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Screening assessment of

trichoderma reesei strain ATCC 74252

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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 Trichoderma reesei ATCC[1] 74252.

Trichoderma reesei (T. reesei) strain ATCC 74252 is a fungus that has characteristics in common with other species of the genus Trichoderma and other strains of the same species. T. reesei, a spore forming fungus, is able to thrive in soil and on decaying plant matter as a major decomposer of plants, and is thus capable of degrading a variety of plant-based substrates. These properties allow for potential uses of T. reesei in fermentation of plant-based feedstocks, and in production of enzymes and biochemicals used in food, animal feed and in health products. T. reesei is widely considered to be a safe production organism due to its long history of safe use for the production of carbohydrase enzymes such as cellulase.

Trichoderma species including T. reesei are capable of producing metabolites called peptaibols. Some T. reesei strains can produce the peptaibol paracelsin as well as other peptaibols. Paracelsin is reported to be harmful to aquatic invertebrates, to mammalian cells, and to mice in experimental conditions where natural barriers were bypassed. Paracelsin has also been reported to have antibiotic and antifungal activity. Paracelsin and other peptaibols production is not thought to occur under the conditions of industry-standard submerged fermentation in which T. reesei strain ATCC 74252 is currently used, but could occur under other growth conditions.

T. reesei as a species is not naturally occurring in Canada. Despite its widespread presence in tropical soils, there are no reports of the species causing adverse effects in aquatic or terrestrial plants or animals in the tropics. In addition, Trichoderma species including T. reesei inhibit various plant pathogens.

There is no evidence in the scientific literature indicating that T. reesei is a human pathogen. T. reesei strain ATCC 74252 is unlikely to cause infection in healthy or debilitated humans and is susceptible to major clinical antifungal drugs that could be used for treatment in the unlikely event of infection. Repeated exposure to commercial enzyme preparations produced by T. reesei and other Trichoderma species infrequently causes allergic reactions in humans.

This assessment considers the aforementioned characteristics of T. reesei strain ATCC 74252 with respect to environmental and human health effects associated

[1] American Type Culture Collection

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 microorganism, the Government launched a mandatory information-gathering survey under section 71 of CEPA, as published in the Canada Gazette, Part I, on October 3, 2009 (section 71 notice). Information submitted in response to the Notice indicates that 10 000 – 100 000 kg of T. reesei strain ATCC 74252 dry cell mass was manufactured in Canada in 2008 for industrial uses.

Based on the information available, it is concluded that T. reesei strain ATCC 74252 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 T. reesei strain ATCC 74252 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.

It is concluded that T. reesei strain ATCC 74252 does not meet any of the criteria set out under section 64 of CEPA.

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1. Introduction

Pursuant to paragraph 74(b) of the Canadian Environmental Protection Act, 1999 (CEPA), the Minister of the Environment and Climate Change 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).[2] Trichoderma reesei (T. reesei) ATCC 74252 was added to the DSL 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 Environment and Climate Change Canada[3] 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 on the 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 T. reesei strain ATCC 74252 are identified as such. Where strain-specific data were not available, surrogate information from the literature was used. When applicable, literature searches conducted on the organism included its synonyms, common and superseded names, and Hypocrea jecorina. Surrogate organisms are identified in each case to the taxonomic level provided by the source. Literature searches were

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

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

conducted using scientific literature databases (SCOPUS, CAB Abstracts, Google Scholar, and NCBI Pubmed), web searches, and key search terms for the identification of human health and environmental hazards, and exposure characteristics. Information identified up to March 2016 was considered for inclusion in this screening assessment.

2. Decisions from domestic and international jurisdictions

2.1 Domestic

The Public Health Agency of Canada (PHAC) assigned the species T. reesei to 'Risk Group 1' (low individual risk, low community risk) for both humans and terrestrial animals (personal communication, PHAC 2014). T. reesei is not considered to be an aquatic animal pathogen, nor a regulated plant pest in Canada by the Canadian Food Inspection Agency (CFIA) (personal communication, CFIA 2014).

Health Canada's List of Permitted Food Enzymes sets out permitted source organisms (including T. longibrachiatum A83 (previously named T. reesei A83) and T. longibrachiatum QM9414 (previously namedT. reesei QM9414) for enzymes that may be used as food additives. As per section B.01.045, Part B, of the Food and Drug Regulations, food additives are required to meet specifications set out in these regulations and where no specifications are set out in Part B, the additive must meet specifications set out in the most recent edition of the Food Chemicals Codex (FCC). For food enzymes, the FCC specifications for enzyme preparations would apply.

T. reesei is listed in the Natural Health Products Ingredients Database with a medicinal role as classified as a natural health product (NHP) substance falling under Schedule 1, item 1 (fungus) of the Natural Health Products Regulations. T. reesei is also listed as a source material for the enzyme cellulase in Health Canada's Cellulase monograph, as well as for the enzymes beta-glucanase, hemicellulose, pectinase, and xylanases in Health Canada's Digestive Enzymes monograph (NHPID 2016).

2.2 International

The United States Environmental Protection Agency (US EPA) put into place a Significant New Use Rule (SNUR) under the Toxic Substances Control Act (TSCA) for use of a genetically modified strain of T. reesei in the production of enzymes for ethanol production. As reviewed under Microbial Commercial Activity Notice (MCAN) J-10-0002, use of the strain for this purpose is not expected to present an unreasonable risk to human health and the environment. The conditions for use specified in the MCAN are those of the typical industry-standard submerged fermentation process for enzyme production, conducted in concordance with the standard industry practices for containment and inactivation. As such, a significant

new use for this SNUR has been defined by the US EPA as: any use of the micro-organism other than uses in fermentation which meet all of the following conditions: submerged fermentation with no solid plant material or insoluble substrate present, and fermentation of solid plant material or insoluble substrate in the fermentation broth which is only initiated after specified inactivation of the micro-organism. These conditions are imposed because strains of T. reesei have been shown to produce the toxic peptaibol paracelsin as a metabolite when used for enzyme production through the fermentation of solid plant-based and insoluble substrates (US EPA 2012a).

Similarly, the US EPA determined that T. reesei QM 6a (the parental strain of T. reesei strain ATCC 74252) and its derivatives meet criteria for recipient microorganisms exempted from full notification and reporting procedures under TSCA for newly manufactured microorganisms going into commerce. The rationale for this decision states that (QM 6a) “will not present an unreasonable risk of injury to health or the environment”. The conditions for this exemption were under the industry-standard submerged fermentation conditions: no solid plant material or insoluble substrate present in the fermentation broth, and the introduced genetic material must be “well-characterized, limited in size, poorly mobilizable, and free of certain sequences” (US EPA 2012b).

3. Hazard assessment

3.1 Characterization of trichoderma reesei

3.1.1 Taxonomic identification and strain history

Binomial name: Trichoderma reesei

Taxonomic designation:

Kingdom: Fungi

Phylum: Ascomycota

Class: Sordariomycetes

Order: Hypocreales

Family: Hypocreaceae

Genus: Trichoderma

Species: Trichoderma reesei

DSL strain: ATCC 74252 (equivalent to ATCC 74444)

Synonyms, common and superseded names:

T. longibrachiatum Rifai was proposed as a synonym of T. reesei Simmons (reviewed in Kuhls et al. 1997; Bissett 1984). T. reesei M2C38 is an alternate strain designation of ATCC 74252 (Environment Canada 2013).

The parental strain of T. reesei strain ATCC 74252, T. reesei QM 6a (ATCC 13631) was deposited as Trichoderma viride (ATCC 2014). Hypocrea jecorina is the teleomorph of T. reesei (reviewed in Kubicek et al. 2003).

Strain history

T. reesei strain ATCC 74252 is a proprietary strain derived from T. reesei RUT-C30 (ATCC 56765). Including T. reesei strain ATCC 74252 and T. reesei RUT-C30, nearly all industrial cellulase producing strains of T. reesei are derivatives of the T. reesei type strain QM 6a (ATCC 13631) (Environment Canada 2013; Nevalainen et al. 1994). ATCC 74252 was originally deposited as a proprietary organism with the American Type Culture Collection as T. longibrachiatum Rifai, after identification by John Bissett, Ministry of Agriculture, Ottawa, Ontario in 1994. In September 1997, ATCC 74252 was renamed T. reesei based on substantial molecular evidence demonstrating that T. longibrachiatum and T. reesei were distinct species. ATCC 74252 was later redeposited with the American Type Culture Collection under ATCC 74444 as T. reesei with an accompanying statement that the two strains were the same (EFSA 2012). For the purposes of this screening assessment report, Trichoderma reesei strain ATCC 74252 will hereafter be referred to using the following name (including the strain number from the original ATCC deposit): T. reesei strain ATCC 74252.

T. reesei QM 6a, the parental strain of T. reesei strain ATCC 74252, was originally isolated from cotton canvas in Bougainville Island, Solomon Islands during World War II (ATCC 2014; Kuhls et al. 1996; Reese 1976).

3.1.1.1 Phenotypic and molecular characteristics

Morphology:

Information provided by the Nominator shows that T. reesei strain ATCC 74252 forms very pale green, thin colonies after 4 days of growth on nutrient agar. T. reesei strain ATCC 74252 is described as having identical morphology, growth and sporulation on potato dextrose agar (PDA), as well as identical hyphal structures to those produced by, T. reesei strain RUT-C30.

A high degree of morphological similarity exists between T. reesei and related Trichoderma species. T. reesei was first described as a distinct species within Trichoderma section Longibrachiatum through morphological examination (Simmons 1977, as reviewed in Bissett 1984); T. reesei can be differentiated from T. longibrachiatum and T. pseudokoningii on the basis that it has the largest conidia and slowest growth rate on special nutrient agar (Samuels et al. 1994).

T. reesei has now been placed in a phylogenetic clade with T. parareesei and T. gracile (Druzhinina et al. 2012, as reviewed in Samuels et al. 2012). Growth patterns of T. reesei, T. parareesei and T. gracile are the same at 25-35^{o}C and 35^{o}C on PDA; however, T. reesei produces fewer conidia on PDA and synthetic nutrient agar (SNA) (Samuels et al. 2012).

Substrate utilization:

Trichoderma species can be distinguished based on substrate utilization. Nutrient utilization patterns for 96 carbon sources can be used in the identification of Trichoderma species (Bochner et al. 2001; Kubicek et al. 2003). For example, gentiobiose and salacine are species-specific carbon sources utilized by two strains of H. jecorina (AF486007 and TUB F-1034) but not by other Trichoderma species tested (Kubicek et al. 2003). Additionally, growth on sucrose can differentiate species within Trichoderma (Kubicek et al. 2003). Initially, based on the behavior of strain QM 6a, Simmons believed that the inability to use sucrose or nitrate was a species descriptor of T. reesei (Simmons 1977, as reviewed in Lieckfeldt et al. 2000). However, as more T. reesei strains were discovered which were capable of growth on sucrose and nitrate as sole carbon and nitrogen sources, this inability to utilize sucrose and nitrate was recognized as a specific feature of QM 6a (Lieckfeldt et al. 2000). Utilization of certain substrates by T. reesei QM 6a and T. longibrachiatum are presented in Table 1-1.

Table 1-1: Comparison of substrate utilization of T. reesei with T. longibrachiatum

Characteristic	T. reesei QM 6a	T. longibrachiatum	References
Growth on sucrose	-	+	Danielson and Davey 1973; Lieckfeldt et al. 2000
Utilization of nitrate	-	+	Danielson and Davey 1973; Lieckfeldt et al. 2000
Utilization of nitrite	-	NT	Lieckfeldt et al. 2000
Growth on salicine	+	-	Atanasova 2014; Kubicek et al. 2003
Growth on gentiobiose	+	-	Atanasova 2014; Kubicek et al. 2003

Characteristic	T. reesei QM 6a	T. longibrachiatum	References
Growth on tagatose	-	NT	Kubicek et al. 2003

(-): Absence of detectable growth or utilization, (+): Presence of detectable growth or utilization, NT: Not Tested

T. reesei RUT-C30 shows reduced growth on linked oligo- and polysaccharides such as dextrin, starch and maltose. Information provided by the Nominator indicates that T. reesei strain ATCC 74252 behaves similarly to T. reesei QM 6a with respect to its inability to utilize sucrose, nitrate, and nitrite as substrates for growth.

Molecular techniques:

T. reesei and T. longibrachiatum can be distinguished using a variety of molecular techniques including DNA fingerprinting (Meyer et al. 1992), DNA hybridization of the cbh2 gene (Morawetz et al. 1992), comparison of coding and flanking regions of the endoglucanase l gene (Gonzalez et al. 1992, as reviewed in Nevalainen et al. 1994) and analysis of internal transcribed spacer regions 1 (ITS1) and 2 (ITS2) of ribosomal RNA gene sequences (Kuhls et al. 1996, as reviewed in Kuhls et al. 1997). Molecular studies using these techniques combined with morphometric and isoenzyme profiles reported by Samuels et al. (1994) produced the conclusion that T. reesei and T. longibrachiatum should be classified as separate species (Kuhls et al. 1996, as reviewed in Kuhls et al. 1997).

Using restriction fragment length polymorphism (RFLP) with oligonucleotide probes to distinguish T. reesei and T. longibrachiatum, homology was ~25% (Meyer et al. 1992). T. reesei and T. longibrachiatum display the following differences between the first introns of their endoglucanase I (egII) genes: 123 base pair (bp) in T. longibrachiatum versus 70 bp in T. reesei, and a 50 bp insertion in the T. longibrachiatum consensus splice signal sequence (a sequence otherwise 80% conserved in T. reesei) (Gonzalez et al. 1992). Analysis of ITS1 and ITS2 regions revealed a 6 bp (2.6%) sequence difference between T. reesei and T. longibrachiatum ITS1 regions (falling within the range of section Longibrachiatum interspecies sequence variability for anamorphic species) (Kuhls et al. 1996, as reviewed in Kuhls et al. 1997).

Molecular evidence also demonstrates that T. reesei is an anamorph of the fungus H. jecorina (Kubicek et al. 2008; Kuhls et al. 1996). T. reesei and H. jecorina ITS rRNA gene sequences are identical. By PCR-fingerprinting, interspecific variation between the two species is equivalent to the intraspecies variation of H. jecorina, suggesting they represent the same organism, and are anamorph/teleomorph with differences only in the mode of reproduction and minor phenotypic characteristics (Kuhls et al. 1996).

TrichOKEY2.0, a molecular Barcode tool, is available online for quick identification of Hypocrea and Trichoderma species using a combination of several oligonucleotides specially allocated within the ITS1 and ITS2 sequences of the rRNA gene cluster (International Subcommission on Trichoderma and Hypocrea Taxonomy). The T. reesei genome has been sequenced (Martinez et al. 2008, as reviewed in Samuels et al. 2012).

3.1.2 Biological and ecological properties

The name T. reesei was accepted over H. jecorina for this anamorph/teleomorph pair (Bissett et al. 2015). Hence literature for both the anamorph and teleomorph were used in this screening assessment where relevant to the characterization of T. reesei strain ATCC 74252.

3.1.2.1 Life cycle

The T. reesei life cycle is typical of Ascomycetes. T. reesei is the anamorph, reproducing asexually, while H. jecorina is the teleomorph, or sexual form, of the species (Kuhls et al. 1996). Asexual reproduction occurs by the production of vegetative reproductive spores, conidia (Nevalainen et al. 1994). Conidia form at 20^{o}C-30^{o}C on SNA and PDA, but poorly at >35^{o}C (Samuels et al. 1998). Transcription of cellulase and hemicellulase genes in T. reesei is triggered by conidiation, which increases the rate of fungal germination once a cellulosic or hemicellulosic carbon source becomes available (Metz et al. 2011).

T. reesei is a heterothallic species, with the industrial strain T. reesei QM 6a possessing the MAT1-2 mating type locus (Seidl et al. 2009). In mating experiments between T. reesei QM 6a and H. jecorina isolates possessing the MAT1-1 locus, sexual fruiting bodies (stromata) with perithecia embedded in the upper surface formed at the interface between mycelia of the two isolates – sexually produced spores (ascospores) were also observed within the perithecia (Seidl et al. 2009; Samuels et al. 1994).

3.1.2.2 Natural occurrence

Trichoderma species are “soilborne, green-spored Ascomyetes” commonly present on decaying plant matter (Schuster and Schmoll 2010). Species of the genus Trichoderma are present in soils in all climates. Typically species in this genus are decomposers of plant matter and have characteristic rapid growth as well as the ability to utilize a diverse array of substrates (Hoyos-Carvajal et al. 2009).

It is rare to isolate the anamorph without the presence of its teleomorph, H. jecorina (Kubicek et al. 2003). T. reesei appears to be specialized in a narrow ecological niche, given its limited conidiation efficiency, low diversity of carbon metabolism between strains, and infrequent detection of T. reesei in nature (Druzhinina et al.

2010). Woody plant material was present at all sites where Samuels et al. isolated H. jecorina specimens (Samuels et al. 1998).

H. jecorina is commonly regarded as a pantropical organism, and is restricted to ±20^{o} latitude around the equator (Samuels et al. 1998). H. jecorina strains (or specimens) have been isolated from:

- Central America and equatorial South America:
    - Serra Araca (Amazonas, Brazil),
    - Combu Island (Belem, Para, Brazil),
    - Sierra de la Neblina (Amazonas, Venezuela),
    - Marino Municipality (Sucre, Venezuela), and
    - Amistad International Park (Puntarenas, Costa Rica),
    - various regions of French Guiana; and
- Oceania:
    - Eastern Dumoga-Bone National Park (North Sulawesi, Indonesia),
    - various regions of North Province of New Caledonia, and
    - Membakut of British North Borneo, (Samuels et al. 1998).

Within these geographic areas, H. jecorina has been widely isolated on plants including bark and decorticated wood of dicotyledonous trees and rarely on palms, as well as on dead cacao brooms in Brazil (Lieckfeldt et al. 2000; Samuels et al. 1998).

H. jecorina has also been isolated in aquatic environments:

- sea mud of the tideland in Lianyungang, Jiangsu, China (Sun et al. 2006; identification methods not disclosed),
- soil of a storage lake in French Guiana (Lieckfeldt et al. 2000).

T. reesei has not been observed to persist or grow in the European environment (Atanasova et al. 2010, as reviewed in Kredics et al. 2014). The northernmost isolation of the anamorph T. reesei to date was in Chitan, Taiwan (Kubicek et al. 2003).

3.1.2.3 Growth parameters

T. reesei is an obligate aerobe (Breakspear and Momany 2007). The ATCC (ATCC 2014) recommends culturing T. reesei on PDA, Emmons' modification of Sabouraud's agar, or Yeast and Mould (YM) agar/broth. The optimal growth temperature of Trichoderma spp. is 25^{o}C-30^{o}C (reviewed in Gams and Bissett 1998). T. reesei can grow across a wide pH range (2.5 to 9) (Adav et al. 2011). All T. reesei strains derived from QM 6a can grow on inorganic media supplemented only with an organic carbon source; no vitamins, amino acids or other growth factors are required (Sternberg 1976).

Based on information provided by the Nominator, T. reesei strain ATCC 74252 grows well at pH 5.5, at 28-30°C on PDA, without light. Spores germinate and form a network of mycelia, which after 6-7 days produce green spores.

3.1.2.4 Survival, persistence and dispersal in the environment

Survival of a derivative of QM 6a (QM 6a#4, which was marked with a recombinant hygromycin-B resistance gene) was followed for 6 months in laboratory-contained, intact soil-core microcosms incubated in a growth chamber (Providenti et al. 2004). Different soil types (sandy loam to clay loam) and the effect of a plant rhizosphere were tested, and after four months, the soil cores were subjected to a simulated over wintering treatment of 2 weeks at 4^{o}C, followed by 3 weeks at -20^{o}C, and followed next by 2 weeks at 4^{o}C.

Results from the soil microcosm studies showed the following:

- T. reesei populations in all soils decreased over 4 months, but QM 6a#4 was still present. Pre-killed T. reesei QM 6a#4 DNA was not detected using PCR after 3 days incubation;
- QM 6a#4 remained viable after over wintering treatment, and
- In the soil with the plant rhizosphere, there was a 3-fold increase in QM 6a#4 after thawing (probably due to nutrient release from dead roots) indicating that T. reesei grew.

These results suggest that although T. reesei is rarely isolated from temperate zones, it is possible that introduced populations could survive and over winter in Canada (Providenti et al. 2004). T. reesei has also been detected in air samples taken in Europe, where it has never been observed to grow, suggesting that long-distance spore dispersal may be possible, but that spores may not be able to establish vegetative growth where conditions are suboptimal (Atanasova et al. 2010, as reviewed in Kredics et al. 2014).

3.1.2.5 Resistance to antifungal agents

Most Trichoderma species are susceptible to the antifungal agents amphotericin B, fluconazole, itraconazole, ketoconazole and miconazole (Kredics et al. 2003). The literature on pathogenic Trichoderma species indicates that treatment of severe Trichoderma infection may in some cases require a combination of treatments, for example, antifungal therapy in association with removal of the source of infection by surgery and treatment of any underlying disease that may have predisposed the patient to infection (Furukawa et al. 1998; Gautheret et al. 1995; Munoz et al. 1997; Richter et al. 1999).

No reports of antifungal susceptibility of T. reesei have been found in the literature; however, there are reports on the antifungal susceptibility of T. longibrachiatum.

Given that these two species are closely related, information on the antifungal susceptibility of T. longibrachiatum has been included for reference (Appendix A, Table A-1). The Nominator has demonstrated that the T. reesei strain ATCC 74252 is resistant to 5-flucytosine and itraconazole but is susceptible to caspofungin and voriconazole. As in the case of T. reesei strain ATCC 74252, T. longibrachiatum is resistant to 5-flucytosine and susceptible to caspofungin and voriconazole. The susceptibility profile of T. longibrachiatum to itraconazole is strain dependent.

3.1.2.6 Pathogenic and toxigenic characteristics

There is limited information on the virulence factors possessed by T. reesei or by species within the genus Trichoderma. Certain species such as T. citrinoviride, T. harzianum, T. koningii, T. viride and T. longibrachiatum have been identified as etiologic agents of a few infections in immunocompromised patients (Kredics et al. 2003).

T. longibrachiatum, which is closely related to T. reesei, possesses potential virulence factors, including:

- The ability to grow at temperatures up to 40^{o}C and over a wide pH range (2-9), making it capable of surviving in a host (Antal et al. 2005), which are traits also exhibited by T. reesei and other species of the Longibrachiatum clade (Richter et al. 1999);
- The ability to use basic amino acids (L-asparagine, aspartic acid, glutamine, glutamic acid, ornithine) as both carbon and nitrogen sources (Antal et al. 2005); and
- Cytotoxicity as measured in compounds produced by T. longibrachiatum clinical isolates UAMH 9515, ATCC 208859, and CM-382 by the boar sperm immobilization assay (Antal et al. 2005).

3.1.2.7 Enzymes produced

Cellulases produced by Trichoderma species have a long history of use in the production of food (Sukumaran et al. 2005). Many enzymes produced by T. reesei are ‘generally recognized as safe’ (GRAS) for use as food ingredients by the United States Food and Drug Administration (U.S. FDA) (U.S. FDA 2014a; U.S. FDA 2014b; U.S. FDA 2014c; U.S. FDA 2014d; U.S. FDA 2014e; U.S. FDA 2014f; U.S. FDA 2014g). T. reesei produces three kinds of enzymes which work together to degrade cellulose into glucose: β-glucosidases, exo-β-1,4 glucanases, and endo-β-1,4-glucanases (Bissett 1979).

T. reesei also produces xylanases, which specifically degrade xylans (hemicelluloses with β-1,4-linked xylopyranose units common to annual plants) (Tenkanen et al. 1992); however, its genome does not include the lignase genes which are essential for the initial digestion of plants (Maheshwari 2008). This may

limit its potential to act as a plant pathogen. T. reesei also produces lignocellulolytic enzymes, however, lignin acts as a barrier to prevent the action of lignocellulolytic enzymes in plants (Dashtban et al. 2009). Endochitinase produced by T. reesei lyses hyphae of the plant pathogenic fungus Ganoderma philipii (Harjono and Widyastuti 2001).

Chymoelastase-like, trypsin-like, and chymotrypsin-like proteases are putative virulence factors of T. longibrachiatum (Kredics et al. 2004). Proteases identified in T. reesei include:

- Trypsin-like serine protease (Dienes et al. 2007).
- Dibasic endopeptidase (Goller et al. 1998).
- Extracellular pepstatin-insensitive, N-chlorosuccinimide sensitive acidic aspartate proteases from T. reesei QM 9414 (Haab et al. 1990).
- Extracellular pepstatin-sensitive acidic aspartate protease in T. reesei D38 (Eneyskaya et al. 1999).

3.1.2.8 Secondary metabolites and mycotoxins:

T. reesei, like other Trichoderma species, produces metabolites called peptaibols also known as a peptaibiotics (Bruckner and Graf 1983). Peptaibols are a family of amphipathic polypeptides produced by soil fungi that contain the amino acid α-aminoisobutyric acid and a C-terminal hydroxylated amino acid (Chugh and Wallace 2001). T. reesei QM 6a, the parental strain of ATCC 74252, and a derived strain, QM9414, produce the peptaibol called paracelsin (Solfrizzo et al. 1994). Both these strains produce other types of peptaibols as well (Degenkolb et al. 2012; Neuhof et al. 2007).

The conditions of growth in which T. reesei produces peptaibiotics/peptaibols are not clearly understood. Conidiation (conidia formation) and factors leading to conidiation have been associated in many Trichoderma spp. with the production of peptaibols. These factors may include growth in the presence of light, growth in the presence of insoluble material, growth on solid substrates, starvation, mechanical injury and old cultures (Degenkolb et al. 2012; Komon-Zelazowska et al. 2007; Röhrich et al. 2014; Solfrizzo et al. 1994). Peptaibol production by Trichoderma species occurs in surface cultures (Berg et al. 2003; Landreau et al. 2002; Rebuffat et al. 1991; Wiest et al. 2002). Production of peptaibols is possible in submerged culture but under specific conditions which typically include complex media. Modification of the fermentation medium and the fermentation conditions (such as pH, temperature, duration, oxygen and presence or absence of insoluble material) could have an effect on the production of peptaibiotics (Brewer et al. 1987).

Industry-standard submerged fermentation conditions with no solid plant material or insoluble substrate present in the fermentation broth are not linked to the production of paracelsin (as reviewed by US EPA 2012b). Industry-standard fermentations are

typically performed in simple media in the absence of light which are conditions that are not thought to elicit the production of paracelsin and other petaibols. No information was found in the scientific literature regarding the stability of paracelsin or other peptaibols in the environment.

Peptaibols can form ion channels in plasma membranes, have antibiotic activity (Chugh and Wallace 2001) and contribute to mycoparasitism by Trichoderma species (Röhrich et al. 2014). Peptaibols from T. longibrachiatum were found to disturb embryogenesis of oysters in nanomolar concentrations (Poirier et al. 2007b). Paracelsin from T. reesei QM 9414 has antibacterial activity against Bacillus subtilis, Micrococcus luteus, Staphylococcus aureus, Streptococcus faecalis, Streptococcus lactis, and Streptococcus thermophiles (Bruckner and Graf 1983) and inhibits Phoma destructiva (Grigoriev et al. 2003). Toxic effects of paracelsin are summarized in Table 1-2. However, one of the toxicity tests was done with culture extract and not paracelsin alone and the authors concluded that some toxicity could be attributed to paracelsin but effects from other components of the extract cannot be excluded.

Table 1-2: Toxic effects of paracelsin

Organism	Toxic Effect	Reference
Human erythrocyte[a]	LC_{50} = 37 µM (equivalent to 71.08 µg/mL)	Bruckner et al. 1984
Mouse	Lethal dose 5 mg/kg (intraperitoneal injection)	Bruckner et al. 1984
Mouse[b]	Toxic dose 20 mg/kg[c]	Grigoriev et al. 2003
PC12 cells (cell line derived from pheochromocytoma of rat adrenal medulla)	LC_{50} = 21.8 µM (equivalent to 34.88 µg/mL)	Raya et al. 1993
Artemia salina (Brine shrimp) larvae 36 hour exposure[d]	LD_{50} = 2.2 µM (equivalent to 4 µg/mL)	Solfrizzo et al. 1994
Artemia salina (Brine shrimp) 24 hour exposure[e]	LC_{50} = 21.26 µM (equivalent to 40.84 µg/mL)	Favilla et al. 2006
Artemia salina (Brine shrimp) 36 hour exposure[e]	LC_{50} = 9.66 µM (equivalent to 18.56 µg/mL)	Favilla et al. 2006
Daphnia magna (Water flea) 24 hour exposure[e]	LC_{50} = 7.70 µM (equivalent to 14.79 µg/mL)	Favilla et al. 2006
Daphnia magna (Water flea) 36 hour exposure[e]	LC_{50} = 5.60 µM (equivalent to 10.76 µg/mL)	Favilla et al. 2006

[a] Induction of hemolysis at LC_{50} dose, indicating toxicity to mammalian cells
[b] Exposure to paracelsin A, an analogue of paracelsin
[c] Induction of hypothermia with spontaneous locomotor activity reduction after a single intraperitoneal administration
[d] Exposure to culture extract of T. reesei
[e] Exposure to a commercial mixture of paracelsin homologs (purity 88.4%)

3.1.3 Effects

3.1.3.1 Environment

Despite a long history of use in large-scale industrial production, no natural infections or toxic effects from T. reesei strains have ever been reported in plants, vertebrates or invertebrates in the environment. Some adverse effects have been reported in an experimental setting, as detailed below.

Microbiota

Trichoderma species are efficient mycoparasites, antagonists and biocontrol agents. Suggested mechanisms of biocontrol and inhibition of soil-borne plant pathogens include antibiosis, lysis, competition, mycoparasitism, promotion of plant growth (Baker 1988; Chet 1987; Henis 1984; Lynch 1990; Papavizas 1985), as well as metabolite secretion. The metabolites secreted by Trichoderma species act as biological fungicides and can kill pathogenic fungi (Navazio et al. 2007; Spiegel and Chet 1998; Vinale et al. 2006; Vinale et al. 2009). Specifically, peptaibols have antibiotic activity and contribute to mycoparasitism by Trichoderma species (Chugh and Wallace 2001; Röhrich et al. 2014). Paracelsin exhibits inhibitory (Grigoriev et al. 2003) and antibacterial (Bruckner and Graf 1983) effects against some micro-organism species.

Cellulases produced by T. reesei strongly inhibit Pepper Mild Mottle Virus infections in plants (Oka et al. 2008). T. reesei was found to be an effective fungal antagonist against the plant pathogens Rhizoctonia solani (Grosch et al. 2006) and Bipolaris oryzae, which causes brown spot disease in rice (Harish et al. 2008). T. reesei is capable of mycoparasitism against Pythium ultimum, an aggressive soilborne plant pathogen (do Nascimento Silva et al. 2009; Harish et al. 2008).

Aquatic plants

No adverse effects of T. reesei in aquatic plants have been reported in the scientific literature.

Terrestrial plants

Trichoderma species enhance systemic resistance of plants (Shoresh et al. 2010; Yedidia et al. 1999), and may also have beneficial effects on plant growth, aiding the growth of roots and protection from toxic chemicals (Harman 2000).

T. reesei is unlikely to be a pathogen of living plants, as its genome does not include the genes required for lignases (essential for the initial digestion of plants) (Maheshwari 2008).

Pathogenicity and toxicity studies were performed by Environment Canada scientists using Red Clover (Trifolium pretense) exposed to T. reesei strain ATCC 74252 cells in field-collected or artificial soil at a concentration of 1.2×10^6 colony forming units (CFU) of T. reesei per gram of soil. Clover seeds were allowed to grow over a period of 14 days. No significant effect of the T. reesei cells was observed on shoot length or mass, or on root length. However, these plants had a significantly greater root mass than control plants, indicating a possible beneficial effect of T. reesei towards roots of this plant.[4] It is not known if paracelsin and other peptaibols were produced in the conditions used in these experiments.

Aquatic vertebrates

No adverse effects of T. reesei in aquatic vertebrates have been reported in the scientific literature.

Terrestrial vertebrates

An extensive search of the scientific literature found no cases of T. reesei infection in terrestrial vertebrates under natural conditions. Under experimental conditions T. reesei can adversely affect immunosuppressed animals including mice, guinea pigs, and rabbits; however, death of some test animals was only observed after intravenous administration of large challenge doses (1.0×10^5 viable spores and 1.0×10^7 viable or killed spores) (Hjortkjaer et al. 1986). Additional information on animal pathogenicity studies using T. reesei is detailed in Section 1.1.3.2 Human Health.

Paracelsin produced by T. reesei is lethal to mice at a dose of 5 mg/kg delivered via intraperitoneal (ip) injection, and paracelsin A (an analogue of paracelsin) is toxic to mice at a dose of 20 mg/kg delivered via ip injection (Bruckner et al. 1984; Grigoriev et al. 2003) (Table 1-2). Toxicity of paracelsin to mammalian cells has also been observed, at LC_{50} values of 71.08 µg/mL and 34.88 µg/mL for human erythrocytes and PC12 cells, respectively (Bruckner et al. 1984; Raya et al. 1993) (Table 1-2). However, ip injection is not a relevant mode of exposure in the environment where oral ingestion is more likely in terrestrial vertebrates. This is particularly important given that peptaibols are highly resistant to proteolytic cleavage and cannot pass through the intestinal wall to reach the blood stream (Degenkolb et al. 2008). Such exposure cannot predict the effect of paracelsin following ingestion in terrestrial vertebrates.

[4] Unpublished data generated by Environment and Climate Change Canada's Ecotoxicology and Wildlife Health Division.

Aquatic invertebrates

Peptaibols and peptaibol-producing Trichoderma spp. isolated in a shellfish farming area in France had high toxicity to mussel larvae (Poirier et al. 2007a), in the first report of peptaibol isolation from naturally-occurring Trichoderma spp. in the environment.

Paracelsin produced by T. reesei could be toxic to Brine Shrip (Artemia salina) and Water Flea (D. magna), as determined through toxicity testing. In A. salina, the LC_{50} of paracelsin is 4 µg/mL for larvae, and the LC_{50} values of a commercial mixture of paracelsin homologs are 40.84 µg/mL and 18.56 µg/mL for 24 hour and 36 hour exposures in adults, respectively (Favilla et al. 2006; Solfrizzo et al. 1994) (Table 1-2). In D. magna the LC_{50} of a commercial mixture of paracelsin homologs is 14.79 µg/mL (24 hour exposure), and 10.76 µg/mL (36 hour exposure) (Favilla et al. 2006) (Table 1-2).

Terrestrial invertebrates

No adverse effects of T. reesei in terrestrial invertebrates have been reported in the scientific literature.

Pathogenicity and toxicity studies were performed by Environment Canada scientists using Collembolan (Folsomia candida)and a species of earthworm Eisenia andrei exposed to T. reesei strain ATCC 74252. Testing on F. candida was conducted over a period of 28 days in field-collected soil (1.1×10^6 T. reesei CFU per gram of soil) and artificial soil (1.4×10^6 T. reesei CFU per gram of soil). The addition of T. reesei strain ATCC 74252 had no significant effect on survival of adult F. candida or on the reproduction of the species. Testing on E. andrei was conducted over a period of 56 days in artificial soil at a concentration of 1.2×10^6 T. reesei cells per gram of soil. The addition of T. reesei strain ATCC 74252 had no significant effect on reproduction of E. andrei or individual mass of the juveniles produced. It is not known if paracelsin and other peptaibols were produced in the conditions used in these experiments.

3.1.3.2 Human health

An extensive search of the scientific literature found that T. reesei is not a known human pathogen. Despite a long history of use, T. reesei has never been implicated in any case report (Degenkolb et al. 2015).

Certain species in the genus Trichoderma, including T. citrinoviride, T. pseudokoningii, T. harzianum, T. koningii, T. viride, and T. longibrachiatum have been identified as etiologic agents in a few case reports of infection in immunocompromised patients (Kredics et al. 2003, as reviewed in Schuster and Schmoll 2010). T. longibrachiatum and H. orientalis are the most common clinical

isolates among Trichoderma spp. (Schuster and Schmoll 2010).T. viride has also been reported to cause respiratory problems in humans due to its production of volatile organic compounds (Larsen et al. 1998). Cases of infection with T. longibrachiatum reported between 1980 and 2013 are summarized in Appendix B. An overall summary of Trichoderma infections in humans is presented in Appendix C. Most Trichoderma infections are reported in individuals with underlying risk factors such as prolonged and severe neutropenia, administration of broad-spectrum antimicrobial agents, steroid therapy, mucosal barrier damage, and organ transplantation (Alanio et al. 2008; Furukawa et al. 1998; Katta et al. 2005; Kredics et al. 2003; Kuhls et al. 1999; Richter et al. 1999). Conventional antifungal therapy can be used in the successful treatment of Trichoderma infections (Espinel-Ingroff et al. 2008; Kratzer et al. 2006). Genencor completed a pathogenicity study of T. reesei strain A83 administered to rats for the U.S. FDA. An ip dose of 2.2×10^7 CFU were given to Sprague Dawley rats. No deaths occurred and no adverse signs were noted cageside or at necropsy (Genencor, 2007). However, in reports of experimental challenge, T. reesei has acted as a pathogen (Hjortkjaer et al. 1986; Nevalainen et al. 1994).

Pathogenicity studies were conducted by Hjortkjaer et al. (1986) involving cortisone-immunosuppressed mice, guinea pigs and rabbits, intravenously (iv) or ip inoculated with 1.0×10^5 or 1.0×10^7 T. reesei spores (viable or dead). Deaths in mice occurred with iv dosages of 1.0×10^7 viable or dead spores. Deaths in rabbits occurred with iv dosages of 1.0×10^5 or 1.0×10^7 viable spores; however, two of these were attributed to spontaneous disease unrelated to the challenge. No effects were seen in groups administered 10^5 viable spores ip. Lesions formed as a result of T. reesei challenge were identified in all three species tested:

- In mice, all challenge groups developed lesions in liver, kidney and lung, while abdominal abscesses were observed in all ip administration groups.
- In rabbits, chronic bronchopneumonia and chronic kidney lesions were observed in groups that received viable spores iv, and peritoneal abscesses were observed in all animals that received 1.0×10^7 viable spores ip, and some that received 1.0×10^7 dead spores ip.
- In guinea pigs, all challenge groups developed interstitial pneumonia, and four animals that received iv 1.0×10^5 or 1.0×10^7 viable spores developed liver necroses.

In all species, T. reesei was recovered from various organs up to 40 days after administration of 1.0×10^5 or 1.0×10^7 viable spores iv or 1.0×10^7 viable spores ip. Notably, all mice dosed iv with live spores exhibited fungal growth in all five organs sampled. Microscopic examination recovered T. reesei hyphae from one rabbit, spores from two mice, and hyphae with spores from a third mouse, all of which received 1.0×10^7 viable spores iv. T. reesei thus has the potential to cause adverse effects and infection in immunocompromised animals; however, this happens only when a large quantity of inoculum is administered (Hjortkjaer et al. 1986).

The toxicity and immunogenicity of enzymes and metabolites produced by T. reesei have the potential to affect human health. Paracelsin produced by T. reesei is lethal to mice at a dose of 5 mg/kg delivered via ip injection, and paracelsin A (an analogue of paracelsin) is toxic to mice at a dose of 20 mg/kg delivered also via ip injection (Bruckner et al. 1984; Grigoriev et al. 2003) (Table 1-2). Toxicity of paracelsin to mammalian cells has also been observed, at LC_{50} values of 71.08 µg/mL and 34.88 µg/mL for human erythrocytes and PC12 cells, respectively (Bruckner et al. 1984; Raya et al. 1993) (Table 1-2). However ip injection is not a relevant mode of exposure where oral ingestion is the most likely route of exposure in vertebrates. This is particularly important given that peptaibols are highly resistant to proteolytic cleavage and cannot pass through the intestinal wall to reach the blood stream (Degenkolb et al. 2008). Such exposure cannot predict the effect of paracelsin following ingestion in terrestrial vertebrates.

The acute oral LD_{50} of a T. reesei cellulase preparation (Celluclast®) is greater than 16, 8 and 5 g/kg body weight in mice, rats and dogs, respectively, and it is classified as of low toxicity based on the US EPA's Globally Harmonized System of Classification and Labelling of Chemicals (GHS) Acute Toxicity Hazard Classification (Health Canada 2014; Hjortkjaer et al. 1986). Hjortkjaer et al. (1986) reviewed the potential for dermal irritation from cellulases derived from a strain of T. reesei. A one-day skin irritation study was conducted on ten healthy adults exposed to various cellulase concentrations. The median irritant dose (ID_{50}) was reported to be approximately 0.75% (w/v), the highest concentration tested. At this dose, five out of ten volunteers showed a reaction (Hjortkjaer et al. 1986). T. reesei β-glucanase had only skin-sensitization potential, and no signs of oral or inhalation toxicity, mutagenic potential, or eye or skin irritancy were found (Coenen et al. 1995).

Like other enzymes produced by industrial micro-organisms, cellulases are potential allergens. Trichoderma-related allergies include dermatitis, rhinitis and asthma resulting from occupational exposure to Trichoderma species and commercial enzyme preparations (Halprin et al. 1973; Hytonen et al. 1994; Ransom and Schuster 1981; Tarvainen et al. 1991), and delayed respiratory distress amongst maintenance workers in a pulp mill wood chip plant (Cohn et al. 1984). In most cases, the patients had previous allergic responses to environmental allergens or also had occupational sensitivities to other enzymes.

In a skin sensitization test performed in 25 adult volunteers over a period of ten days, each individual received a forearm patch containing 0.3 g of the cellulase applied under an occlusive dressing for a 48-hour period. After a 10-day rest period, a challenge patch was applied for 48 hours. Observation immediately after removal and 24 hours later revealed no instances of contact sensitization (Hjortkjaer et al. 1986).

3.2 Hazard severity

Since the 1960s Trichoderma species have been used commercially to produce carbohydrases for applications in the food, animal feed, health product and forestry industries. Based on this industrial experience of safe use, T. reesei is currently considered by many authors in the scientific literature to be a safe production organism (Miettinen-Oinonen and Suominen 2002; Nevalainen et al. 1994; Sukumaran et al. 2005).

3.2.1 Environment

The environmental hazard potential of T. reesei strain ATCC 74252 is assessed to be low-medium based on the following considerations:

1. T. reesei acts as a biological fungicide inhibiting fungal plant pathogens by various mechanisms, including: antibiosis, lysis, competition/antagonism, mycoparasitism, metabolite secretion, and in plants, enhancement of systemic resistance and growth promotion. Paracelsin produced by T .reesei QM 9414 has antibacterial and antifungal activity.
2. T. reesei has not been reported to act as an aquatic or terrestrial plant pathogen and may lack enzymes necessary for pathogenicity. It may, however, have positive effects on plant growth and systemic resistance to infection and toxic chemicals. Pathogenicity and toxicity testing using T. reesei strain ATCC 74252 has shown a lack of deleterious effects towards T. pratense and has shown a potential beneficial effect of the strain on T. pratense roots.
3. There are no reports in the scientific literature with respect to adverse effects in aquatic vertebrates.
4. An extensive search of the scientific literature found no cases of adverse effects or T. reesei infection in terrestrial vertebrates under natural conditions. Under experimental conditions T. reesei can cause adverse effects in immunosuppressed animals including mice, guinea pigs, and rabbits, however, deaths were only observed after ip administration of large challenge doses (1.0×10^5 viable spores and 1.0×10^7 viable or killed spores). Paracelsin produced by T. reesei is toxic to mammalian cells and mice.
5. T. reesei has not been observed to adversely affect aquatic invertebrates; however, paracelsin produced by T. reesei is toxic to A. salina larvae and adults, and D. magna adults. Peptaibol compounds, like paracelsin, also have a high toxicity to mussel larvae. It is not known if T. reesei strain ATCC 74252 produces peptaibols such as paracelsin, but it is reasonable to assume that it can, as this is an attribute of strain QM 6a, from which it was derived.
6. In spite of its natural presence in tropical soils, an extensive search of the scientific literature found no cases of adverse effects or T. reesei infection in terrestrial invertebrates. Pathogenicity and toxicity testing using T. reesei

strain ATCC 74252 has shown a lack of deleterious effects towards F. candida and E. andrei adults and juveniles.

3.2.2 Human health

The human hazard potential of T. reesei strain ATCC 74252 is assessed to be low based on the following considerations:

1. There are no reported cases in the literature of human infections caused by T. reesei.
2. Closely related species such as T. longibrachiatum have caused infections, but only in severely immunocompromised individuals.
3. Under experimental conditions T. reesei can cause adverse effects in immunosuppressed animals including mice, guinea pigs, and rabbits, however, deaths were only observed after iv administration of large challenge doses (1.0×10^5 viable spores and 1.0×10^7 viable or killed spores).
4. In the unlikely event of infection with T. reesei strain ATCC 74252, it is susceptible to antifungal treatments that have been clinically proven to be effective against other Trichoderma species.
5. While T. reesei has not been observed to adversely affect humans; paracelsin produced by T. reesei is toxic to mammalian cells and mice in experimental conditions where natural barriers were bypassed. It is not known if T. reesei strain ATCC 74252 produces paracelsin, but it is reasonable to assume that it can, as this is an attribute of strain QM 6a, from which it was derived.
6. T. reesei has a long history of use in enzyme production without incidents of infection; however, repeated occupational exposure to commercial enzyme preparations (e.g., cellulases) produced by T. reesei have led to Trichoderma-related allergies such as dermatitis, rhinitis and asthma.

Hazards related to micro-organisms used in the workplace should be classified 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 Hazardous Products Regulations, which is part of the regulatory framework for the Workplace Hazardous Materials Information System (WHMIS) for products intended for workplace use.

4. Exposure assessment

4.1 Sources of exposure

This assessment considers exposure to T. reesei strain ATCC 74252 resulting from its addition to consumer or commercial products and its use in industrial processes in Canada.

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 T. reesei strain ATCC 74252 was in commercial use in 2006. 1 000 – 10 000 kg of T. reesei strain ATCC 74252 dry cell mass was produced in 2006 for industrial uses.

The 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 T. reesei strain ATCC 74252, whether alone, in a mixture or in a product. In 2008, 10 000 – 100 000 kg of T. reesei strain ATCC 74252 dry cell mass was produced for industrial uses.

A search of the public domain (safety data sheets (SDS), literature and patents) revealed the following consumer, commercial and industrial applications of other strains of T. reesei. These represent potential uses of T. reesei strain ATCC 74252, as this strain is likely to share the characteristics (modes of action) with other commercialized T. reesei strains:

Use as a production organism for the following:

- enzymes used in the degradation of cellulosic biomass (Merino et al. 2008; Peciulyte et al. 2014; Schuster and Schmoll 2010);
- enzyme used for food and feed additives (Galante et al. 1998; Nevalainen et al. 1994; Schuster and Schmoll 2010; Rhode Jr. et al. 1998);
- enzyme such as alpha glucanases used for oral care (Kim et al. 2009);
- enzymes used in mashing and filtration step in brewing (Elvig and Festersen 2005);
- meat tenderization enzymes (Robbins et al. 1986);
- enzymes used for treating cotton fabrics (Clarkson et al.1992);
- proteins used in the textile, detergent and pulp and paper industries (Baeck et al. 2000; Foreman et al. 2010);
- production of glycoproteins, antimicrobial proteins, heterologous proteins (calf chymosin) and immunologically active antibody fragments (Bobrowicz et al. 2007; Harkki et al. 1989; Masri et al. 2013; Nevalainen et al. 2005 Nyyasonen et al. 1993; Uusitalo et al. 1991);

- production of fuels/alcohols (Bonaccorsi et al. 2006);
- fungicidal protective coatings and fungicides (Batdorf and Brendle 1998; Schuster and Schmoll 2010); and
- synthesizing silver nanoparticles (Mansoori 2013; Vahabi and Dorcheh 2014).

T. reesei is recognized as source(s) of the enzyme preparations of protease aminopeptidase, as well as of the carbohydrase cellulase, beta-glucanase, beta-d-glucosidase, and hemicellulose and pentosanase in the Food Chemicals Codex (FCC 2016)

Furthermore, T. reesei have the potential to be used in biodegration of polycyclic aromatic hydrocarbons and in bioremediation (Cocaign et al. 2013; Zafra and Cortés-Esponosa 2015). In addition, T. reesei strain ATCC 28217 is listed in a patent for microbial pesticides as one of many micro-organism strains intended for direct application to soils, plants and seeds (Bok et al. 1996).

Although T. reesei strain ATCC 74252 is on the DSL and can be used in Canada without prior notification, it is a proprietary strain to which only the nominating company has access. As a result, the application of T. reesei strain ATCC 74252 for any identified potential uses in Canada is limited relative to publicly available strains.

4.2 Exposure characterization

T. reesei strain ATCC 74252 is a proprietary strain only available through the Nominator, and is thus less likely than publicly available strains to be used in potential applications identified in Section 2.1 Sources of Exposure; however, as the Nominator could sell the strain or use it differently, potential future exposure scenarios have also been considered. T. reesei strain ATCC 74252 is most likely to be used as an industrial production organism. Releases from production facilities are expected to be limited by the application of good manufacturing practices, in which measures should be taken to minimize release of production micro-organisms.

4.2.1 Environment

The overall environmental exposure estimation for T. reesei strain ATCC 74252 is low. based on responses to the section 71 notice in which reported uses were limited to industrial processes within a contained facility. The industrial process currently used with T. reesei strain ATCC 74252 is not expected to release paracelsin in the environment.

Should potential uses identified in Section 2.1 be realized in Canada, T. reesei strain ATCC 74252 could be applied as bioremediation, biodegradation or pesticidal strain. If so, the living organism could be released into the environment during application to soils, plants and seeds, exposing terrestrial plants and invertebrates, and to a lesser extent terrestrial vertebrates feeding at the site of application, and aquatic

species via runoff from treated plants and soils. The extent of environmental exposure to T. reesei strain ATCC 74252 will depend on the quantity released, and on its survival, persistence and dispersal in the receiving environment. Pesticidal products containing T. reesei strain ATCC 74252 for use in Canada would be subject to environmental and human health assessment and registration under the Pest Control Products Act.

Although Trichoderma species are common soil borne fungi that are found across the planet, T. reesei is known to be a pantropical organism that is specialized in narrow habitats, restricted to ±20° latitude around the equator. T. reesei strain ATCC 74252 has an optimal growth temperature of 28-30°C. Soil microcosm studies have shown that T. reesei could survive cold periods (4°C to – 20°C) for 7 weeks with added nutrients, indicating that it could overwinter where winters are mild in Canada. Nevertheless, spore dispersal documented in European air samples has not led to the establishment of T. reesei in Europe, so it is likely that introduced populations would not persist in the long term. In addition, maintenance of high numbers of introduced microorganisms beyond background levels is unlikely due to natural competition with naturally occurring microorganisms in the environment (Dobbs and Hinson 1953).

4.2.2 Human

The overall human exposure estimation for T. reesei strain ATCC 74252 is low based on responses to the section 71 notice in which reported uses were limited to industrial processes within a contained facility.

Should potential uses identified in Section 2.1 be realized in Canada, T. reesei strain ATCC 74252 could be applied as a bioremediation, biodegradation or pesticidal strain. If so, it could be released into the environment during application to soils, plants and seeds. The extent of human exposure to T. reesei strain ATCC 74252 would depend on the quantity released, and the proximity of bystanders to the site of application. Exposure is not expected from the use of natural health products containing enzymes produced from T. reesei, enzyme preparations are expected to be meet finished product specifications.

5. Risk characterization

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

Hazard has been estimated for T. reesei strain ATCC 74252 to be low-medium for the environment, because of the potential for paracelsin and other peptaibols production under certain growth parameters, and low for human health.

Environmental and human exposure to T. reesei strain ATCC 74252 or paracelsin is not currently expected because current uses of T. reesei strain ATCC 74252 occur in a contained facility under growth parameters which are not known to lead to the production of paracelsin and other peptaibols. The risk associated with current uses is therefore 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 or altered growth parameters). T. reesei strain ATCC 74252 is a proprietary strain, which has limited potential for increased use relative to publicly available strains; however, there remains the possibility that the Nominator could undertake or sell T. reesei strain ATCC 74252 for new activities.

The potential hazard associated with T. reesei strain ATCC 74252 is related to its presumed ability to produce paracelsin or other peptaibols, which have a potential toxicity to aquatic invertebrates and mammals. Paracelsin and other peptaibols are not known to be produced under current growth conditions, which adhere to the following parameters: submerged fermentation in the absence of light with no solid plant material or insoluble substrate present, and fermentation of solid plant material or insoluble substrate in the fermentation broth which is only initiated after specified inactivation of T. reesei. Paracelsin or other peptaibols could, however, be produced in the event that other growth parameters are used. These different growth conditions may include submerged liquid fermentation in the presence of insoluble material, as well as solid state fermentation. Release of large quantities of paracelsin or other peptaibols into the environment could pose a potential risk to aquatic invertebrates.

Should T. reesei strain ATCC 74252 be developed as a pesticidal strain, there is potential for widespread environmental release. In Canada, microbial pest control agents and end-use pesticide products containing T. reesei strain ATCC 74252 would be subject to registration under the Pest Control Products Act. These uses of T. reesei strain ATCC 74252 would therefore undergo a complete environmental and human health risk assessment, and any necessary risk mitigation measures would be applied by the Pest Management Regulatory Agency.

6. Conclusion

Based on the information presented in this screening assessment, it is concluded that Trichoderma reesei strain ATCC 74252 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 T. reesei strain ATCC 74252 does not meet any of the the criteria set out in section 64 of the CEPA.

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APPENDICES

Appendix A: Antifungal resistance and susceptibility of T. longibrachiatum

Table A-1: Minimum inhibitory concentrations (MIC) for T. longibrachiatum strains

Antifungal agent	MIC (µg/mL)	Interpretation based on reports in literature	References
5-fluorocytosine	≥64	Resistant	Furukawa et al. 1998; Munoz et al. 1997; Myoken et al. 2002; Trabelsi et al. 2010
Akacid Plus®	0.06 - 0.5	Sensitive	Kratzer et al. 2006
Amphotericin B	0.016 - 8	Sensitive to Intermediate to Resistant (Strain Dependent)	Antal et al. 2005; Espinel-Ingroff 2001a; Espinel-Ingroff 2001b; Furukawa et al. 1998; Kratzer et al. 2006; Molnár-Gábor et al. 2013; Munoz et al. 1997; Myoken et al. 2002; Richter et al. 1999; Santillan Salas et al. 2011; Seguin et al. 1995; Tanis et al. 1995; Trabelsi et al. 2010
Caspofungin	≤0.5	Sensitive	Molnár-Gábor et al. 2013; Santillan Salas et al. 2011; Trabelsi et al. 2010
Chlorhexidine Digluconate	1 - 8	Sensitive to Intermediate (Strain Dependent)	Kratzer et al. 2006
Fluconazole	Most >64, 12.5, 16	Resistant	Antal et al. 2005; Furukawa et al. 1998; Kratzer et al. 2006; Molnár-Gábor et al. 2013; Munoz et al. 1997; Myoken et al. 2002; Richter et al. 1999; Seguin et al. 1995
Itraconazole	0.3 - >32	Sensitive to Intermediate to Resistant (Strain Dependent)	Antal et al. 2005; Espinel-Ingroff 2001a; Hennequin et al. 2000; Molnár-Gábor et al. 2013; Munoz et al. 1997; Myoken et al. 2002; Seguin et al. 1995

Antifungal agent	MIC (µg/mL)	Interpretation based on reports in literature	References
Ketoconazole	0.008 - 1	Sensitive	Antal et al. 2005
Posaconazole	0.5 - ≥2	Sensitive to Intermediate to Resistant (Strain Dependent)	Santillan Salas et al. 2011; Trabelsi et al. 2010
Voriconazole	0.5 - 2	Sensitive	Espinel-Ingroff 2001a; Espinel-Ingroff 2001b; Kratzer et al. 2006; Myoken et al. 2002; Santillan Salas et al. 2011; Trabelsi et al. 2010

Appendix B: Case reports of infection caused by T. longibrachiatum strains

Table B-1: Summary of case reports of human infection with T. longibrachiatum

Immune status	Reaction	Treatment	Outcome	Reference
Unknown; history of atopy and asthma	Allergic fungal sinusitis	Allergen immunotherapy and oral itraconazole, corticosteroids and intranasal itraconazole	Resolved	Tang et al. 2003
Immunocompromised; organ transplantation	Acute invasive sinusitis	Amphotericin B and sinus operations for debridement	Resolved	Furukawa et al. 1998
Immunocompromised; allogeneic bone marrow transplantation	Disseminated infection	Amphotericin B, itraconazole	Fatal	Richter et al. 1999
Immunocompromised; cardiac surgery	Mediastinitis and peritonitis	Caspofungin and voriconazole, amphotericin deoxycholate	Fatal	Santillan Salas et al. 2011
Unknown; chronic otitis	Otitis externa	Amoxicillin and topical ofloxacin, nystatin and polymyxin B and oxytetracycline	Resolved	Hennequin et al. 2000
Immunocompromised; severe aplastic anemia and prolonged neutropenia	Invasive skin and subcutaneous infection	Amphotericin B, Amphotericin B lipid complex, bone marrow transplant	Resolved	Munoz et al. 1997
Immunocompromised; acute lymphoblastic leukemia and severe neutropenia	Invasive pulmonary infection	Voriconazole and Caspofungin	Resolved	Alanio et al. 2008
Immunocompromised; leukemia and prolonged neutropenia	Brain abscess	Neurosurgical resection, itraconazole and amphotericin B	Resolved	Seguin et al. 1995
Immunocompromised; malignant lymphoma and neutropenia	Necrotizing stomatitis	Amphotericin B and itraconazole	Fatal	Myoken et al. 2002
Immunocompromised; continuous ambulatory peritoneal dialysis	Fungal peritonitis	Amphotericin B	Fatal	Tanis et al. 1995
Immunocompromised;	Subcapsular	Surgical	Resolved	Chouaki et al.

Immune status	Reaction	Treatment	Outcome	Reference
liver transplantation	hepatic collection	debridement, povidone iodine		2002
Immunocompromised; lung transplantation	Infection found in bronchoalveolar lavage, pleural drains	Lipid-associated amphotericin B	Fatal	Chouaki et al. 2002
Immunocompromised; renal transplantation	Skin infection	Voriconazole	Resolved	Trabelsi et al. 2010
Immunocompromised; bone marrow transplantation	Invasive infection	Liposomal amphotericin B, flucytosine	Fatal	Gautheret et al. 1995[a]
Immunocompetent	Sinusitis sphenoidalis	Surgery, steroid nasal lavage, amphotericin B, suction	Resolved	Molnár-Gábor et al. 2013

[a]Reidentified as T. longibrachiatum in (Kuhls et al. 1999) via DNA fingerprinting and ITS comparison.

Appendix C: Case reports of infection caused by Trichoderma species

Table C-1: Reported cases of Trichoderma species isolated from humans (adapted from Sandoval-Denis et al. 2014)

Species	Number of isolates from Superficial	Number of isolates from Respiratory	Number of isolates from Deep tissue
T. longibrachiatum	3	10	6
T. citrinoviride	3	5	5
T. bissettii	4	3	2
T. orientale	1	3	4
H. lixit/T. harzianum	2	3	1
T. koningiopsis	1	1	0
T. asperelloides	1	0	0
T. erinaceum	1	0	0
T. sinuosum	1	0	0
T. asperellum	0	1	0
T. gansii	0	1	0
T. atroviride	0	0	1