984, and December 31, 1986, and it entered or was released into the environment without being subject to conditions under CEPA 1999 or any other federal or provincial legislation.

Responses to a 2007 voluntary questionnaire sent to a subset of key biotechnology companies in Canada, combined with information obtained from other federal government regulatory and non-regulatory programs, indicate that a very small amount of *P. stutzeri* ATCC 17587 was imported into Canada for research and development in the 2006 reporting year.

The Government conducted a mandatory information-gathering survey under section 71 of CEPA 1999 (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. stutzeri* ATCC 17587, whether alone, in a mixture, or in a product. No commercial or consumer activities using *P. stutzeri* ATCC 17587 were reported in response to the section 71 Notice.

P. stutzeri is metabolically diverse which could make it of commercial interest in a variety of industries, particularly in water treatment and degradation of xenobiotic compounds. A search of the public domain yielded the following ongoing consumer, commercial and industrial applications of other naturally-occurring strains of *P. stutzeri*:

- Bioremediation of light crude oil in a contaminated sandy beach in Nova Scotia (Lee and Levy, 1987).

- Water restoration, including lakes, decorative and irrigation ponds, aquariums, and cattle dugouts (Product Sheet A, 2012; Product Sheet B, 2012; Product Sheet C, 2013).
- Septic tank cleaner and deodorizer (Product Sheet A, 2012; Product Sheet B, 2012).
- Treatment of grease traps and sewer lines (Product Sheet D, 2009; Product Sheet E, 2013).
- Enhanced nitrification in wastewater treatment plants (Product Sheet A, 2012; Product Sheet E, 2013; Product Sheet C, 2013).
- Agricultural odour control and solid waste management (Product Sheet A, 2012).
- Biocleaning of nitrate alterations on wall paintings (Bosch-Roig et al., 2013).
- Component of a compost tea inoculum (Product Sheet F, 2013).
- Production of glucan 1,4-alpha-maltotetrahydrolase for use as food processing aid (FSANZ, 2010).
- Production of lipase for use in food, cosmetics and health industry, fine chemicals, and flavours and fragrances (Product Sheet G, 2013).

Some potential uses of other naturally-occurring *P. stutzeri* identified from patent submissions include:

- Oil recovery (Fallon et al., 2012 US Patent 20120277126; Keeler et al., 2013 US Patent 8357526, CIPO CA2717852; Choban et al., 2010 CIPO CA 2758325; Alsop et al., 2012 CIPO CA 28184400; Durham and Harless, 2012 CIPO CA 2812167).
- As a component of a microbial consortium used for the recovery of precious metals, such as gold and silver (Brierley and Kulpa, 1993 US Patent 5244493).
- Removal of heavy metals, such as selenium, in contaminated wastewater streams (Borg et al., 2012 US Patent 8323497).
- Biological treatment of wastewater to remove tertiary butyl alcohol (Insell, 1992 CIPO CA 1304031).
- Production of esterase and xylanase for use in bioethanol production (Henderson and Higgins, 2007 US Patent 20070190627; Bhosle and Giriyan, 2003 US Patent 20030008379).
- Production of lipolytic enzymes, such as lipase, for use in the manufacture of detergents and processing of textiles (Mikkelsen et al., 2012 US Patent 8329632; Daimon and Uyama, 2001 CIPO CA2413838 US Patent 6797010; Lund et al., 1998 US Patent 6077316; Mao, 2000 WO/2000/040551; Farin et al., 1993 CIPO CA 1313360).
- Production of amylase for use as a food additive (Kragh and Soerensen, 2005 WO/2005/003339, CIPO CA 2531647).
- Production of purine arabinosides that may be used as agricultural chemicals or medicinal ingredients (Utagawa et al., 1982 CIPO CA 1120876).

- o Production of phosphorylase for use in the production of 7-hydroxyguanine, an active ingredient in anti-tumour agents (Kitahara et al., 1989 CIPO CA 1263946).

2.2 Exposure characterization

2.2.1 Environment

The environmental exposure for *P. stutzeri* ATCC 17587 is estimated to be low based on the absence of responses to the Section 71 Notice, suggesting that this strain is no longer used in consumer or commercial products or for industrial processes in Canada.

Nevertheless, environmental exposure scenarios, in the event that consumer, commercial or industrial activities with *P. stutzeri* ATCC 17587 resume, have been considered along with persistence and survival properties of this micro-organism.

The magnitude of plant and animal exposure to the *P. stutzeri* ATCC 17587 will depend on its persistence and survival in the environment. *P. stutzeri* does not form spores, so it cannot survive for long periods without nutrients or resist extremes of temperature, radiation or chemicals (reviewed in Setlow, 2006). However, *P. stutzeri* is metabolically versatile and is expected to readily colonize new terrestrial environments. Xiang et al. (2010) investigated the persistence of *P. stutzeri* ATCC 17587 in microcosm soil and reported that populations of this strain dominated, following an initial adaptation period after inoculation, and remained stable with a relatively high abundance of 10^7 CFU/g dry soil throughout the soil incubation experiment. In addition, naturally-occurring *P. stutzeri* strains have been isolated from various aquatic ecosystems. Lalucat et al. (2006) suggested that *P. stutzeri* is a true marine species because of its ability to tolerate high salt concentration. Marine strains of *P. stutzeri* are located in the water column and in sediments where they play a role in nitrogen cycling. Several strains have also been isolated from contaminated groundwater and wastewaters, thus confirming that *P. stutzeri* can survive in freshwater habitats (reviewed in Lalucat et al., 2006; Criddle et al., 1990; Dybas et al., 1995). Overall, *P. stutzeri* ATCC 17587 should be able to survive and persist in most terrestrial, aquatic and marine environments.

The following exposure scenarios are based on known uses of other strains and probable future uses as described in Section 2.1 Sources of exposure. Uses such as bioremediation, agricultural odour control, solid waste management, composting and disposal of solid wastes from industrial uses of the organism are likely to introduce *P. stutzeri* ATCC 17587 to terrestrial ecosystems. Terrestrial invertebrates living in the soils at the site of application or disposal and plants growing in treated soils are likely to be the most directly exposed. Vertebrates could ingest *P. stutzeri* ATCC 17587 while feeding on plants or invertebrates growing in treated or contaminated soils.

Aquatic and marine species may come into contact with *P. stutzeri* ATCC 17587 from runoff subsequent to terrestrial application and from the direct application of *P. stutzeri* ATCC 17587 to water bodies for uses such as water restoration (fresh and salt water), wastewater treatment, or disposal of wastewater from applications such as recovery of oil and metals or the manufacture of enzymes, detergents and food additives.

Aquatic applications could also expose terrestrial species. For example, grazing animals could ingest *P. stutzeri* ATCC 17587 subsequent to its use in water restoration in cattle dugouts, and plants and soil invertebrates could be exposed subsequent to the treatment of irrigation ponds.

In the event that consumer, commercial or industrial activities resume, the environmental exposure to *P. stutzeri* ATCC 17587 will likely increase. The environmental compartments and species that will be exposed to the DSL strain will depend on the uses outlined in the exposure scenarios described above.

2.2.2 Human

Based on the absence of consumer or commercial activity in Canada according to the Section 71 Notice, the overall human exposure estimation for *P. stutzeri* ATCC 17587 is low. Nevertheless, given the range and scale of known and potential applications of the species *P. stutzeri* listed in Section 2.1 Sources of exposure, there is potential for an increase in human exposure to products containing *P. stutzeri* ATCC 17587, and exposure scenarios arising from these products have been considered.

Should products containing *P. stutzeri* ATCC 17587 become available in Canada, human exposure could be greatest through the use of consumer products intended for the treatment of aquariums and decorative ponds, degreasing of kitchen drains, cleaning and deodorizing of septic tanks, and composting. Handling and application of such products would be expected to result in direct exposure to the skin and through inhalation of aerosolized droplets or dusts containing *P. stutzeri* ATCC 17587 in the lungs.

Secondary to product application, residual *P. stutzeri* ATCC 17587 on surfaces and in reservoirs such as treated drains could result in dermal exposure, exposure through inadvertent ingestion where the organism persists on food preparation surfaces, and inhalation where aerosols are generated (e.g., from kitchen garbage disposal units). Since *P. stutzeri* ATCC 17587 is expected to persist following application, such exposures may be temporally distant from the time of application.

Should commercial products containing *P. stutzeri* ATCC 17587 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 moderate.

Human exposure to bodies of water and soil treated with *P. stutzeri* ATCC 17587, (e.g., through recreational activities), could also result in exposure of the skin and eyes, as well as inadvertent ingestion, however dilution of the product is expected to significantly reduce exposure relative to household application scenarios.

Indirect exposure to *P. stutzeri* ATCC 17587 in the environment subsequent to its use in oil recovery, water and wastewater treatment, soil bioremediation, 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 organism enters 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.

In the event that the potential consumer, commercial or industrial uses of *P. stutzeri* ATCC 17587 are realized, human exposure through the exposure scenarios described above can be expected and could include direct, possibly repeated, exposure to concentrated preparations of *P. stutzeri* ATCC 17587.

B.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. stutzeri* ATCC 17587 to be low for the environment and low-medium for human health. Environmental and human exposure to *P. stutzeri* ATCC 17587 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.

The determination of risk from current uses is followed by consideration of the estimated hazard in relation to foreseeable future exposures (from new uses). If a risk may be associated with new uses or activities, the government can take action through the application of the Significant New Activity provisions to require an assessment of these new uses or activities before they begin.

Nevertheless, *P. stutzeri* ATCC 17587 has useful properties that could result in environmental and human exposure to products containing this strain in the future. The risk from foreseeable potential uses of *P. stutzeri* ATCC 17587 remains low given that there is no evidence of effects to human health or of adverse ecological effects at the population level for environmental species in spite of widespread distribution of *P. stutzeri* in the environment and of history of industrial, environmental, and commercial uses.

In susceptible humans, *P. stutzeri* can act as an opportunistic pathogen. Compared with other closely-related opportunistic *Pseudomonas* pathogens, the incidence of nosocomial (hospital-acquired) or secondary infection due to *P. stutzeri* in individuals with compromised immunity and underlying medical conditions is low. Only three reports of *P. stutzeri* infection in otherwise healthy individuals were found in the literature. In the unlikely event of infection with *P. stutzeri* ATCC 17587 arising from its use in industrial processes, consumer or commercial products, a number of clinically-relevant antibiotics are effective against *P. stutzeri* ATCC 17587. The risk from foreseeable potential uses of *P. stutzeri* ATCC 17587 to the environment and general population and is expected to be low.

C.Conclusion

Based on responses to the section 71 Notice in 2009, it is concluded that *P. stutzeri* ATCC 17587 is not entering the environment in a quantity or concentration or under conditions that:

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

Therefore, it is concluded that this substance does <u>not</u> meet the criteria as set out in section 64 of the CEPA 1999.

D.References

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APPENDICES

E. Characterization *P. stutzeri* ATCC 17587

Table A-1: Colony morphology of *P. stutzeri* ATCC 17587 on nutrient agar at various temperatures and incubation periods

Characteristic[a]	Nutrient agar (ATCC) 37°C 24 h	Nutrient agar 28°C 24 h	Nutrient agar 28°C 48 h	Nutrient agar 37°C 24 h	Nutrient agar 37°C 48 h
Size	0.5 mm	0.5 mm	a) 2 mm b) 11 mm	a) 1 mm b) 10 mm	a) 4 mm b) 25 mm
Form	a) circular b) irregular rhizoid	circular	a) circular b) circular	a) circular b) irregular	a) circular b) circular
Elevation	a) convex b) raised	no data	flat	flat	flat
Margin	a) entire	no data	a) entire b) filiform	entire	a) imperfect entire b) filiform
Surface	a) smooth	no data	a) moist b) spongy	a) smooth b) spongy	a) umbonate b) spongy
Opacity	a) translucent b) translucent	no data	a) semi-translucent b) translucent	a) opaque b) semi-translucent	a) semi-translucent b) translucent
Chromogenesis	colourless	colourless	colourless	colourless	colourless
Comments	2 phenotypes	too small to discern detail	2 phenotypes	2 phenotypes	2 phenotypes

[a] Unpublished data generated by Health Canada's Healthy Environments and Consumer Safety Branch

Table A-2: Colony morphology of *P. stutzeri* ATCC 17587 on Tryptic Soy Broth (TSB) at various temperatures and incubation periods

Characteristic[a]	TSB 28°C 24 h	TSB 28°C 48 h	TSB RT 7 d	TSB 37°C 24 h	TSB 37°C 48 h
Size	0.5 mm	2–3 mm	5 mm	1-3 mm	a) 3 mm b)11 mm
Form	circular	circular	circular - irregular	circular	a) circular b) irregular
Elevation	no data	flat	raised - convex - umbonate	flat	raised
Margin	no data	entire	entire-undulate	entire	a) entire b) undulate
Surface	no data	moist	moist	small smooth larger and wrinkled	a) moist b) dry and winkled
Opacity	no data	semi-translucent	opaque	transparent	semi-translucent
Chromogenesis	no data	colourless	tan-gold, beige non fluorescent	colourless	light tan, beige
Comments	too small to discern detail	none	non fluorescent	2 phenotypes	2 phenotypes

[a] Unpublished data generated by Health Canada's Healthy Environments and Consumer Safety Branch

Table A-3: Growth of *P. stutzeri* ATCC 17587 in liquid media at various temperature

Medium	28°C	32°C	37°C	42°C
Tryptic Soy Broth	+	+	+	+
Sheep Plasma	~	~	~	-
Fetal Bovine Serum	+	+	+	~
Dulbecco's Modified Eagles Medium (mammalian cell culture)	v	-	-	-

+ Growth - No growth ~ Low level of growth v Variable

Data generated by Health Canada's Healthy Environments and Consumer Safety Branch.
Growth of *P. stutzeri* ATCC 17587 in broth culture was measured by increase in absorbance at 500 nm, in four different growth media and over a range of temperatures. Concentration of bacteria at time zero was $1×10^6$ CFU/mL.

Table A-4: Growth characteristics of *P. stutzeri* on solid media at 37°C

Medium	37°C
Nutrient	+
Growth on Starch [a]	+
Starch Hydrolysis [a]	+
Maconkey Agar [b]	-
Lysine Iron [c]	+ (no lysine decarboxylase detected)
Triple Sugar Iron - w phenol red [d]	+ (no gas, no hydrogen sulfide detected)
Mannitol Egg Yolk Polymyxin supplements [e]	-
Mannitol Salt Agar [f]	-
Citrate [g]	-
Urea [h]	+ (no urea hydrolysis)

\+ Growth - No growth

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

[a] Differential medium that tests the ability of an organism to grow on starch and to produce extracellular enzymes that hydrolyze starch

[b] Detection of coliform organisms in milk and water; tests for ability of organism to ferment lactose

[c] Simultaneous detection of lysine decarboxylase and formation of hydrogen sulfide in the identification of *Enterobacteriaceae*, in particular *Salmonella* and *Arizona* according to Edwards and Fife.

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

[e] *B. cereus* selective agar

[f] Isolation and differentiation of *Staphylococci*

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

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

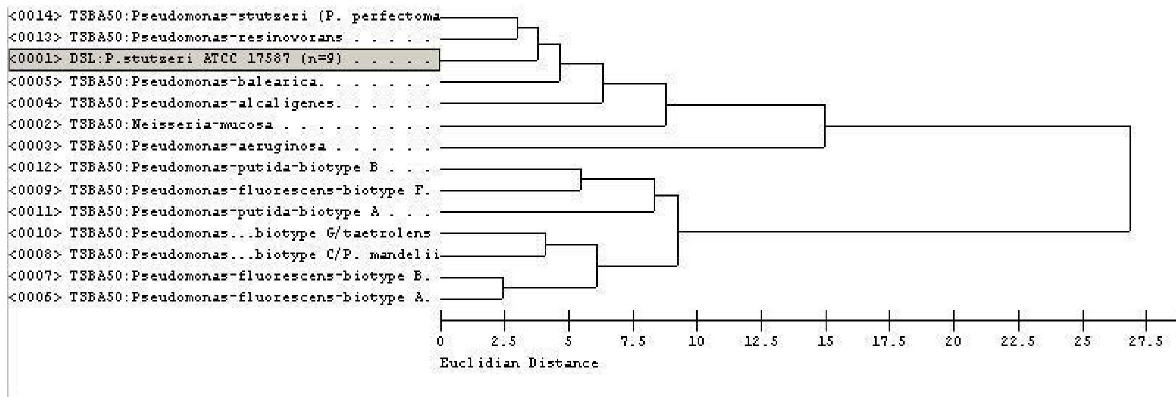

Figure A-1: Fatty Acid Methyl Ester (FAME) Analysis of *P. stutzeri* ATCC 17587 using MIDI TSBA50 Environmental Database

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

Figure A-1 shows the relatedness of DSL strain *P. stutzeri* ATCC 17587 to nine *Pseudomonas* species (*P. stutzeri*, *P. resinovorans*, *P. balearica*, *P. alcaligenes*, *P. aeruginosa*, *P. putida*, *P. fluorescens*, *P. taertolens*, and *P. mandeii*) and *Neisseria mucosa* according to their cellular fatty acid compositional similarity using GC-FAME and the Sherlock® MIDI Microbial Identification System. Based on the dendrogram, the DSL *P. stutzeri* strain is more closely related to another strain of *P. stutzeri, P. resinovoarans*, *P. balearica*, *P. alcaligenes*, *N. mucosa* and *P. aeruginosa*, than to *P. fluorescens* and *P. putida.*

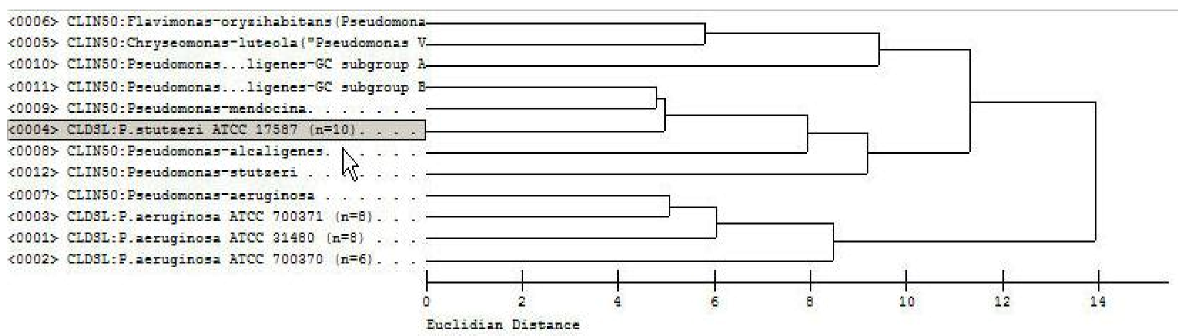

Figure A-2: Fatty Acid Methyl Ester (FAME) Analysis of *P. stutzeri* ATCC 17587 using MIDI Clinical Database

[a] Data generated by Heath Canada's Environmental Health Science and Research Bureau

Figure A-2 shows the relatedness of DSL strain *P. stutzeri* ATCC 17587 to *P. aeruginosa*, *P. ligenes, P. alcaligenes*, *Flavimonas oryzihabitans* and *Chrymseomonas luteola* according to their cellular fatty acid compositional similarity using GC-FAME and the Sherlock® MIDI Microbial Identification System. Based on the dendrogram, the DSL *P. stutzeri* strain is more closely related to *P. ligenes* and *P. mendocina*, than to *P. aeruginosa*.

APPENDIX B: Select Mobile Elements in *P. stutzeri*

Mobile elements	Strain	Characteristic and function	Reference
Insertion Sequence ISPst6 (4 copies)	ATCC 17587 (clinical isolate)	– recognizable by their imperfect inverted repeat (pseudo-palindrome) structure with two characteristic “flipped-out” positions – targets recombination site *attC* – interaction between ISPst6 and *attC* has been used as a model in investigating evolutionary processes in pseudomonads	Tetu and Holmes, 2008
Insertion Sequence ISPst9	CCUG 29243 (marine isolate)	– catabolic gene inactivation of naphthalene pathway	Christie-Oleza et al., 2008
Insertion Sequence ISPs1	OX1 (sludge isolate)	– catabolic gene inactivation of meta and para-xylene pathways	Bolognese et al., 1999
Class 1 Integron	ATCC 17588 (clinical isolate) CCUG 29243 (marine isolate) DSM 10701 (soil isolate) DSM 4166 (soil isolate) CCBH 4919 (clinical isolate)	– contains bla_{IMP-16} gene – codes for imipenemase-type metallo-β-lactamases that catalyze the hydrolysis of a broad range of β-lactams, including carbapenem – possibly acquired under selective pressure of antibiotic exposure in the hospital environment	Winsor et al., 2011 The Uniprot Consortium Lee et al., 2004 Carvalho-Assef et al., 2010 Yan et al., 2001
Tn501-like transposon	OX1 (environmental isolate)	– organo-mercury detoxification through the activity of mercuric reductase (*merA*)	Reniero et al., 1998