Final Screening Assessment for

Pseudomonas fluorescens ATCC 13525

Environment Canada

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Synopsis

Pursuant to paragraph 74(*b*) of the *Canadian Environmental Protection Act, 1999* (CEPA 1999), the Ministers of the Environment and of Health have conducted a screening assessment on *Pseudomonas fluorescens* ATCC 13525.

P. fluorescens is considered to be a ubiquitous bacterium and it has the ability to adapt to and thrive in soil and on plants and aqueous surfaces. Multiple potential uses of *P. fluorescens* in various industrial and commercial sectors exist. These include pulp and paper and textile processing, in municipal and industrial wastewater treatment, for waste degradation, particularly in petroleum refineries, bioremediation and biodegradation, as well as in commercial and household drain cleaners and degreasers, enzyme and chemical production, septic tank additives and general cleaning and odour-control products. Other uses include pest control, plant growth promotion and use as an anti-frost agent on plants.

There is no evidence from the scientific literature to suggest that *P. fluorescens* ATCC 13525 is likely to have a significant impact on animal and plant populations in the environment.

There have been no reported infections in humans attributed specifically to the strain *P. fluorescens* ATCC 13525. Information from the scientific literature indicates that some strains of *P. fluorescens* are unlikely to infect the general Canadian population, but that they can infect humans with compromised immunity, and human outbreaks, associated with contaminated medical devices and fluids, have been reported. *P. fluorescens* can also grow at temperatures typical of refrigerated storage, a characteristic which has enabled it to proliferate in stored blood products and cause sepsis in transfused patients.

This assessment considered human and environmental exposure to *P. fluorescens* ATCC 13525 from its deliberate use in household or commercial products or in industrial processes in Canada. The government launched a mandatory information-gathering survey (notice) under section 71 of CEPA 1999 as published in the Canada Gazette I on October 3rd, 2009 (hereafter “the s. 71 notice”). Information submitted in response to the notice indicates that 100 to 1000 kg of *P. fluorescens* ATCC 13525 were imported into or manufactured in Canada in 2008.

Based on the information available *P. fluorescens* ATCC 13525 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. *P. fluorescens* ATCC 13525 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].

This screening assessment considers hazard information obtained from the public domain as well as from unpublished research data and comments from researchers in related fields. Exposure information was obtained from the public domain, as well as 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 titled "Framework on the Science-Based Risk Assessment of Micro-organisms under the *Canadian Environmental Protection Act, 1999*".

Data that are specific to *Pseudomonas fluorescens* strain ATCC 13525 are identified as such and include results of laboratory analyses conducted at Health Canada[2]. Where data concerning the particular strain were not available, surrogate information from the scientific literature of other *P. fluorescens* strains and the genus *Pseudomonas* was used. Surrogate organisms are identified in each case to the taxonomic level provided by the source. Information identified as of March 2014 was considered for inclusion in this report. Literature searches were conducted using scientific literature databases (SCOPUS, Google Scholar, and CABI), web searches and key search terms for the identification of human health and environmental hazards of each of the DSL strains assessed in this report.

[1] A determination of whether one or more of the criteria of section 64 of CEPA 1999 are met is based upon an assessment of potential risks to the environment and/or to human health associated with exposure in the general environment. For humans, this includes, but is not limited to, exposure from air, water and the use of products containing the substances. A conclusion under CEPA 1999, on *Pseudomonas fluorescens* strain ATCC 13525, is not relevant to, nor does it preclude, an assessment against the hazard criteria for Workplace Hazardous Materials Information System (WHMIS) that are specified in the Controlled Products Regulations for products intended for workplace use.

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

1. Hazard Assessment

1.1 Characterization of *Pseudomonas fluorescens*

1.1.1 Identification, Taxonomy and Strain History

Pseudomonas fluorescens is a Gram-negative, obligate aerobic, motile, rod-shaped bacterium. It grows at neutral pH and has an optimal growth temperature of 25-30^{o}C (Palleroni, 1984), with growth possible as low as 4^{o}C. *P. fluorescens* does not form spores or other survival structures and does not grow under acidic conditions (<pH 4.5) (Holt, 1994). Nutritional demands of *P. fluorescens* are modest, and so it can survive and multiply for months in moist environments. Most strains are strictly aerobic chemo-organotrophs requiring both oxygen and organic carbon for growth (Holt, 1994). Descriptions of the colony morphology of *P. fluorescens* ATCC 13525 are outlined in Table 1-1.

Table 1-1 Colony morphology of *P. fluorescens* ATCC 13525

Characteristic	TSB agar after 7 days of growth at room temperature[a]	Nutrient agar/broth at 26^{o}C[b]
Shape	Circular	Spreading
Size (mm) diameter	4	No data
Margin	Entire	Undulate
Elevation	Raised	Flat
Colour	Cream-beige	Glistening
Texture	Moist	Smooth or rough
Opacity	Semi-translucent	Transparent
UV fluorescence	Yes	Yes
Pigment	Diffusing yellow pigment	A yellow-green fluorescent pigment is produced on some media

a Data generated by Health Canada's Environmental Health Science and Research Bureau
b ATCC description

Health Canada scientists independently characterized *P. fluorescens* ATCC 13525 using growth kinetics at different temperatures (Appendix 1), growth on different media at 28°C and 37°C (Appendix 2) and fatty acid methyl-ester (FAME) analysis (Appendix 3). Other phenotypic methods, such as the API biochemical tests, can be used for the rapid identification of *P. fluorescens* in medical and agroalimentary settings (Bodilis et al., 2004), but these techniques do not differentiate the DSL-listed strain from other *P. fluorescens* strains.

The genus *Pseudomonas* is one of the most diverse bacterial genera, and its taxonomy has undergone many changes. Earlier studies resulted in the division of the *P. fluorescens* group into 5 biovars (I-V, synonym of biotypes A, B, C, F and G) based on phenotypic characteristics such as metabolic tests, fatty acid composition and protein profiles (Palleroni, 2005). *P. fluorescens* ATCC 13525 is the type strain of biovar I (Palleroni, 2005). Phenotypic characteristics central to the identification and differentiation of the various *P. fluorescens* biovars are presented in Table 1-2.

Table 1-2 General phenotypic characteristics of *P. fluorescens* biovars[a]

Characteristics /Substrates Used for Growth	Biovar I	Biovar II	Biovar III	Biovar IV	Biovar V
Denitrification	-[b]	+[c]	+	+	-
Levan Formation	+	+	-	+	-
L-Arabinose	+	+	d[d]	+	d
Sucrose	+	+	-	+	d
Saccharate	+	+	d	+	d
Propionate	+	-	d	+	+
Butyrate	-	d	d	+	d
Sorbitol	+	+	d	+	d
Adonitol	+	-	d	-	d
Propylene glycol	-	+	d	-	d
Ethanol	-	+	d	-	d

a Adapted from Bergey's Manual of Systematic Bacteriology (Palleroni, 2005)
b -, negative in most strains
c +, positive in most strains
d d, different strains give different reactions

The taxonomy of the *P. fluorescens* group is continually under review. While phenotypic characteristics and the biovar I-V designation are still valid for *P. fluorescens* identification, molecular characterization is used more reliably to demonstrate the phylogenetic relationships and variations between *P. fluorescens* strains and among closely related *Pseudomonas* species. These include full genomic sequence analyses (Paulsen et al., 2005; Silby et al., 2009) and 16S–23S rDNA intergenic spacer-restriction fragment length polymorphism (ITS-RFLP) analyses (Milyutina et al., 2004; Scarpellini et al., 2004). For instance, phylogenetic analyses of the *Pseudomonas* species performed by Mulet et al. (2010) using four housekeeping genes (16S rRNA, *gyrB, rpoB,* and *rpoD)* have shown that the *P. fluorescens* group consists of 9 subgroups, namely *P. fluorescens, P. mandelii*, *P. corrugata, P. gessardii*, *P. fragi, P. jessenii*, *P. koreensis, P. chloroaphis* and

P. asplenii. The *P. fluorescens* subgroup is composed of 20 species, including the *P. fluorescens* type strain, ATCC 13525.

Pseudomonas aeruginosa, a known opportunistic human and animal pathogen, is the pathogen that is most closely related to, but is distinct from, *P. fluorescens*. Phylogenetic trees based on 16S rDNA sequences of *Pseudomonas* species show that, while the *P. fluorescens* group is not closely clustered with the *P. aeruginosa* group, the two are nevertheless related (Palleroni, 2005).

The genome size of *P. fluorescens* varies from approximately 6.4 Mb to 7.07 Mb (Paulsen et al., 2005; Silby et al., 2009). Comparative studies of three fully sequenced saprophytic *P. fluorescens* (Pf-5, SBW25 and Pf0-1) revealed an unexpectedly high degree of diversity (Silby et al., 2009), relative to other *Pseudomonas* species, which are more conserved. These three strains of *P. fluorescens* share only 61.4% of their gene content compared with five sequenced genomes of *P. aeruginosa* which share 80% to 90% of their gene content (Mathee et al., 2008; Silby et al., 2009).

Health Canada scientists independently demonstrated 100% homology of the 16S rRNA gene sequence of the DSL *P. fluorescens* strain to *P. fluorescens* ATCC 13525 sequences in the proprietary MicroSeq® ID library and 99% homology to four other library entries (*P. fluorescens* ATCC 17572, *P. veronii* DSM 11331, *P. marginalis* ATCC 10844, and *P. tolaasii* ATCC 33618). In consensus sequence comparisons with the NCBI Blast (National Center for Biotechnology Information Basic Local Alignment Search Tool), ATCC 13525 matched most closely with two *P. fluorescens* strains (SBW25 and WH6) and an unidentified *Pseudomonas* sp. shotgun sequence. Subsequent matches featured *P. syringae* and *P. savastanoi*.

The following are superseded names for *P. fluorescens*: "*Bacillus fluorescens liquefaciens* Flügge 1886, "*Bacillus Fluorescens*" Trevisan 1889, "*Bacterium fluorescens*" (Trevisan 1889) Lehmann and Neumann 1896 and "*Liquidomonas fluorescens*" (Trevisan 1889) Orla-Jensen 1909 (Skerman et al., 1980). According to Hugh et al. (1964), ATCC 13525 was isolated in 1951 from water works pre-filter tanks in Reading, England. *P. fluorescens* ATCC 13525 is recognized by different accession numbers in other culture collections, for example it is referred to as DSM 50090 from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH and as NCCB 76040 from the Netherlands Culture Collection of Bacteria.

1.1.2 Biological and Ecological Properties

1.1.2.1 Biocontrol and plant growth promotion

P. fluorescens has been recognized as beneficial to plant growth (Kloepper et al., 1988; Weller and Cook, 1986). The majority of fluorescent pseudomonads produce complex peptidic siderophores called pyoverdines or pseudobactins, which are very efficient iron scavengers. *P. fluorescens* can enhance plant growth through production of siderophores which efficiently complex environmental iron, making it unavailable to other components of the soil microflora.

Certain strains of *P. fluorescens* produce molecules that make them ideal candidates for biocontrol against a wide spectrum of species including weeds (downy brome and annual bluegrass), aphids, termites, nematodes and zebra mussels, and various insects (see Appendix 4) as well as against a variety of pests in wheat, sugar beets, chickpea, tomato, cotton and cabbage (Kamilova et al., 2006; Khan et al., 2006; Schmidt et al., 2004; Someya et al., 2007; Srivastava et al., 2001; Weller, 2007). Some isolates have also been shown to suppress infections from *Aeromonas hydrophila* in fish (Das et al., 2006).

Below are some examples of strains, different from *P. fluorescens* ATCC 13525 on the DSL, in which biocontrol characteristics have been identified. There are no available data comparing the DSL strain, *P. fluorescens* ATCC 13525 with the biocontrol strains reported below. In addition, the DSL strain has not been reported in the literature as a candidate for biocontrol or able to produce the following toxins or metabolites.

Toxins:

- *P. fluorescens* ATCC 55799 (referred to as CL145A) was assessed by the Pest Management Regulatory Agency (PMRA) in 2012 for use as biopesticide for the control of zebra mussels. It produces a toxin which is a heat labile, protein-like secondary metabolite. The mode of action of *P. fluorescens* ATCC 55799 is intoxication (i.e., not infection); mussel death appears to be directly linked to the selective destruction of the digestive tract through lysis of the epithelial cells (Molloy et al., 2013a; Molloy et al., 2013b).

- *P. fluorescens* strain MSS-1 produces a mosquitocidal exotoxin which is lethal by oral ingestion to mosquitoes (Pushpanathan and Pandian, 2008; Rajan and Pandian, 2008a; Rajan and Pandian, 2008b). The toxin may be synergetic with other compounds present in the culture supernatant, since it could not be purified (Murty et al., 1994).

- In plants, Johnson et al. (1993) reported that *P. fluorescens* strain D7 can interfere with growth of downy brome through the activity of a phytotoxin. It was suggested that the D7 phytotoxin expresses its activity on the root surface, because the effect is reversible when the seedlings are removed from it.

Other metabolites:

- Two deleterious rhizosphere strains identified as *P. fluorescens* biotype A (synonym of biovar 1) (Banowetz et al., 2008) produced a Germination Arrest Factor (GAF), that blocks the germination of Annual Bluegrass and a large number of grassy weeds. Culture filtrates inhibited germination in a developmentally-specific manner, arresting germination immediately after emergence of the coleorhizae and plumule and causing chlorosis of the plumule.

- *P. fluorescens* CHA0 is able to produce hydrogen cyanide under *in-vitro* conditions in termite (*Odontotermes obesus*) colonies. This secondary metabolite

is an important determinant of the biocontrol ability of *P. fluorescens* CHA0 towards termites (Devi and Kothamasi, 2009).

Other mechanisms, separate from those that are mediated by specific toxins or metabolites, have been reported for biocontrol such as colonization of the host organism. For example, *P. fluorescens* MF0 shows virulence towards the fruit fly by its ability to evade its host immune system, growing rapidly within the insect (de Lima Pimenta et al., 2006). In the case of use as an antagonist of plant pathogens (bacteria, fungi or nematodes), the control of root diseases by some stains of *P. fluorescens* involves a combination of complementary mechanisms. The most prominent mechanisms are antibiosis towards plant pathogens, degradation of virulence factors produced by pathogens and induction of defence mechanisms in host plants. Efficient competition for colonization sites and micro- and macro-nutrients in the rhizosphere is an important prerequisite for effective biocontrol (Compant et al., 2005; Haas and Défago, 2005; Lugtenberg et al., 2001; Van Loon et al., 1998). *P. fluorescens* ATCC 31948 (referred to as A506) was assessed by the Pest Management Regulatory Agency (PMRA) in 2010 for use as biopesticide for the control of fire blight on apples and pears. It competes with the plant pathogen, *Erwinia amylovora*, for the same ecological niche (PMRA-HC, 2010).

1.1.2.2 Biogeochemical cycling and metal resistance

Pseudomonads play a role in biogeochemical cycling as ecologically important micro-organisms in soil and water that assist in the degradation of many soluble compounds derived from the plant and animal materials (Palleroni, 1981).

P. fluorescens is also well known for its tolerance to metals (Appanna et al., 1996; Lemire et al., 2010).

1.1.2.3 Pathogenic and Toxigenic Characteristics

Environment

No relevant reports were found in the publicly available literature, which investigated specifically the potential pathogenicity traits of *P. fluorescens* ATCC 13525 towards plants or animals. However, *P. fluorescens,* as a species, is known to produce a variety of enzymes, toxins, and other metabolites (Appendix 5).

Pathogenicity and toxicity testing studies on terrestrial organisms were performed by scientists at Environment Canada laboratories using the DSL-listed *P. fluorescens* ATCC 13525. Results of 21-day toxicity testing study using soil invertebrates, springtail (*Folsomia candida*) and earthworm (*Eisenia andrei*) exposed to *P. fluorescens* ATCC 13525 at 10^8 CFU/g dry soil and 10^6 CFU/g dry soil respectively, demonstrate no adverse effect on adult mortality or juvenile reproduction. Plant testing using red fescue grass (*Festuca rubra*) grown with *P. fluorescens* ATCC 13525 (10^{10} CFU/g dry soil) demonstrated no adverse effect on seedling emergence, shoot and root length, or shoot dry mass; however, a significant decrease in root dry mass was observed, (33% reduction) when exposed

to ATCC 13525, relative to control growth (Princz, 2010). This result was not characterized further but it might be explained by the fact that *P. fluorescens* is capable of producing inhibitory metabolites that reduce root elongation, as is the case for strain A313 (Åström et al., 1993).

In the absence of other published test data on the DSL-listed strain ATCC 13525, data from pathogenicity and toxicity testing on non-target species of other *P. fluorescens* strains, were reviewed as surrogate information. These data were provided to support the registration of these strains as biopesticides in Canada (PMRA-HC, 2010; PMRA-HC, 2012) and the United States (USEPA, 2009) (Appendix 6). Testing results of ATCC 55799 showed no evidence of mortality in the ciliate (*Colpidium colpoda)* or the cladoceran (*Daphnia magna)* and no mortality or adverse effects in terrestrial organisms. However, mortality was observed in four fish species in experiments with high concentrations, not recommended for the intended pesticidal use (Appendix 7).

An acute toxicity study was performed with another biocontrol strain, *P. fluorescens* ATCC 31948 (PMRA-HC, 2010). No signs of phytopathogenicity were observed when vascular plants[3] were exposed to 10^6 or 10^8 CFU/mL of *P. fluorescens* ATCC 31948. However, the test protocol was not done according to guidelines as the test doses were below the maximum label rate (3.7×10^9 CFU/mL).

P. fluorescens ATCC 55799 and ATCC 31948 were observed to be not toxic to rats when exposed via oral or pulmonary route nor was it infective after intravenous injection. Mice were exposed to *P. fluorescens* ATCC 31948 via an intraperitoneal injection. Despite the study not meeting the recommended length of 21 days, general signs of toxicity were observed including scruffy coats, discharge from eyes, lethargy and diarrhea which may be attributed an immune reaction caused by the lipopolysaccharide.

There are no available data comparing *P. fluorescens* ATCC 13525 with the above two biocontrol strains (ATCC 31948 or ATCC 55799). In addition, the DSL strain has not been reported in the literature as a candidate for biocontrol or able to produce toxins and metabolites identified in other *P. fluorescens* stains. On this basis, we consider that *P. fluorescens* ATCC 13525 is unlikely to be toxic towards terrestrial or aquatic organisms.

Human Health

P. fluorescens is a psychrotrophic organism that is capable of proliferation at low temperatures typical of refrigerated storage (de Lima Pimenta et al., 2003; Gennari and Dragatto, 1992; Prescott et al., 2005). It is known as a spoilage organism, but not a pathogen, in refrigerated foods; however *P. fluorescens* can grow rapidly in refrigerated stored blood products. A seven to eight log increase in *P. fluorescens* cell number was observed after only one week of incubation at 4^oC (Khabbaz et al.,

[3] Vascular plants tested include barley, maize, oats, sorghum, wheat, broccoli, cabbage, cauliflower, cucumber, snapbean, citrus, peach, pear, tomato and tobacco.

1984). *P. fluorescens* contamination of blood products is strongly associated with post-transfusional sepsis (Gibaud et al., 1984; Gibb et al., 1995; Gibb, 2000; Khabbaz et al., 1984; Murray et al., 1987; Pappas et al., 2006; Scott et al., 1988), a rare, but often fatal systemic inflammatory response to bacteria in the bloodstream (Gottlieb, 1993; reviewed in Guinet et al., 2011; Riedemann et al., 2003). It is characterized by a rapid onset of symptoms, including fever, neutrophilia, rapid heart rate and loss of blood pressure, occurring during or immediately following transfusion of contaminated blood products (Brecher and Hay, 2005; Guinet et al., 2011; Murray et al., 1987).

Lipopolysaccharide (LPS) is a major structural component of the cell wall of all Gram negative bacteria, including *P. fluorescens*. In sepsis, LPS stimulates the innate immune response. In the tissues, this response acts effectively to contain infection, but in the bloodstream the widespread activation of phagocytes and the resulting cytokine cascade is catastrophic, inducing widespread endothelial cell activation, disseminated intravascular coagulation and organ dysfunction (reviewed in Guinet et al., 2011; Riedemann et al., 2003; Vincent, 2002). The structure of LPS affects its potency (reviewed in Netea et al., 2002), and in *P. fluorescens* LPS structure is known to be influenced by growth temperature (Picot et al., 2004); however it is unclear whether structural changes observed at low growth temperatures increase the potency of *P. fluorescens* LPS as an inflammatory mediator.

P. fluorescens has also been implicated as the causative agent of infection, mainly in patients with compromised immune function (de Lima Pimenta et al., 2003; Kanj et al., 1997; Nelson et al., 1991; Pappas et al., 2006). Unbroken skin and mucous membranes are the principle barriers against microbial invasion (reviewed in Ki and Rotstein, 2008). There is no evidence in the scientific literature that *P. fluorescens* has specific mechanisms for bypassing physical, chemical or lymphoid barriers to infection at epithelial or mucosal surfaces, and cases are usually associated with injury or medical interventions that breach normal physical barriers (Grice et al., 2008; Roth and James, 1988; Segre, 2006). Its abundance in the environment (Chapalain et al., 2008; Rebière-Huët et al., 2002) and its occurrence as a commensal member of the normal body flora (Chapalain et al., 2008; Madi et al., 2010; Stenhouse and Milner, 1992; Sutter et al., 1966; Wei et al., 2002) allow it to readily take advantage of such breaches.

Once entry into the host is achieved, adhesion to a host cell is the earliest step in the establishment of infection through colonisation of the host tissues (de Lima Pimenta et al., 2003; Hahn, 1997; Picot et al., 2001). Adhesion requires interaction between molecules present on the surfaces of the micro-organism and the host cell, and this interaction determines the tissue tropism of the ensuing infection (de Lima Pimenta et al., 2003). Like *P. aeruginosa*, *P. fluorescens* uses many different factors as adhesins including exopolysaccharides, lipopolysaccharides (LPS) and outer membrane proteins (reviewed in de Lima Pimenta et al., 2003; Dé et al., 1997; Picot et al., 2003), pili, flagellar proteins (reviewed in de Lima Pimenta et al., 2003; reviewed in Hahn, 1997), and porins (Rebière-Huët et al., 2002). Some of these are known to adhere to the human extracellular matrix protein fibronectin (de Lima Pimenta et al., 2003; Rebière-Huët et al., 2002). Fibronectin binding is an important

mechanism in the pathogenicity of *P. fluorescen*s, as fibronectin is involved in many cellular processes including tissue repair (To and Midwood, 2011), and is more available where tissue damage has occurred. As a consequence, breaches in the natural physical barrier, not only permit entry, but also favour colonization and infection.

The infectious potential of *P. fluorescens* in neurons was investigated because the closely-related opportunistic pathogen *P. aeruginosa* has been implicated in central nervous system infections with high rates of morbidity and mortality (Picot et al., 2001). The adherence of *P. fluorescens* to nerve and glial cells is mediated by LPS and generally causes apoptosis (Veron et al., 2008). In one study, adherence of *P. fluorescens* to cortical neurons and glial cells from rat neonate cell lines was comparable to that of *P. aeruginosa* (Picot et al., 2001). *P. fluorescens* has specific physiological characteristics, such as the expression of acetylcholinesterase (Rochu et al., 1998), γ-aminobutyric acid (GABA) aminotransderase, and high affinity GABA binding protein (Guthrie et al., 2000), which make it particularly harmful to nervous tissues (reviewed in Picot et al., 2004). It is able to exert cytotoxic effects that can induce cell death most likely through an apoptotic mechanism. *P. fluorescens* shows most features of an opportunistic pathogen in nerve cells and could therefore behave as a pathogen in certain situations (Picot et al., 2001). Nevertheless, *P. fluorescens* has been implicated as the cause of meningitis in only one report (Sarubbi et al., 1978).

Following adhesion, changes in enzyme production and protein secretion, including the expression of virulence factors, are regulated by quorum sensing. It is a cell-to-cell signalling system that functions through the secretion of diffusible autoinducer molecules that, when sensed by cell-surface receptors of other bacterial cells in the vicinity, regulates gene expression (Singh et al., 2010). Quorum sensing functions similarly in most Gram-negative bacteria, acting like a ‘switch’ to regulate expression of virulence factors to adapt to changes in environmental conditions (Singh et al., 2010). This mechanism enables bacteria to track changes in cell population density by monitoring the flux in autoinducer concentration, and associated coordination of gene expression (Swem et al., 2009). The two-component GacS-GacA regulatory system, which is conserved among Gram-negative bacteria like *P. fluorescens,* plays a role in quorum sensing and can determine virulence or biocontrol activities at the protein translation level (Blumer et al., 1999). In *P. fluorescens*, the GacS-GacA system controls the expression of extracelluar products such as antibiotics, exoenzymes, and hydrogen cyanide (Blumer et al., 1999).

P. fluorescens secretes an exotoxin related to the β-exotoxin of *Bacillus thuringiensis* (Picot et al., 2001); exoenzymes, including proteases (Koka and Weimer, 2000; Liao and McCallus, 1998; Sacherer et al., 1994); lipases (Dieckelmann et al., 1998) and a cholinesterase-like enzyme (Rochu et al., 1998); and other toxins and secondary metabolites reported in Appendix 5. Haemolysis has been observed in some, mainly clinical, strains of *P. fluorescens* (Sperandio et al., 2010). No haemolytic activity was observed when *P. fluorescens* ATCC 13525 was plated on blood sheep agar in tests conducted at Health Canada.

Quorum sensing is also important in biofilm formation. Biofilms have been extensively reported as a mechanism of pathogenicity in Pseudomonads through the adhesion to and colonization of biotic surfaces as well as abiotic surfaces (O'Toole and Kolter, 1998). *P. fluorescens* has been reported to form biofilms (Costerton et al., 1999; Heffernan et al., 2009). Once formed, biofilms may be more resistant to antimicrobial agents and could contribute to persistent and chronic bacterial infections (Costerton et al., 1999). *P. fluorescens* biofilms, which colonised catheter lumens, were implicated as a contributing factor in a series of delayed onset bloodstream infections as a result of exposure to contaminated heparin flush (CDC, 2006).

Some strains of *P. fluorescens* have been implicated in the pathogenesis of Crohn's Disease (CD) and Inflammatory Bowel Disease (IBD) (Sandborn, 2007; Wei et al., 2002). Certain *P. fluorescens* strains carry the I2 antigen, which is a T-cell superantigen that induces the proliferative immune response and IL-10 secretion by CD4+ T-cells that is associated with CD and IBD (Cuffari, 2009; Dalwadi et al., 2001; Wei et al., 2002). *P. fluorescens* ATCC 13525 has not been associated with CD, and it is unknown whether it carries I2.

1.1.3 Effects

1.1.3.1 Environment

P. fluorescens is a naturally occurring micro-organism that may occasionally be associated with some effects in plants and animals but, under normal circumstances, is unlikely to be a serious hazard.

No relevant reports, in the publicly available literature, specifically investigated the potential adverse effects of *P. fluorescens* ATCC 13525 towards plants or animals. However, the literature search performed with *P. fluorescens* at the species level, excluding the biocontrol context, revealed several cases of pathogenesis where *P. fluorescens* either caused a secondary infection or was found in the context of routine monitoring of diseases (see Appendix 8). Stress, compromised natural defenses, or comorbidity with primary viral or fungal infections are generally preconditions to the reported *P. fluorescens* infections.

- Organisms in experiments that bypassed natural barriers, received *P. fluorescens* via injections (in parenchymal/vascular plant tissue; intraperitoneal and hemocoelic injections in animals), application to burns and cuts, prickling and puncturing.
- No relevant adverse effects in plants or animals were found in the publicly available literature, which were specifically attributed to *P. fluorescens* ATCC 13525. The involvement of other *P. fluorescens* strains in adverse effects in plants or animals have been reported (Appendix 8).
- *P. fluorescens* can infect a wide range of animals including horses (Sarasola et al., 1992), chickens (Lin et al., 1993), marine turtles (Glazebrook and Campbell, 1990), and many fish and invertebrate species. However, because it is unable to

grow at elevated temperatures (Palleroni, 1992), it is likely to be rarely an opportunistic pathogen for warm-blooded animals.

- *P. fluorescens* is considered a secondary invader of damaged fish tissues, but may also be a primary pathogen of fish (Roberts and Horne, 1978; Stoskopf, 1993). The species causes bacterial tail rot and can affect freshwater and saltwater fish throughout the world (Stoskopf, 1993). Adverse effects associated with *P. fluorescens* in fish species often appear to be linked to stress from transportation or cultivation of fish. Carson and Schmidtke (1993) showed that *P. fluorescens* strain 92/3556, isolated from diseased fish in a commercial hatchery fed with river water, may be an opportunistic pathogen of cold-stressed Atlantic salmon with depressed immune functions. Farmed European Eel may harbour *P. fluorescens* that can act as opportunistic pathogens capable of infection and causing mortality (Esteve et al., 1993). This was the same conclusion for other types of fish in the context of investigations for cause of mortality of rainbow trout and Scottish rainbow trout outbreaks (Roberts and Horne, 1978; Sakai et al., 1989) and in tilapia, raised in indoor hatcheries (Okaeme, 1989).

In conclusion, while several studies have shown that different strains of *P. fluorescens* have toxigenic and pathogenic potential, it is important to note that adverse effects reported in Appendices 6 to 8 are mostly attributable to other strains and are not expected to occur to biota in the environment in Canada from *P. fluorescens* ATCC 13525.

1.1.3.2 Human Health

Human infections have been mainly nosocomial in immune compromised or seriously ill patients, some of which resulted in serious and occasionally fatal disease. It is less virulent than other pseudomonads, such as *P. aeruginosa* (Foulon et al., 1981; Pappas et al., 2006).

P. fluorescens has also been reported as the causative agent in a diverse array of infections, many involving immune compromised patients, or those with debilitating co-morbidities, and with injury or medical interventions that breach normal physical barriers or introduce *P. fluorescens*-contaminated fluids into the body (Burgos et al., 1996; Carpenter and Dicks, 1982; Dalamaga et al., 2005; Essex et al., 2004; Foulon et al., 1981; Kitzmann et al., 2008; Manfredi et al., 2000; Nelson et al., 1991; Pappas et al., 2006; Rais-Bahrami et al., 1990; Rutenburg et al., 1958; Sarubbi et al., 1978). *Pseudomonas* infections, as a primary cause of sepsis in neonates, are increasingly recognized. Most are associated with *P. aeruginosa*, but one case of *P. fluorescens* sepsis resulting in the death of a neonate was reported (Rais-Bahrami et al., 1990). *P. fluorescens*-contaminated amphotericin B was implicated in one case of nosocomially-acquired meningitis and bacteraemia (Sarubbi et al., 1978). Pelviperitonitis caused by *P. fluorescens* was diagnosed in a patient wearing an intrauterine device (Foulon et al., 1981). A patient with positive HIV status was diagnosed with a *P. fluorescens* infection likely the result of the central venous catheter (Nelson et al., 1991). *P. fluorescens* endophthalmitis was reported in two

patients: one secondary to trauma (Essex et al., 2004), the other associated with a corneal ulcer (Kitzmann et al., 2008). Urinary tract infections caused by *P. fluorescens* (Carpenter and Dicks, 1982; McLean and Nickel, 1991; Rutenburg et al., 1958) are often related to bladder catheterisation (Carpenter and Dicks, 1982). However, a cancer patient, with no history of catheterisation was also diagnosed with a urinary tract infection caused by *P. fluorescens* (Pappas et al., 2006). The death of a cystic fibrosis patient 18 months post-lung transplantation was attributed to pericarditis caused by *P. fluorescens* (Kanj et al., 1997). Other causes of *P. fluorescens* infection include anabolic androgen steroid use (Kienbacher et al., 2007) and a dog bite which resulted in cutaneous abscess and recurrent bacteraemia (Dalamaga et al., 2005).

P. fluorescens was identified as the causative agent of infection in two outbreaks of *P. fluorescens* bacteremia associated with the presence of indwelling devices and contaminated fluids (e.g. heparin/saline flushes) (Gershman et al., 2008). One, reported in Taiwan (Hsueh et al., 1998), involved four patients with underlying malignancies and was associated with the use of Port-A-Cath implants. All recovered with treatment. The second, reported in the United States, originated from a single contaminated heparin/saline flush product distributed to several States (Gershman et al., 2008). Approximately 80 patients, being treated for various conditions, were infected. All recovered after antibiotic treatment.

In vitro and in vivo tests were conducted by Health Canada's scientists to evaluate the potential of *P. fluorescens* ATCC 13525 to cause cytotoxicity and adverse immune effects. Results indicate no evidence of cytotoxic effects on human colonic cells (HT29) after 6, 12 and 24 hours of exposure. No haemolytic activity was observed on sheep blood agar. BALB/c mice exposed to 1×10^6 CFU/µL of ATCC 13525 by endotracheal instillation showed no changes in behavior and physical appearance. No significant increase in lung granulocytes, or lung and blood cytokines, or in serum amyloid A were observed over the one week sampling period. All bacteria were cleared 96h and 168h after exposure from lungs, trachea and esophagus. In the absence of complete pathogenicity/toxicity test data on *P. fluorescens* ATCC 13525, data on other strains was considered as surrogate information. Health Canada's Pest Management Regulatory Agency (PMRA) has registered two strains of *P. fluorescens* (ATCC 55799 and ATCC 31948) as microbial pest control agents under the strain designations CL45A and A506, respectively. Studies reviewed by PMRA as part of their decisions found that neither strain was toxic or pathogenic in standard acute pathogenicity/toxicity tests. *P. fluorescens* ATCC 55799 was observed to be not toxic to rats when exposed via oral (2.42×10^7 CFU/kg bw) or pulmonary (3.4×10^8 CFU/animal) route nor was it infective after intravenous injection (4.7×10^6 CFU/mL to 1.95×10^7 CFU/mL). No significant adverse effects were reported when a mixture (*P. fluorescens* ATCC 31928 and other *P. fluorescens* strains) or *P. fluorescens* AGS 3001.2 (a strain similar to *P. fluorescens* ATCC 31928) was administered orally to rats. *P. fluorescens* ATCC 31948 was observed to be not toxic to rats when exposed via oral (8.4×10^{10} CFU/animal) route. In mice exposed to *P. fluorescens* ATCC 31948 via intraperitoneal injection (2.0×10^8 CFU/animal) there were no mortalities, but

general signs of toxicity were observed including scruffy coats, discharge from eyes, lethargy and diarrhea. The study did not meet the recommended 21 day observation period to assess infectivity. It is likely that the observed signs of toxicity can be attributed to an immune reaction to the lipopolysaccharide. These studies have been included in Appendix 7 (PMRA-HC, 2010; PMRA-HC, 2012).

The most serious effect of *P. fluorescens* on human health is associated with its ability to proliferate in blood products stored at refrigeration temperatures. Post-transfusional sepsis (Gibaud et al., 1984; Gibb et al., 1995; Gibb, 2000; Khabbaz et al., 1984; Murray et al., 1987; Pappas et al., 2006; Scott et al., 1988) occurs rarely, but is fatal in approximately 60% of reported cases, and affects people of all ages and states of health.

1.1.3.3 Antibiotic Susceptibility

Antibiotics used successfully to treat the *P. fluorescens* infections described above include amikacin, aminoglycosides, ampicillin, ceftazimide, cefazolin, ciprofloxacin, chloramphenicol, gentamicin, methotrexate, moxifloxacin, prednisone, tetracycline, ticarcillin, ticarcillin/clavulanate, tobramycin, and vancomycin. Table 1-3 represents an antibiogram generated by Health Canada for the characterization of *P. fluorescens* ATCC 13525.

Table 1-3 Minimal Inhibitory Concentration (MIC) for *P. fluorescens* ATCC 13525[a]

Antibiotic	MIC (µg/mL)
Amoxycillin	> 50
Amphotericin	> 50
Aztreonam	> 50
Cephotaxime	> 50
Doxycycline	0.8 +/- 0.4
Erythromycin	> 50
Gentamicin	2.3 +/- 2.3
Nalidixic acid	24
Trimethoprim	> 50
Vancomycin	> 50

a Work conducted using TSB-MTT liquid assay method to characterize the *P. fluorescens* DSL strain (Seligy et al., 1997). The reported values are based on a minimum of three independent experiments. Values correspond to the minimal inhibitory concentration (µg/ml) for select *P. fluorescens* (20, 000 CFU/well) grown in the presence of antibiotic for 24 hrs. at 37°C.

MIC tests performed by Sader and Jones (2005) on *P. fluorescens* clinical isolates indicate that the most active compound was amikacin (MIC_{50}, 2 mg/l; 88.5% susceptible), followed by cefepime (MIC_{50}, 4 mg/l; 84.2% susceptible), which showed the lowest resistance rate (6.3%). Other compounds with reasonable activity against *P. fluorescens* include tobramycin, imipenem, polymyxin B and ceftazidime.

All micro-organisms contain components, such as lipopolysaccharides, antigens, toxins and enzymes, which may act as potential sensitizers. Though *P. fluorescens* possesses antigens which are known to cause hypersensitivity (Bernstein et al., 1995; Fishwick et al., 2005; Skorska et al., 2005; Yadav et al., 2003), there are no reported cases directly implicating allergenicity of *P. fluorescens* ATCC 13525 in healthy individuals.

1.2 Hazard Severity

P. fluorescens, as a species, is a well characterized micro-organism. 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. fluorescens* in soil and water and the use of certain strains for biocontrol against specific pest organisms, only a few reports were found regarding the pathogenic and toxigenic potential of some strains of the species. Stress, compromised natural defenses or comorbidity with primary viral or fungal infections are generally preconditions to the reported *P. fluorescens* infections. No relevant reports, in the publicly available scientific literature, specifically investigated the potential adverse effect of *P. fluorescens* ATCC 13525 towards plants or animals. Furthermore, there have been no reports regarding the pathogenic or toxigenic potential of *P. fluorescens* ATCC 13525, nor has it been associated with toxins or metabolites that may lead to adverse effects.

Therefore, environmental hazard severity for *P. fluorescens* ATCC 13525 is estimated to be low.

Despite adverse effects reported at the species level, there have been no reported infections attributed specifically to *P. fluorescens* ATCC 13525 in the scientific literature. Its inability to grow at normal human body temperature may limit its ability to invade and cause disease in immune competent individuals. The human health hazard for *P. fluorescens* ATCC 13525 to the general population is estimated to be low. Nonetheless, there are reports of adverse effects including *P. fluorescens* nosocomial infections and sepsis resulting from contaminated medical devices and blood products, suggesting a hazard to individuals undergoing medical treatment. The human health hazard associated with these individuals is estimated to be medium. The overall human hazard severity for *P. fluorescens* ATCC 13525 is therefore estimated to be low-medium.

2. Exposure Assessment

2.1 Sources of Exposure

In 2007, a voluntary questionnaire was sent to a subset of key biotechnology companies. These results combined with information obtained from other federal government regulatory and non-regulatory programs indicate that 10,000 to 100,000 kg of products potentially containing *P. fluorescens* ATCC 13525 (formulation and

concentration unknown) were imported into or manufactured in Canada in 2006-2007.

In 2009, the government conducted a mandatory information-gathering Notice (survey) under section 71 of CEPA 1999 as published in the Canada Gazette Part 1 on October 3rd, 2009 (hereafter "s. 71 Notice"). The s. 71 Notice applied to any persons who, during the 2008 calendar year, manufactured or imported a DSL substance, whether alone, in a mixture, or in a product. Anyone meeting these reporting requirements was legally obligated to respond. Respondents were required to submit information on the industrial sector, uses and any trade names associated with products containing these strains, as well as the quantity and concentration of the strain imported or manufactured in the 2008 calendar year. A variety of environmental, industrial and household applications using *P. fluorescens* ATCC 13525 were reported in response to the s. 71 Notice. According to information submitted, 100 to1000 kg of *P. fluorescens* ATCC 13525 was imported into or manufactured in Canada in 2008.

The focus of this screening assessment is exposure to *P. fluorescens* ATCC 13525 from its deliberate use in consumer or commercial products and industrial processes.

The species *P. fluorescens* has properties that make it of commercial interest in a variety of industries. *P. fluorescens* has been shown to have the ability to degrade a wide variety of compounds, including; 3-chlorobenzoic acid (Fava et al., 1993); naphthalene, phenantrene, fluorine and fluoranthene (Weissenfels et al., 1990); chlorinated aliphatic hydrocarbons (Vandenbergh and Kunka, 1988); styrene (Baggi et al., 1983); and pure hydrocarbons and crude oil (Janiyani et al., 1993). *P. fluorescens* can also be used in biosensor applications. For example, the recombinant *P. fluorescens* strain HK9, which lights up in the presence of contaminants such as PAHs (due to insertion of *lux* genes), allows easy detection of bioavailable fractions of pollutants in soils and sediments (King et al., 1990).

A search of the public domain (internet, patent databases) suggests multiple potential uses, including in pulp and paper and textile processing, municipal and industrial wastewater treatment, waste degradation, particularly in petroleum refineries, bioremediation and biodegradation as well as in commercial and household drain cleaners and degreasers, enzyme and chemical production, septic and RV tank additives and general cleaning and odour-control products. For agricultural applications, *P. fluorescens* has been used for pest control, for plant growth and disease suppression and as an anti-frost agent.

Due to expanding commercialization of products potentially containing *P. fluorescens,* there is a likelihood of an increase in the use and release of *P. fluorescens* ATCC 13525 in the environment through human activity (Chatzipavlidis et al, 2013).

2.2 Exposure Characterization

2.2.1 Environment

Based on reported uses identified through Section 71, the most likely routes of introduction of *P. fluorescens* ATCC 13525 into the environment could be directly and indirectly into water and soil.

P. fluorescens is a normal inhabitant of the soil rhizosphere and several studies were reported about the survival and persistence of different strains. *P. fluorescens* cells are able to withstand low temperatures, and could survive better at 4°C than at 15°C or 27°C following introduction into natural soil (George et al., 1999) and *P. fluorescens* R2f survived above 10^7 CFU/g dry soil for up to 84 days in loamy sand microcosms when encapsulated (Van Elsas et al., 1992), while free cells declined below 10^5 cells/g dry soil after 21 days. A study by Troxler et al. (1997) showed that under aerobic conditions, numbers of culturable *P. fluorescens* CHA0 cells declined in effluent water, from 10^7 to approximately 10^2, in the span of 115 days. The authors hypothesized that the decline was due to competition-antagonism with indigenous micro-organisms or grazing by protozoa.

Persistence data was specifically performed by Environment Canada on *P. fluorescens* ATCC 13525 in agricultural soil. After inoculation of soil with live cells, it was found that the DNA from this strain persisted for up to 28 days (Xiang et al., 2010). The persistence observed may or may not be from viable cells. However, given the ubiquity of the species, one could assume that this strain is also able to survive for considerable lengths of time in soil and other media even if there is no evidence of proliferation.

The above information indicates that in most exposure scenarios, *P. fluorescens* ATCC 13525 is theoretically able to survive and persist in the environment but maintenance of high numbers beyond those of background are unlikely, due to competition (Leung et al., 1995) and microbiotasis (Van Veen et al., 1997), which is an inhibitory effect of soil that results in the rapid decline of populations of introduced bacteria. Competition and competitive niche exclusion are likely to limit the growth of introduced pseudomonad inoculants. Competitors are likely to include closely related pseudomonads and other bacteria able to compete for the same ecological niches with similar nutritional requirements (Lindow, 1992).

No relevant reports concerning persistence in the environment of toxins produced by *P. fluorescens* have been found.

Thus, the overall **environmental exposure** to *P. fluorescens* strain ATCC 13525 is estimated to be **medium.**

2.2.2 Humans

Hazards related to micro-organisms used in the workplace should be classified accordingly under the Workplace Hazardous Materials Information System (WHMIS)[4].

Human exposure is expected to be greatest through the use of consumer or commercial products containing *P. fluorescens* ATCC 13525. For consumer products, the routes and extent of user and bystander exposure will depend on the application method, the concentration of *P. fluorescens* ATCC 13525 in the product, and the amount of product applied. During product application, dermal exposure is likely for products applied by hand, and inhalation of aerosols or dust containing *P. fluorescens* ATCC 13525 is expected for products with spray or powder formulations. Secondary to product application, residual *P. fluorescens* ATCC13525 on surfaces and in reservoirs such as treated drains could result in dermal exposure, as well as inadvertent ingestion where the organism persists on food preparation surfaces, and inhalation, where aerosols are generated (e.g., kitchen garbage disposal units). Since *P. fluorescens* ATCC 13525 is expected to persist following application, such exposures may be temporally distant from the time of application.

For commercial products, the general population could be exposed as bystanders during product application. The route and extent of exposure will depend on the application method, the concentration of *P. fluorescens* ATCC 13525 in the product, the amount of product applied, and proximity to the site application. The general population could also come into contact with residual *P. fluorescens* ATCC 13525 on treated surfaces.

Use of *P. fluorescens* ATCC 13525 in waste and wastewater treatment or in industrial processes, may introduce the organism into bodies of water and soil. Nevertheless, human exposure to the strain through the environment is expected to be low. While large inputs of DSL-listed *P. fluorescens* ATCC 13525 into the environment could result in concentrations greater than background levels of *P. fluorescens*, high numbers of vegetative cells are unlikely to be maintained in water and in soil due to competition (Leung et al. 1995) and microbiostasis (van Veen et al. 1997), as previously mentioned. Moreover, drinking water treatment processes are expected to effectively eliminate these micro-organisms and so limit their ingestion in drinking water.

The overall **human exposure** estimation for *P. fluorescens* ATCC 13525 is **medium**. Given the range and scale of intended and potential uses, there is reason to expect that the quantity of *P. fluorescens* ATCC 13525 released into the Canadian

[4] A determination of whether one or more of the criteria of section 64 of CEPA 1999 are met is based upon an assessment of potential risks to the environment and/or to human health associated with exposure in the general environment. For humans, this includes, but is not limited to, exposure from air, water and the use of products containing the substances. A conclusion under CEPA 1999, on these substances, 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.

environment will be greater than that reported in response to the section 71 Notice. In addition, specific exposure scenarios, such as the use of consumer cleaning products containing *P. fluorescens*, could result in direct, possibly repeated, exposure to larger quantities of the micro-organism.

3. Decisions from other Jurisdictions

In Canada, the Pest Management Regulatory Agency (PMRA), under the authority of the *Pest Control Products Act* (PCPA) and Regulations, as well as the United States Environmental Protection Agency (USEPA) granted full registration for the sale and use of *P. fluorescens* strain (ATCC 31948, biovar 1) microbial pest control agents (MPCA) against a fireblight pathogen. Another strain of *P. fluorescens* (ATCC 55799, biovar 1, inactivated) was approved for use as a MPCA against zebra and quagga mussels by both the PMRA and the USEPA. An evaluation by PMRA of available scientific information found that, under the approved conditions of use, the products do not present an unacceptable risk to human health or the environment. Infectivity and toxicity testing for the latter strain is included in Appendix 7 (PMRA-HC, 2010; PMRA-HC, 2012). *P. fluorescens* was considered a plant pest of quarantine importance in the Commonwealth of Dominica in 2005 (Regulated Pests of member countries of the International Plant Protection Convention).

P. fluorescens is considered a Risk Group 1 animal and human pathogenic by the Public Health Agency of Canada. It is not considered to be an animal pathogen, nor does the Canadian Food Inspection Agency require a plant protection permit to import this organism into Canada (CFIA, Import Permit Office - Plant Health Program). The Public Health Agency of Canada considers *P. fluorescens* to be a Risk Group 1 bacterium, based on the fact that it is an opportunistic human pathogen, capable of causing human infection, but unlikely to do so in healthy individuals.

4. 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. fluorescens* ATCC 13525 to be low and 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.

Based on the considerations outlined above, the risk to the environment from current and foreseeable future uses of *Pseudomonas fluorescens* ATCC13525 is expected to be low.

Based on the low level of human health hazard of *P. fluorescens* ATCC 13525 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. fluorescens* ATCC 13525.

It is therefore proposed to conclude that *P. fluorescens* ATCC 13525 does not meet any of the criteria set out in section 64 of CEPA 1999.

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 to require assessment of these new activities before they begin.

The risk to the environment from foreseeable future uses is expected to be low. Aquatic and terrestrial species may be exposed to the DSL strain when used in activities at higher concentrations compared to what would be expected in a naturally-occurring microbial community. Nevertheless, no evidence of adverse effects have been reported at the population or ecosystem levels in Canada that can be specifically attributed to *P. fluorescens* ATCC 13525 and the dilution factor of products containing the DSL strain is expected to be significant, so the concentrations required to see any potential adverse effects are not anticipated to be reached.

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. fluorescens ATCC 13525 has properties that make it suitable for use in a range of products, and there is reason to expect new uses of *P. fluorescens* ATCC 13525 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.

5. Conclusion

Based on responses to the 2009 s. 71 Notice, it is concluded that *P. fluorescens* ATCC 13525 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 those substances do not meet the criteria as set out in section 64 of the CEPA 1999.

6. References

Aalten, P.M., Vitour, D., Blanvillain, D., Gowen, S.R., and Sutra, L. (1998). Effect of rhizosphere fluorescent Pseudomonas strains on plant-parasitic nematodes Radopholus similis and Meloidogyne spp. Lett. Appl. Microbiol. *27,* 357-361.

Ahmed, S.M. (1992). Clinical microbiological examinations and prevention of saprolegniasis infection in *Mormyrus kannume*.*27,* 357-361.

Anson, A.E. (1982). A Pseudomonad Producing Orange Soft Rot Disease in Cacti. Phytopathol. *103,* 163-172.

Appanna, V.D., Gazsó, L.G., and St. Pierre, M. (1996). Multiple-metal tolerance in Pseudomonas fluorescens and its biotechnological significance. J. Biotechnol. *52,* 75-80.

Åström, B., Gustafsson, A., and Gerhardson, B. (1993). Characteristics of a plant deleterious rhizosphere pseudomonad and its inhibitory metabolite(s).*74,* 20-28.

Baggi, G., Boga, M.M., Catelani, D., Galli, E., and Treccani, V. (1983). Styrene catabolism by a strain of *Pseudomonas fluorescens*. Syst Appl Micriobiol *4,* 141-147.

Bale, M.J., Fry, J.C., and Day, M.J. (1988). Transfer and occurrence of large mercury resistance plasmids in river epilithon. Appl. Environ. Microbiol. *54,* 972-978.

Banowetz, G.M., Azevedo, M.D., Armstrong, D.J., Halgren, A.B., and Mills, D.I. (2008). Germination-Arrest Factor (GAF): Biological properties of a novel, naturally-occurring herbicide produced by selected isolates of rhizosphere bacteria. Biol. Control *46,* 380-390.

Barker, G.A., Smith, S.N., and Bromage, N.R. (1991). Commensal bacteria and their possible relationship to the mortality of incubating salmonid eggs. J. Fish Dis. *14,* 199-210.

Baruah, N.D., and Prasad, K.P. (2001). Efficacy of levamisole as an immunostimulant in *Macrobrachium rosenbergii* (de Man).*14,* 199-210.

Bateman, D.F., and Millar, R.L. (1966). Pectic enzymes in tissue degradation.*4,* 119-145.

Bergen, T. (1981). Human- and animal-pathogenic members of the genus Pseudomonas. In The Prokaryotes - A Handbook on Habitats, Isolation and Identification of Bacteria, Starr, M. P., Stolp, H., Truper, H. G., Balows, A. and Schegel, H. G. eds., (Berlin: Springer-Verlag)

Bernstein, D.I., Lummus, Z.L., Santilli, G., Siskosky, J., and Bernstein, I.L. (1995). Machine operator's lung. A hypersensitivity pneumonitis disorder associated with exposure to metalworking fluid aerosols. Chest *108,* 636-641.

Betterley, D.A., and Olson, J.A. (1989). Isolation, Characterization and Studies of Bacterial Mummy Disease of *Agaricus brunnescens.*.*12,* 679-688.

Bezanson, G.S., MacInnis, R., Potter, G., and Hughes, T. (2008). Presence and potential for horizontal transfer of antibiotic resistance in oxidase-positive bacteria populating raw salad vegetables. Int. J. Food Microbiol. *127,* 37-42.

Blumer, C., and Haas, D. (2000). Mechanism, regulation, and ecological role of bacterial cyanide biosynthesis. Arch. Microbiol. *173,* 170-177.

Blumer, C., Heeb, S., Pessi, G., and Haas, D. (1999). Global GacA-steered control of cyanide and exoprotease production in Pseudomonas fluorescens involves specific ribosome binding sites.*96,* 14073-14078.

Bodilis, J., Calbrix, R., Guérillon, J., Mérieau, A., Pawlak, B., Orange, N., and Barray, S. (2004). Phylogenetic Relationships between Environmental and Clinical Isolates of

Pseudomonas fluorescens and Related Species Deduced from 16S rRNA Gene and OprF Protein Sequences. Syst. Appl. Microbiol. *27,* 93-108.

Bopp, L.H., Chakrabarty, A.M., and Ehrlich, H.L. (1983). Chromate resistance plasmid in *Pseudomonas fluorescens*. J. Bacteriol. *155,* 1105-1109.

Brecher, M.E., and Hay, S.N. (2005). Bacterial Contamination of Blood Components. Clin Microbiol Rev *18,* 195-204.

Burgos, F., Torres, A., Gonzalez, J., Puig de la Bellacasa, J., Rodriguez-Roisin, R., and Roca, J. (1996). Bacterial colonization as a potential source of nosocomial respiratory infections in two types of spirometer. Eur. Respir. J. *9,* 2612-2617.

Cantore, P.L., and Iacobellis, N.S. (2008). Head Rot of Cauliflower Caused by *Pseudomonas fluorescens* in Southern Italy. In. *Pseudomonas syringae* Pathovars and Related Pathogens Identification.69-72.

Carpenter, E.M., and Dicks, D. (1982). Isolation of *Pseudomonas fluorescens* after suprapubic catheterisation. J. Clin. Pathol. *35,* 581.

Carson, J., and Schmidtke, L.M. (1993). Opportunistic infection by psychrotrophic bacteria of cold-comprised Atlantic salmon.*13,* 49-52.

Castric, P.A. (1983). Hydrogen cyanide production by *Pseudomonas aeruginosa* at reduced oxygen levels. Can. J. Microbiol. *29,* 1344-1349.

CDC. (2006). Update: Delayed onset *Pseudomonas fluorescens* bloodstream infections after exposure to contaminated heparin flush--Michigan and South Dakota, 2005-2006. MMWR Morb. Mortal. Wkly. ReP. *55,* 961-963.

Chandrasekaran, S., and Lalithakumari, D. (1998a). Maintenance of a *Pseudomonas fluorescens* plasmid in heterologous hosts: metabolic burden as a more reliable variable to predict plasmid instability. Indian. J. ExP. Biol. *36,* 693-698.

Chandrasekaran, S., and Lalithakumari, D. (1998b). Plasmid-mediated rifampicin resistance in *Pseudomonas fluorescens*. J. Med. Microbiol. *47,* 197-200.

Chapalain, A., Rossignol, G., Lesouhaitier, O., Merieau, A., Gruffaz, C., Guerillon, J., Meyer, J., Orange, N., and Feuilloley, M.G.J. (2008). Comparative study of 7 fluorescent pseudomonad clinical isolates. Can. J. Microbiol. *54,* 19-27.

Chatzipavlidis, I., Kefalogianni, I,. Venieraki, A. and Holzapfel, W. (2013). Status and Trends of the Conservation and Sustainable Use of Microorganisms in Agroindustrial Processes. Commission on Genetic Resources for Food and Agriculture, Food and Agriculture Organization of the United Nations.

Compant, S., Duffy, B., Nowak, J., Clément, C., and Barka, E.A. (2005). Use of plant growth-promoting bacteria for biocontrol of plant diseases: Principles, mechanisms of action, and future prospects. Appl. Environ. Microbiol. *71,* 4951-4959.

Corotto, L.V., Wolber, P.K., and Warren, G.J. (1986). Ice nucleation activity of *Pseudomonas fluorescens*: mutagenesis, complementation analysis and identification of a gene product. EMBO J. *5,* 231-236.

Costerton, J.W., Stewart, P.S., and Greenberg, E.P. (1999). Bacterial Biofilms: A Common Cause of Persistent Infections.*284,* 1318.

Cottyn, B., Heylen, K., Heyrman, J., Vanhouteghem, K., Pauwelyn, E., Bleyaert, P., Van Vaerenbergh, J., Hofte, M., De Vos, P., and and Maes, M. (2009). *Pseudomonas cichorii* as

the casual agent of midrib rot, an emerging disease of greenhouse-grown butterhead lettuce in Flanders.*32,* 211-225.

Csaba, G., Prigli, M., Békési, L., Kovács-Gayer, É., Bajmóczy, E., and Fazekas, B. (1984). Septicaemia in silver carp (*Hypophthalmichthys molitrix* val.) and bighead carp (*Aristichthys nobilis* rich.) caused by *Pseudomonas fluorescens.*.75-84.

Cuffari, C. (2009). Diagnostic Considerations in Pediatric Inflammatory Bowel Disease Management. Gastroenterol. Hepatol. *5,* 775-783.

Daane, L., Molina, J., Berry, E., and Sadowsky, M. (1996). Influence of earthworm activity on gene transfer from *Pseudomonas fluorescens* to indigenous soil bacteria. Appl. Environ. Microbiol. *62,* 515-521.

Dalamaga, M., Karmaniolas, K., Chavelas, C., Liatis, S., Matekovits, H., and Migdalis, I. (2005). *Pseudomonas fluorescens* cutaneous abscess and recurrent bacteremia following a dog bite. Int. J. Dermatol. *44,* 347-349.

Dalwadi, H., Wei, B., Kronenberg, M., Sutton, C.L., and Braun, J. (2001). The Crohn's Diease-Associated Bacterial Protein I2 Is a Novel Enteric T Cell Superantigen. Immun. *15,* 149-158.

Das, B.K., Samal, S.K., Samantaray, B.R., Sethi, S., Pattnaik, P., and Mishra, B.K. (2006). Antagonistic activity of cellular components of Pseudomonas species against Aeromonas hydrophila. Aquaculture *253,* 17-24.

de Lima Pimenta, A., Di Martino, P., Le Bouder, E., Hulen, C., and Blight, M.A. (2003). In vitro identification of two adherence factors required for in vivo virulence of *Pseudomonas fluorescens*. Microbes Infect. *5,* 1177-1187.

de Lima Pimenta, A., Di Martino, P., and Blight, M.A. (2006). Positive correlation between in vivo and in vitro assays for the evaluation of Pseudomonas virulence. Res. Microbiol. *157,* 885-890.

Dé, E., Orange, N., Saint, N., Guérillon, J., De Mot, E., and Molle, G. (1997). Growth and temperature dependence of channel size of the major outer-membrane protein (OprF) in psychrontrophic *Pseudomonas fluorescens* stains. Microbiol. *143,* 1029.

Demaneche, S., Kay, E., Gourbiere, F., and Simonet, P. (2001). Natural transformation of *Pseudomonas fluorescens* and *Agrobacterium tumefaciens* in soil. Appl. Environ. Microbiol. *67,* 2617-2621.

Devi, K.K., and Kothamasi, D. (2009). *Pseudomonas fluorescens* CHA0 can kill subterranean termite *Odontotermes obesus* by inhibiting cytochrome c oxidase of the termite respiratory chain. FEMS Microbiol. Lett. *300,* 195-200.

Dieckelmann, M., Johnson, L.A., and Beacham, I.R. (1998). The diversity of lipases from psychrotrophic strains of *Pseudomonas*: a novel lipase from a highly lipolytic strain of *Pseudomonas fluorescens*. J Appl Microbiol *85,* 527.

Doi, O., and Nojima, S. (1971). Phospholipase C from *Pseudomonas fluorescen*s. Biochim. Biophys. Acta Lipids Lipid Metab. *248,* 234-244.

Dufour, D., Nicodème, M., Perrin, C., Driou, A., Brusseaux, E., Humbert, G., Gaillard, J.-., and Dary, A. (2008). Molecular typing of industrial strains of Pseudomonas spp. isolated from milk and genetical and biochemical characterization of an extracellular protease produced by one of them. Int. J. Food Microbiol. *125,* 188-196.

Ellis, R.J., Lilley, A.K., Lacey, S.J., Murrell, D., and Godfray, H.C.J. (2007). Frequency-dependent advantages of plasmid carriage by Pseudomonas in homogeneous and spatially structured environments. ISME J. *1,* 92-95.

Essex, R.W., Charles, P.G., and Allen, P.J. (2004). Three cases of post-traumatic endophthalmitis caused by unusual bacteria. Clin. Experiment. Ophthalmol. *32,* 445-447.

Esteve, C., Biosca, E.G., and Amaro, C. (1993). Virulence of *Aeromonas hyrophila* and some other bacteria isolated from European eels *Anguilla anguilla* reared in fresh water.*16,* 15-20.

Farajzadeh, D., Aliasgharzad, N., Sokhandan Bashir, N., and Yakhchali, B. (2010). Cloning and characterization of a plasmid encoded ACC deaminase from an indigenous *Pseudomonas fluorescens* FY32. Curr. Microbiol. *61,* 37-43.

Fava, F., Gioia, D.D., and Marchetti, L. (1993).
Characterization of a pigment produced by *Pseudomonas fluorescens* during 3-chloroenzoate co-metabolism. Chemosphere *27,* 825-835.

Fishwick, D., Paul, T., Elms, J., Robinson, E., Crook, B., Gallagher, F., Lennox, R., and Curran, A. (2005). Respiratory symptoms, immunology and organism identification in contaminated metalworking fluid workers. What you see is not what you get.*55,* 238-241.

Flores-Vargas, R.D., and O'Hara, G.W. (2006). Isolation and characterization of rhizosphere bacteria with potential for biological control of weeds in vineyards. J. Appl. Microbiol. *100,* 946-954.

Folsom, D., and Friedman, B.A. (1959). *Pseudomonas fluorescens* in relation to certain diseases of potato tubers in Maine. Am. Potato J. *36,* 90-97.

Foulon, W., Naessens, A., Lauwers, S., Volckaert, M., Devroey, P., and Amy, J.J. (1981). Pelvic inflammatory disease due to *Pseudomonas fluorescens* in patient wearing an intrauterine device. Lancet *2,* 358-359.

Fuchs, A. (1965). The transeliminative breakdown of Na-polygalacturonate by *Pseudomonas fluorescens*. Antonie Van Leeuwenhoek J. Microbiol. Serol. *31,* 323-340.

Gandhi, P.I., and Gunasekaran, K. (2008). Biopesticide seed treatment for the management of sucking pests in bhendi.*95,* 225-229.

Gennari, M., and Dragatto, F. (1992). A study of the incidence of different fluorescent *Pseudomoas* species and biovars in the microflora of fresh and spoiled meat and fish, raw milk, cheese, soil and water. J Appl Bacteriol *72,* 281-288.

George, S.E., Nelson, G.M., Kohan, M.J., Brooks, L.R., and Boyd, C. (1999). Colonization and clearance of environmental microbial agents upon intranasal exposure of strain C3H/HeJ mice. J. Toxicol. Environ. Health A *56,* 419-431.

Gershman, M.D., Kennedy, D.J., Noble-Wang, J., Kim, C., Gullion, J., Kacica, M., Jensen, B., Pascoe, N., Saiman, L., McHale, J.*, et al.* (2008). Multistate outbreak of Pseudomonas fluorescens bloodstream infection after exposure to contaminated heparinized saline flush prepared by a compounding pharmacy.*47,* 1372-1379.

Gibaud, M., Martin-Dupont, P., Dominguez, M., Laurentjoye, P., Chassaing, B., and Leng, B. (1984). *Pseudomonas fluorescens* septicemia following transfusion of contaminated blood: Septicémie à *Pseudomonas fluorescens* après transfusion de sang contaminé.*13,* 2583-2584.

Gibb, A.P. (2000). Bacterial contamination of donated blood. Rev. Med. Microbiol. *11,* 179-187.

Gibb, A.P., Martin, K.M., Davidson, G.A., Walker, B., and Murphy, W.G. (1995). Rate of growth of *Pseudomonas fluorescens* in donated blood. J. Clin. Pathol. *48,* 717-718.

Glazebrook, J.S., and Campbell, R.S.F. (1990). A survey of the diseases of marine turtles in northern Australia. I. Farmed turtles. Dis. Aquat. Org. *9,* 83-95.

Gottlieb, T. (1993). Hazards of Bacterial Contamination of Blood Products. Anaesth Intens Care *21,* 20-23.

Grice, E.A., Kong, H.H., Renaud, G., Young, A.C., NISC Comparative Sequencing Program, Bouffard, G.G., Blakesley, R.W., Wolfsberg, T.G., Turner, M.L., and Segre, J.A. (2008). A diversity profile of the human skin microbiota. Genome Res. *18,* 1043-1050.

Gross, H., and Loper, J.E. (2009). Genomics of secondary metabolite production by Pseudomonas spp. Nat. Prod. Rep. *26,* 1408-1446.

Guinet, F., Carniel, E., and Leclerq, A. (2011). Transfusion-Transmisitted *Yersernia entercolitica* Sepsis. Emerg Infect *53,* 583-591.

Guo, Q., Guo, D., Zhao, B., Xu, J., and Li, R. (2007). Two cyclic dipeptides from *Pseudomonas fluorescens* GcM5-1A carried by the pine wood nematode and their toxicities to Japanese black pine suspension cells and seedlings in vitro. J. Nematol. *39,* 243-247.

Guthrie, G.D., Nicholson-Guthrie, C.S., and Leary Jr., H.L. (2000). A Bacterial High-Affinity GABA Binding Protein: Isolation and Characterization. Biochem. Biophys. Res. Commun. *268,* 65-68.

Haas, D., and Défago, G. (2005). Biological control of soil-borne pathogens by fluorescent pseudomonads. Nat. Rev. Microbiol. *3,* 307-319.

Hahn, H.P. (1997). The type-4 pilus is the major virulence-associated adhesin of *Pseudomonas aeruginosa* – a review. Gene *192,* 99-108.

Hammer, P., Hill, D., Lam, S., Van Pee, K., and Ligon, J. (1997). Four genes from *Pseudomonas fluorescens* that encode the biosynthesis of pyrrolnitrin. Appl. Environ. Microbiol. *63,* 2147-2154.

Han, Z.M., Hong, Y.D., and Zhao, B.G. (2003). A study on pathogenicity of bacteria carried by pine wood nematodes. J. Phytopathol. *151,* 683-689.

Hearn, E.M., Dennis, J.J., Gray, M.R., and Foght, J.M. (2003). Identification and Characterization of the emhABC Efflux System for Polycyclic Aromatic Hydrocarbons in *Pseudomonas fluorescens* cLP6a. J. Bacteriol. *185,* 6233-6240.

Heffernan, B., Murphy, C.D., and Casey, E. (2009). Comparison of Planktonic and Biofilm Cultures of *Pseudomonas fluorescens* DSM 8341 Cells Grown on Fluoroacetate. Appl Environ Microbiol *75,* 2899.

Heinaru, E., Vedler, E., Jutkina, J., Aava, M., and Heinaru, A. (2009). Conjugal transfer and mobilization capacity of the completely sequenced naphthalene plasmid pNAH20 from multiplasmid strain *Pseudomonas fluorescens* PC20. FEMS Microbiol. Ecol. *70,* 563-574.

Hildebrand, P.D. (1989). Surfactant-like characteristics and identity of bacteria associated with broccoli head rot in Atlantic Canada. Can. J. Plant Pathol. *11,* 205-214.

Holder-Franklin, M.A., and Franklin, M. (1993). River bacteria time series analysis: a field and laboratory study which demonstrates aquatic ecosystems health. J Aquatic Ecosyst Health *2,* 251-259.

Holt, J.G. (1994). Bergey's Manual of Determinate Bacteriology.

Hsueh, P.R., Teng, L.J., Pan, H.J., Chen, Y.C., Sun, C.C., Ho, S.W., and Luh, K.T. (1998). Outbreak of *Pseudomonas fluorescens* bacteremia among oncology patients. J. Clin. Microbiol. *36,* 2914-2917.

Huether, J.P., and McIntyre, G.A. (1969). Pectic enzyme production by two strains of *Pseudomonas fluorescens* associated with the pinkeye disease of potato tubers. Am. Potato J. *46,* 414-423.

Hugh R, Guarraia G, Hat H. (1964). The Proposed Neotype Strains of *Pseudomonas fluorescens* (Trevisan) Migula 1895.*14,* 145-155.

Hwang, S.F., Howard, R.J., and Goatcher, L. (1989). Bacteria associated with crown and root rot of sainfoin in southern Alberta. Can. Plant Dis. Survey *69,* 5-8.

Iwalokun, B.A., Akinsinde, K.A., Lanlenhin, O., and Onubogu, C.C. (2006). Bacteriocinogenicity and production of pyocins from Pseudomonas species isolated in Lagos, Nigeria.*5,* 1072-1077.

Jackson, T.A., and McNeill, M.R. (1998). Premature death in parasitized *Listronotus bonariensis* adults can be caused by bacteria transmitted by the parasitoid *Microctonus hyperodae.* Biocontrol Sci. Technol. *8,* 389-396.

James, R.R., and Lighthart, B. (1992). The effect of temperature, diet, and larval instar on the susceptibility of an aphid predator, *Hippodamia convergens* (Coleoptera: Coccinellidae), to the weak bacterial pathogen *Pseudomonas fluorescens*. J. Invertebr. Pathol. *60,* 215-218.

Janiyani, K.L., Wate, S.R., and Joshi, S.R. (1993). Morphological and biochemical characteristics of bacterial isolates degrading crude oil. J Environ Sci Health Part A: Environ Sci Eng *28,* 1185-1204.

Johnson, B.N., Kennedy, A.C., and Ogg Jr, A.G. (1993). Suppression of downy brome growth by a rhizobacterium in controlled environments. Soil Sci. Soc. Am. J. *57,* 73-77.

Kamilova, F., Kravchenko, L.V., Shaposhnikov, A.I., Makarova, N., and Lugtenberg, B. (2006). Effects of the tomato pathogen *Fusarium oxysporum* f. sp. *radicis-lycopersici* and of the biocontrol bacterium *Pseudomonas fluorescens* WCS365 on the composition of organic acids and sugars in tomato root exudate. Mol. Plant-Microbe Interact. *19,* 1121-1126.

Kanj, S.S., Tapson, V., Davis, R.D., Madden, J., and Browning, I. (1997). Infections in patients with cystic fibrosis following lung transplantation. Chest *112,* 924-930.

Khabbaz, R.F., Arnow, P.M., and Highsmith, A.K. (1984). *Pseudomonas fluorescens* bacteremia from blood transfusion. Am. J. Med. *76,* 62-68.

Khan, M.R., Fischer, S., Egan, D., and Doohan, F.M. (2006). Biological control of fusarium seedling blight disease of wheat and barley. Phytopathology *96,* 386-394.

Ki, V., and Rotstein, C. (2008). Bacterial skin and soft tissue infections in adults: A review of their epidemiology, pathogenesis, diagnosis, treatment and site of care. Can. J. Dis. Med. Microbiol. *19,* 173-184.

Kienbacher, G., Maurer-Ertl, W., Glehr, M., Feiert, C., and Leithner, A. (2007). A case of a tumorsimulating expansion caused by anabolic androgen steroids in body building.*21,* 195-198.

King, J., Digrazia, P., Applegate, B., Burtage, R., Sanseverino, J., Dunbar, P., Larimer, F., and Sayler, G. (1990). Rapid, sensitive bioluminescent reporter technology for naphthalene exposure and biodegradation. Sci. Total Environ. *249,* 778-780.

Kitzmann, A.S., Goins, K.M., Syed, N.A., and Wagoner, M.D. (2008). Bilateral herpes simplex keratitis with unilateral secondary bacterial keratitis and corneal perforation in a patient with pityriasis rubra pilaris. Cornea *27,* 1212-1214.

Kloepper, J.W., Hume, D.J., Scher, F.M., Singleton, C., Tipping, B., Laliberte, M., Frauley, K., Kutchaw, T., Simonson, C., Lifshitz, R., Zaleska, I., and Lee, L. (1988). Plant growth promoting rhizobacteria on canola (rapeseed). Plant Dis *72,* 42-46.

Koka, R., and Weimer, B.C. (2000). Isolation and characterization of a protease from *Pseudomonas fluorescens* RO98. J. Appl. Microbiol. *89,* 280-288.

Lemire, J., Auger, C., Bignucolo, A., Appanna V. P., and Appanna, V.D. (2010). Metabolic strategies deployed by *Pseudomonas fluorescens* to combat metal pollutants: Biotechnological prospects. In Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology, Méndez-Vilas, A. ed., (Badajoz, Spain: FORMATEX RESEARCH CENTER) pp. 177-187.

Lenz, A.P., Williamson, K.S., Pitts, B., Stewart, P.S., and Franklin, M.J. (2008). Localized gene expression in *Pseudomonas aeruginosa* biofilms. Appl. Environ. Microbiol. *74,* 4463-4471.

Leung, K., Trevors, J.T., and Lee, H. (1995). Survival of and lacZ expression recombinant *Pseudomonas* strains introduced into river water microcosms. Can. J. Microbiol. *41,* 461-469.

Li, B., Yu, R.R., Yu, S.H., Qiu, W., Fang, Y., and Xie, G.L. (2009). First Report on Bacterial Heart Rot of Garlic Caused by *Pseudomonas fluorescens* in China. *25,* 91-94.

Liao, C.H., and McCallus, D.E. (1998). Biochemical and genetic characterization of an extracellular protease from *Pseudomonas fluorescens* CY091. Appl. Environ. Microbiol. *64,* 914-921.

Lilley, A.K., and Bailey, M.J. (1997). Impact of plasmid pQBR103 acquisition and carriage on the phytosphere fitness of *Pseudomonas fluorescens* SBW25: Burden and benefit. Appl. Environ. Microbiol. *63,* 1584-1587.

Lin, M.Y., Cheng, M.C., Huang, K.J., and Tsai, W.C. (1993). Classification, pathogenicity and drug susceptibility of hemolytic gram-negative bacteria isolated from sick or dead chickens.*37,* 6-9.

Lindow, S.E. (1992). Ice- strains of *Pseudomonas syringae* introduced to control ice nucleation active strains on potato. In Biological Control of Plant Diseases, Tjamos, E. S., Papavizas, G. C. and Cook, R. J. eds., (New York: Plenum Press)

Lindow, S.E. (1987). Competitive Exclusion of Epiphytic Bacteria by Ice *Pseudomonas syringae* Mutants. Appl. Environ. Microbiol. *53,* 2520-2527.

Liu, P.V. (1964). Pathogenicity of *Pseudomonas fluorescens* and related pseudomonads to warm-blooded animals.*41,* 150-153.

Loper, J.E., Henkels, M.D., Shaffer, B.T., Valeriote, F.A., and Gross, H. (2008). Isolation and identification of rhizoxin analogs from Pseudomonas fluorescens Pf-5 by using a genomic mining strategy. Appl. Environ. Microbiol. *74,* 3085-3093.

Lugtenberg, B.J.J., Dekkers, L., and Bloemberg, G.V. (2001). Molecular determinants of rhizosphere colonization by *Pseudomonas*. Ann. Rev. Phytopathol. *39,* 461-490.

Madi, A., Lakhdari, O., Blottière, H.M., Guyard-Nicodème, M., Le Roux, K., Groboillot, A., Svinareff, P., Doré, J., Orange, N., Feuilloley, M.G.J., and Connil, N. (2010). The clinical

Pseudomonas fluorescens MFN1032 strain exerts a cytotoxic effect on epithelial intestinal cells and induces Interleukin-8 via the AP-1 signalling pathway. BMC Microbiol. *10,* 215-223.

Manfredi, R., Nanetti, A., Ferri, M., and Chiodo, F. (2000). Pseudomonas Organisms Other than *Pseudomonas aeruginosa* as Emerging Bacterial Pathogens in Patients with Human Immunodeficiency Virus Infection.*9,* 79-87.

Marchand, S., Vandriesche, G., Coorevits, A., Coudijzer, K., De Jonghe, V., Dewettinck, K., De Vos, P., Devreese, B., Heyndrickx, M., and De Block, J. (2009). Heterogeneity of heat-resistant proteases from milk *Pseudomonas* species. Int. J. Food Microbiol. *133,* 68-77.

Mathee, K., Narasimhan, G., Valdes, C., Qiu, X., Matewish, J.M., Koehrsen, M., Rokas, A., Yandava, C.N., Engels, R., Zeng, E*., et al.* (2008). Dynamics of *Pseudomonas aeruginosa* genome evolution. Proc. Natl. Acad. Sci. U. S. A. *105,* 3100-3105.

Maunsell, B., Adams, C., and O'Gara, F. (2006). Complex regulation of AprA metalloprotease in *Pseudomonas fluorescens* M114: Evidence for the involvement of iron, the ECF sigma factor, PbrA and pseudobactin M114 siderophore. Microbiology *152,* 29-42.

Mavrodi, D.V., Loper, J.E., Paulsen, I.T., and Thomashow, L.S. (2009). Mobile genetic elements in the genome of the beneficial rhizobacterium *Pseudomonas fluorescens* Pf-5. BMC Microbiol. *9,* 8.

Mayer, D. (2009). *Daphnia magna* Acute Toxicity Tests. MOI 401 *Pseudomonas fluorescens* CL 145A. *Volume 4 of 6 submission.,*

McLean, R.J.C., and Nickel, J.C. (1991). Bacterial Colonization Behaviour: a New Virulence Strategy for Urinary Infections? Med Hypotheses *36,* 269.

Menn, F., Applegate, B.M., and Sayler, G.S. (1993). NAH plasmid-mediated catabolism of anthracene and phenanthrene to naphthoic acids. Appl. Environ. Microbiol. *59,* 1938-1942.

Mezghani-Abdelmoula, S., Khemiri, A., Lesouhaitier, O., Chevalier, S., Orange, N., Cazin, L., and Feuilloley, M.G. (2004). Sequential activation of constitutive and inducible nitric oxide synthase (NOS) in rat cerebellar granule neurons by *Pseudomonas fluorescens* and invasive behaviour of the bacteria. Microbiol. Res. *159,* 355-363.

Mezghani-Abdelmoula, S., Chevalier, S., Lesouhaitier, O., Orange, N., Feuilloley, M.G.J., and Cazin, L. (2003). *Pseudomonas fluorescens* lipopolysaccharide inhibits both delayed rectifier and transient A-type K+ channels of cultured rat cerebellar granule neurons. Brain Res. *983,* 185-192.

Michel-Briand, Y., and Baysse, C. (2002). The pyocins of *Pseudomonas aeruginosa*. Biochimie *84,* 499-510.

Milyutina, I.A., Bobrova, V.K., Matveeva, E.V., Schaad, N.W., and Troitsky, A.V. (2004). Intragenomic heterogeneity of the 16S rRNA-23S rRNA internal transcribed spacer among *Pseudomonas syringae* and *Pseudomonas fluorescens* strains. FEMS Microbiol. Lett. *239,* 17-23.

Molloy, D.P. (2004). Factors Affecting Zebra Mussel Kill by the Bacterium *Pseudomonas fluorescens*.

Molloy, D.P., Mayer, D.A., Gaylo, L.E., Karatayev, A.Y., Presti, K.T., Sawyko, P.M., Morse, J.T., and Paul, E.A. (2013a). Non-target trials with *Pseudomonas fluorescens* strain CL145A, a lethal control agent of dreissenid mussels (Bivalvia: Dreissenidae). Management of Biological Invasion *4,*

Molloy, D.P., Mayer, D.A., Gaylo, M.J., Morse, J.T., Presti, K.T., Sawyko, P.M., Karatayev, A.Y., Burlakova, L.E., Laruelle, F., Nishikawa, K.C., and Griffin, B.H. (2013b). *Pseudomonas fluorescens* strain CL145A – A biopesticide for the control of zebra and quagga mussels (Bivalvia: Dreissenidae). J Invertebr Pathol 1-11.

Moon, C.D., Zhang, X.-., Matthijs, S., Schäfer, M., Budzikiewicz, H., and Rainey, P.B. (2008). Genomic, genetic and structural analysis of pyoverdine-mediated iron acquisition in the plant growth-promoting bacterium *Pseudomonas fluorescens* SBW25. BMC Microbiology *8,*

Moore, G.E. (1972). Pathogenicity of ten strains of bacteria to larvae of the southern pine beetle. J. Invertebr. Pathol. *20,* 41-45.

Mulet, M., Lalucat, J., and García-Valdés, E. (2010). DNA sequence-based analysis of the *Pseudomonas* species. Environ. Microbiol. *12,* 1513-1530.

Murray, A.E., Bartzokas, C.A., Shepherd, A.J., and Roberts, F.M. (1987). Blood transfusion-associated *Pseudomonas fluorescens* septicaemia: is this an increasing problem? J. Hosp. Infect. *9,* 243-248.

Murty, M.G., Srinivas, G., and Sekar, V. (1994). Production of a mosquitocidal exotoxin by a *Pseudomonas fluorescens* strain. J. Invertebr. Pathol. *64,* 68-70.

Murugesan, N., and Kavitha, A. (2009). Seed treatment with *Pseudomonas fluorescens,* plant products and synthetic insecticides against leafhopper, *Amrasca devastans* (Distant) in cotton. *2,* 22-25.

Neher, T.M., and Lueking, D.R. (2009). Pseudomonas fluorescens ompW: plasmid localization and requirement for naphthalene uptake. Can. J. Microbiol. *55,* 553-563.

Nejad, P., Ramstedt, M., and Granhall, U. (2004). Pathogenic ice-nucleation active bacteria in willows for short rotation forestry. For. Pathol. *34,* 369-381.

Nelson, M.R., Shanson, D.C., Barter, G.J., Hawkins, D.A., and Gazzard, B.G. (1991). Pseudomonas septicaemia associated with HIV. AIDS *5,* 761-763.

Netea, M.G., van Deuren, M., Kullberg, B.J., Cavaillon, J.M., and Van der Meer, J.W.M. (2002). Does the shape of lipid A determine the interaction of LPS with Toll-like receptors? TRENDS Immunol. *23,* 135-139.

Nowak-Thompson, B., Chaney, N., Wing, J.S., Gould, S.J., and Loper, J.E. (1999). Characterization of the Pyoluteorin Biosynthetic Gene Cluster of *Pseudomonas fluorescens* Pf-5. J. Bacteriol. *181,* 2166-2174.

O'Donnell, K.J., and Williams, P.A. (1991). Duplication of both xyl catabolic operons on TOL plasmid pWW15. J. Gen. Microbiol. *137,* 2831-2838.

Okaeme, A.N. (1989). Bacteria associated with mortality in tilapias, *Heterobranchus bidorsalis*, and *Clarias lazera* in indoor hatcheries and outdoor ponds. Journal of Aquaculture in the Tropics.*4,* 143-146.

Ostland, V.E., Byrne, P.J., Lumsden, J.S., MacPhee, D.D., Derksen, J.A., Haulena, M., Skar, K., Myhr, E., and Ferguson, H.W. (1999). Atypical bacterial gill disease: A new form of bacterial gill disease affecting intensively reared salmonids. J. Fish Dis. *22,* 351-358.

O'Toole, G.A., and Kolter, R. (1998). Initiation of biolfilm formation in *Pseudomonas fluorescens* WDS365 proceeds via multiple, convergent signalling pathways: a genetic analysis. Mol. Microbiol. *28,* 449-461.

Padmanabhan, V., Prabakaran, G., Paily, K.P., and Balaraman, K. (2005). Toxicity of a mosquitocidal metabolite of *Pseudomonas fluorescens* on larvae & pupae of the house fly, *Musca domestica*. Indian J. Med. Res. *121,* 116-119.

Palleroni, N.J. (1984). Genus I. *Pseudomonas* Migula 1984, 237AL. In Bergey's Manual of Systematic Bacteriology, Krieg, N. R., and Holt, J. G. eds., (Baltimore: Williams & Wilkins) pp. 141–199.

Palleroni, N.J. (2005). *Pseudomonadaceae*. In *Bergey's Manual of Systematic Bacteriology*, Brenner, D. J., Kreig, N. R. and Staley, J. T. eds., pp. 323-379.

Palleroni, N.J. (1981.). Introduction to the family *Pseudomonadaceae*. In *The Prokaryotes - A Handbook on Habitats, Isolation and Identification of Bacteria.*, Starr, M. P., Stolp, H., Truper, H. G., Balows, A. and Schegel, H. G. eds., (Berlin: Springer-Verlag) pp. 655-665.

Palleroni, A. (1992). In Human and Animal Pathigenic Pseudomonds; The Procaryotes : A Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identication, Applications, (New York: Springer-Verlag) pp. 3086-3103.

Pappas, G., Karavasilis, V., Christou, L., and Tsianos, E.V. (2006). *Pseudomonas fluorescens* infections in clinical practice. Scand. J. Infect. Dis. *38,* 68-70.

Paulsen, I.T., Press, C.M., Ravel, J., Kobayashi, D.Y., Myers, G.S.A., Mavrodi, D.V., DeBoy, R.T., Seshadri, R., Ren, Q., Madupu, R*., et al.* (2005). Complete genome sequence of the plant commensal *Pseudomonas fluorescens* Pf-5. Nat. Biotechnol. *23,* 873-878.

Péchy-Tarr, M., Bruck, D.J., Maurhofer, M., Fischer, E., Vogne, C., Henkels, M.D., Donahue, K.M., Grunder, J., Loper, J.E., and Keel, C. (2008). Molecular analysis of a novel gene cluster encoding an insect toxin in plant-associated strains of *Pseudomonas fluorescens.* Environ. Microbiol. *10,* 2368-2386.

Pessi, G., and Haas, D. (2000). Transcriptional control of the hydrogen cyanide biosynthetic genes hcnABC by the anaerobic regulator ANR and the quorum-sensing regulators LasR and RhlR in *Pseudomonas aeruginosa*. J. Bacteriol. *182,* 6940-6949.

Peters, M., Jogi, E., Suitso, I., Punnisk, T., and Nurk, A. (2001). Features of the replicon of plasmid pAM10.6 of *Pseudomonas fluorescens.* Plasmid *46,* 25-36.

Picot, L., Abdelmoula, S.M., Merieau, A., Leroux, P., Cazin, L., Orange, N., and Feuilloley, M.G. (2001). *Pseudomonas fluorescens* as a potential pathogen: adherence to nerve cells. Microbes Infect. *3,* 985-995.

Picot, L., Chevalier, S., Mezghani-Abdelmoula, S., Merieau, A., Lesouhaitier, O., Leroux, P., Cazin, L., Orange, N., and Feuilloley, M.G. (2003). Cytotoxic effects of the lipopolysaccharide from *Pseudomonas fluorescens* on neurons and glial cells. Microb. Pathog. *35,* 95-106.

Picot, L., Mezghani-Abdelmoula, S., Chevalier, S., Merieau, A., Lesouhaitier, O., Guerillon, J., Cazin, L., Orange, N., and Feuilloley, M.G. (2004). Regulation of the cytotoxic effects of *Pseudomonas fluorescens* by growth temperature. Res. Microbiol. *155,* 39-46.

PMRA-HC. (2012). Proposed Registration Decision (PRD2012-12): *Pseudomonas fluorescens* strain CL145A. Pest Management Regulatory Agency

PMRA-HC (2010). Evaluation Report (ERC2010-07): *Pseudomonas fluorescens* Strain A506. Pest Managemetn Regulatory Agency

Prabakaran, G., Hoti, S.L., and Paily, K.P. (2009). Development of cost-effective medium for the large-scale production of a mosquito pupicidal metabolite from *Pseudomonas fluorescens* Migula. Biol. Control *48,* 264-266.

Prabakaran, G., Paily, K.P., Padmanabhan, V., Hoti, S.L., and Balaraman, K. (2003). Isolation of a *Pseudomonas fluorescens* metabolite/exotoxin active against both larvae and pupae of vector mosquitoes. Pest Manag. Sci. *59,* 21-24.

Prescott, L.M., Harley, J.P., and Klein, D.A. (2005). Microbiology, 6th ed.

Princz, J. (2010). Pathogenicity and Toxicity of Risk Group II Microbial Strains on Territrial Organisms. Special report prepared by Environment Canada

Pushpanathan, M., and Pandian, R.S. (2008). Management of dengue and chikungunya vectors *Aedes aegypti* (Linn) and *Aedes albopictus* (Skuse) (Diptera: Culicidae) by the exotoxin of *Pseudomonas fluorescens* Migula (Pseudomonadales: Pseudomonadaceae).*2,* 74-103.

Rais-Bahrami, K., Platt, P., and Naqvi, M. (1990). Neonatal pseudomonas sepsis: even early diagnosis is too late. Clin. Pediatr. *29,* 444.

Rajan, V.V., and Pandian, R.S. (2008a). Larvicidal and pupicidal activities of the exotoxin of *Pseudomonas fluorescens* (Migula) isolates against the brain fever vectors, *Armigeres subalbatus* Coquillett and *Culex tritaeniorhynchus* Giles (Diptera: Culicidae). *2,* 32-40.

Rajan, V.V., and Pandian, R.S. (2008b). Mosquitocidal properties of the natural isolates of *Pseudomonas fluorescens* Migula (Pseudomonadales: Pseudomonadaceae).*2,* 220-229.

Rebière-Huët, J., Guérillon, J., de Lima Pimenta, A., Di Martino, P., Orange, N., and Hulen, C. (2002). Porins of *Pseudomonas fluorescens* MFO as fibronectin-binding proteins. FEMS Microbiol. Lett. *215,* 121-126.

Riedemann, N.C., Guo, R.F., and Ward, P.A. (2003). The enigma of sepsis. J Clin Invest *112,* 460-467.

Roberts, R.J., and Horne, M.T. (1978). Bacterial meningitis in farmed rainbow trout *Salmo gairdneri* Richardson affected with chronic pancreatic necrosis.*1,* 157-164.

Rochu, D., Rothlisberger, C., Taupin, C., Renault, F., Gagnon, J., and Masson, P. (1998). Purification, molecular characterization and catalytic properties of a *Pseudomonas fluorescens* enzyme having cholinesterase-like activity. Biochim. Biophys. Acta *1385,* 126-138.

Roth, R.R., and James, W.D. (1988). Microbial ecology of the skin. Ann. Rev. Microbiol. *42,* 441-464.

Rutenburg, A.M., Koota, G.M., and Schweinburg, F.B. (1958). The Efficacy of Kanamycin in the Treatment of Surgical Infections. Ann NY Acad Sci *76,* 348.

Ryall, B., Davies, J.C., Wilson, R., Shoemark, A., and Williams, H.D. (2008). *Pseudomonas aeruginosa,* cyanide accumulation and lung function in CF and non-CF bronchiectasis patients. Eur. Respir. J. *32,* 740-747.

Sacherer, P., Défago, G., and Hass, D. (1994). Extracellular protease and phopholipase C are controlled by the global regulatory gene *gacA* in the biocontrol strain *Pseudomonas fluorescens* CHA0. FEMS Microbiol Lett *116,*

Sadanandane, C., Reddy, C.M.R., Prabakaran, G., and Balaraman, K. (2003). Field evaluation of a formulation of *Pseudomonas fluorescens* against *Culex quinquefasciatus* larvae and pupae. Acta TroP. *87,* 341-343.

Sader, H.S., and Jones, R.N. (2005). Antimicrobial susceptibility of uncommonly isolated non-enteric Gram-negative bacilli. Int. J. Antimicrob. Agents *25,* 95-109.

Sakai, M., Atsuta, S., and Kobayashi, M. (1989). *Pseudomonas fluorescens* isolated from the diseased rainbow trout, Oncorhynchus mykiss. KITAZATO ARCH. EXP. MED. *62,* 157-162.

Sandborn, W.J. (2007). Clinical Perspectices in Crohn's Disease: Now and in the Future. Rev. Gastroenterol. Disord. *7,* S1-S2.

Sarasola, P., Taylor, D.J., Love, S., and McKellar, Q.A. (1992). Secondary bacterial infections following an outbreak of equine influenza. Vet. Rec. *131,* 441-442.

Sarubbi, F.A.J., Wilson, B., Lee, M., and Brokopp, C. (1978). Nosocomial meningitis and bacteremia due to contaminated amphotericin B. JAMA *239,* 416-418.

Saygili, H., Aysan, Y., Sahin, F., Ustun, N., and Mirik, M. (2004). Occurrence of pith necrosis caused by Pseudomonas fluorescens on tomato plants in Turkey. Plant Pathol. *53,* 803.

Scarpellini, M., Franzetti, L., and Galli, A. (2004). Development of PCR assay to identify *Pseudomonas fluorescens* and its biotype. FEMS Microbiol. Lett. *236,* 257-260.

Schmidt, C.S., Agostini, F., Simon, A.-., Whyte, J., Townend, J., Leifert, C., Killham, K., and Mullins, C. (2004). Influence of soil type and pH on the colonisation of sugar beet seedlings by antagonistic Pseudomonas and Bacillus strains, and on their control of Pythium damping-off. Eur. J. Plant Pathol. *110,* 1025-1046.

Scott, J., Boulton, F.E., Govan, J.R., Miles, R.S., McClelland, D.B., and Prowse, C.V. (1988). A fatal transfusion reaction associated with blood contaminated with *Pseudomonas fluorescens.* Vox Sang. *54,* 201-204.

Segre, J.A. (2006). Epidermal barrier formation and recovery in skin disorders. J. Clin. Invest. *116,* 1150-1158.

Seligy, V.L., Beggs, R.W., Rancourt, J.M., and Tayabali, A.F. (1997). Quantitative bioreduction assays for calibrating spore content and viability of commercial *Bacillus thuringiensis* insecticides.*18,* 370-378.

Sellwood, J.E., Ewart, J.M., and Buckler, E. (1981). New or unusual records of plant diseases and pests. *30,* 179-180.

Sezen, K., and Demirbag, Z. (1999). Isolation and insecticidal activity of some bacteria from the hazelnut beetle (*Balaninus numcum* L.).*34,* 85-89.

Siddiqui, I.A., Haas, D., and Heeb, S. (2005). Extracellular protease of *Pseudomonas fluorescens* CHA0, a biocontrol factor with activity against the root-knot nematode Meloidogyne incognita. Appl. Environ. Microbiol. *71,* 5646-5649.

Silby, M., Cerdeno-Tarraga, A., Vernikos, G., Giddens, S., Jackson, R., Preston, G., Zhang, X., Moon, C., Gehrig, S., Godfrey, S.*, et al.* (2009). Genomic and genetic analyses of diversity and plant interactions of *Pseudomonas fluorescens*. Genome Biol. *10,* R51.

Singh, G., Wu, B., Baek, M.S., Camargo, A., Nguyen, A., Slusher, N.A., Srinivasan, R., Weiner-Kronish, J.P., and Lynch, S.V. (2010). Secretion of *Pseudomonas aeruginosa* type III cytotoxins is dependent on pseudomonas quinlone signal concentration. Microbiol Pathog *49,* 169.

Skerman, V.B.D., McGowan, V., and Sneath, P.H.A. (1980). Approved lists of bacterial names. Int. J. Syst. Bacteriol. *30,* 225-420.

Skorska, C., Sitkowska, J., Krysinska-Traczyk, E., Cholewa, G., and Dutkiewicz, J. (2005). Exposure to airborne microorganisms, dust and endotoxin during processing of valerian roots on farms.*12,* 119-126.

Smit, E., Van Elsas, J.D., Van Veen, J.A., and De Vos, W.M. (1991). Detection of plasmid transfer from *Pseudomonas fluorescens* to indigenous bacteria in soil by using bacteriophage φR2f for donor counterselection. Appl. Environ. Microbiol. *57,* 3482-3488.

Someya, N., Tsuchiya, K., Yoshida, T., Noguchi, M.T., and Sawada, H. (2007). Encapsulation of cabbage seeds in alginate polymer containing the biocontrol bacterium *Pseudomonas fluorescens* strain LRB3W1 for the control of cabbage soilborne diseases. Seed Sci. Technol. *35,* 371-379.

Sperandio, D., Rossingnol, G., Guerillon, J., Connil, N., Orange, N., Feuilloley, M.G.J., and Merieau, A. (2010). Cell-associated hemolysis activity in the clinical strain of *Pseudomonas fluorescens MFN1032*. BMC Microbiol. *10,*

Spök, A., and Klade, M. (2009). Environmental, Health and Legal Aspects of Cleaners Containing Living Microbes as Active Ingredients. IFZ 1-17.

Srivastava, A.K., Singh, T., Jana, T.K., and Arora, D.K. (2001). Induced resistance and control of charcoal rot in *Cicer arietinum* (chickpea) by *Pseudomonas fluorescens.* Can. J. Bot. *79,* 787-795.

Stenhouse, M.A., and Milner, L.V. (1992). A survey of cold-growing gram-negative organisms isolated from the skin of prospective blood donors. Transfus. Med. *2,* 235-237.

Stoskopf, M.K. (1993). Bacterial diseases of goldfish, koi, and carP. In Fish Medicine, (Philadelphia, PA: W.B. Saunders Co.) pp. 473-475.

Sutter, V.L., Hurst, V., and Landucci, A.O.J. (1966). Pseudomonads in Human Saliva. J. Dent. Res. *45,* 1800-1803.

Swem, L.R., Swem, D.L., O'Loughlin, C.T., Gatmaitan, R., Zhao, B., Urlich, S.M., and Bassler, B.L. (2009). A Quorum-Sensing Antagonist Targets Both Membrane-Bound and Cytoplasmic Receptors and Controls Bacterial Pathogenicity. Mol Cell *35,* 143.

Takase, H., Nitanai, H., Hoshino, K., and Otani, T. (2000). Impact of siderophore production on *Pseudomonas aeruginosa* infections in immunosuppressed mice. Infect. Immun. *68,* 1834-1839.

Tayabali, A.F., Nguyen, K.C., and Seligy, V.L. (2010). Early murine immune responses from endotracheal exposures to biotechnology-related *Bacillus* strains. Toxicol. Environ. Chem.

To, W.S., and Midwood, K.S. (2011). Plasma and cellular fibronectin: Distinct and independent functions during tissue repair.*4,*

Trevors, J.T., Van Elsas, J.D., Starodub, M.E., and Van Overbeek, L.S. (1990). *Pseudomonas fluorescens* survival and plasmid RP4 transfer in agricultural water. Water Res. *24,* 751-755.

Troxler, J., Azelvandre, P., Zala, M., Défago, G., and Haas, D. (1997). Conjugative transfer of chromosomal genes between fluorescent pseudomonads in the rhizosphere of wheat. Appl. Environ. Microbiol. *63,* 213-219.

USEPA. (2009). *Pseudomonas Fluorescens;* Receipt of Application for Emergency Exemption, Solicitation ofPublic Comment [EPA-HQ-OPP-2009-0803; FRL-8796-5]. Federal Register *74,* 58287-58289.

Van Elsas, J.D., Trevors, J.T., Jain, D., Wolters, A.C., Heijnen, C.E., and van Overbeek, L.S. (1992). Survival of, and root colonization by, alginate-encapsulated *Pseudomonas fluorescens* cells following introduction into soil. Biol Fertil Soils *14,* 14-22.

Van Elsas, J.D., Trevors, J.T., Starodub, M.E., and Van Overbeek, L.S. (1990). Transfer of plasmid RP4 between pseudomonads after introduction into soil; Influence of spatial and temporal aspects of inoculation. FEMS Microbiol. Ecol. *73,* 1-12.

Van Loon, L.C., Bakker, P.A.H.M., and Pieterse, C.M.J. (1998). Systemic resistance induced by rhizosphere bacteria. Ann. Rev. Phytopathol. *36,* 453-483.

Van Veen, J.A., Van Overbeek, L.S., and Van Elsas, J.D. (1997). Fate and activity of microorganisms introduced into soil. Microbiol. Mol. Biol. Rev. *61,* 121-135.

Vandenbergh, P.A., and Kunka, B.S. (1988). Metabolism of volatile chlorinated Aliphatic Hydrocarbons by *Pseudomonas fluorescens*. Appl Environ Microbiol *54,* 2578-2579.

Vandenbergh, P.A., and Cole, R.L. (1986). Plasmid Involvement in Linalool Metabolism by *Pseudomonas fluorescens*. Appl. Environ. Microbiol. *52,* 939-940.

Vasudevan, N., Bharathi, S., and Arulazhagan, P. (2007). Role of plasmid in the degradation of petroleum hydrocarbon by *Pseudomonas fluorescens* NS1. J. Environ. Sci. Health. A. Tox. Hazard. Subst. Environ. Eng. *42,* 1141-1146.

Veremeichenko, S.N., Vodyanik, M.A., and Zdorovenko, G.M. (2005). Structural characteristics and biological properties of *Pseudomonas fluorescens* lipopolysaccharides. Appl. Biochem. Microbiol. *41,* 365-371.

Veremeichenko, S.N., and Zdorovenko, G.M. (2008). Specific structural features and immunomodulatory properties of the lipopolysaccharides of Pseudomonas bacteria. Appl. Biochem. Microbiol. *44,* 571-579.

Veron, W., Orange, N., Feuilloley, M.G.J., and Lesouhaitier, O. (2008). Natriuretic peptides modify *Pseudomonas fluorescens* cytotoxicity by regulating cyclic nucleotides and modifying LPS structure. BMC Microbiol *8,*

Vincent, J.L. (2002). Sepsis definitions. The Lancet *2,* 135.

Wei, B., Huang, T., Dalwadi, H., Sutton, C.L., Bruckner, D., and Braun, J. (2002). *Pseudomonas fluorescens* encodes the Crohn's disease-associated I2 sequence and T-cell superantigen. Infect. Immun. *70,* 6567-6575.

Weissenfels, W., Beyer, M., and Klein, J. (May 30-31, 1989) Paper presented at Bacterial degradation of naphthalene, phenathrene, fluorene and fluoranthine by pure strains. (Frankfurt, Germany: Frankfurt, Germany).

Weller, D.M., and Cook, R.J. (1986). Increased growth of wheat by seed treatments with fluorescent pseudomonads, implications of Pythium control. Can J Plant Pathol *8,* 328-334.

Weller, D.M. (2007). *Pseudomonas* biocontrol agents of soilborne pathogens: Looking back over 30 years. Phytopathology *97,* 250-256.

Wilson, M.J., Glen, D.M., Hughes, L.A., Pearce, J.D., and Rodgers, P.B. (1994). Laboratory tests of the potential of entomopathogenic nematodes for the control of field slugs (Deroceras reticulatum). J. Invertebr. Pathol. *64,* 182-187.

Xiang, S., Cook, M., Saucier, S., Gillespie, P., Socha, R., Scroggins, R., and Beaudette, L.A. (2010). Development of amplified fragment length polymorphism-derived functional strain-specific markers to assess the persistence of 10 bacterial strains in soil microcosms. Appl. Environ. Microbiol. *76,* 7126-7135.

Yadav, J.S., Khan, I.U.H., Fakhari, F., and Soellner, M.B. (2003). DNA-Based Methodologies for Rapid Detection, Quantification, and Species- or Strain-Level Identification of Respiratory Pathogens (Mycobacteria and Pseudomonads) in Metalworking Fluids. Appl. OccuP. Environ. Hyg. *18,* 966-975.

Yildiz, H.Y. (1998). Effects of experimental infection with pseudomonas fluorescens on different blood parameters in carp (Cyprinus carpio L.). Isr. J. Aquacult. Bamidgeh *50,* 82-85.

Youard, Z.A., Mislin, G.L.A., Majcherczyk, P.A., Schalk, I.J., and Reimmann, C. (2007). *Pseudomonas fluorescens* CHA0 produces enantio-pyochelin, the optical antipode of the pseudomonas aeruginosa siderophore pyochelin. J. Biol. Chem. *282,* 35546-35553.

Zhang, W., Hu, Y., Wang, H., and Sun, L. (2009). Identification and characterization of a virulence-associated protease from a pathogenic *Pseudomonas fluorescens* strain. Vet. Microbiol. *In Press, Corrected Proof.*

Appendix 1: Characteristics of *P. fluorescens* ATCC 13525 – Growth Kinetics

Growth kinetics was investigated using Dulbecco's Modified Eagle Medium, Trypticase Soy Broth and Fetal Bovine Serum at various temperatures. Each table entry shows whether growth (increase in absorbance at 500nm) occurs at different temperatures (28, 32, 37, 42^{o}C). Measurements were taken at OD 500nm every 15min over a 24h period.

Table A1-1 Growth kinetics of *P. fluorescens* ATCC 13525[a]

Medium	28°C	32°C	37°C	42°C
Trypticase Soy Broth	+[b]	~[c]	-[d]	-
Sheep Plasma	(+)[e]	(+)	-	-
Fetal Bovine Serum	~	-	-	-
Dulbecco's Modified Eagles Medium (mammalian cell culture)	(+)	-	-	-

a Data generated by Health Canada's Healthy Environments and Consumer Safety Branch
b +, growth
c ~, low level growth
d -, no growth
e (+), delayed growth (after 15h)

Appendix 2: Characteristics of *P. fluorescens* ATCC 13525 - Growth on Different Media at 28°C and 37°C (48 hours)

Table A2-1 Growth of *P. fluorescens* ATCC 13525 on different media[a]

Medium	28°C	37°C
Nutrient	+[b]	-[c]
TSB[d]	+	-
Starch[e]-Growth	NT[f]	-
Starch[e]-Hydrolysis	NT	-
Maconkey Agar[g]	+	-
Lysine Iron[h]	+	-
Triple Sugar Iron - w phenol red[i]	+	-
Mannitol Egg Yolk Polymyxin supplements[j]	+	-
Mannitol[k]	-	-
Citrate[l]	+	-
Urea[m]	+	-

a Data generated by Heath Canada's Environmental Health Science and Research Bureau
b +, positive growth
c -, negative growth
d Trypticase soy broth, an all-purpose medium
e Differential medium that tests the ability of an organism to produce extracellular enzymes that hydrolyze starch
f NT, not tested
g Detection of coliform organisms in milk and water; tests for ability of organism to ferment lactose
h 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
i Gram-negative enteric bacilli based on glucose, lactose, and sucrose fermentation and hydrogen sulfide production
j *B. cereus* selective agar
k Isolation and differentiation of *Staphylococci*
l Citrate utilization test, ability to use citrate as the sole carbon source
m Screening of enteric pathogens from stool specimens - urea metabolism

Appendix 3: Characteristics of *P. fluorescens* ATCC 13525 – Fatty Acid Methyl Ester (FAME) Analysis

Data presented shows the best match between the sample and the environmental MIDI databases, along with the number of matches (fraction of total number of tests) and the fatty acid profile similarity index (in parentheses; average of all matches). MIDI is a commercial identification system that is based on the gas chromatographic analysis of cellular fatty acid methyl esters

Table A3-1 Environmental MIDI database results for *P. fluorescens* ATCC 13525[a]

Species Matches	Fatty Acid Profile Similarity Index (average of all matches)
P. fluorescens biotype A	6/22 (0.888)
P. fluorescens biotype B	6/22 (0.800)
P. putida biotype A	1/22 (0.805)
P. putida biotype B	4/22 (0.229)
Corynebacterium diphtheriae-gravis & mitis	1/22 (0.729)
Pseudoalteromonas nigrifaciens	1/22 (0.117)
No Match	3/22

a Data generated by Health Canada's Healthy Environments and Consumer Safety Branch.

Appendix 4: LD_{50} Values for Toxins Produced by Some Strains of *P. fluorescens*

Table A4-1 LD_{50} values for toxins produced by some strains of *P. fluorescens*

Substance	Organism	LD_{50} or LC_{50}	Strain
LPS[a]	10- to 12- week-old BALB/c mice (sensitized to *D*-galactosamine)	LD_{50} 7500 ng/mouse	ATCC 13525 (IMV 4125)
P. fluorescens (Fit Toxin producing strains)[b]	*Galleria mellonella* *Manduca sexta*	LD_{50} 1.8 x 10^2 cells LD_{50} 9.8 x 10^2 cells LD_{50} 4 x 10^3 cells	CHA0 Pf-5 CHA0 and Pf-5
Unidentified[c] 44 kDa protein (VCRC B426)[d]	*Anopheles stephensi (*larvae)	LC_{50} 70.4 µg protein ml^{-1}	*P. fluorescens* Migula
Unidentified[c] 44 kDa protein (VCRC B426)[d]	*Culexquinquefasciatus* (larvae)	LC_{50}511.5 µg protein ml^{-1}	*P. fluorescens* Migula
Unidentified[c] 44 kDa protein (VCRC B426)[d]	*Aedes aegypti* (larvae)	LC_{50} 757.3 µg protein ml^{-1}	*P. fluorescens* Migula
Unidentified[c] 44 kDa protein (VCRC B426)[d]	*Anopheles stephensi (*pupae)	LC_{50} 2.0 µg protein ml^{-1}	*P. fluorescens* Migula
Unidentified[c] 44 kDa protein (VCRC B426)[d]	*Culexquinquefasciatus* (pupae)	LC_{50} 9.4 µg protein ml^{-1}	*P. fluorescens* Migula
Unidentified[c] 44 kDa protein (VCRC B426)[d]	*Aedes aegypti* (pupae)	LC_{50}19.2 µg protein ml^{-1}	*P. fluorescens* Migula
Unidentified[c] 44 kDa protein (VCRC B426)[e]	*Musca domestica* (net mortality of larvae and pulpae all together)	LC_{50} 8.25 µg protein ml^{-1}	*P. fluorescens* Migula

a (Veremeichenko et al., 2005)
b (Péchy-Tarr et al., 2008)
c Proprietary information; toxin name undisclosed
d (Prabakaran et al., 2003)
e (Padmanabhan et al., 2005)

Appendix 5: Toxin and Secondary Metabolite Production

Table A5-1 List of toxins and secondary metabolites produced by *P. fluorescens*

Toxins	Actions	References
Lipopolysaccharide (LPS), endotoxin	• LPS plays a leading role in the infectious process • LPS is a complex amphiphilic molecule essential for outer membrane functions, particularly during host-pathogen interactions • Major virulence factor responsible for membrane depolarisation in cerebellar granule neurons. Causes the reduction of two of the major voltage-dependant potassium currents • *P. fluorescens* can bind to glial cells and its LPS will modulate potassium channels in target cells. LPS induces the expression of a nitrite oxide synthase (NOS) associated to apoptosis. Cells invasion and cytotoxicity are not mutually exclusive events • 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	(Mezghani-Abdelmoula et al., 2004; Mezghani-Abdelmoula et al., 2003; Picot et al., 2003; Picot et al., 2004; Veremeichenko et al., 2005; Veremeichenko and Zdorovenko, 2008)
AprX	• Virulence factor that contributes to bacterial infection • Heat resistant extracellular alkaline metalloprotease of the serralysin family • Involved in nutrient utilization; ability to degrade proteins in the environment • Possesses two conserved binding domains (Zn^{2+} and Ca^{2+}) • Associated with strain linked to spoilage of milk and dairy products	(Dufour et al., 2008; Marchand et al., 2009; Zhang et al., 2009)
AprA (alkaline protease)	• Extracellular protease (EDTA-sensitive) • Anti-nematode factor	(Lenz et al., 2008; Maunsell

Toxins	Actions	References
	• Serralysin-type metalloprotease • *apra* genes are regulated by quorum sensing	et al., 2006; Siddiqui et al., 2005)
Phospholipase C	• Substrate specificity different from phospholipase C from either *Cl. welchii* or *B. cereus* • Phosphatidyl ethanolamine is hydrolyzed more easily than other phospholipids	(Doi and Nojima, 1971)
Unidentified 44 kDa protein (VCRC B426)	• A bacterial metabolite in simulated field conditions; mode of action unknown • Approximate molecular mass of 44 kDa with temperature stability at 120 ºC for 20 min • Nontoxic to mammals • Caused significant mortality of *Culex quinquefasciatus* pupae and suppression of adult emergence • VCRC B426 in a 0.09% emulsifiable concentration showed reduction of 80% in pupal density for *Culex quinquefasciatus*	(Murty et al., 1994; Padmanabhan et al., 2005; Prabakaran et al., 2003; Prabakaran et al., 2009; Sadanandane et al., 2003)
Hydrogen cyanide	• Inhibitor of plants roots and a broad-spectrum of compounds • 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 • 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 secreted in the extracellular environment and resulting in a ferri-pyoverdine complex that will be	(Gross and Loper, 2009; Moon et al., 2008)

Toxins	Actions	References
	transported back into the bacteria by a cell surface receptor protein • Virulence factor in *P. aeroginosa*	
Pyochelin	• Iron-scavenging metabolites with a very different scaffold than the pyoverdines • *P. fluorescens* produced a stereoisomer of pyochelin, named *enantio*-pyochelin • *Enantio*-pyochelin enables the bacteria to sequestered iron in an available form for them but not for competing bacteria • Virulence factor in *P. aeroginosa*	(Gross and Loper, 2009; Takase et al., 2000; Youard et al., 2007)
Pseudomonine	• Secondary siderophore but function like a siderophore • Consist of salicylic acid and two heterocyclic amino acids	(Reviewed in Gross and Loper, 2009)
Pyocins	• Pyocins are antibacterial agents (active against closely related species or strains) usually associated with *P. aeruginosa* which exist in the three type (R, F and S) • Putative F- and R- pyocins appear to be ubiquitously distributed among strains of *P. fluorescens* • 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)
Fit toxin (*P. fluorescens* insecticidal toxin)	• Toxin produced only by the strain CHA0 and Pf-5 which have an anti-insect activity related to the insecticidal toxin Mcf (causes caterpillars to become floppy) from *Photorhabdus luminescens*	(Péchy-Tarr et al., 2008)

Toxins	Actions	References
Rhizoxin analogs (such as (WF-1360 F, 22Z-WF-1360 F, WF1360 C, WF-1360 B and Rhizoxin D)	• 16-member macrolide that exhibits phytotoxic, antifungal and antitumor activities (reported to be produced by strain Pf-5) • Activity related to the binding of those molecules to β-tubilin which will interfere with microtubule dynamics during mitosis	(Gross and Loper, 2009; Loper et al., 2008)
Cyclic lipopeptides (viscosin, massetolide, orfamide ,etc.)	• Class of compounds with diverse structure containing a fatty acyl residue 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 • Lower surface tension and altering cellular membranes integrity by interaction due to their amphiphilic properties • Increase the bioavailability of water-insoluble substrates, promote cellular swarming and enhance virulence or antagonism against other microorganism	(Reviewed in Gross and Loper, 2009)
Pyrrolnitrin	• Strong antifungal activity, compound use as a topical antimycotic in human • Inhibitor of fungal respiratory chain	(Reviewed in Gross and Loper, 2009)
Phenazines	• Over 50 compounds in this large family of colorful nitrogen-containing tricyclic molecules • Antibiotic, antitumor and antiparasitic activity • Activity due to interaction with polynucleotides, topoisomerase inhibition and the generation of free radical • Intracellular signals have an influence on transcriptional regulation and broad effect on bacterial physiology and fitness	(Reviewed in Gross and Loper, 2009)
2-4-	• Biologic controls against plants disease	(Gross and

Toxins	Actions	References
diacetylphlorogluciol	produced by a subset of *P. fluorescens*. • Toxic effect on a range of plant pathogenic fungi • Antibacterial, anti-helmintic and phytotoxic properties at high concentration • DAPG triggers systemic resistance of plants against disease	Loper, 2009)
Pyoluteorin	• Substance composed of a bichlorinated pyrrole linked to a resorcinol moiety • Shows antifungal properties • Moderate plant disease cause by fungal pathogen such as Oomycetes fungi	(Hammer et al., 1997; Nowak-Thompson et al., 1999)
Cyclo(-Pro-Val-) and cyclo(-Pro-Tyr-)	• Phytotoxic activity	(Guo et al., 2007)

Appendix 6: Strains of *P. fluorescens* used as biocontrol agents against plants and invertebrates

Table A6-1 *P. fluorescens* strains used as a biocontrol agent against plants

Target	Strains	Reference
Downy brome (*Bromus tectorum)* seeds	*P. fluorescens* strain D7	(Johnson et al., 1993)
Annual bluegrass (*Poa annua* L.)	*Different P. fluorescens* isolates	(Banowetz et al., 2008)

Table A6-2 *P. fluorescens* strains used as a biocontrol agent against invertebrates

Target	Strains	Reference
Aphid (*Aphis gossypii*) Leaf hopper (*Amrasca biguttula*), whitefly (*Bemisia abaci*) -nymphs and adults	No strain designation provided	(Gandhi and Gunasekaran, 2008)
Argentine stem weevil (*Listronotus bonariensis)* - *a*dults collected from field populations	No strain designation specified	(Jackson and McNeill, 1998)
Fruit Fly (*Drosophila melanogaster)*	*P. fluorescens* MF0	(de Lima Pimenta et al., 2006)
Hazelnut beetle (*Balaninus nucum, Curculio nucum*)	No strain designation specified	(Sezen and Demirbag, 1999)
Lady beetle (*Hippodamia convergens*)	No strain designation specified	(James and Lighthart, 1992)
Leafhopper (*Amrasca devastans*) (Distant)	Pf- 1®	(Murugesan and Kavitha, 2009)
Mosquitoes (*Culex quinquefasciatus, Anopheles stephensi, Aedes aegypti*) *a*nd other species (late second instar stage larvae)	*P. fluorescens* strain MSS-1, isolated from diseased mosquito larvae	(Murty et al., 1994)
Subterranean termite (*Odontotermes obesus*)	*P. fluorescens* CHA0	(Devi and Kothamasi, 2009)
Southern pine beetle (*Dendroctonus frontalis*)	No strain designation	(Moore, 1972)

Target	Strains	Reference
Burrowing nematode (*Radopholus similis*)Females and juveniles, root-knot nematode (*Meloidogyne* spp.) 2nd stage juveniles	3 *P. fluorescens* strains	(Aalten et al., 1998)
Zebra mussel (*Dreissena polymorpha, D. Bugensis*)	*P. fluorescens* strain ATCC 55799	(Molloy, 2004) See also (Mayer, 2009)
Daphnid (*Daphnia magna*)	*P. fluorescens* ATCC 55799 (dead cells)	(Mayer, 2009)
Giant freshwater prawn (*Macrobrachium rosenbergii*)	No strain designation provided	(Baruah and Prasad, 2001)
Field slug (*Deroceras reticulatum*)	• No strain designation specified; isolated from a cadaver of *D. reticulatum* following death by an infection of *Phasmarhabditis hermaphrodita* • Cultures of *P. fluorescens* were grown in nutrient broth for 24 h at 22˚C	(Wilson et al., 1994)

Appendix 7: Pathogenicity and Toxicity Studies of other Strains of *P. fluorescens*

Table A7-1 Toxicity and infectivity studies of *P. fluorescens* ATCC 55799 (CL145A)

Test	Test Organism	Test Substance or Concentration	LD_{50}, LC_{50}, EC_{50}
Acute oral toxicity [a]	Mallard ducks	2000 mg/kg bw (inactive)[b]	LD_{50} >2000 mg/kg bw/ kg bw
Toxicity studies (4-day static renewal) [a]	Rainbow trout (*Oncorhynchus mykiss*)	25, 50, 100, 200 or 400 mg a.i/L (inactive)	59.09 mg a.i./L[c]
Toxicity studies (4-day static renewal) [a]	Fathead minnow (*Pimephales promelas*)	100, 200, 400 or 600 mg a.i/L (inactive)	LD_{50}: 569.9 mg a.i./L
Toxicity studies (30-day static renewal) [a]	Fathead minnow (*Pimephales promelas*)	3.8×10^9 CFU/mL (live)	LC_{50}: 6.9×10^6 CFU/mL
Toxicity studies (30-day static renewal) [a]	Fathead minnow (*Pimephales promelas*)	3.8×10^3 CFU/mL (inactive)	LC_{50} could not be calculated due to relatively low mortality rates
Toxicity studies (4-day static renewal) [a]	Chinook salmon (*Oncorhynchus tshawytscha*)	100, 200, 400 or 800 mg a.i./L (inactive)	LC_{50}: 183.5 mg a.i./L
Toxicity studies (4-day static renewal) [a]	Sacramento split tail (*Pogonichthys macrolepidotus*)	50, 100, 200, 400, 800 and 1600 mg a.i./L (inactive)	LC_{50}: 137.6 mg a.i./L
Toxicity study (48-hour static) [a]	Shrimp (*Hyalella azteca*)	25, 50, 100 or 200 ppm (inactive with active biotoxin)	LC_{50} >200 ppm
Toxicity study (48-hour static) [a]	Shrimp (*Hyalella azteca*)	25, 50, 100 or 200 ppm (inactive and heat-treated with inactive biotoxin)	LC_{50} >200 ppm
Toxicity study (10-day static [a])	*Daphnia magna*	200 ppm (killed, irradiated)	LC_{50} >200 ppm (48 hours)
Toxicity study (2-day static) [a]	*Daphnia magna*	15.625, 31.25, 62.5, 125, and 250 mg a.i./L (inactive)	EC_{50}: 143.59 mg a.i./L
Acute oral toxicity study	Sprague-Dawley rats	2.42×10^7 CFU/kg bw	LD_{50} >2.42×10^7 CFU/kg bw
Acute pulmonary	Sprague-Dawley	3.4×10^8	LD_{50} >3.4×10^8

Test	Test Organism	Test Substance or Concentration	LD_{50}, LC_{50}, EC_{50}
infectivity and toxicity	rats	CFU/animal (0.1 mL dose)	CFU/animal
Acute inhalation study	Sprague-Dawley rats	Aerosolised dose of 2.25 mg/L (CFU/animal could not be determined)	LC_{50} >2.25 mg/L
Acute intravenous infectivity study	Sprague-Dawley rats	Doses ranged from 4.7×10^6 CFU/mL to 1.95×10^7 CFU/mL	Non-infective to rats

a Adapted from (PMRA-HC, 2012)
b Inactive/killed (use in end-use product)
c Active ingredient (a.i.)

Table A7-2 Toxicity and infectivity studies of *P. fluorescens* ATCC 31948 (A506)

Test	Test Organism	Test Substance or Concentration	LD_{50}, LC_{50}, EC_{50}
Acute oral toxicity [a]	Sprague-Dawley rats	5.0 g/kg bw[b]	LD_{50} >5.0 mg/kg bw
Acute oral toxicity [a]	Sprague-Dawley rats	8.4×10^{10} CFU/animal[c]	LD_{50} >8.5×10^{10} CFU/animal (no mortality, no significant toxicity)
Acute pulmonary toxicity [a]	Sprague-Dawley rats	5.3 mg/L[d]	LD_{50} >5.3 mg/kg bw
Acute infectivity (intraperitoneal injection) [a]	Swiss Webster mice	2.0×10^8 CFU/animal[c]	No mortalities, general signs of toxicity (scruffy coats, discharge from eyes, lethargy, diarrhea)
Contact toxicity [a]	Italian honeybees (*Apis mellifera*)	5 µL at 1.03×10^5 CFU/bee, 2.06×10^5 CFU/bee, 4.12×10^5 CFU/bee, 8.25×10^5 CFU/bee, or 1.65×10^6 CFU/bee[c]	LC_{50} > 1.65×10^6 CFU/bee[e]
Acute toxicity [a,f]	Vascular plants[g]	10^6 or 10^8 CFU/mL[b], or both	No signs of phyto-pathogenicity (doses below the max. label rate of 3.7×10^9 CFU/mL)

a Adapted from (PMRA-HC, 2010)
b MPCA, microbial pest control agent
c Testing was conducted with Frostbans A, a mixture containing the MPCA, as well as other *Pseudomonas* species which are known to be more pathogenic than the MPCA (e.g., *Pseudomonas syringae*), rather than with Blightban A506 (ATCC 31948) itself
d Testing was conducted with *Pseudomonas fluorescens* strain AGS3001.2, a strain considered equivalent to the MPCA (*Pseudomonas fluorescens* ATCC 31948) rather than with the MPCA itself
e Cause of death not established, increase in mortality coincided with a decline in food supply. Infectivity was not assessed.
f Inoculation methods: puncture wound, leaf swab, hole punch, and leaf infiltration, depending on host.
g Tested plants: barley, maize, oats, sorghum, wheat, broccoli, cabbage, cauliflower, cucumber, snapbean and citrus, peach, pear, tomato and tobacco.

Appendix 8: Adverse effects associated with other strains of *P. fluorescens*[5]

Table A8-1 Adverse effects reported in plants

Organism	Strain	Adverse effects/disease symptoms	Reference
Sainfoin (*Onobrychis viciaefolia*)	*P. fluorescens* strains SA-2-1, SB-1-1, SC-4-1, SD-2-4 and SF-2-1	Crown and root rot	(Hwang et al., 1989)
Cacti	Atypical strain of *P. fluorescens* (biotype II of Buchan & Gibbons, 1974)	Orange soft rot	(Anson, 1982)
Willow (*Salix viminalis*)	No strain designation specified	Necrotic tissue damage (discolouration, necrosis of the bark or glassy appearance of the woody tissue)	(Nejad et al., 2004)
Black Pine (*Pinus thunbergii*)	*P. fluorescens* biotype I and biotype II	Wilting or browning[a]	(Han et al., 2003), see also (Guo et al., 2007)

a Pine wood nematodes were also implicated in the disease progression

Table A8-2 Adverse effects reported in vertebrates

Organism	Isolation, challenge method[a] and strain	Adverse effects/disease symptoms	Reference
Atlantic salmon (*Salmo salar*)	*P. fluorescens* strain 92/3556 isolated from fish that demonstrated languid swimming behaviour, congestion at the base of the fins, tail erosion, and with patches of haemorrhagic petechiation on the flank	• *P. fluorescens* strain 92/3556 did not cause re-infection in healthy fish • ***P. fluorescens* strain 92/3556 may be considered to be an opportunisitic pathogen of cold-stessed fish with depressed immune function**	(Carson and Schmidtke, 1993)
Elephantsnout	*P. fluorescens* (no	Invasion of fish with	(Ahmed,

[5] Adverse effects excluding those reported in a biocontrol context.

Organism	Isolation, challenge method[a] and strain	Adverse effects/disease symptoms	Reference
fish (*Mormyrus kannume*)	strain designation) was isolated from diseased fish	*P. fluorescens* may have enhanced saprolegnia infection	1992)
European eel (*Anguilla anguilla*) and rainbow trout (*Oncorhynchus mykiss*)[b]	*P. fluorescens* (no strain designation) isolated from diseased eel and fish	• Cutaneous petechiation (ventral) and ulceration • Recovered from kidneys and liver of dead (challenged) fish	(Esteve et al., 1993)[c]
Rainbow trout (*Oncorhynchus mykiss*), Tilapia spp.[d]	*P. fluorescens* (no strain designation) isolated from kidneys of diseased fish	• Hemorrhage at the base of fin and anal regions • Petechia of organs (especially the intestine) • Mortality was reported	(Sakai et al., 1989) See also (Barker et al., 1991; Ostland et al., 1999)
Scottish rainbow trout (*Salmo gairdneri*)	*P. fluorescens* (no strain designation) isolated from diseased (chronic infectious pancreatic necrosis) and dead fish	• *P. fluorescens* did not cause re-infection in healthy fish; likely an opportunistic pathogen in this case	(Roberts and Horne, 1978)
Tilapia spp. (*Clarias lazera*, *Heterobranchus bidorsalis*)	*P. fluorescens* (no strain designation) isolated by tissue swab	• *P. fluorescens* was associated with fin rot, dropsy, pop-eye and haemorrhagic septicaemia of freshwater fish • May contribute to mortality, especially in stress conditions • Did not cause re-infection in healthy fish • Likely an opportunistic pathogen	(Okaeme, 1989)
Carp (*Cyprinus carpio* L.)	Intraperitoneal injection of *P. fluorescens* (no strain designation)	Significant reduction in hematocrit and erythrocyte counts; elevation in leukocrit and total leukocyte numbers; decrease in plasma protein and albumin; and lowering of plasma	(Yildiz, 1998) See also (Csaba et al., 1984) (silver carp and bighead carp in

Organism	Isolation, challenge method[a] and strain	Adverse effects/disease symptoms	Reference
		electrolytes (Na^+, K^+, Ca^{2+} & P)	farms).
Chicken	*P. fluorescens* (no strain designation) isolated from clinical sick or dead chickens and inoculated by intraperitoneal injection in health chickens	Mortalities as a result of bacterial pneumonia, sinusitis, and/or septicemia were reported	(Lin et al., 1993)
Marine turtles (*Chelonia mydas* and *Eretmochelys imbricate)*	*P. fluorescens* (no strain designation) isolated from diseased turtles	• Associated with ulcerative dermatitis, stomatitis, blepharitis and shell disease; rhinitis; broncho-pneumonia; kerato-conjunctivitis; adenitis; peritonitis; septicaemia-toxaemia; and osteomyelitis • **Re-infection not performed to determine pathogenicity in healthy turtles**	(Glazebrook and Campbell, 1990)
Horse	*P. fluorescens* (no strain designation) isolated from clinically diseased horses during an outbreak of equine influenza virus E2/F Influenza A/Equi-2/	Re-infection not performed to determine pathogenicity in healthy horses	(Sarasola et al., 1992)
Mice (*Mus musculus*)	A superficial burn lesion was made at the base of the mouse's tail and subsequently inoculated with *P. fluorescens* strain 8/3	• *P. fluorescens* was isolated from the lesion but was not isolated from the organs • Study demonstrated that *P. fluorescens* did not readily cause infection in the mice	(Liu, 1964)

a Challenge method provided if applicable
b Fish were challenged
c Authors indicate that *P. fluorescens* is likely a secondary invader that took advantage of damaged tissues and suggest that it acts as an opportunistic pathogen
d Diseased fish (not challenged)