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Final Screening Assessment for

Arthrobacter globiformis strain ATCC 8010

Environment and Climate Change Canada

Health Canada

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Synopsis

Pursuant to paragraph 74(b) of the Canadian Environmental Protection Act, 1999 (CEPA), the Minister of the Environment and the Minister of Health have conducted a screening assessment of Arthrobacter globiformis (A. globiformis) strain ATCC 8010.

A. globiformis strain ATCC 8010 is a soil bacterium that has characteristics in common with other strains of the species. A. globiformis is nutritionally versatile and reported to be ubiquitous in freshwater, saltwater and soils. The characteristics of A. globiformis strain ATCC 8010 make it suitable for use in food production, biocontrol, probiotic use in humans and animals, biodegradation and water and wastewater treatment.

There are no reported adverse effects in terrestrial or aquatic plants, invertebrates or vertebrates, or infections in humans associated with this specific Domestic Substances List strain, or other strains of A. globiformis.

This assessment considers the aforementioned characteristics of A. globiformis strain ATCC 8010 with respect to environmental and human health effects associated with consumer and commercial product use and in industrial processes subject to CEPA, including releases to the environment through waste streams and incidental human exposure through environmental media. To update information about current uses of this micro-organism, the Government of Canada launched a mandatory information-gathering survey under section 71 of CEPA, as published in the Canada Gazette, Part I, on October 3, 2009 (section 71 notice). Information submitted in response to this section 71 notice indicates that A. globiformis strain ATCC 8010 was not imported into or manufactured in Canada in 2008.

Based on the information available, it is concluded that A. globiformis strain ATCC 8010 does not meet the criteria under paragraph 64(a) or (b) of CEPA as it is not entering the environment in a quantity or concentration or under conditions that have or may have an immediate or long-term harmful effect on the environment or its biological diversity or that constitute or may constitute a danger to the environment on which life depends. It is also concluded that A. globiformis strain ATCC 8010 does not meet the criteria under paragraph 64(c) of CEPA as it is not entering the environment in a quantity or concentration or under conditions that constitute or may constitute a danger in Canada to human life or health.

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Introduction

Pursuant to paragraph 74(b) of the Canadian Environmental Protection Act, 1999 (CEPA), the Minister of the Environment and the Minister of Health are required to conduct screening assessments of living organisms added to the Domestic Substances List (DSL) by virtue of section 105 of the Act to determine whether they present or may present a risk to the environment or human health (according to criteria set out in section 64 of CEPA).[1] Arthrobacter globiformis (A. globiformis) strain ATCC 8010 was added to the DSL in 1997 under subsection 25(1) of CEPA (1988) and the DSL under subsection 105(1) of CEPA (1999) because it was manufactured in or imported into Canada between January 1, 1984 and December 31, 1986.

This screening assessment considers hazard information obtained from the public domain and from unpublished research data generated by Health Canada[2] research scientists, as well as comments from scientific peer reviewers. Exposure information was obtained from the public domain and from a mandatory CEPA section 71 notice published in the Canada Gazette, Part I, on October 3, 2009. Further details on the risk assessment methodology used are available in the Risk Assessment Framework document Framework for Science-Based Risk Assessment of Micro-Organisms Regulated Under the Canadian Environmental Protection Act, 1999 (Environment Canada and Health Canada 2011).

In this screening assessment, data that are specific to the DSL-listed strain A. globiformis strain ATCC 8010 are identified as such. Where strain-specific data were not available, surrogate information from literature searches was used. When applicable, literature searches conducted on the organism included its synonyms and common and superseded names. Surrogate organisms are identified in each case to the taxonomic level provided by the source. Literature searches were conducted using scientific literature databases (i.e., SCOPUS, CAB Abstracts, Agricola, Google Scholar, NCBI PubMed and FreePatentsOnline), web searches and key search terms for the identification of human health and environmental hazards. Information identified up to October 2015 was considered for inclusion in this screening assessment.

[1] A determination of whether one or more criteria of section 64 of CEPA are met is based on an assessment of potential risks to the environment and/or to human health associated with exposure in the general environment. For humans, this includes, but is not limited to, exposure from air, water and the use of products containing the substances. A conclusion under CEPA may not be relevant to, nor does it preclude, an assessment against the criteria specified in the *Hazardous Products Regulations*, which is part of the regulatory framework for the Workplace Hazardous Materials Information System, 2015 (WHMIS 2015) for products intended for workplace use.

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

Decisions from domestic and international jurisdictions

Domestic

The Public Health Agency of Canada (PHAC) assigned A. globiformis (as a species) to Risk Group 1 (low individual, low community risk) for humans and terrestrial animals (PHAC, personal communication). The Canadian Food Inspection Agency has not listed A. globiformis as a regulated plant pest in Canada by the or as an agent causing reportable or notifiable diseases affecting terrestrial and aquatic animal health (CFIA 2015a, 2015b, 2015c).

A. globiformis is listed in the Natural Health Products Ingredients Database (NHPID) with a medicinal role (NHPID 2017). However, it is not listed in the Licensed Natural Health Products Database as being present as such in currently licensed natural health products (LNHPD 2017).

No pesticides are currently registered under the Pest Control Products Act of the Pest Management Regulatory Agency (PMRA) that contain A. globiformis as an active ingredient (PMRA 2015).

International

The International Dairy Federation and the European Food and Feed Cultures Association, which are responsible for creating and updating an authoritative list of micro-organisms with documented uses in food, have listed A. globiformis as a microbial food culture (MFC) used in dairy fermentations and cheese production (IDF Bulletin 2012; Hansen 2011). Use of A. globiformis as a MFC still requires a pre-market safety assessment by the European Food Safety Authority (IDF 2012). No other regulatory decisions by other international governments or organizations were identified for A. globiformis.[3]

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

1. Hazard assessment

1.1 Characterization of Arthrobacter globiformis

1.1.1 Taxonomic identification and strain history

1.1.1.1 Identification

Binomial name Arthrobacter globiformis

Taxonomic designation

Kingdom Bacteria

Phylum Actinobacteria

Class Actinobacteria

Order Micrococcales

Family Micrococcaceae

Genus Arthrobacter

Species Arthrobacter globiformis (validated 1980)

DSL Strain ATCC 8010 (type strain)

Common/Superseded names: Bacterium globiforme; Achromobacter globiformis; Mycobacterium globiforme; Arthrobacter globiforme; Corynebacterium globiforme (reviewed in Conn and Dimmick 1947 and Skerman et al. 1980).

Strain history

In 1925, N.R. Smith (U.S. Department of Agriculture) deposited Arthrobacter globiformis strain ATCC 8010 as Achromobacter globiforme into the American Type Culture Collection (ATCC) (Stackebrandt and Kandler 1979). In 1928, H.J. Conn (New York State Agriculture Experiment Station) described the bacterium as Bacterium globiforme. There were unsuccessful attempts to classify this micro-organism as a species of Mycobacterium or Corynebacterium. Finally, the genus Arthrobacter was proposed to represent all species that, like A. globiformis, were non-flagellate, rod-shaped and produced “arthrospores” (the coccoid phase of the rod-coccus life cycle) (Conn and Dimmick 1947). Skerman et al. (1980) then listed

Arthrobacter globiforme as Arthrobacter globiformis, the name that has been used since then (Busse et al. 2015).

The strain has been deposited to various culture collections under the following designations: AS 1.1894 = ATCC 8010 = BCRC (formerly CCRC) 10598 = CCUG 581 = CCUG 12157 = CCUG 28997 = CIP 81.84 = DSM 20124 = HAMBI 88 = HAMBI 1863 = IAM 12438 = ICPB 3434 = IFO (now NBRC) 12137 = JCM 1332 = LMG 3813 = NCIMB 8907 = NRIC 0151 = NRRL B-2979 = VKM Ac-1112 (Euzeby 1997).

1.1.1.2 Phenotypic and molecular characteristics

The genus Arthrobacter has more than 60 recognized species (Busse et al. 2015; Busse 2015; Euzeby 1997). Members of the genus Arthrobacter share many characteristics. A polyphasic approach including morphology, combined with other defining characteristics of the species, as well as sequence analysis, is therefore needed to unequivocally differentiate A. globiformis from other Arthrobacter species.

A. globiformis is the type species of the genus, and A. globiformis strain ATCC 8010 is the type strain of the species (Busse et al. 2015). The phenotypic characteristics of A. globiformis strain ATCC 8010 purchased from ATCC and tested by Health Canada scientists are consistent with the descriptions found in the literature. A. globiformis strain ATCC 8010 is a non-motile, Gram-positive bacterium, appearing as rods (0.8-1.2 µm × 1.0-8.0 µm) during exponential growth and as cocci during stationary phase (0.6-1.0 µm) and forming circular white colonies with no distinct pigmentation when grown on yeast extract-peptone media. Growth was observed at 28°C and 32°C when tested on various liquid media (Table A-1, Appendix A) but no growth was observed at 37°C or above. On trypticase soy agar medium, growth was observed at 28°C, 32°C and 37°C, with limited growth at 40°C only after seven days. No growth was observed at 42°C (Table A-2, Appendix A).

The species can also be identified by its unique cell wall peptidoglycan interpeptide bridge comprising type A3α, Lys–Ala3 (lysine-alanine) and the menaquinone MK-9(H_2) as its principal isoprenoid quinone system. Respiration is aerobic, with no production of acid or gas when grown in medium containing glucose, mannose, lactose, galactose and glycerol. A. globiformis strain ATCC 8010 is reported to grow in the presence of nitrate, glucose and pyruvate as carbon sources carrying out anaerobic respiratory nitrate reduction with fermentation of carbon sources. It also grows anaerobically in the absence of nitrate, with glucose and pyruvate as carbon sources (Busse et al. 2015).

The draft genome of A. globiformis strain ATCC 8010 consists of 4.95 Mbp, with 4529 coding sequences, 3 rRNAs, 5 tRNAs and a GC% content of 66.2%. Plasmid content has not been determined. A. globiformis can also be identified using 16S rRNA (GenBank Accession number X80736.1) gene sequence analysis. The full genome sequence of A. globiformis strain ATCC 8010 is publicly available (NCBI BioProject accession PRJDA71847; NCBI Reference sequence

NZ_BAEG01000000), and a comprehensive whole genome sequence hybridization and analyses of copy number variations and polymorphisms in various genomic regions may reveal further genetic differences between A. globiformis strain ATCC 8010 and other strains of A. globiformis, as well as other Arthrobacter species.

Based on a polyphasic approach combining phylogeny, 16S rRNA gene sequence similarities, peptidoglycan composition and quinone systems, A. globiformis has been grouped within the newly proposed A. globiformis group (also called Arthrobacter sensu stricto rRNA cluster 1), along with three recognized species: A. pascens, A. humicola and A. oryzae (Busse et al. 2015). Three additional species—A. crystallopoietes, A. ramosus and A. methylotrophus—have been tentatively assigned to this group, but due to discrepancies in the 16S rRNA gene sequences and the phenotypic properties, they are not yet conclusively assigned to the Arthrobacter sensu stricto rRNA cluster 1 (Busse et al. 2015). Characteristics of A. globiformis with those of other recognized species of the Arthrobacter sensu stricto rRNA cluster 1 have been compared (Table 1-1).

Table 1-1 Comparative features of A. globiformis with other species in the Arthrobacter sensu stricto rRNA cluster 1

Characteristics[a]	A. globiformis	A. humicola	A. oryzae	A. pascens
Motility	-	+	+	-
Growth temperature range (º C)	-5 to 32	4 and 34	4 and 34	N/A
Peptidoglycan type and interpeptide bridge	A3α, Lys–Ala_3	A3α Lys–$Ala_{>2}$	A3α, Lys–$Ala_{>2}$	A3α, Lys–Ala_2
Major fatty acids	$C_{15:0}$ anteiso, [$C_{15:0}$ iso], ($C_{16:0}$ iso, $C_{17:0}$ anteiso)	$C_{15:0}$ anteiso, [$C_{17:0}$ anteiso $C_{16:0}$ iso, $C_{15:0}$ iso)	$C_{15:0}$ anteiso, ($C_{17:0}$ anteiso)	$C_{15:0}$ anteiso, [$C_{17:0}$ anteiso], ($C_{15:0}$ iso)
NaCl range for growth (% w/v)	0-5	0-3	0-2	0-5
Cell wall sugars	Gal[b], Glc[c]	Gal, Rha	Gal, Glc	Gal, Glc
Utilization of L-arabinose	+	-	-	+
Nitrate reductase	-	-	+	-
Pyrrolidonyl arylamidase	-	-	+	-
Acid phosphatase	-	+	+	-
α-Galactosidase	-	+	-	+
β-Glucuronidase	-	w	+	-
α-Mannosidase	+	w	-	+

[a] Adapted from Busse et al. 2015
Ala = Alanine
- = negative
+ = positive
[b] = Galactose
[c] = Glucose
Lys = Lysine
w = weakly positive
N/A = Not available

Arthrobacter species belong to different proposed phylogenetic Arthrobacter groups and sub-clades and their differential characteristics have been thoroughly reviewed by Busse et al. (2015). While there have been no reports of species belonging to the Arthrobacter sensu stricto rRNA cluster 1 causing adverse effects in plants, animals or humans, other Arthrobacter species have been implicated in opportunistic human infections or isolated from veterinary sources. These include: A. albus, A. creatinolyticus, A. cumminsii, A. luteolus, A. oxydans, A.sanguinis, A. scleromae, A. woluwensis, A. gandavensis, A. nasiphocae and A. equi (Collins et al. 2002; Mages et al. 2008; Storms et al. 2003; Yassin et al. 2011). These species, however, belong to different proposed phylogenetic groups and sub-clades and have differential characteristics (Busse et al. 2015). A phylogenetic analysis by Health Canada scientists using publicly available 16S rRNA gene sequences of A. globiformis strain ATCC 8010 and other Arthrobacter species demonstrate that A. globiformis strain ATCC 8010 clusters closely with two of the members of the Arthrobacter sensu stricto rRNA cluster 1 (i.e., A. pascens strain DSM 20545 and Arthrobacter sp. strain DSM 20546, also named as A. ramosus) and with A. oryzae and A. humicola as the closest phylogenetic neighbouring tree. Based on the tree topology, A. globiformis strain ATCC 8010 is not closely related to potential opportunistic pathogens of the species (Figure 1-1).

is

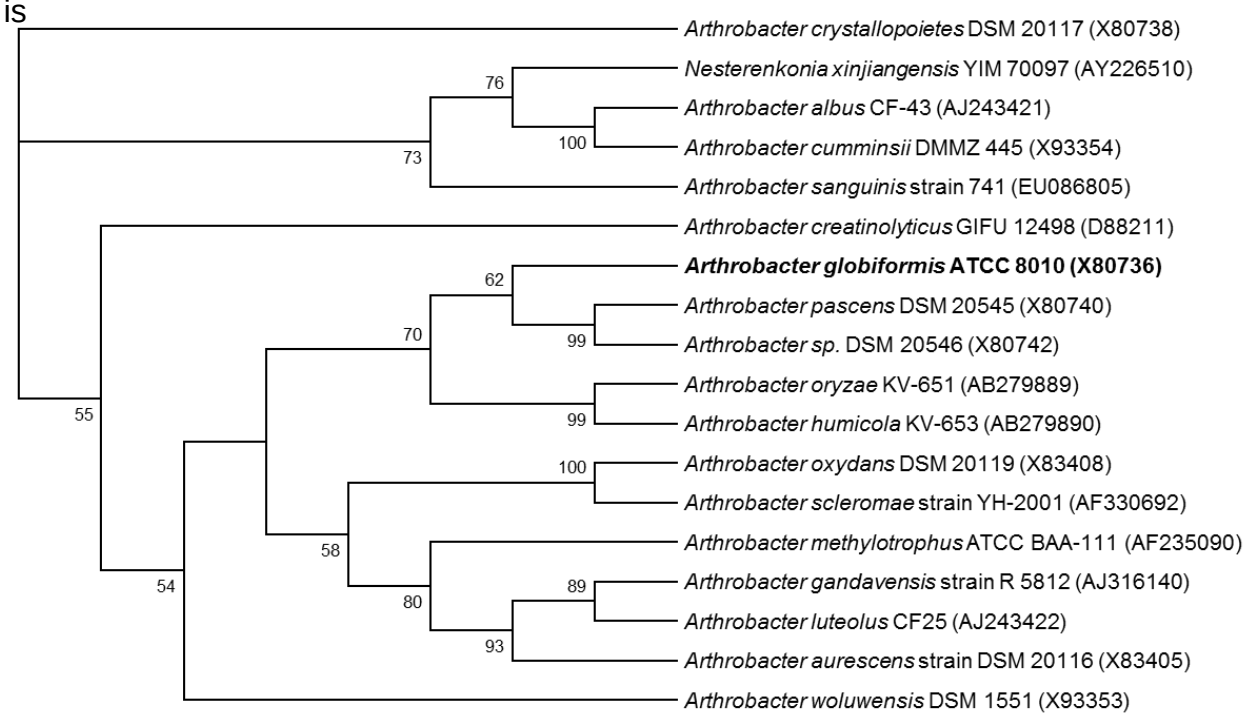

Figure 1-1: Maximum likelihood phylogenetic tree generated using 16S rRNA sequences of A. globiformis strain ATCC 8010 and other Arthrobacter species of veterinary and clinical relevance

The phylogenetic tree was constructed first by alignment of the sequences by the MUSCLE method and then analyzed with the Tamura-Nei distance model within the MEGA version 7 platform (Kumar et al. 2016). Bootstrap values of 50% and higher are shown at the nodes. Percentages are based on 500 re-samplings.

1.1.2 Biological and ecological properties

1.1.2.1 Natural occurrence

Various Arthrobacter species have been isolated from a range of ecological niches including soils, fresh and sea water, human skin, oil, brine, plants, air, sewage and activated sludge, mural paintings, stainless steel plumbing systems, and clinical specimens, as well as from extreme environments, such as arctic ice and the deep subsurface of Earth (Wietz et al. 2012; reviewed in Busse and Wieser 2014 and Busse et al. 2015). The species A. globiformis is reported to be ubiquitous in fresh water, salt water and soils (Goodfellow et al. 2012; Rayes 2013; Feiler 2013; Mulder et al. 1966). The DSL strain A. globiformis strain ATCC 8010 was isolated from soil (Conn and Dimmick 1947; ATCC 2015).

1.1.2.2 Growth and metabolism

A. globiformis strain ATCC 8010 is nutritionally versatile and is capable of surviving in different environments (Jones and Keddie 2006). When glucose or pyruvate is the

only carbon substrate present, this facultative anaerobe proceeds with lactate, acetate or ethanol fermentation (Eschbach et al. 2003). It also undergoes anaerobic respiration, with the reduction of the final electron acceptor nitrate, resulting in ammonification but not denitrification (Eschbach et al. 2003).

As reported above, Health Canada scientists found that A. globiformis strain ATCC 8010 can grow in liquid cultures at 29°C and 32°C, but not at higher temperatures (i.e., 37°C and above) (Table A-1, Appendix A). The optimum growth temperature of the species is 25-30°C (Busse et al. 2015). However, certain strains can grow at temperatures between 5°C and 32°C (Berger et al. 1997). A. globiformis can grow in nutrient broth containing up to 5% w/v NaCl (Arora and Jain 2013; Goodfellow et al. 2012). Likewise, the DSL strain A. globiformis strain ATCC 8010 exhibited high metabolic activity at tested concentrations of 1% and 4% w/v NaCl, but not at 8% w/v NaCl in testing by Health Canada scientists.

1.1.2.3 Survival, persistence and dispersal in the environment

Cocci are a common growth form of A. globiformis in soil (Luscombe and Gray 1971; Luscombe and Gray 1974). Artificially inoculated rod-shaped cells of A. globiformis (strain NCIMB 10683) transformed into cocci or short rods one to two weeks post-inoculation and did not revert to rod forms in either sterile or non-sterile soils (Luscombe and Gray 1974; Mansoor and Gray 1995). A. globiformis (strain NCMBI 10683) inoculated into sterile and non-sterile wheat soils showed a gradual increase in biovolume of cells (i.e., 10 fold in non-sterile soil and 200 fold in sterile soil) for one to three weeks post-inoculation, but significantly declined by the end of the experiment (i.e., 50 days), although complete loss of cell biovolume was not reported (Mansoor and Gray 1995).

A. globiformis is generally considered a dominant bacterium in unamended soils because of its efficient utilization of minimum substrate concentrations. In sterile dry soils of 40% water-holding capacity, A. globiformis dominated compared to certain Pseudomonas species. A. globiformis also showed enhanced resistance to drying in soils, and at low temperatures (i.e., 10°C), it displayed an extended lag phase (Salonius et al. 1970). However, in mixed cultures, certain Pseudomonas species are suggested to compete with A. globiformis, possibly due to the ability of Pseudomonas species to take up more oxygen under sterile soil conditions, as well as their ability to lower the pH and produce some uncharacterized water-soluble pigments in liquid broth culture conditions (Salonius et al. 1970; Labeda et al. 1976).

A. globiformis is a non-spore forming bacterium, but it uses a number of strategies to resist unfavourable environmental conditions. Under nutrient limited conditions, it can form dormant, non-culturable, cyst-like resting cells (CRCs) (Mulyukin et al. 2009). Reactivation of growth was observed—from 10 to 44% of CRCs—only when plated on certain media containing antioxidants, resulting in the formation of microcolonies (Mulyukin et al. 2009). As a response to osmotic stress, A. globiformis can also enter a myceloid phase, wherein it can form a multicellular structure

consisting of irregularly-shaped cells held together by interactions between their cell walls (Malwane and Deutch 1999; Deutch and Perera 1992). Myceloid structures are also formed when salt concentration increases or essential growth factors like biotin or manganese are limited (Deutch and Perera 1992). Cells in the myceloid phase are more resistant to other environmental stresses, such as temperature and ultraviolet radiation, than those not in the myceloid phase (Malwane and Deutch 1999). A. globiformis is also known to tolerate nutrient starvation, osmotic stress, extreme temperatures and drought by the production and intracellular accumulation of organic osmolytes, such as glycine betaine (Fan et al. 2004; Kempf and Bremer 1998), trehalose and glycogen (Zevenhuizen 1992). These osmoprotectants are known to counteract the loss of water in a hypertonic environment without interrupting cellular functions (Kempf and Bremer 1998) and to stabilize protein and cell structure during abiotic stresses (Wang et al. 2010).

1.1.2.4 Horizontal gene transfer

There are no reports to suggest the presence of plasmids in A. globiformis strain ATCC 8010, although other strains of A. globiformis are reported to have them (Brandsch and Decker 1984; Turnbull et al. 2001; Gillespie 2011). There is no evidence to suggest it is capable of natural transformation.

A. globiformis has been reported to support the growth of bacteriophages under laboratory conditions when the soil media is nutritionally amended with glucose or sucrose, maintained at 60% water-holding capacity, and incubated with rod-stage host cells. Bacteriophages for A. globiformis strain ATCC 8010 are rarely detected in soils, and the indigenous host cells are suggested to be in an insensitive state (e.g., non-synchronous spheroidal outgrowth phase) to viral infection (Casida and Liu 1974; Einck et al. 1973). There is no evidence of phage-mediated transduction reported for A. globiformis.

Members of the Arthrobacter genus are capable of degrading a wide variety of synthetic organic compounds, including aliphatic, aromatic, and polycyclic aromatic compounds (Busse and Wieser 2014). Horizontal gene transfers are suggested as factors contributing to this enormous degradation potential of Arthrobacter species, and this notion is strengthened by the fact that degradation genes and catabolic pathways for many xenobiotics are often plasmid encoded in this genus (Busse and Wieser 2014).

1.1.2.5 Resistance to antibiotics, metals and chemical agents

Several Arthrobacter species are reported to tolerate and/or degrade heavy metals, hydrocarbons, herbicides, fertilizers and radiation (Busse and Wieser 2014; Busse et al. 2015); however, there is no report attributing such properties to A. globiformis strain ATCC 8010.

Another strain of A. globiformis (strain D47) was able to metabolize phenylurea herbicides (e.g., linuron, diuron, monolinuron, metoxuron, and isoproturon) (Turnbull

et al. 2001). Likewise, A. globiformis strain KZT1 was capable of degrading chlorinated benzoic acids (CBA) (intermediates in the metabolism of herbicides and chlorinated biphenyls), and the degradative genes were found to be encoded in the plasmid pBS1501 (Zaitsev et al. 1991). A different study suggests that CBA-degrading genes can also be located on the chromosomes, as evidenced in A. globiformis strain HR19, which is devoid of any plasmid (Yi et al. 2000). In a laboratory study, a strain of A. globiformis isolated from contaminated soil from a decommissioned bulk gas station site at Hendon, Saskatchewan, was capable of degrading phenanthrene (Fernet 2008). This strain reduced the total petroleum hydrocarbon concentration in soil by 45%; and when soils were amended with manure and inoculated with phenanthrene-degrading A. globiformis, the hydrocarbon contaminant concentration decreased in soil by approximately 33% (Fernet 2008).

The antimicrobial susceptibility patterns of several Arthrobacter species, including A. globiformis strain ATCC 8010, are available in the literature (Funke et al. 1996). A. globiformis strain ATCC 8010 was not found to be multi-drug resistant based on 16 antimicrobials tested. It was reported to be susceptible to erythromycin, gentamicin, imipenem, meropenem, penicillin, rifampin, tetracycline and vancomycin. A. globiformis was found to be resistant to sulfonamides, the second most commonly used antibiotics in veterinary medicine after tetracyclines (Bialk-Bielinska et al. 2011).

Health Canada's in vitro antibiotic susceptibility study using the microdilution method showed that the DSL strain, A. globiformis strain ATCC 8010, was susceptible to most antibiotics tested, except for cefotaxime, trimethoprim/sulfamethoxazole and ciprofloxacin (with intermediary resistance) (Table 1-2). Three other methods (Remel Synergy Quad plate, antibiotic strips and Sensititre™ Gram-positive all-in-one format (GPALL1F) plates) were also used to determine the antibiotic susceptibility profile of A. globiformis strain ATCC 8010 (Table B-1 and Table B-2, Appendix B). Based on Remel quad plates testing, A. globiformis strain ATCC 8010 was found to be sensitive to gentamicin, streptomycin and vancomycin (Table B–1, Appendix B). Using the antibiotic strips and Sensititre™ GPALL1F plates methods, it was found to be sensitive to several antibiotics tested, except for oxacillin, ciprofloxacin and levofloxacin, which were least effective in curtailing the growth of A. globiformis strain ATCC 8010 (Table B–2, Appendix B).

Table 1-2: Antimicrobial susceptibility of A. globiformis strain ATCC 8010 determined using the microdilution method

Antibiotics	Microdilution method (MIC in µg/mL)[a]	Microdilution method (MIC in µg/mL)[b]	Breakpoints[c] MIC (µg/mL)	Interpretation of results for both studies (a/b)
Amoxicillin	1.2 ± 0.6	0.5	NA[d]	NA
Ampicillin	NA[c]	0.5	NA	NA

Antibiotics	Microdilution method (MIC in µg/mL)[a]	Microdilution method (MIC in µg/mL)[b]	Breakpoints[c] MIC (µg/mL)	Interpretation of results for both studies (a/b)
Cefalothin	NA	1.0	NA	NA
Cefotaxime	6 ± 0	NA	S[e]≤1 I[f]=2 R[g]≥4	R/NA
Ceftriaxone	NA	8.0	S≤1 I=2 R≥4	NA/R
Cefuroxime	NA	4.0	NA	NA
Chloramphenicol	NA	4.0	NA	NA
Ciprofloxacin	1.5 ± 0	4	S≤1 I=2 R≥4	I/R
Clindamycin	NA	1.0	S≤0.5 I=1-2 R≥4	NA/I
Doxycycline	<0.37	NA	S≤4 I=8 R≥16	S/NA
Erythromycin	<0.37	0.25	S≤0.5 I=1 R≥2	S/S
Gentamicin	0.56 ± 0.27	1	S≤4 I=8 R≥16	S/S
Imipenem	NA	1.0	S≤4 I=8 R≥16	NA/S
Meropenem	2.3 ± 1.1	NA	S≤4 I=8 R≥16	S/NA
Nalidixic acid	18 ± 8.5	NA	NA	NA
Penicillin	NA	0.25	S≤1 I=2 R≥4	NA/S
Rifampin	NA	≤0.03	S≤1 I=2 R≥4	NA/S
Teichoplanin	NA	0.06	NA	NA
Tetracycline	NA	0.25	S≤4 I=8 R≥16	NA/S

Antibiotics	Microdilution method (MIC in µg/mL)[a]	Microdilution method (MIC in µg/mL)[b]	Breakpoints[c] MIC (µg/mL)	Interpretation of results for both studies (a/b)
Tigecycline	NA	NA	NA	NA
Trimethoprim/ sulfamethoxazole	4.5 ± 1.7	NA	S≤2/38 R≥4/76	R/NA
Vancomycin	<0.37	0.25	S≤1	S/S

[a] Data generated by Health Canada's Environmental Health Science and Research Bureau. Results represent means of three experiments;
[b] Adapted from (Funke et al. 1996).
[c] Clinical breakpoints taken from CLSI M45-A for Coryneforms, information and interpretive criteria for broth microdilution susceptibility testing (NCCLS 2001).
NA, Test or interpretive data not available.
S, Susceptible
I, Intermediate
R, Resistant

1.1.2.6 Pathogenic and toxigenic characteristics

Neither A. globiformis strain ATCC 8010 nor any other strain of the species is reported to be pathogenic or toxigenic to aquatic plants, invertebrates, vertebrates or humans. As a result, there are no reports on mechanisms of virulence in A. globiformis.

Stainless steel plumbing systems and distribution pipes of domestic drinking-water facilities have been reported to harbour biofilms of Arthrobacter species (Percival et al. 1998). However, there is no report of biofilm formation by either A. globiformis strain ATCC 8010 or any other strain of the species.

Based on in vitro studies by Health Canada scientists, A. globiformis strain ATCC 8010 does not grow in liquid culture at temperatures above 32°C (Table A-1). It produces phospholipases, but not proteases, and no hemolytic activity was observed when it was grown on sheep blood agar for 48 hours at 37°C (, Table C-1, Appendix C). The ability of A. globiformis strain ATCC 8010 to be toxic and to activate pro-inflammatory cytokines in HT29 human colonic epithelial cells and J774A.1 mouse macrophage cells were also tested in vitro by measuring bioreductivity upon exposure for 4 and 24 hours. A. globiformis strain ATCC 8010 was not toxic (Figure C-1, Appendix C), and no significant activation of pro-inflammatory cytokines was observed in either mammalian cell type (Figure C-2 and Figure C-3, Appendix C).

Like other bacteria, A. globiformis may contain other bacterial components, such as cell wall proteins, antigens and enzymes, which may act as potential sensitizers. Sensitization or allergic reactions to micro-organisms could occur via dermal and respiratory routes in frequently exposed or susceptible individuals (Martel et al. 2010; Ring et al. 1992).

1.1.3 Effects

1.1.3.1 Environment

Certain Arthrobacter species have been isolated from animals, their environments or from veterinary clinical material (e.g., A. equi from genital swabs of a horse; A. stackebrandtii from poultry litter; A. citreus from chicken feces; A. gandavensis from mammary and uterine infections in cattle) (Busse et al. 2015). However, an in-depth scientific literature search on A. globiformis and its synonyms yielded no reports of the presence of virulence factors or evidence of pathogenicity or toxicity to terrestrial and aquatic plants, vertebrates or invertebrates.

The ability of A. globiformis to suppress other bacteria has been reported (Rayes 2013). For example, A. globiformis is used as a probiotic in shrimps, for its ability to inhibit infection with pathogenic Vibrio harveyi, both in vitro and in vivo. Several strains of A. globiformis isolated from maize rhizosphere were able to suppress growth of the plant pathogen Fusarium verticillioides in vitro (Cavaglieri et al. 2004). Also, the presence of root-associated, endophytic, native A. globiformis strains is suggested to contribute to plant resistance to phytopathogenic Ralstonia species (Feng et al. 2013; Upreti and Thomas 2015).

1.1.3.2 Human health

Despite the widespread presence of A. globiformis in various environments, there are no reported infections, toxicity or adverse immune effects in humans specific to the DSL strain A. globiformis strain ATCC 8010. One report from Poland documents occupational exposure to barley and barley dust containing A. globiformis, along with other bacteria, being associated with extrinsic allergic alveolitis (hypersensitivity pneumonitis, granulomatous pneumonitis) among agricultural workers. The contribution of A. globiformis to the reaction was not elucidated (Milanowski et al. 1998).

Certain other Arthrobacter species have been implicated in opportunistic human infections predominantly in immunocompromised individuals and those with underlying medical conditions. Infections reported include bacteremia, postoperative endophthalmitis, Whipple’s disease-like syndrome, phlebitis, endocartitis and a catheter-related blood and urinary tract infection (Busse et al. 2015; Bernasconi et al. 2004; Goodfellow et al. 2012; Shin et al. 2006; Park et al. 2012). The majority of Arthrobacter infections are attributed to A. cumminsii, A. oxydans, and A. aurescens, although a few other species (A. albus, A. creatinolyticus, A. luteolus, A. sanguinis, A. scleromae and A. woluwensis) have also been isolated from clinical sources (Mages et al. 2008; Wauters et al. 2000; Pasciak et al. 2010). These species have been recovered from human blood, skin infections, surgical wounds, urinary tracts and inflamed ocular cavities (Funke et al. 1996; Mages et al. 2008; Wauters et al. 2000). None of the above reports identified A. globiformis as an etiological agent in disease in humans.

1.2 Hazard severity

A combination of morphological, biochemical, and molecular studies allow the DSL strain A. globiformis strain ATCC 8010 to be reliably identified and differentiated from other Arthrobacter species, including those species belonging to the same Arthrobacter sensu stricto rRNA cluster 1.

The environmental hazard potential of A. globiformis strain ATCC 8010 is assessed to be low, because A. globiformis is a common soil bacterium with widespread distribution and a history of use in aquatic invertebrates as a probiotic and in plants as a biocontrol against microbial plant pathogens. However, it has not been implicated in infections or other adverse effects in terrestrial and aquatic plants, vertebrates or invertebrates.

The human hazard potential of A. globiformis strain ATCC 8010 is assessed to be low, because it is not known to have pathogenic or toxigenic characteristics, and there are no known instances of human infection with A. globiformis or any of its synonyms. In the unlikely event of infection, clinically-relevant antibiotics are available. Cases of human infection have been attributed to other Arthrobacter species. However, these cases are rare and occurred in immunocompromised individuals. Furthermore, A. globiformis strain ATCC 8010 was not toxic to HT29 human colonic epithelial cells or J774A.1 mouse macrophage cells upon 48-hour exposure, and no significant activation of pro-inflammatory cytokines was observed in either cell.

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

2. Exposure assessment

2.1 Sources of exposure

This screening assessment considers exposure to A. globiformis strain ATCC 8010 resulting from its uses in consumer, commercial and industrial applications in Canada.

A. globiformis strain ATCC 8010 was nominated for use in consumer and commercial products.

[4] A determination of whether one or more criteria of section 64 of CEPA are met is based on an assessment of potential risks to the environment and/or to human health associated with exposure in the general environment. For humans, this includes, but is not limited to, exposure from air, water and the use of products containing the substances. A conclusion under CEPA may not be relevant to, nor does it preclude, an assessment against the criteria specified in the *Hazardous Products Regulations*, which is part of the regulatory framework for the Workplace Hazardous Material s Information (WHMIS) for products intended for workplace use.

Responses to a voluntary questionnaire sent in 2007 to a subset of key biotechnology companies, combined with information obtained from other federal regulatory and non-regulatory programs, indicate that 10 000 to 100 000 kg of products potentially containing A. globiformis strain ATCC 8010 were imported into or manufactured in Canada in the 2006 reporting year.

The Government of Canada conducted a mandatory information-gathering survey under section 71 of CEPA, as published in the Canada Gazette, Part I, on October 3, 2009 (section 71 notice). The section 71 notice applied to any person who, during the 2008 calendar year, manufactured or imported A. globiformis strain ATCC 8010, whether alone, in a mixture or in a product. No commercial or consumer activities using A. globiformis strain ATCC 8010 were reported in response to this section 71 notice.

The 2007 and 2009 surveys differed significantly in target and scope of the surveyed population. This partially explains the large difference in quantities reported. In this screening assessment, results from the 2009 survey were used to estimate exposure from current uses because it requested information on uses of the micro-organism strain that is listed on the DSL, whereas the 2007 survey asked about uses of the products that had been associated with the micro-organism at the time it was nominated to the DSL. Because product formulations may have changed, information from the 2009 survey may more accurately represent current uses. Uses reported in the 2007 voluntary survey were also considered in the assessment of potential uses.

Although no uses were reported for A. globiformis strain ATCC 8010 in the mandatory survey, it is available for purchase from the ATCC. As it is listed on the DSL and so can be used in Canada without prior notification, it could be an attractive choice for commercialization. A search of the public domain (e.g., material safety data sheets, literature and patents) revealed the following potential consumer, commercial and industrial applications of other strains of A. globiformis:

- Microbial food culture in dairy fermentations and cheese production (IDF Bulletin 2012; Hansen 2011);
- Probiotic use in natural health products for humans (Product Sheet A, 2015);
- Production of coproporphyrin III dyes for food and beverages (Kojima et al. 1982);
- Production of erythorbic acid and enzymes like D-glucose to 2-ketogluconic acid (Xue et al. 2015);
- Biological control of plant pathogens such as Fusarium or Ralstonia species (Cavaglieri et al. 2004; Upreti and Thomas 2015);
- Probiotic use in shrimps for protection against bacterial disease (Rayes 2013);
- Biological degreasing, bioremediation of grease and oil, and sludge treatment (Product Sheet B, 2015);
- Bioremediation of chromium-contaminated areas (Tsibakhashvili et al. 2008);

- Biodegradation of glyphosate-based herbicides and synthetic tanning agent (Song and Burns 2005; Sumalan et al. 2009);
- Biodegradation of petroleum-contaminated areas (Fernet 2008); and
- Water and wastewater treatment (Amy et al. 2008; Insell 1987; reviewed in Vymazal and Kröpfelová 2008).

These represent possible uses of the DSL strain, as A. globiformis strain ATCC 8010 is likely to share the characteristics with other commercialized strains.

2.2 Exposure characterization

2.2.1 Environment

Based on the absence of consumer or commercial activity in Canada according to the section 71 notice, the estimated environmental exposure to A. globiformis strain ATCC 8010 is low.

Given the range and scale of known and potential applications of the species A. globiformis listed in Section 2.1, there is potential for an increase in environmental exposure to A. globiformis strain ATCC 8010, and exposure scenarios arising from these uses have been considered in this screening assessment, along with the persistence and survival properties of this micro-organism.

Terrestrial species, including plants, invertebrates and vertebrates, are expected to be exposed to A. globiformis strain ATCC 8010 through uses such as application to agricultural fields and crops as a biocontrol agent, biodegradation of herbicides and bioremediation. Note that exposure to the DSL strain as a pesticide would be considered as part of a pesticide registration administered by PMRA. No pesticides are currently registered under the Pest Control Products Act that contain A. globiformis as an active ingredient (PMRA 2015).

Aquatic species, including plants, invertebrates and vertebrates, are expected to be exposed to A. globiformis strain ATCC 8010 through uses such as degreasing, biodegradation of oil, treatment of sludge, water and wastewater, and releases from fermentation industries involved in production of dairy and cheese products, as well as food and beverage dyes and enzymes. In addition, aquatic species could be exposed to run-off subsequent to application to soil and bioremediation sites. Feed supplements and probiotic use in aquaculture could expose aquatic invertebrates and vertebrates to high concentrations of A. globiformis strain ATCC 8010.

The extent of exposure to A. globiformis strain ATCC 8010 will depend on the quantity released, its persistence in the receiving environment, and the proximity of environmental species to the sites of application or disposal.

Given the nutritional flexibility and ability of the species to survive unfavourable conditions, A. globiformis strain ATCC 8010 will likely be able to survive the

environmental stresses common in soil. Large or repeated releases into the environment from anthropogenic sources could temporarily elevate environmental concentrations. Nevertheless, high numbers of cells from such sources are unlikely to be maintained in water or soil. Competition and microbiostasis are likely to reduce the population to pre-release background levels (van Veen et al. 1997).

2.2.2 Human

Considering the absence of consumer or commercial activity in Canada according to the section 71 notice, human exposure to A. globiformis strain ATCC 8010 is estimated to be low. Nevertheless, given the range and scale of known and potential applications of the species A. globiformis listed in Section 2.1, there is potential for an increase in human exposure, and exposure scenarios arising from these uses have been considered in this screening assessment. The following human exposure scenarios are therefore considered based on the potential uses of A. globiformis strain ATCC 8010.

The highest potential for direct human exposure would occur should probiotics or microbial food cultures containing A. globiformis strain ATCC 8010 become available in Canada. A. globiformis is listed in the NHPID with a medicinal role for use as medicinal ingredient in natural health products. Direct human exposure could also occur if A. globiformis strain ATCC 8010 is used in consumer products for biological degreasing, biodegradation of oil, and treatment of sludge, water and wastewater. Use of A. globiformis strain ATCC 8010 in waste and wastewater treatment or in industrial processes may introduce the micro-organism into bodies of water and soil. Human exposure to bodies of water and soil treated with A. globiformis strain ATCC 8010 (e.g., through recreational activities or surface run-off into domestic wells or by ingestion) could also result in exposure of the skin, eyes and gastrointestinal tract. However, dilution of the micro-organism in water and rapid decline in soil population levels are expected to significantly reduce exposure relative to household application scenarios.

The general population could be exposed as bystanders during the application of A. globiformis strain ATCC 8010 for agricultural uses and for treatment of water and wastewater. The general population could also come into contact with residual A. globiformis strain ATCC 8010 on surfaces treated with commercial products. The route and extent of exposure will depend on the nature of the product, the application method, the concentration of A. globiformis strain ATCC 8010 in the product, the amount of product applied, and the proximity of the bystander to the site of application. However, the extent of exposure is not expected to be greater than direct exposure during the product application.

In the event that the micro-organism enters municipal drinking water treatment systems through release from potential uses, drinking water treatment processes (e.g., coagulation, flocculation, ozonation, filtration and chlorination) are expected to effectively eliminate this micro-organism, and so limit ingestion.

3. Risk characterisation

In this screening assessment, risk is characterized according to a paradigm whereby a hazard and exposure to that hazard are both required for there to be a risk. The risk assessment conclusion is based on the hazard and on what is known about exposure from current uses.

Hazard for A. globiformis strain ATCC 8010 has been estimated to be low for both the environment and human health. Based on responses to the section 71 notice, the exposure to living A. globiformis strain ATCC 8010 from its use in industrial processes or commercial applications in Canada is low for both the environment and human health.

The overall risk associated with current uses is estimated to be low for both the environment and human health.

The determination of risk from current uses is followed by consideration of the estimated hazard in relation to foreseeable future exposures (from new uses). A. globiformis strain ATCC 8010 has useful properties that may increase environmental and human exposure to these strains in the future. The risk from foreseeable potential uses of A. globiformis strain ATCC 8010 nevertheless remains low given that there is no evidence of adverse ecological effects at the population level for environmental species or of adverse effects on human health.

4. Conclusion

Based on the information presented in this screening assessment, it is concluded that A. globiformis strain ATCC 8010 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;
- 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 A. globiformis strain ATCC 8010 does not meet the criteria set out in section 64 of the CEPA.

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Appendices

Appendix A: Characteristics of A. globiformis strain ATCC 8010

Table A-1: Growth of A. globiformis strain ATCC 8010 in liquid media at various temperatures measured by absorbance at 500 nm (OD 500)

Medium used	Room temperature	28°C	32°C	37°C	40°C	42°C
TSB[a]	0	0.27	0.24	0	0	0
Nutrient	0.13	0.31	0.38	0	0	0
BHI[b]	0	0.15	0.15	0	0	0
SAB[c]	0	0	0	0	0	0
YPD[d]	0	0.21	0.12	0	0	0
10% FBS[e]	0	0.14	0	0	0	0
10% Sheep Serum	0	0	0	0	0	0
DMEM with FBS and glutamine[f]	0	0	0	0	0	0

Data generated by Health Canada's Environmental Health Science and Research Bureau. Growth of A. globiformis strain ATCC 8010 in broth culture, as measured by changes in absorbance at 500 nm, in different growth media and over a range of temperatures: Concentration of bacteria at time zero was 1×10^4 CFU/mL. Measurements were taken after 48-hour period with a multi-well spectrophotometer.

[a] TSB: Trypticase soy broth
[b] BHI: Brain-heart infusion broth
[c] SAB: Sabouraud dextrose broth
[d] YPD: Yeast extract - peptone-dextrose broth
[e] FBS: Fetal bovine serum
[f] DMEM: Dulbecco's Modified Eagle Medium

Table A-2: Growth characteristics of A. globiformis strain ATCC 8010 on tryptic soy broth (TSB) agar media at various temperatures

Time	Room temperature	28°C	32°C	37°C	40°C	42°C
24 hours	-	-	-	-	-	-
48 hours		-	-	-	-	-
3 days	-	+ (1 mm)	+ (1 mm)	+ (1 mm)	-	-
5 days	+ (0.5 mm)	+ (1 mm)	+ (1 mm)	+ (1 mm)	-	-
7 days	+ (2 mm)	+ (3 mm)	+ (3 mm)	+ (3 mm)	+ (3 mm)	-

Data generated by Health Canada's Environmental Health Science and Research Bureau. Growth of A. globiformis strain ATCC 8010 in solid media was examined at 24 hours, 48 hours, and 7 days at various temperatures.

– = no growth
+ = growth (millimeter diameter of growth)

Appendix B: Antibiotic susceptibility profile of A. globiformis strain ATCC 8010

Table B-1: Antimicrobial susceptibility of A. globiformis strain ATCC 8010 using Remel Synergy Quad Plate method in BHI agar medium

Antibiotics (concentration)	Growth measured at 37°C, 24 hours [a]
No antibiotic	Positive
Gentamicin (500 µg/mL)	Negative
Streptomycin (2000 µg/mL)	Negative
Vancomycin (6 µg/mL)	Negative

Data generated by Health Canada's Environmental Health Science and Research Bureau.
[a] Results represent means of three experiments

Table B-2: Antimicrobial susceptibility of A. globiformis strain ATCC 8010 determined using antibiotic strips and Sensititre™ Gram-positive plate format

Antibiotics	Etest ® Antibiotic strips (MIC in µg/mL)[a]	Sensititre™ Gram-positive format (MIC in µg/mL)[a,b]
Amoxicillin	0.5 ± 0	NA
Ampicillin	NA	1.7± 0.76
Cefalothin	NA	NA
Cefotaxime	6.7 ± 2.3	NA
Chloramphenicol	NA	<2.0
Ciprofloxacin	2.2 ± 1.8	2.0 ± 0
Clindamycin	0.10 ± 0.03	<0.5
Daptomycin	NA	2.0 ± 0
Erythromycin	NA	<0.25
Gentamicin	0.33 ± 0.14	<2.0
Imipenem	0.12 ± 0.11	NA
Levoflxacin	3.33 ± 1.15	4.0 ± 0
Linezoid	1 ± 0.87	2.0 ± 0
Meropenam	0.67 ± 0.29	NA
Moxifloxacin	NA	2.0 ± 0
Oxacillin	5.33 ± 2.31	4.0 ± 0
Penicillin	NA	0.83 ± 0.29
Rifampin	NA	<0.5
Tetracycline	1.17 ± 0.76	<2.0
Tigecycline	0.5 ± 0	0.25 ± 0
Trimethoprim/ sulfamethoxazole	NA	<0.5/9.5
Vancomycin	0.5 ± 0	0.83 ± 0.29

[a] Data generated by Health Canada's Environmental Health Science and Research Bureau. Results represent means of three experiments;

[b] Adapted from (Funke et al. 1996).
NA, Test or interpretive data not available.
±, plus or minus
<, less than

Appendix C: Characterization of potential virulence factors and pathogenicity of A. globiformis strain ATCC 8010

Table C-1: Growth characteristics of A. globiformis strain ATCC 8010 on selective solid media to determine potential virulence properties in vitro[a]

Tests on solid media plates	Result
Egg-yolk reaction (phospholipase)/protease (48h@37°C)	Positive/Negative
Skim milk agar clearing (protease) (48h@37°C)	Growth but no lysis (Negative)
Hemolysis on sheep blood agar (48h@37°C)	Negative

[a]Data generated by Health Canada's Environmental Health Science and Research Bureau. Growth was examined at 48 hours at 37°C.

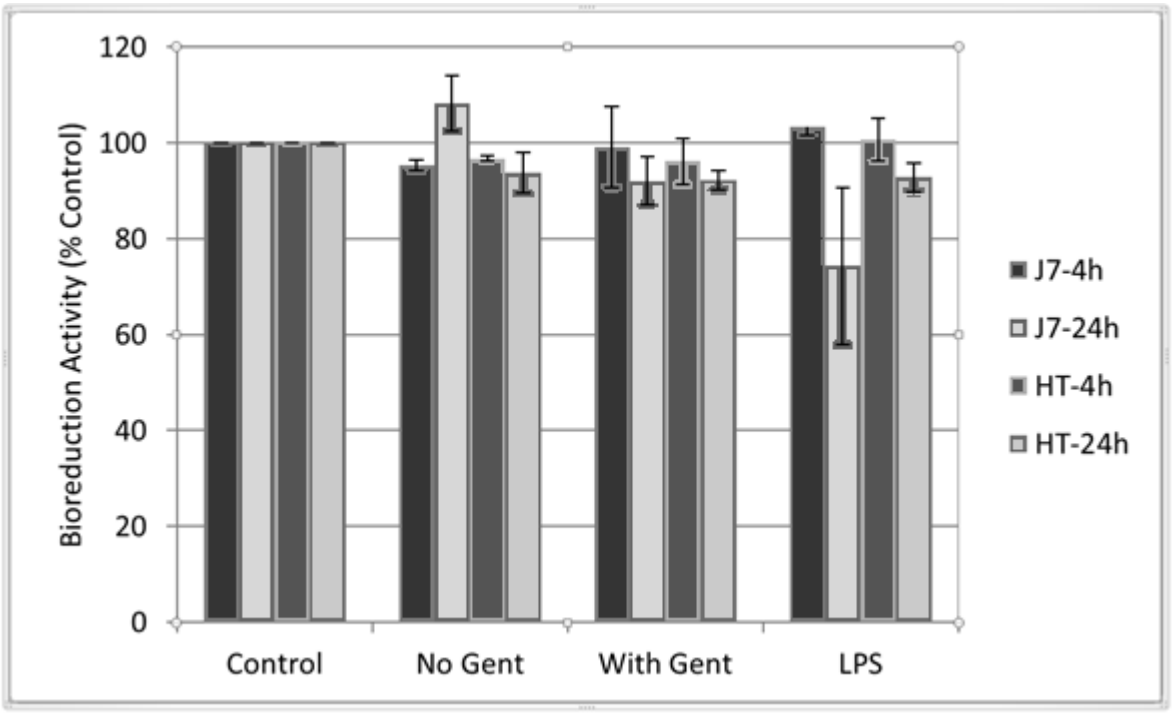

Figure C-1: In vitro cytotoxicity testing using bioreduction activity (viability) of mouse macrophage cells (J774A.1) and human colon epithelial cells (HT29) upon exposure to the DSL strain A. globiformis strain ATCC 8010 and the positive control, lipopolysacharides (LPS), for 4 hours and 24 hours, with or without gentamycin (With Gent; No Gent) treatment.

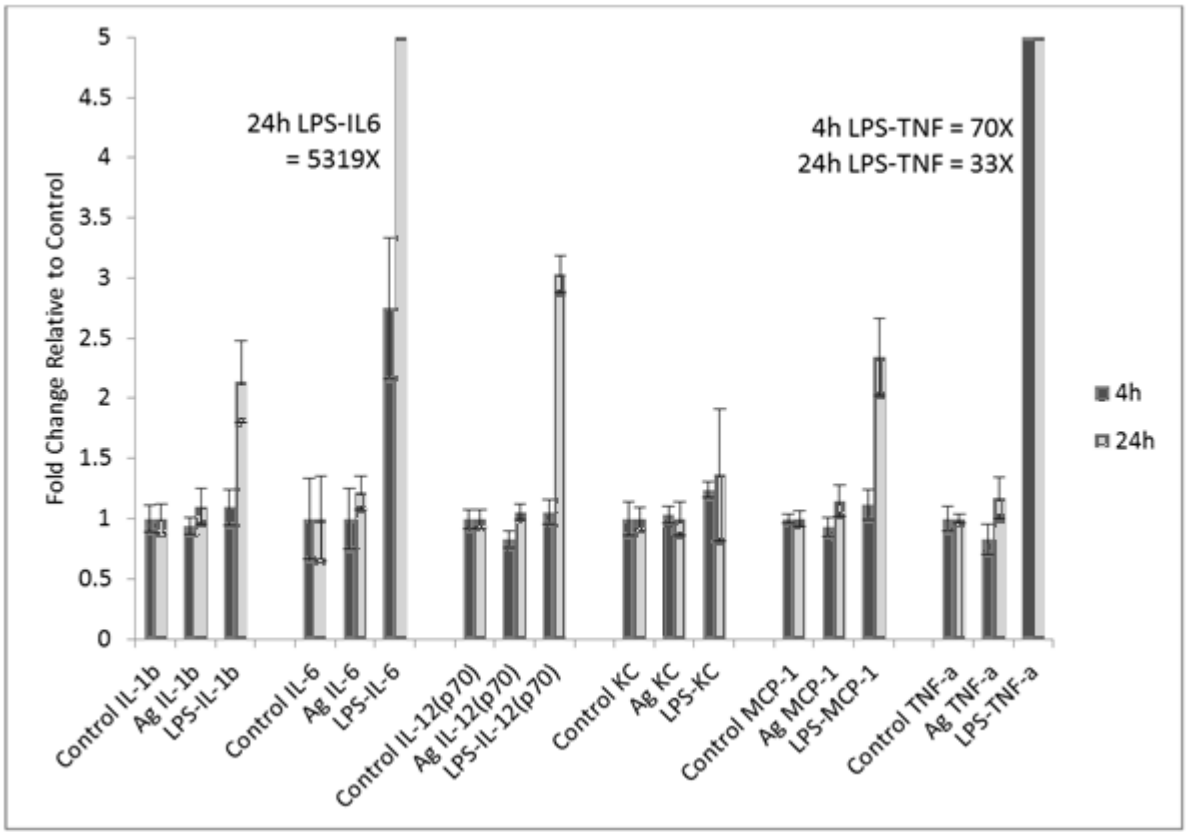

Figure C-2: Pro-inflammatory response of mouse macrophage cells (J774A.1) upon exposure to A. globiformis strain ATCC 8010 by measuring the cytokines IL-1b, IL-6, IL-12 (p70), KC, MCP-1, TNF- α in comparison with the positive control, lipopolysaccharides (LPS), for 4 hours and 24 hours.

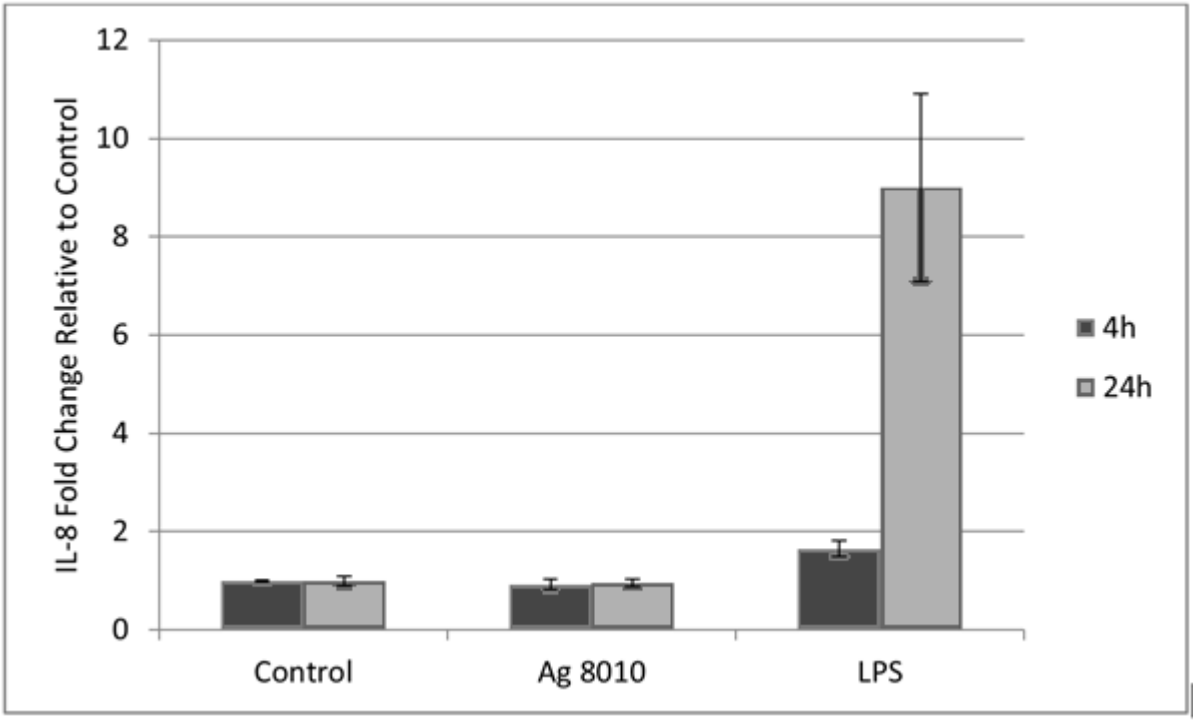

Figure C-3: Pro-inflammatory response of human colon epithelial cells (HT29) upon exposure to A. globiformis strain ATCC 8010 (Ag 8010) by measuring the cytokines IL-8, in comparison with the positive control, lipopolysaccharides (LPS), for 4 hours and 24 hours