Draft Screening Assessment of
Cellulomonas biazotea ATCC 486

Environment 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 Cellulomonas biazotea strain ATCC\(^1\) 486.

*C. biazotea* strain ATCC 486 is a soil bacterium that has characteristics in common with other strains of the species. The characteristics of *C. biazotea* make it suitable for use in animal feed supplements, fertilizers, biodegradation and biofuel production.

There are no reported adverse effects in terrestrial or aquatic plants, invertebrates or vertebrates or infections in humans associated with these specific DSL strains or other strains of *C. biazotea*.

This assessment considers the aforementioned characteristics of *C. biazotea* strain ATCC 486 with respect to environmental and human health effects associated with consumer and commercial product use and industrial processes subject to CEPA, including releases to the environment through waste streams and incidental human exposure through environmental media. To update information about current uses 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 the section 71 Notice indicates that *C. biazotea* strain ATCC 486 was not imported into or manufactured in Canada in 2008.

Based on the information available, it is proposed to conclude that *C. biazotea* strain ATCC 486 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 proposed to conclude that *C. biazotea* strain ATCC 486 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.

\(^1\) American Type Culture Collection
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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).  

C. biazotea ATCC 486 was added to the DSL 2005 under subsection 105(1) of CEPA because it was manufactured in or imported into Canada between January 1, 1984, and December 31, 1986, and it entered or was released into the environment without being subject to conditions under CEPA or any other federal or provincial legislation.

This screening assessment considers hazard information obtained from the public domain and from unpublished research data generated by Health Canada 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 report, data that are specific to the DSL-listed strain C. biazotea ATCC 486 are identified as such. Where strain-specific data were not available, surrogate information from literature searches was used. When applicable, literature searches conducted on the organism included its synonyms, and common and superseded names. Surrogate organisms are identified in each case to the taxonomic level provided by the source. Literature searches were conducted using scientific literature databases (SCOPUS, CAB Abstracts, Agricola, Google Scholar and NCBI PubMed and FreePatentsOnline), web searches and key search terms for the identification of human health and environmental hazards. Information identified up to June 2015 was considered for inclusion in this report.

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

3 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 *C. biazotea* (as a species) to Risk Group 1 for humans and terrestrial animals (personal communication, PHAC 2015). *C. biazotea* is not listed as a regulated plant pest in Canada (CFIA 2015a) or as an agent causing reportable or notifiable diseases affecting terrestrial and aquatic animal health (CFIA 2015b, 2015c).

International

Under the German Technical Rules for Biological Agents (TRBA), *C. biazotea* DSM 20112 (ATCC 486) is designated as biosafety level 1 (DSMZ 2015).

No other regulatory decisions by other international governments or organizations were identified for *C. biazotea*.4

4 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 *Cellulomonas biazotea*

1.1.1 Taxonomic identification and strain history

1.1.1.1 Identification

**Binomial name:** *Cellulomonas biazotea*

**Taxonomic designation (reviewed in Stackebrandt and Schumann 2012):**

- **Kingdom:** Bacteria
- **Phylum:** Actinobacteria
- **Class:** Actinobacteria
- **Order:** Actinomycetales
- **Family:** *Cellulomonadaceae*
- **Genus:** *Cellulomonas*
- **Species:** *Cellulomonas biazotea* (Kellerman et al. 1913) Bergey et al. 1923 (reviewed in Clark 1953; Stackebrandt and Kandler 1979)

**DSL strains:** ATCC 486 (type strain)

**Synonyms, common and superseded names:**

- “Bacillus biazoteus” (Kellerman et al. 1913) (ATCC 2008); “Proteus cellulomonas var. biazoteus” (Clark 1953)

**Strain history:**

*Cellulomonas biazotea* ATCC 486 was first isolated from soil in Utah, in the United States by N.R. Smith (designated as strain 127). The strain was deposited to the ATCC in 1950 (ATCC 2008; NCTC 2013).

The genus, including the type species *C. biazotea*, was initially classified within the Corynebacteriaceae (Clark 1953; Stackebrandt and Kandler 1979). However, it is distinct from other corynebacteria, such as *Corynebacterium diphtheriae*, based on
the ability to degrade cellulose (Clark 1953; da Silva and Holt 1965; Jones 1975) and the high guanine-cytosine (G+C)% content of its DNA (Stackebrandt and Kandler 1979). The diphtheria toxin, present in three species of the Corynebacterium genus, is absent in other coryneform bacteria such as C. biazotea and other Cellulomonas species (Bernard 2012). C. biazotea was moved to a new family Cellulomonadaceae in 1991, with Cellulomonas as the type genus.

The strain has been deposited to various culture collections under the following designations: 127, AJ 1569, AS 1.1899, BCRC 14864, CCC 14864, CCRC 14864, CFBP 4269, CGMCC 1.1899, CIP 82.11, CIP 82.11T, DSM 20112, IAM 12106, IFM 10509, IFO 12680, IMET 10473, JCM 1340, KCTC 1370, LMG 16695, N. R. Smith 127, N.R.Smith127, NBRC 12680, NCAIM B.01385, NCDO 1654, NCFB 1654, NCIB 8077, NCIM 2550, NCIMB 8077, NCTC 10823, NRS-127, PTCC1256, QM B-525, QMB-525, Smith 127, Suzuki CNF 024, VKM Ac-1410.

1.1.1.2 Phenotypic and molecular characteristics

Cells are motile with one to four polar flagella (Thayer 1984). The availability of nutrients may affect motility. Chemotaxis has been observed in another species of this genus (Hsing and Canale-Parola 1992). Cellulomonas gelida ATCC 488 displays chemotaxis towards plant and fungal cell wall polysaccharides. Motility is not present when distilled water is used in the medium (Thayer 1984). The cell wall peptidoglycan contains ornithine and MK-9 (H4) as the major menaquinone (Ahmed et al. 2014; Stackebrandt and Kandler 1979).

Colonies of C. biazotea appear smooth, glistening and yellow-white on yeast agar (Stackebrandt and Kandler 1979). Production of a number of carotenoids results in the formation of yellowish pigment (Weeks et al. 1980).

Cellulomonas species are aerobic or facultatively anaerobic (Ahmed et al. 2014; Stackebrandt and Kandler 1979; Suzuki et al. 1981).

Within the genus Cellulomonas, only two species, Cellulomonas denverensis and Cellulomonas hominis, have been implicated in human infection as rare opportunistic pathogens, with a total of only five clinical cases (Salas et al. 2014). C. biazotea can be differentiated from these species based on growth temperature and biochemical characteristics (Table 1-1).

Table 1-1: Biochemical characteristics of C. biazotea, the closely related C. fimi and two medically relevant Cellulomonas species

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>C. biazotea</th>
<th>C. fimi ATCC 484</th>
<th>C. denverensis ATCC BAA-788</th>
<th>C. hominis ATCC 51964</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth at 25°C</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Growth at 45°C</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lysis of cellulose&lt;sup&gt;c&lt;/sup&gt;</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>---</td>
<td>---</td>
<td>----</td>
<td>---</td>
</tr>
<tr>
<td>Gelatin hydrolysis&lt;sup&gt;c&lt;/sup&gt;</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Xylose fermentation&lt;sup&gt;c&lt;/sup&gt;</td>
<td>+/-&lt;sup&gt;e&lt;/sup&gt;</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>DNase&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-&lt;sup&gt;c&lt;/sup&gt;, weak&lt;sup&gt;f&lt;/sup&gt;</td>
<td>-</td>
<td>ND</td>
<td>+</td>
</tr>
<tr>
<td>Nitrate reduction&lt;sup&gt;e&lt;/sup&gt;</td>
<td>+</td>
<td>+/-&lt;sup&gt;g&lt;/sup&gt;</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

ND: No data

<sup>a</sup> (Brown et al. 2005)
<sup>b</sup> (Sukapure et al. 1970)
<sup>c</sup> (Funke et al. 1995)
<sup>e</sup> In contrast to Clarke (1953), Funke et al. (1995) reported that C. biazotea can ferment xylose.
<sup>f</sup> Environmental Health Science and Research Bureau research scientists
<sup>g</sup> In contrast to Funke et al. (1995), Yoon et al. (2008) reported a negative result for nitrate reduction for C. fimi (same strain used).

+: positive
-: negative

Phylogenetic analysis shows that C. biazotea clusters distantly from these species. It is most closely related to Cellulomonas fimi, exhibiting 99.7% 16S rRNA similarity (Rainey, Weiss, Stackebrandt 1995). Health Canada research scientists constructed several phylogenetic trees to demonstrate the relationships among Cellulomonas species (Figure 1-1 and Figure 1-2). A maximum likelihood phylogenetic tree was generated using 16S rRNA sequences of C. biazotea and Cellulomonas species of environmental and clinical relevance (Figure 1-1). A similar tree was constructed using Bacillus subtilis as the out-group (Figure 1-2).
Figure 1-1: Maximum likelihood phylogenetic tree generated using 16S rRNA sequences of *C. biazotea* and *Cellulomonas* species of environmental and clinical relevance

The phylogenetic tree was generated by Environmental Health Science and Research Bureau based on publicly available 16S ribosomal RNA gene sequences by alignment of the sequences using the MUSCLE method and then analyzed with the Tamura-Nei distance model within the MEGA version 6 platform (Tamura et al. 2013). Bootstrap values of 50% and higher are shown at the nodes. Percentages are based on 500 re-samplings.
Figure 1-2: Maximum likelihood phylogenetic tree generated using 16S rRNA sequences of *C. biazotea* and *Cellulomonas* related species of environmental and clinical relevance

The phylogenetic tree was generated by Environmental Health Science and Research Bureau based on publicly available 16S ribosomal RNA gene sequences by alignment of the sequences using the MUSCLE method and then analyzed with the Tamura-Nei distance model within the MEGA version 6 platform (Tamura et al. 2013). 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

*C. biazotea* has been isolated from various habitats, including:

- soils (Clark 1953);
- the rhizosphere, root surface, and interior of canola (*Brassica napa*) (de Freitas and Germida 1998);
- bagasse (Parvez et al. 2004);
- landfills (Pourcher et al. 2001);
- the gut of the wood-eating termite *Centrocestus formosanus* (Femi-Ola et al. 2013); and
- human fecal isolate (Lagier et al. 2012)

1.1.2.2 Survival, persistence and dispersal in the environment

In low nutrient conditions, particularly low nitrogen availability, *Cellulomonas* species typically respond by forming biofilms. Cells within the biofilm increase carbon uptake in order to create a capsule of beta(1-3) glucan. Once favourable environmental conditions, such as increased nitrogen availability, are restored, this capsule is used as a carbon source (Young et al. 2012).

Levels of cellulolytic bacteria such as *C. biazotea* declined over time in a French landfill, from an average of 12.9 x 10^7 CFU per gram dry weight in a one-year-old refuse sample to 0.56 x 10^7 CFU per gram dry weight in a sample taken from five-year-old refuse. Cellulose availability in the environment promoted survival of a complex flora of cellulolytic bacteria (Pourcher et al. 2001).

Members of the *Cellulomonas* genus were found to persist for at least 2 to 4 weeks at different times of the year when 1-mL aliquots of bacterial isolates were inoculated into streambed sediments in 2.95-L flowing-water microcosms. Bacteria from sediment samples were enumerated after detachment of cells from the sediment using sonication and separation by centrifugation, demonstrating a recovery of 68.3 ± 15.0% of the bacteria initially inoculated. This persistence within the stream sediments is particularly notable since these organisms are not typically components of aquatic microflora (Bott and Kaplan 1991).

1.1.2.3 Growth parameters

*C. biazotea* grows well between temperatures of 20°C and 37.5°C, while optimal growth occurs between 28°C and 33°C (Clark 1953).
When grown on cellobiose, glucose and xylose, *C. biazotea* NIAB442 had a shorter lag period and doubling time and the highest specific growth rate when compared to three other members of the *Cellulomonas* genus (Rajoka and Malik 1986). The doubling times of *C. biazotea* NIAB442 are compared to the closely related *C. fimi* NIAB444 (Table 1-2).

Growth characteristics on TSB-agar and in liquid medium are detailed in Table A-2, Table A-3, Table A-4 and Table A-5.

**Table 1-2: Comparison of doubling times (hours) of *C. biazotea* and *C. fimi* when grown on different media**

<table>
<thead>
<tr>
<th>Nutrient source</th>
<th><em>C. biazotea</em> NIAB442</th>
<th><em>C. fimi</em> NIAB444</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellobiose</td>
<td>2.9</td>
<td>5.3</td>
</tr>
<tr>
<td>Glucose</td>
<td>4.0</td>
<td>4.8</td>
</tr>
<tr>
<td>Xylose</td>
<td>3.3</td>
<td>5.0</td>
</tr>
</tbody>
</table>

*(Rajoka and Malik 1986)*

**1.1.2.4 Role in nutrient cycling**

*Cellulomonas* is distinguished from related genera by its ability to degrade cellulose aerobically and anaerobically (Clark 1953), resulting in the fermentation of cellulose to CO₂ gas (Young et al. 2012).

Similar to most species of *Cellulomonas*, *C. biazotea* is capable of reducing nitrate to nitrite (Clark 1953). There is a general inability to fix nitrogen (N₂) among *Cellulomonas* species (Young et al. 2012).

The presence of the beta-glucosidase gene in *C. biazotea* confers the ability to hydrolyze esculin and cellobiose (Parvez et al. 2004). This enzyme is most active at 38°C and pH 6.6 and is inhibited by 0.5 mM MnCl₂ and by 0.5 M NaCl (Siddiqui et al. 1997).

**1.1.2.5 Resistance to Antibiotics, Metals and Chemical Agents**

*Cellulomonas* is typically susceptible to rifampin, tetracycline and vancomycin (Funke et al. 1997).

Strains of *C. biazotea* isolated from the gut of wood-eating termites demonstrated resistance to cotrimoxazole, cloxacillin, erythromycin, gentamicin, augmentin, streptomycin, tetracycline and chloramphenicol (Femi-Ola et al. 2013). The antibiotic susceptibility profiles of *C. biazotea* ATCC 486 and the closely related *C. fimi* ATCC 484 have been compared (Table 1-3).
Table 1-3: A comparison of the minimum inhibitory concentrations (MIC, µg/mL) of antibiotics against *C. biazotea* ATCC 486 and *C. fimi* ATCC 484

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th><em>C. biazotea</em> ATCC 486</th>
<th><em>C. fimi</em> ATCC 484</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin</td>
<td>2 (I)</td>
<td>2 (I)</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>2 (I)</td>
<td>2 (I)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>4 (R)</td>
<td>4 (R)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>16 (R)</td>
<td>32 (R)</td>
</tr>
<tr>
<td>Penicillin G</td>
<td>1 (S)</td>
<td>0.06 (S)</td>
</tr>
<tr>
<td>Rifampin</td>
<td>0.06 (S)</td>
<td>≤0.03 (S)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>0.25 (S)</td>
<td>0.5 (S)</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>0.06 (S)</td>
<td>0.06 (S)</td>
</tr>
</tbody>
</table>

aData from Funke et al. (1995)
S: susceptible
I: intermediate
R: resistant
≤: less than or equal to

1.1.2.6 Pathogenic and toxigenic characteristics

*C. biazotea* forms biofilms in oligotrophic conditions, particularly when nitrogen is limiting. The addition of nitrogen to biofilm cultures induces dissolution of beta(1-3) glucan capsules and produces motile cells (Young et al. 2012). This contributes to persistence in the environment, though there is no evidence of pathogenic or toxic effects produced as a result.

No other pathogenic or toxic characteristics pertaining to *C. biazotea* have been reported in the literature. Other species of the *Cellulomonas* genus including *C. fimi* were evaluated for toxigenic characteristics. *Cellulomonas* cell proteins were not reported to cause mutagenic or teratogenic effects (Dey and Fields 1995).

1.1.3 Effects

1.1.3.1 Environment

Plants

*C. biazotea* was isolated from the rhizosphere, root surface and interior of canola (*Brassica napa*), where it did not cause infection (de Freitas and Germida 1998).

No adverse effects on aquatic or terrestrial plants implicating the DSL strains have been reported in the literature.
Invertebrates

*C. biazotea* was isolated from the gut of *Centrocestus formosanus*, a wood-eating termite, where it is a commensal organism and has a role in the breakdown of cellulosic materials (Femi-Ola et al. 2013).

No adverse effects of *C. biazotea* in aquatic or terrestrial invertebrates have been reported in the literature.

Vertebrates

No adverse effects of *C. biazotea* in aquatic or terrestrial vertebrates have been reported in the literature.

1.1.3.2 Human health

*Cellulomonas* species are rarely pathogenic in humans. Some species such as *C. hominis* and *C. denverensis* may act as opportunistic pathogens and are considered to be medically relevant (Bernard 2012). These species, though related to *C. biazotea*, are distantly spaced in a phylogenetic tree based on 16S rRNA gene sequence (Figure 1-2). As of 2014, there have been five case reports in which a species of this genus has been cultured from an active human infection (Salas et al. 2014). The details of these cases are provided in Appendix B.

There are no reports of infection, toxicity or adverse immune effects in humans specific to *C. biazotea* or to the DSL strains.

1.2 Hazard severity

The environmental and human health hazard severity for *C. biazotea* ATCC 486 is assessed to be low because: (1) no adverse effects in environmental species or humans, attributed to *C. biazotea* ATCC 486 or its close relatives, have been reported; and (2) in the unlikely event of infection in humans, clinically relevant antibiotics are available.

Hazards related to micro-organisms used in the workplace should be classified accordingly under the Workplace Hazardous Materials Information System (WHMIS).5

5 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 Controlled Products Regulations or the Hazardous Products Regulations, which is part of the regulatory framework for the Workplace Hazardous Materials Information System (WHMIS) for products intended for workplace use.
2. Exposure Assessment

2.1 Sources of exposure

This assessment considers exposure to *C. biazotea* ATCC 486 resulting from its addition to consumer or commercial products and its use in industrial processes in Canada.

*C. biazotea* ATCC 486 was nominated to the DSL for use in consumer and commercial products.

Responses to a voluntary questionnaire sent in 2007 to a subset of key biotechnology companies, combined with information obtained from other federal government regulatory and non-regulatory programs, indicate that a very small amount of *C. biazotea* ATCC 486 was imported into Canada for research and development in the 2006 reporting year.

The federal government 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 persons who, during the 2008 calendar year, manufactured or imported *C. biazotea* ATCC 486, whether alone, in a mixture, or in a product. No commercial or consumer activities using *C. biazotea* ATCC 486 were reported in response to the section 71 notice.

Although no uses were reported for *C. biazotea* ATCC 486 during the mandatory survey, it is available for purchase from the ATCC. Given that it is on the DSL and can therefore be used in Canada without prior notification, it could be an attractive choice for use in products that could be commercialized. A search of the public domain (MSDS, literature and patents) revealed the following potential consumer, commercial and industrial applications of other strains of *C. biazotea* (these represent possible uses of strain ATCC 486 because it is likely to share characteristics (modes of action) with other commercialized *C. biazotea* strains):

- a source of single-cell protein for use as a supplementary animal feed (Rajoka 2005);
- fermenting systems involved in the production of nitrogenated fertilizer (Domínguez et al. 2003);
- biofertilizer for canola (de Freitas and Germida 1998);
- waste and wastewater treatment, including:
  - degradation of carbohydrates in complex wastes (Chaudhary et al. 1997);
  - biological degradation of fatty substances and starches trapped in grease tanks (Bald et al. 1996);
- degradation of undesirable constituents of wastewater (e.g., cellulose fibres) to make the water suitable for agricultural uses (Erickson and Worne 1979);
- phosphorus removal from wastewater (Udaka and Shoda 1980);
- hydrogen energy production via waste fermentation (Saratale et al. 2010); and
- biofuel and electricity-producing fuel cells (Reguera et al. 2013).

### 2.2 Exposure characterization

#### 2.2.1 Environment

Based on the absence of consumer and commercial activity in Canada according to the section 71 notice, the estimated environmental exposure to *C. biazotea* ATCC 486 is low.

Given the range and scale of known and potential applications of the species *C. biazotea* listed in Section 2.1, there is potential for an increase in environmental exposure to *C. biazotea* ATCC 486, and exposure scenarios arising from these uses have been considered in this assessment, along with the persistence and survival properties of this micro-organism.

Use of this micro-organism as a biofertilizer is likely to introduce (or increase existing population levels of) *C. biazotea* ATCC 486 in terrestrial ecosystems. Terrestrial invertebrates living in the soils at the site of application and treated plants are likely to be the most directly exposed. Vertebrates could ingest *C. biazotea* ATCC 486 while feeding on plants or invertebrates growing in soil treated with these bacteria. Use of the DSL strains in animal feeds would result in a direct exposure to terrestrial or aquatic vertebrate or invertebrate species fed with the supplement, and possibly indirect exposure to other environmental species if these strains survive passage through the digestive tracts of these animals. It should be noted that the use of these bacteria in fertilizers or animal feeds, including their environmental and indirect human health assessment, is regulated in Canada by the Canadian Food Inspection Agency under the *Fertilizers Act* and *Feeds Act*, respectively.

Aquatic and marine species may come into contact with *C. biazotea* ATCC 486 from runoff subsequent to terrestrial application of fertilizer products, and from the direct application of *C. biazotea* ATCC 486 to water bodies for uses such as water treatment (fresh and salt water), release of effluents from wastewater treatment, or disposal of wastewater from applications such as the manufacture of fertilizers and biofuels.

Aquatic applications could also expose terrestrial species. For example, grazing animals could ingest *C. biazotea* ATCC 486 subsequent to its use in restoration of a
water source, and plants and soil invertebrates could be exposed as a result of the use of treated water for irrigation.

*C. biazotea* is metabolically versatile and is capable of forming biofilms (Young et al. 2012), traits which enable it to readily colonize and survive in new terrestrial and aquatic environments. *C. biazotea* does not form spores, and competition and microbiostasis are likely to prevent the persistence of introduced populations above background levels (van Veen et al. 1997).

### 2.2.2 Human

Based on the absence of consumer or commercial activity in Canada according to the section 71 notice, the human exposure estimation for *C. biazotea* ATCC 486 is low. Nevertheless, given the range and scale of known and potential applications of the species *C. biazotea* listed in Section 2.1, there is potential for an increase in human exposure, and human exposure scenarios arising from these uses have been considered in this assessment.

Potential uses of *C. biazotea* ATCC 486 identified in Section 2.1 are largely commercial or industrial and, as such, direct exposure of the general population in Canada is not expected to increase significantly should such uses occur in Canada. There is potential for some increase in exposure through environmental media from uses of *C. biazotea* ATCC 486 in processing of animal feed supplements, application of fertilizers containing this strain, waste and wastewater treatment, and energy and biofuel production.

The general population could be exposed as bystanders during the application of commercial products. The extent of bystander exposure will depend on the mode of application, the volume applied, and the proximity of bystanders to the site of application, but in general is expected to be low.

In the event that the organism enters municipal drinking water treatment systems through releases into drinking water sources, it is expected to be effectively eliminated from drinking water by the water treatment process, which utilizes one or more of the following methods: coagulation, flocculation, ozonation, filtration, ultraviolet radiation and chlorination.

### 3. Risk Characterisation

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

Hazard for \textit{C. biazotea} ATCC 486 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 \textit{C. biazotea} ATCC 486 is not currently expected, so 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). \textit{C. biazotea} ATCC 486 has useful properties that may result in its use in new products that could lead to increased environmental and human exposure to this strain in the future. The risk from foreseeable potential uses of \textit{C. biazotea} ATCC 486 is low because there is no evidence of adverse ecological effects at the population level for environmental species or of adverse effects on human health.

\textbf{4. Conclusion}

Based on the information presented in this draft screening assessment, it is proposed to conclude that \textit{C. biazotea} ATCC 486 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 proposed to conclude that \textit{C. biazotea} ATCC 486 does not meet the criteria set out in section 64 of the CEPA.
5. References


ATCC. 2008. Cellulomonas biazotea (kellerman et al.) bergey et al. (ATCC 486).


Udaka S, Shoda M, inventors; President of Nagoya University (Aichi, JP), assignee. 1980. Method of cleaning phosphorus-containing waste water by microorganisms.


Appendices

Appendix A: Characteristics of C. biazotea and related species

Table A-1: DNA G+C% content and 16S rRNA GenBank accession numbers of C. biazotea, the closely related C. fimi and two medically relevant Cellulomonas species

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>C. biazotea ATCC 486</th>
<th>C. fimi ATCC 484</th>
<th>C. denverensis ATCC BAA-788</th>
<th>C. hominis ATCC 51964</th>
</tr>
</thead>
<tbody>
<tr>
<td>G+C content (%)</td>
<td>71.5-75.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>74.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>68.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>70°, 73-76&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>16S rRNA (GenBank accession number)</td>
<td>X83802&lt;sup&gt;e&lt;/sup&gt;</td>
<td>X83803&lt;sup&gt;e&lt;/sup&gt;</td>
<td>AY501362&lt;sup&gt;c&lt;/sup&gt;</td>
<td>X82598&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> (Stackebrandt and Kandler 1979)
<sup>b</sup> (Christopherson et al. 2013)
<sup>c</sup> (Brown et al. 2005)
<sup>d</sup> (Funke et al. 1995)
<sup>e</sup> (Rainey et al. 1995)

Table A-2: Growth characteristics of C. biazotea ATCC 486 on tryptic soy broth agar at various temperatures

<table>
<thead>
<tr>
<th>Time&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Room Temp</th>
<th>28°C</th>
<th>32°C</th>
<th>37°C</th>
<th>40°C</th>
<th>42°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 d</td>
<td>No growth</td>
<td>0.1 mm</td>
<td>0.3 mm</td>
<td>0.1 mm</td>
<td>No growth</td>
<td>No growth</td>
</tr>
<tr>
<td>7 d</td>
<td>0.5 mm</td>
<td>1.5 mm</td>
<td>1.5 mm</td>
<td>1.5 mm</td>
<td>No growth</td>
<td>No growth</td>
</tr>
</tbody>
</table>

Data from Health Canada scientists
<sup>a</sup> No growth was observed after 24 hours, 48 hours or 3 days at any temperature

Table A-3: Liquid growth characteristics of C. biazotea ATCC 486 (5 × 10<sup>4</sup> CFU/mL) based on optical density at 500 nm after 48 hours

<table>
<thead>
<tr>
<th>Temperature&lt;sup&gt;a&lt;/sup&gt;</th>
<th>TSB&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Nutrient</th>
<th>BHI&lt;sup&gt;c&lt;/sup&gt;</th>
<th>SAB&lt;sup&gt;d&lt;/sup&gt;</th>
<th>YPD&lt;sup&gt;e&lt;/sup&gt;</th>
<th>10% FBS&lt;sup&gt;f&lt;/sup&gt;</th>
<th>10% Sheep Serum</th>
<th>DMEM&lt;sup&gt;g&lt;/sup&gt; with FBS and Glutamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Room temperature</td>
<td>0.09</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>28°C</td>
<td>0.60</td>
<td>0.32</td>
<td>0.43</td>
<td>0</td>
<td>0</td>
<td>0.06</td>
<td>0.07</td>
<td>0</td>
</tr>
<tr>
<td>32°C</td>
<td>0.65</td>
<td>0.39</td>
<td>0.40</td>
<td>0</td>
<td>0</td>
<td>0.17</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>37°C</td>
<td>0</td>
<td>0.09</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.17</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Data from Health Canada scientists
<sup>a</sup> No growth was observed at temperatures above 40°C
<sup>b</sup> Tryptic soy broth
<sup>c</sup> Brain heart infusion
<sup>d</sup> Sabouraud
<sup>e</sup> Yeast extract-peptone-dextrose
<sup>f</sup> Fetal bovine serum
<sup>g</sup> Dulbecco’s modified eagle medium
Table A-4: Growth characteristics of *C. biazotea* ATCC 486 on solid media at 37°C in 48 hours

<table>
<thead>
<tr>
<th>Tests</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg-yolk reaction (phospholipase)/protease</td>
<td>Growth but negative/negative</td>
</tr>
<tr>
<td>Skim milk agar clearing (protease)</td>
<td>Growth but no lysis (negative)</td>
</tr>
<tr>
<td>Starch hydrolysis</td>
<td>Positive</td>
</tr>
<tr>
<td>Hemolysis</td>
<td>Negative</td>
</tr>
<tr>
<td>Invasive growth on sheep blood agar</td>
<td>Negative</td>
</tr>
</tbody>
</table>

Data from Health Canada scientists

Table A-1: Growth characteristics of *C. biazotea* ATCC 486 on selective agar plates at 37°C in 48 hours

<table>
<thead>
<tr>
<th>Selective Agar Plates</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bc selective agar (growth/reaction)</td>
<td>Positive/positive</td>
</tr>
<tr>
<td>Cetrimide agar</td>
<td>No growth</td>
</tr>
<tr>
<td>DMEM(^a) agar (DMEM high glucose with 10% FBS(^b), glutamine and 1.5% agar)</td>
<td>growth</td>
</tr>
<tr>
<td>Mannitol-salt agar</td>
<td>No growth</td>
</tr>
<tr>
<td>MacConkey agar</td>
<td>No growth</td>
</tr>
<tr>
<td>SAB(^c) dextrose</td>
<td>No growth</td>
</tr>
</tbody>
</table>

Data from Health Canada scientists

\(^a\) Dulbecco’s modified eagle medium (glucose concentration 4500 mg/L)
\(^b\) Fetal bovine serum
\(^c\) Sabouraud
Appendix B: Isolation of *Cellulomonas* species from humans

Table B-1: Description of *Cellulomonas* species infections in humans

<table>
<thead>
<tr>
<th>Country</th>
<th>Patient history</th>
<th>Infection type</th>
<th><em>Cellulomonas</em> species</th>
<th>Synopsis</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>United States</td>
<td>• 70 y.o. male</td>
<td>Endophthalmitis</td>
<td>Not speciated</td>
<td>Infection resolved after IV treatment with vancomycin and ceftazidime</td>
<td>(reviewed in Sharma et al. 2008)</td>
</tr>
<tr>
<td></td>
<td>• 78 y.o. female • Back injury and intermittent lower back pain for 10 years</td>
<td>Endocarditis, osteomyelitis</td>
<td>Not speciated</td>
<td>Infection resolved after IV treatment with penicillin, gentamicin and oral treatment with TMP-SMX</td>
<td>(Lai et al. 2009)</td>
</tr>
<tr>
<td>China</td>
<td>• Uterine myoma resulting in a total hysterectomy • Moderate to severe mitral regurgitation (MR) with a history of paroxysmal atrial fibrillation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Japan</td>
<td>• 82 y.o. female • The patient had been noted as previously healthy</td>
<td>Cholecystitis, bacteremia</td>
<td><em>C. denverensis</em></td>
<td>Infection resolved after IV treatment with ampicillin-sulbactam</td>
<td>(Ohtaki et al. 2009)</td>
</tr>
<tr>
<td>Slovenia</td>
<td>• 30 y.o. male • Intravenous drug user • Chronic hepatitis C</td>
<td>Endocarditis</td>
<td>Not speciated</td>
<td>Infection resolved after IV treatment with cefotaxime and gentamicin; valve perforation complication</td>
<td>(Logar and Lejko-Zupanc 2013)</td>
</tr>
<tr>
<td>United States</td>
<td>• 82 y.o. male • Peptic ulcer disease • Cholecystectomy • Billroth I procedure • Interstitial lung disease and peripheral vascular disease</td>
<td>Ascending cholangitis, bacteremia</td>
<td>Not speciated</td>
<td>Infection resolved after IV treatment with vancomycin IV and oral treatment with piperacillin-tazobactam</td>
<td>(Salas et al. 2014)</td>
</tr>
<tr>
<td>Canada</td>
<td>No data</td>
<td>Isolated from cerebrospinal fluid</td>
<td><em>C. hominis</em></td>
<td>Clinical significance unknown</td>
<td>(Brown et al. 2005)</td>
</tr>
<tr>
<td>United States</td>
<td>No data</td>
<td>Isolated from pilonidal cyst</td>
<td><em>C. hominis</em></td>
<td>Clinical significance unknown</td>
<td>(Brown et al. 2005)</td>
</tr>
<tr>
<td>Country</td>
<td>Patient history</td>
<td>Infection type</td>
<td>Cellulomonas species</td>
<td>Synopsis</td>
<td>Reference</td>
</tr>
<tr>
<td>-----------</td>
<td>-----------------</td>
<td>-----------------------------------------------------</td>
<td>----------------------</td>
<td>---------------------------------------------</td>
<td>----------------------------</td>
</tr>
<tr>
<td>United States</td>
<td>No data</td>
<td>Isolated from lip wound</td>
<td>C. hominis</td>
<td>Clinical significance unknown</td>
<td>(Brown et al. 2005)</td>
</tr>
<tr>
<td>United States</td>
<td>No data</td>
<td>Isolated from blood and hemograft heart valve</td>
<td>C. denverensis</td>
<td>Clinical significance unknown</td>
<td>(Brown et al. 2005)</td>
</tr>
</tbody>
</table>