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Final Screening Assessment for *Candida utilis* ATCC 9950

Environment and Climate Change Canada
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Synopsis

Pursuant to paragraph 74(*b*) of the *Canadian Environmental Protection Act*, 1999 (CEPA 1999), the Minister of Environment and Climate Change and the Minister of Health have conducted a screening assessment of *Candida utilis* ATCC 9950.

C. utilis ATCC 9950 is a yeast that has characteristics in common with other strains of the species *C. utilis*. *C. utilis* can adapt to varying conditions and thrives in soil and water. Multiple potential uses of *C. utilis* in consumer, industrial, commercial and agricultural sectors exist. These include production of food, natural health products, feeds, biochemicals used in cosmetics and therapeutic drugs, bioremediation and wastewater treatment.

C. utilis has an established history of use as a feed supplement in aquaculture, swine, poultry, and livestock diets, yet only two incidents of infection in vertebrates have been attributed to *C. utilis*. In both cases, the affected animals had pre-existing conditions and the infections were effectively treated with antifungals. No reports in the literature showed significant effects of *C. utilis* in terrestrial or aquatic plants or invertebrates. Certain strains of *C. utilis* have anti-algal, antibacterial and anti-fungal properties, which allow its use as a biocontrol agent against pest micro-organisms.

Although *C. utilis* has also been extensively used in the food industry, the incidence of human infection with *C. utilis* is exceedingly low. There have been no reported human infections attributed specifically to the *Domestic Substances List* (DSL) strain *C. utilis* ATCC 9950; however, some strains of *C. utilis* can act as opportunistic pathogens in susceptible individuals, particularly those who have a weakened immune system or underlying medical conditions.

This assessment considers the aforementioned characteristics of *C. utilis* ATCC 9950 with respect to environmental and human health effects associated with consumer and commercial product use and industrial processes subject to CEPA 1999, including releases to the environment through waste streams and incidental human exposure through environmental media. A conclusion under CEPA 1999 on this living organism has no bearing on and does not preclude assessments, authorized under *the Food and Drugs Act*, of products produced by or containing *C. utilis* ATCC 9950. *C. utilis* ATCC 9950 was nominated to the DSL for use in the food industry. To update information on current uses, the Government launched a mandatory information-gathering survey under section 71 of CEPA 1999 (section 71 notice) as published in the *Canada Gazette*, Part I, on October 3, 2009. Information submitted in response to the notice indicates that *C. utilis* ATCC 9950 was imported into Canada in 2008 for use in food production and processing. No uses related to consumer products were reported in Canada.

Considering all available lines of evidence presented in this screening assessment, there is low risk of harm to organisms and the broader integrity of the environment from *C. utilis* ATCC 9950. It is concluded that *C. utilis* ATCC 9950 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.

Based on the information presented in this screening assessment, it is concluded that *C. utilis* ATCC 9950 does not meet the criteria under paragraph 64(c) of CEPA as it is not entering the environment in a quantity or concentration or under conditions that constitute or may constitute a danger in Canada to human life or health.

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Introduction

Pursuant to paragraph 74*(b)* of the *Canadian Environmental Protection Act, 1999* (CEPA 1999), the Minister of Environment and the Minister of Health are required to conduct screening assessments of those 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 as set out in section 64 of CEPA 1999).[1] *Candida utilis* ATCC 9950 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 and entered or was released into the environment without being subject to conditions under CEPA 1999 or any other Act of Parliament or of the legislature of a province.

This screening assessment considers hazard information obtained from the public domain and from unpublished research data, as well as comments from scientific peer reviewers. Exposure information was also obtained from the public domain and from a mandatory CEPA 1999 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 entitled "Framework on the Science-Based Risk Assessment of Micro-organisms under the *Canadian Environmental Protection Act, 1999*" (Environment Canada and Health Canada 2011).

In this report, data that are specific to the DSL-listed strain, *C. utilis* ATCC 9950, are identified as such. Strain-specific data include information from the Nominator, the American Type Culture Collection (ATCC) and unpublished data generated by Health Canada[2] and Environment Canada[3] research scientists. Where strain-specific data were not available, surrogate information from literature searches was used. When applicable, literature searches conducted on the organism included its synonyms, and common and superseded names. Surrogate organisms are identified in each case to the taxonomic level provided by the source. Literature searches were conducted using scientific literature databases (SCOPUS, CAB Abstracts, and NCBI PubMed), web searches, and key search terms for the identification of human health and environmental hazards. Information identified up to June 2014 was considered for inclusion in this screening assessment report.

[1] A determination of whether one or more criteria of section 64 of CEPA 1999 are met is based on an assessment of potential risks to the environment and/or to human health associated with exposure in the general environment. For humans, this includes, but is not limited to, exposure from air, water and the use of products containing the substances. A conclusion under CEPA 1999 may not be relevant to, nor does it preclude, an assessment against the criteria specified in the *Controlled Products Regulations*, which is part of the regulatory framework for the Workplace Hazardous Materials Information System (WHMIS) for products intended for workplace use.
[2] Testing conducted by Health Canada's Environmental Health Science and Research Bureau
[3] Testing conducted by Environment Canada's Biological Methods Division

Decisions from Domestic and International Jurisdictions

Domestic

The Public Health Agency of Canada (PHAC) assigned *C. utilis* (as a species) to Risk Group 1 (low individual and community risk) for both humans and terrestrial animals (personal communication, PHAC 2014).

The Canadian Food Inspection Agency (CFIA) does not consider *C. utilis* as a regulated plant pest in Canada (personal communication, CFIA 2014). In addition, single ingredient feeds containing dehydrated Yeast Torula (common name for *C. utilis*), are exempt from the registration requirement under Part I, Schedule IV of the *Feeds Regulations* (CFIA 2014).

C. utilis, listed as dried Torula yeast, is an approved non-medicinal ingredient in the Natural Health Products Ingredients Database (NHPID). It can be safely used provided the total folic acid content of the yeast does not exceed 0.04 milligram per gram of yeast as permitted by the U.S. FDA and it must also comply with the monograph for Yeast, dried in the Food Chemicals Codex (Health Canada 2015).

International

In 2013, the United States Food and Drug Administration (U.S. FDA) under Section 172.896 of the Code of Federal Regulations permitted the use of dried *C. utilis* (also referred as Torula yeast) as a food additive for human consumption provided that the total folic acid of the yeast does not exceed 0.04 mg/g of yeast (U.S. FDA 2013).

Hazard Assessment

1.1 Characterization of *Candida utilis*

1.1.1 Taxonomic identification and strain history

Binomial name *Candida utilis* (*C. utilis)*

Taxonomic designation

Kingdom	Fungi
Phylum	Ascomycota
Subphylum	Saccharomycotina
Class	Saccharomycetes
Order	Saccharomycetidae
Family	*Saccharomycetales*
Genus	*Candida*
Species	*utilis* (Henneberg) Lodder et Kreger-van Rij (1952)
Strain	ATCC 9950 (equivalent to NRRL Y-900, CBS 5609, DSM 2361, NBRC 0988)

Superseded names: *Torula utilis* Henneberg, *Saccharomyces jadinii* (Sartory, R. Sartory, Weill & J. Meyer), *Torulopsis utilis* (Henneberg) Lodder var. *utilis*

Anamorph of: *Hansenula jadinii* (Sartory, R. Sartory, Weill & J. Meyer), *Pichia jadinii* (Sartory et al.) Wickerham, *Lindnera jadinii* (A. & R. Satory, Weill & J. Meyer), *Cyberlindnera jadinii* (R. Sartory, R. Sartory, Weill & J. Meyer).

C. utilis is an asexual non-filamentous ascomycete. It is the asexual (anamorph) state of *Pichia* (*Hanensula, Cyberlindnera*) *jadinii* (Kurtzman et al. 1979; Yamada et al. 1995). Regardless of the reproductive state, the names *P. jadinii* and *C. utilis* have been used synonymously in numerous publications (Kurtzman 1988, Barnett 2004).

Common names: Torula Yeast, Fodder Yeast

Strain history

According to the United States Department of Agriculture's ARS Culture Collection, the organism was originally isolated from animal fodder (USDA ARS 2014). It was deposited at the USDA ARS Culture Collection (NRRL) as NRRL Y-900 and to the American Type Culture Collection as ATCC accession number 9950. The strain was also deposited in various culture collections with the following designations: CCRC 20325, IFO 0988, NCYC 707, NRCC 2721, VTT C-78085.

1.1.1.2 Phenotypic and molecular characteristics

The genus *Candida* contains over 150 species (Barnett et al. 2000; Dorko et al. 2000). With the exceptions of *C. glabrata* and *C. krusei*, almost all of the pathogenic *Candida* species, including *C. albicans*, *C. tropicalis, C. dubliniensis,* and *C. parapsilosis*, belong in a single *Candida* clade characterized by the unique translation of CUG codons as serine rather than leucine (Butler et al. 2009). *C. utilis,* on the other hand, is a member of a distinct clade separate from other *Candida* yeasts including those commonly associated with human disease (Buerth et al. 2011; Tomita et al. 2012).

The purpose of this section is to describe methodologies that can be used to distinguish between *C. utilis* and other *Candida* species, particularly *C. albicans* which is a commensal gut yeast in humans but is also the most common cause of opportunistic fungal infection. A polyphasic approach is important in generating a robust taxonomic identification that allows for clear differentiation of *C. utilis* from closely-related pathogenic *Candida* species.

In clinical settings, culture-based rapid identification methods based on biochemical and metabolic endpoints, such as the *Candida* ID, API *Candida*, API 20C, API ID 32C, VITEK System, and RapID Yeast Plus (Barnett et al. 2000; Fricker-Hidalgo et al. 2001; Hata et al. 2007; Verweij et al. 1999) are often used for the diagnostic identification of *Candida* and other clinically relevant yeasts.

Phenotypic properties comparing *C. utilis* ATCC 9950 with *C. albicans* are summarized in Tables 1-1 and 1-2. Morphology of *C. utilis* on chromogenic agar, corn meal-Tween 80 agar and in a germ tube test is distinctly different (Table 1-1).

Table 0-1: Morphological properties of *C. utilis* ATCC 9950

Characteristic	***C. utilis* ATCC 9950 (DSL strain)**[a]	***C. albicans* ATCC MYA-2786 (equivalent to SC5314)**[a]
Chromogenic agar	Circular, pink, glossy	Circular, green smooth
Corn meal-Tween 80 agar	Pseudohyphae No chlamydospore	Hyphae Chlamydospores
Germ tube test	Negative	Positive
Czapek agar	3 mm diameter, circular, entire, flat-convex, white/off-white, moist, opaque	1 mm diameter, circular, entire, flat-convex, white/off-white, opaque

[a] Unpublished data generated by Health Canada's Healthy Environments and Consumer Safety Branch. Refer to Appendix A Figure A-1 for growth on Corn Meal Tween agar and Table A-1 for growth on various media at temperatures

In contrast to *C. albicans, C. utilis* does not form chlamydospores (Kondo et al. 1995; NCYC 2014) (shown in Figure A-1), which when present in *C. albicans* are developed at true hyphal extremities. The presence of chlamydospores is the basis of a method for rapid presumptive identification of *Candida* species infection: the germ tube test (Sheppard et al. 2008).

In contrast to the true hyphae seen in *C. albicans*, *C. utilis* (including ATCC 9950) produces only simple pseudohyphae (Kurtzman et al. 1979) (Refer to Figure A-1). Structurally, hyphae are narrower and more uniform than pseudohyphal buds, which have a constriction between the mother and daughter cells (reviewed in Sudbery et al. 2004).

Table 0-2: Biochemical properties of *C. utilis* ATCC 9950

Characteristic	***C. utilis* ATCC 9950 (DSL strain)**	***C. albicans* ATCC MYA-2786 (equivalent to SC5314)**
Growth at 37°C on YPD	Yes (CBS-KNAW, 2014)	Yes (Thewes et al. 2008)
Growth at 42°C on YPD	No (CBS-KNAW, 2014)	Yes (Lorenz and Fink, 2001)
Crabtree effect (production of ethanol in aerobic condition)	Negative (Tomita et al. 2012)	Negative (Helmerhorst et al. 2006)
Trehalose assimilation	Negative[a]	Positive[a]
Maltose assimilation	Negative[a]	Positive[a]
Sucrose assimilation	Positive[a]	Positive[a]
L-rhamnose assimilation	Negative (Kurtzman et al. 1979)	Negative (CBS-KNAW, 2014)

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

Fatty acid compositional analysis, shown in Figure1-1, was conducted by Health Canada scientists on *C. utilis* ATCC 9950 using GC-FAME and the Sherlock® MIDI Microbial Identification System.

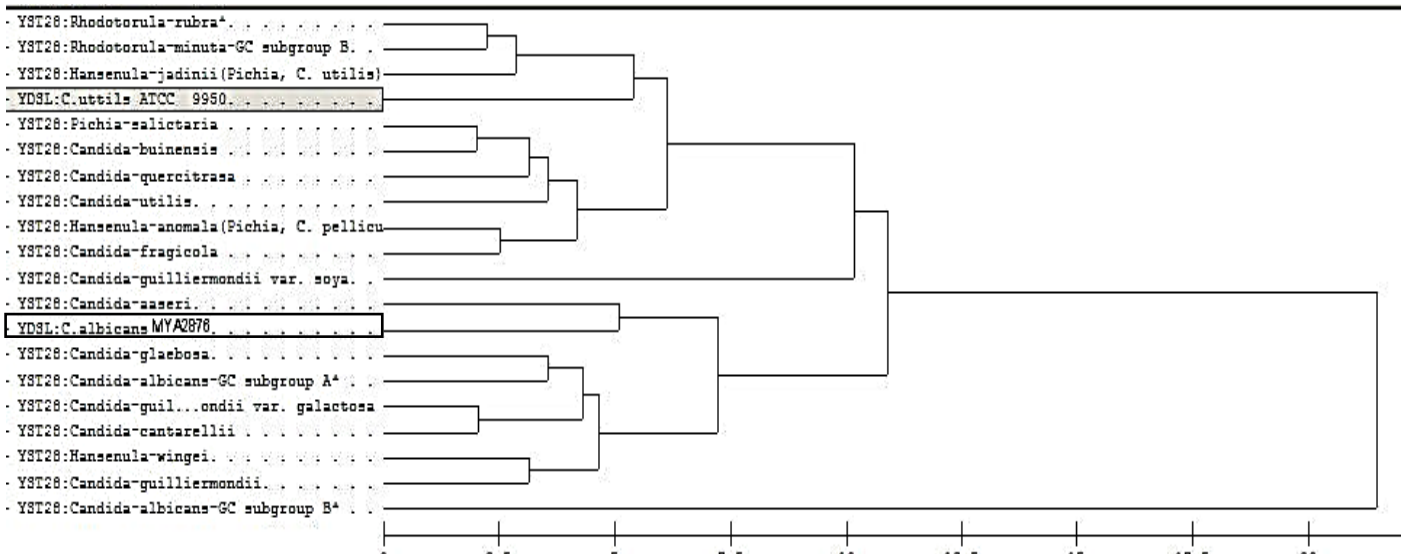


Figure 0-1: Fatty acid methyl ester (FAME) analysis of *C. utilis* ATCC 9950 using MIDI YST28 Yeast Database

Based on cellular fatty acid composition, the DSL *C. utilis* strain, ATCC 9950, is not closely related to *C. albicans*. Similarly, Singh et al. (2010) demonstrated that *C. utilis* has a very distinctive phospholipid profile compared to *C. albicans* using electrospray ionization tandem mass spectrometry.

The secreted proteome of *C. utilis* ATCC 9950, grown on SD medium, was analyzed by Buerth et al. (2011) using mass spectroscopy. A total of 37 proteins were identified and compared with the secreted proteome of *Saccharomyces cerevisiae, C. albicans* and *C. glabrata.* Most of the *C. utilis* ATCC 9950 secreted proteins, such as glucanases, glucanosyltransferase, chitin transglycosylase, and thioredoxin, are homologous and function similarly to those secreted by *C. albicans* and *C. glabrata*. These proteins are used for cell wall assembly and maintenance, and stress response. Others are unique to *C. utilis* ATCC 9950, and their presence could be helpful in its identification. They include proteins associated with carbon metabolism and nitrate assimilation, such as invertase and asparaginase, respectively (Buerth et al. 2011). Proteins related to pathogenicity in *C. albicans*, such as aspartyl proteases and the Op4 protein, were not detected in *C. utilis* ATCC 9950 (Buerth et al. 2011). For more details about the role of these proteins in pathogenicity, refer to section 1.2.9.

Phylogenetic analysis was conducted by Health Canada scientists in 2013 on *C. utilis* ATCC 9950 and clinically significant *Candida* species using publicly available *Candida* 18S and 28S rRNA gene sequences and on *C. utilis* ATCC 9950 sequences generated in-house. The resulting dendrogram (Figure 1-2) demonstrates the divergence between *C. utilis* ATCC 9950 and pathogenic *Candida* species, such as *C. albicans*, *C. dubliniensis*, *C. tropicalis,* and *C. parapsilosis*, and to a lesser extent *C. glabrata*. This finding is consistent with others reported in the scientific literature (Buerth et al. 2011; Tomita et al. 2012).

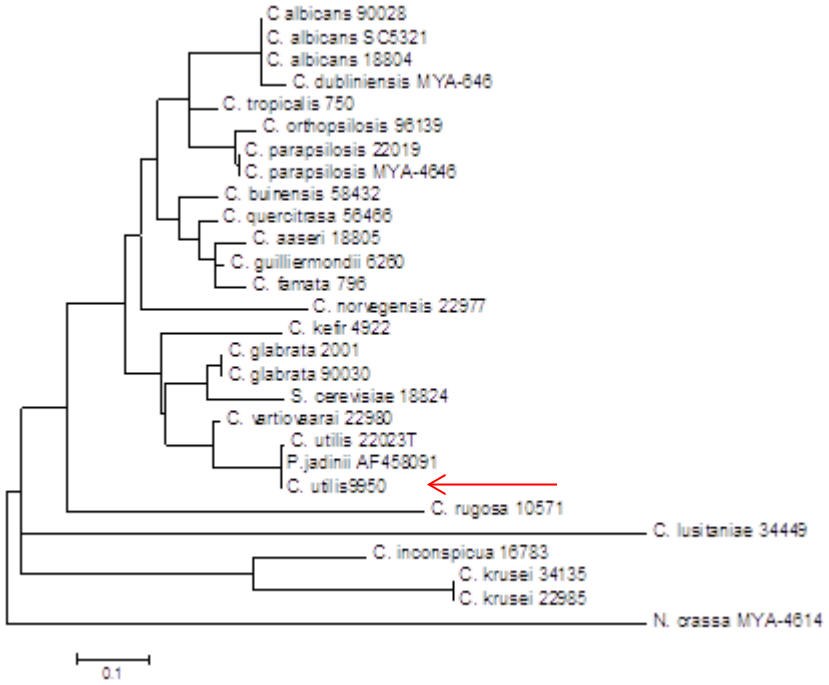


Figure 0-2: Phylogenetic analysis of select *Candida* species based on the alignment of the ITS1 and ITS2 sequences of the 18S and 28S rRNA genes

The genome of *C. utilis* ATCC 9950 was sequenced by Buerth et al. (2011) and Tomita et al. (2012). The genomic composition determined from the shotgun sequence determined by Tomita et al.(2012) is summarized in Table 1-3.

Table 0-3: Molecular properties of *C. utilis* ATCC 9950 predicted from shotgun sequences

Characteristic	*C. utilis* ATCC 9950[a]
Genome size	14.6 Mb
Predicted number of genes	8864
Assembled chromosomes	13
Unmapped chromosomes	1
G+C content (%)	45.36
Coding (%)	59.2
tRNA genes	191
rRNA genes	27
Ploidy	Tetraploid
Genomic Sequence Accession Numbers	BAEL01000001 – BAEL01001163

[a] Adapted from Tomita et al. (2012)

The large subunit (LSU) D2 rDNA and ITS1-5.8S rDNA-ITS2 regions from *C. utilis* ATCC 9950 were sequenced by Health Canada researchers based on methods by Chen et al. (2000) and Ciardo et al. (2006) and were used as query sequences against the fungal database of the Applied BioSystems Microseq and the DNA databases of the National Center for Biotechnology Information (NCBI) using the

Basic Local Alignment Search Tool (BLAST). Using the LSU D2 region sequence for comparison in the Microseq database, *C. utilis* ATCC 9950 is 99.8% homologous to its teleomorph *Pichia jadinii* (type strain ATCC 18201). NCBI BLAST sequence alignments of the ITS1-5.8S rDNA-ITS2 region show 95% and 100% rDNA homology to *P. jadinii* type strain.

Other molecular techniques that can be used to complement phenotypic methods and to reliably distinguish closely-related *Candida* species include:

- PCR assay using universal fungal primers and species-specific probes targeting the ITS2 region (Elie et al. 1998)
- 18S rRNA sequencing from species with characteristic phenotypes, such as the production of ubiquinone (Suzuki and Nakase 2002)
- multilocus sequence typing (MLST) of housekeeping genes (reviewed in Spampinato and Leonardi 2013; Bougnoux et al. 2004; Odds and Jacobsen 2008; Tavanti et al. 2005)
- microsatellite length polymorphism of short tandem repeats (Botterel et al. 2001; Enache-Angoulvant et al. 2010; Garcia-Hermoso et al. 2010; L'Ollivier et al. 2012; Tavanti et al. 2003)

Currently published methods do not permit distinction between different *C. utilis* strains.

1.2 Biological and ecological properties

1.2.1 Natural occurrence

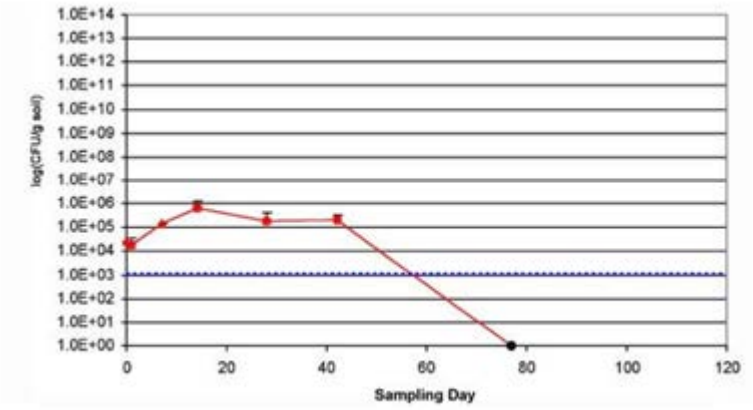
Environmental strains of *C. utilis* have been isolated from various geographic locations and habitats including:

- phyllosphere of the halophyte *Halocnemum strobilaceum* (type of shrub) in the southern coast of Kuwait (Al-Mailem et al. 2010),
- soil (Atuanya and Oseghe 2006; Pan et al. 2009; Zheng et al. 2001),
- eutrophic lakes in Olsztyn, Poland from a depth of up to 30 cm and 4.5 m away from the shore (Biedunkiewicz et al. 2013),
- air (Pisman and Somova 2003)
- water from melted icicles in north-eastern Poland (Biedunkiewicz and Ejdys 2011),
- deep well in Qena, Egypt (Mohawed 1994),
- hydrophyte wastewater treatment plant in Nowa Słupia, Poland (Biedunkiewicz and Ozimek 2009),
- landfill leachate, activated sludge flocs and in sewage treatment batch reactor outflow in Poland (Szyłak-Szydłowski and Kornikłowicz-Kowalska 2012), and
- wastewater from cassava processing plants in Nigeria (Arotupin 2007).

Occurrence of other *Candida* species, such as *C. albicans*, *C. glabarata*, *C. guillermondii, C. parapsilosis*, *C. tropicalis, C. solani* and *C. zeylanoides* in

aquatic habitats (i.e. fresh water, estuaries, marine water, sewage, and polluted water) was reported in the literature (Ahearn et al. 1968; Buck 1977; Buck and Bubucis 1978; Cook and Schlitzer 1981; reviewed in Jones and Schmitt 1978). Given that most *Candida species* are considered commensal gut yeasts in humans, it has been speculated that their isolation from aquatic environments likely results from contamination with human or animal excrement (Ahearn et al. 1968).

A soil persistence study on *C. utilis* ATCC 9950 undertaken by Environment Canada in 2005 used strain specific non-coding amplified fragment length polymorphism to estimate changes in concentration of the micro-organism. The study showed that if released into soil at an initial density of 10^4 CFU/g soil, the *C. utilis* ATCC 9950 population increases within 15 days to a density of 10^6 CFU/g soil. The population persists for up to 42 days, then declines to concentrations below the detection limit of 10^3 CFU/g soil (Figure 1-3) (personal communication, Beaudette 2014).



---- indicates the detection limit of qPCR

Figure 0-3: Persistence of *C. utilis* ATCC 9950, based on qPCR analyses of extractable soil DNA

1.2.2 Growth conditions

C. utilis is a facultative anaerobe. On sugars, fermentation occurs only under anaerobic conditions and growth is slow (Kurtzman et al. 1979; Visser et al. 1990; Franzblau and Sinclair 1983; Ordaz et al. 2001; Weusthuis et al. 1994). Under aerobic conditions, no accumulation of ethanol is observed (referred to as the Crabtree-effect) (Kaliterna et al. 1995; Tomita et al. 2012).

C. utilis ATCC 9950 has a growth temperature range of 10°C to 39°C with optimal growth observed at 35°C (NCYC 2014). Growth kinetics of *C. utilis* ATCC 9950 on various growth media at different temperatures was conducted at Health Canada and is shown in Appendix A Table A-1.

C. utilis is adaptable to a wide range of substrates and conditions. It thrives in environments that are rich in sugars such as xylose, hexose and pentose (Buerth et al. 2011; Chakravorty et al. 1962; Chang 1985; Kurtzman et al. 1979; Lee and Kyun

Kim 2001). *C. utilis*, including ATCC 9950, is generally cultivated on by-products containing free sugars, such as wastes from food and beverage manufacturing (Gold et al. 1981).

C. utilis also grows on organic acids and alcohols. Diauxic growth pattern was observed on *C. utilis* strain CBS621 on mixtures of sugars and ethanol (Weusthuis et al. 1994). During growth on glucose and ethanol, both substrates are utilized simultaneously, but when the yeast is provided with mixtures of maltose and ethanol, the latter substrate is preferentially utilized.

The organism thrives on media supplemented with nitrogen compounds, such as urea, ammonium salts, pyrimidine, and various amino acids (Buerth et al. 2011). *C. utilis* also grows well in sulfur-rich environments. When cultivated for single-cell protein biomass, *C. utilis* is often grown in sulfite liquor generated from pulp and paper processing (Gold et al. 1981; Streit et al. 1987).

Certain strains of *C. utilis* can grow on a wide range of aliphatic and aromatic hydrocarbons as sole sources of carbon and energy (Al-Mailem et al. 2010).

C. utilis strains that have been used in industrial fermentation processes have been exposed to fluctuations in temperature, oxygen concentration, osmotic pressure, pH, nutrient availability and salts. The following examples illustrate the extent to which *C. utilis* strains tolerate fluctuations in different parameters:

- Viability of *C. utilis* strain RD898 decreased with increasing osmotic pressure, where 15-26 MPa osmotic pressure caused a loss of 40% of the population at 22°C (Mille et al. 2005);
- *C. utilis* strain HP-P1 grew well in salinities between 1 to 2M NaCl, but did not tolerate salinities greater than 2M NaCl (Al-Mailem et al. 2010);
- *C. utilis* strain WSH 02–08 was not affected by extremes in pH from 5.5 to 10.5 (Nie et al. 2005).*C. utilis* ATCC 60459 tolerated temperatures up to 50°C when a circadian-like rhythm of the light-dark cycle is in place; survival was markedly decreased in constant darkness (Lapena et al. 2006). The authors speculated that the transient development of resistance to various stresses for this strain under the stationary growth phase was in response to light-induced generation of reactive oxygen species; and
- Metabolism of *C. utilis* ATCC 60560 was affected relatively quickly following a 15 minute interruption of air supply (Vraná and Sobotka 1989).

1.2.3 Nutrient cycling

Nitrate assimilation is a unique characteristic of *C. utilis* (Tomita et al. 2012). The *C. utilis* ATCC 9950 genome sequence revealed the existence of a contiguous nitrate/nitrite assimilation gene cluster which is considered to be responsible for the nitrate assimilation phenotype in *C. utilis* (Tomita et al. 2012).

C. utilis strain F87 can control eutrophication by converting nutrients such as nitrogen and phosphorus into microbial protein, simultaneously inhibiting the growth of the algae *Microcystis aeruginosa* (Kong et al. 2013). The authors showed that *C. utilis* strain F87 was better able to absorb ammonia nitrogen and phosphorus than *M. aeruginosa* in water, and the growth of *M. aeruginosa* was inhibited due to the lack of nutrients.

1.2.4 Biocontrol

Various *C. utilis* strains have antibacterial and antifungal properties that are expressed either through the secretion of enzymes or interaction with other micro-organisms:

- *C. utilis* strain PYCC 3671 showed potential "killer phenotype" against a variety of *Candida* and non-*Candida* species (Antunes and Aguiar 2012);
- When used to ferment the biomass of *Ecklonia cava* (brown algae), *C. utilis* strain KCTC 11355 exhibited an antibiotic property against methicillin-resistant *Staphylococcus aureus* (Eom et al. 2013);
- *C. utilis* strain TISTR 5001, in combination with plant extracts, was used as a fungicide against post-harvest green mold rot on tangerines caused by *Penicillium digitatum* (Sukorinia et al. 2013);
- At a concentration of 10^8 CFU/mL, *C. utilis* strain MPPLY-001 alone or in combination with chitosan effectively controlled tomato fruit rot caused by *Alternaria alternata* and *Geotrichum candidum* (Sharma et al. 2006);
- A consortium containing *C. utilis* was found to inhibit fire blight disease caused by *Erwinia amylovora* in apples (Martinez et al. 2008);
- Certain strains of *C. utilis* have been utilized as biocontrol agents against post-harvest bacterial and fungal pathogens in citrus fruits (Sukorinia et al. 2013), tomatoes (Sharma et al. 2006), and apples (Martinez et al. 2008).
- *C. utilis* acted as a probiotic, enhancing protection against pathogenic bacteria *Pasteurella haemolityca* and *Vibrio alginolyticus* for *Artemia nauplii* (brine shrimp) larvae (Abdelkarim et al. 2010); and
- *C. utilis* provided moderate protection to rainbow trout against the fish pathogen *Aeromonas salmonicida* (Siwicki et al. 1994).

A consortium containing bacteria and fungi, including *C. utilis*, has been shown to promote growth on hydroponic *Lolium perenne L*. (turf grass), as well as to reduce tearing out of the grass (Gaggìa et al. 2013).

1.2.5 Biosorption of metals

The capacity of naturally-occurring strains of *C. utilis* to adsorb certain metals has been investigated. A few examples are listed below:

- *C. utilis* ATCC 9950 adsorbed 5.42 mg magnesium/g dry cell mass (Blazejak et al. 2008) and 181.7 mg of zinc/g dry cell mass (Ahmad et al. 2013);

- *C. utilis* strain RIBM C8 adsorbed cadmium 8.7 mg/g dry cell mass at pH 5.4 and 28 mg/g dry cell mass at pH 5.5 (Kujan et al. 2006);
- Intact and dehydrated *C. utilis* cells were utilized to uptake hexavalent chromium from an aqueous solution in the presence of other metals (Muter et al. 2002); and
- Failla et al. (1976) demonstrated the presence of a highly specific zinc ion transporter in *C. utilis* strain NRRL-Y-7634, which allows for the uptake, solubilization, transport and storage of zinc ions from the environment.

1.2.6 Susceptibility to chemical agents and antifungals

Certain strains of *C. utilis* are susceptible to acriflavine (Keyhani et al. 2009), and agricultural fungicides such as cymoxanil, penconazol, and dichlofluanid (Ribeiro et al. 2000).

Susceptibility of other *Candida* species to chemical disinfectants has been reported in the literature. Jones and Schmitt (1978) investigated the effect of chlorination on the survival of *C. albicans.* A cell concentration of 10^5 CFU/mL exposed to 2 ppm chlorine with a 30 minute contact period was reduced 10^1 CFU/mL, and 4, 8, 16, and 25-ppm treatments were lethal. *C. albicans* is also susceptible to quaternary ammonium compounds, such as benzalkomium chloride and cetrimide (Gupta et al. 2002; reviewed in McDonnell and Russell 1999). Moreover, *C. albicans* is susceptible to ozone, a gas commonly used in purification of drinking water, at an average concentration of 0.064 mg O^3/L (Restaino et al. 1995).

Antifungal susceptibility patterns of *C. utilis* isolated from clinical specimens indicate that *C. utilis* is susceptible to echinocandins (caspofungin, micafungin), triazoles (itraconazole, fluconazole, voriconazole, ketoconazole, posaconazole), and amphotericin B (Fleck et al. 2007; Pfaller et al. 2011; Tortorano et al. 2004). As shown in Table 1-4, antibiotic susceptibility testing conducted by Health Canada in 2013 indicates that *C. utilis* ATCC 9950 is susceptible to amphotericin B, nystatin, clotrimazole, isoconazole, micafungin, terbinafine, and 5-fluorocystosine + amphotericin B, which is consistent with the findings in the literature for the treatment of *Candida* infections.

Table 0-4: Minimal inhibitory concentrations of antifungal agents for *C. utilis* ATCC 9950

Antifungal	MIC (µg/mL)[a]
Amphotericin B	4.5 ± 1.7
Nystatin	3.8 ± 1.5
5-Fluorocytosine	>24
5-Fluorocytosine + Amphotericin B	1.1 ± 0.4
Clotrimazole	1.1 ± 0.4
Isoconazole	0.75
Intraconazole	15 ± 6
Micafungin	0.37

Terbinafine	1.3 ± 0.4
Griseofulvin	>24

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

1.2.7 Pathogenic and toxigenic properties

C. utilis has relatively low virulence compared to pathogenic species of *Candida*, presumably because it lacks one or more of the virulence factors described below that contribute to the pathogenicity of *C. albicans*.

Morphogenesis

Morphogenesis (switching from cellular to hyphal to filamentous growth forms, and switching between opaque and white cell and colony phenotypes) is an important part of the pathogenic life cycle of *C. albicans* (Nadeem et al. 2013; Nie et al. 2010; Ramírez-Zavala et al. 2008; Vylkova et al. 2011; Whiteway and Bachewich 2007). An extensive search of the scientific literature provided no evidence to suggest that *C. utilis* can undergo morphogenesis, either from white to opaque phenotype or from cellular to hyphal growth.

Adherence

In *C*. *albicans*, adherence to host endothelial and epithelial cells is mediated by a range of cell wall glycoproteins (Hoyer 2001; Kinneberg et al. 1999; Staab et al. 1996). These surface adhesins also contribute to fungal antigenic variation (Liu and Filler 2011) and formation of biofilms (Nobile et al. 2008).*C. utilis* may share some glycoproteins with *C*. *albicans*, as there is antigenic cross-reactivity between the hyphal wall protein of *C*. *albicans* and *C*. *utilis* (Laín et al. 2007); however, in general these glycoproteins are specific to *C*. *albicans* based on antigenic specificity.

Quorum Sensing

Quorum sensing molecules, such as farnesol and tyrosol, are involved in regulating virulence in *C*. *albicans* (Cremer et al. 1999; Westwater et al. 2005). An extensive search of the scientific literature provided no evidence to suggest that *C. utilis* ATCC 9950 produces these quorum sensing molecules.

Biofilm

C. utilis forms biofilms in clinical settings. Paulitsch et al. (2009) reported that *C. utilis* was recovered from 1% of indwelling devices collected from intensive care units in Austria. Using scanning electron microscopy, the isolated *C. utilis* cells were described to be in an early stage of biofilm formation (i.e. mainly yeast cells in a basal layer). *In vitro* biofilm formation by a clinical isolate of *C. utilis* was enhanced in the presence of heparin preservative in the culture medium (Sabouraud Dextrose Broth at 37ºC for 6 h; while EDTA was shown to reduce *C. utilis* biofilm formation (Al Akeel et al. 2013). Biofilms are relevant to pathogenicity because they exhibit increased drug resistance relative to planktonic cells and can seed bloodstream

infections that result in life-threatening systemic disease (reviewed in Finkel and Mitchell 2010).

Proteolytic enzymes and phospholipases

Production of proteolytic enzymes, such as aspartic protease (Sap), is considered crucial for *C. albicans* to cause epithelial tissue damage, tissue penetration during disseminated infection, and evasion and destruction of macrophages (Albrecht et al. 2006; reviewed in Gropp et al. 2009; Hube and Naglik, 2001; Naglik et al. 2004; Schaller et al. 1999). These enzymes are also associated with hyphal formation and phenotypic switching (Naglik et al. 2003). Aspartic proteases were not detected in *C. utilis* ATCC 9950 (Buerth et al. 2011). In *C*. *albicans*, extracellular phospholipases have been linked to adherence and membrane disruption during host cell invasion (Barrett-Bee et al. 1985; Ibrahim et al. 1995; Leidich et al. 1998). An extracellular phospholipase B has been identified in *C*. *utilis* (Fujino et al. 2006; Buerth et al. 2011).

Antimicrobial resistance

Induction of efflux pumps, encoded by the transporter genes *cdr1, cdr2,* and *mdr1,* has have been implicated in azole resistance in *Candida albicans* (Franz et al. 1998; Prasad et al. 1995; Riggle and Kumamoto 2006; Sanglard et al. 1997; Wirsching et al. 2000). Up-regulation of these genes in *C. albicans* biofilms has been related to intrinsic resistance of sessile cells to fluconazole compared with planktonic cells (Ramage et al. 2002). An extensive search of the scientific literature provided no evidence to suggest that the genome of *C. utilis* ATCC 9950 contains genes that have been associated with antimicrobial resistance.

Others

C. utilis ATCC 9950 is a tetraploid yeast (Ikushima et al. 2009; Tomita et al. 2012). In the mouse model of infection, *C. albicans* tetraploids were reported to be less virulent than diploids, either because they are inherently less able to survive host defenses, or may be outcompeted by the parental diploids, or they rapidly return to the diploid state via random chromosome loss (Ibrahim et al. 2005; Bennett and Johnson 2003).

The ability to grow optimally at normal human body temperature (37°C) and pH and to thrive at febrile temperatures also contributes to the incidence of *C. albicans* infections in humans. Generally, prolific hyphal growth requires a temperature of 37°C (reviewed in Sudbery 2011; Heilmann et al. 2011). Although *C. utilis* ATCC 9950 can grow at temperatures up to 39°C, its optimal growth is reported to be at 35°C.

A pH of 7.4 was found to be best suited for hyphal induction *in vitro* (Nadeem et al. 2013). Nonetheless, *C. albicans* is known to colonize and infect anatomical sites of diverse pH, including the mildly acidic mouth, skin and vagina, neutral intestine and blood, and highly acidic stomach (reviewed in Davis 2003; Vylkova et al. 2011).

In vitro and *in vivo* tests conducted on *C. utilis* ATCC 9950 at Health Canada found:

- minimal cytotoxic effects on HT29 human colonic epithelial cells and J774A.1 macrophages after 2, 4 and 6 h of exposure to *C. utilis* ATCC 9950 with decreased cytotoxicity after 24 h;
- no hemolytic activity on sheep blood agar after 48 h at 28°C and 37°C;
- in four BALB/c mice per treatment, 1.0 x 10^6 CFU/25 µL exposed by endotracheal instillation showed, no changes in behaviour or physical appearance over a one week monitoring period, no significant increase in lung granulocytes and pro-inflammatory cytokines, or blood cytokine marker levels one week after exposure; and rapid clearance of *C. utilis* ATCC 9950 from the lungs, with dramatically reduced fungal cell numbers after 2 h and no yeast cells detected one week post exposure.

1.3 Effects

1.3.1 Environment

No reports have been specifically attributed to the DSL strain *C. utilis* ATCC 9950 in the scientific literature. Nonetheless, there have been rare reports of naturally-occurring infection in terrestrial mammals with other strains of *C. utilis*.

Plants
No reports in the literature implicate *C. utilis* in adverse effects in terrestrial or aquatic plants.

Certain strains of C. utilis have been used as biocontrol agents against disease-causing agents in plants (Section 1.2.4) without adverse effect in treated plants, which further supports its non-pathogenic nature in plants.

Invertebrates

No reports in the literature implicate *C. utilis* in adverse effects in terrestrial or aquatic invertebrates. Instead,

- Lehner (1983) found that *C*. *utilis* is a suitable pollen substitute in the diets of *Apis melllifera* (honeybees). In a similar study, an increase in bee population was observed in colonies fed with the yeast (Peng et al. 1984);
- *C*. *utilis* has been used successfully either as a feed supplement or as algae substitutes in the diets of Sydney rock oysters (Brown et al. 1996; Nell 1985; Nell et al. 1996) and American oysters (Epifanio 1979; Urban and Langdon 1984) with no adverse effects reported; and
- live and freeze-dried *C. utilis* was successfully used as alternative food source for *Penaeus japonicas* (penaeid shrimp) with no adverse effect on growth or survival (Rahman 1996).

Vertebrates

No adverse effects on fish were attributed to *C. utilis* in the following feeding studies. Instead,

- substitution of animal protein with 30% inactive *C. utilis* in the diet of *Oreochromis mossambicus* Peters (tilapia) and *Salmo salar* (Atlantic salmon) pre-smolts (Olvera-Novoa et al. 2002; Øverland et al. 2013) enhanced growth;
- likewise, *C. utilis* protein supplement in the diet of Atlantic salmon counteracted intestinal inflammation arising from the conventional diet containing extracted soybean meal (Grammes et al. 2013);
- Al-Hafedh and Alam (2013) reported that crude protein from *C. utilis* could also be used to replace fishmeal in Nile tilapia fingerlings without adverse effect on fish growth performance or feed utilization;
- *C. utilis* (viability unknown) was found to be a suitable feed for *Cyprius carpio* (carp) larvae, either alone or in combination with fish meal (Hecht and Viljoen 1982);
- in *Oncorhynchus mykiss* (rainbow trout), diet supplemented with dried *C. utilis* stimulated growth and improved feed conversion rate (Stevens and Truog 1957);
- similarly, Martin et al. (1993) deemed dried *C. utilis* a good substitute for traditional sources of protein in rainbow trout; and
- diet containing dried *C. utilis* resulted in immunostimulation in rainbow trout and protection against *Aeromonas salmonicida* (Siwicki et al. 1994).

In the following feeding studies on poultry, swine and cattle no adverse effects were observed due to the presence of dried *C. utilis* in the diet. Instead,

- chickens exposed to diet supplemented with probiotics containing *C. utilis, Bacillus subtilis* and *Lactobacillus acidophilus* in drinking water at 4.0×10^9 CFU/chicken/day for three days showed enhanced intestinal mucosal immune response up to 10 days post-exposure (Yurong et al. 2005);
- Rodríguez et al. (2013) reported that *C. utilis* was effectively used to replace 20% of the soybean meal protein in broiler chicken diet;
- *C. utilis* was used as protein substitute for up to 66% of total protein in pig feed for 8 days with no effect on weight or feed conversion (Mora et al. 2012);
- bulls exposed to an herb diet supplemented with *Saccharomyces cerevisiae* and 15×10^{11} CFU *C. utilis*/g feed showed an increase in body weight (Mahyuddin and Winugroho 2010); and
- one study reported that as a feed additive, the purified *C. utilis* cell wall elicited an immune response in germ-free piglets following a 54-day feeding experiment (Fencl et al. 1982). Histological analyses indicate an increase in immunoglobulin levels resulting from the stimulation of intestinal lymphatic apparatus of the jejunum and colon. Nevertheless, the animals were in good clinical condition throughout the study.

In a 4-week mouse study, Uetsuka et al. (1976) reported no mortalities in 80 mice dosed intravenously with 2.0 x 10^8 CFU/mL *C. utilis* strain FO-0639. Eight of the 40 mice had mild pyelitis, while three developed encephalitis.

In fifteen cortisone-treated (immunosuppressed) and untreated female Swiss Webster mice exposed intravenously to 1.0 x 10^7 CFU *C. utilis* strain ATCC 20248, no evidence of hyphae formation, inflammatory response, or tissue invasion, and no deaths were observed during the 30-day study (Holzschu et al. 1979). *C utilis* at concentrations of 10^4 - 10^6 CFU/g tissue was recovered from the brain of mice sacrificed at day 6. Histopathological examination at day 30 showed persistence of the fungus in the kidney of the cortisone-treated mice (density of 1.70 x 10^3 CFU/g), but no signs of renal infection were observed. The authors considered that the recovery of residual fungus from the brains of mice sacrificed at day 6 was a function of the inoculation route rather than a neurotropic effect. The large volume of blood directed anteriorly from the heart could have carried the yeast to the brain where they may have been mechanically trapped in small vessels.

Two cases of arthritic joint infection caused by *C. utilis* in horses have been reported. *C. utilis* was isolated from synovial fluid samples of a three-year-old Standard bred filly with a history of bacterial infectious arthritis (Cohen et al. 2008). *C. utilis* infection was effectively treated with the combination of fluconazole, amphotericin B, and arthroscopic debridement. Hepworth (2012) reported a case of equine septic arthritis due to *C. utilis.* As with the previous case, the horse had a history of joint problems and had received corticosteroid treatments. *C. utilis* was also cultured from synovial fluid samples. The fungal infection was effectively treated with fluconazole. Cohen et al. (2008) attributed the ability of *C. utilis* to thrive in the joint, an area generally considered inhospitable for fungal growth, to the combination of antibiotics and local immune suppression with corticosteroid from previous treatments.

C. utilis has been sporadically isolated from cows with mastitis. A strain of *C. utilis* was isolated among other bacteria and yeasts (2.4% of total microbial load) from infected cows in Turkey (Turkylmaz and Kaynarca 2010). Similarly, Wawron et al. (2010) reported that *C. utilis* was the least common yeast isolated from infected udder secretions of cows in Poland, comprising of only 0.67% (1of 150 isolated species). However, unlike other *Candida* species, such as *C. krusei*, *C. albicans*,

C. *guilliermundi*, *C. kefyr* and *C. rugosa*, *C. utilis* has not been implicated as an etiological agent of bovine mastitis (Costa et al. 1993; dos Santos and Marin 2005; Farnsworth and Sorensen 1972; Şeker 2010; Watts 1988; Zhou et al. 2013). Infections are often attributed to poor animal hygiene, the excessive and erratic use of antimicrobials and immunosuppressive drugs, and presence of other chronic diseases (reviewed in Watts 1988).

1.3.2 Humans

Among all the *Candida* species, *C. albicans*, *C. glabrata, C. tropicalis* and C*. parapsilosis* together account for approximately 95% identifiable *Candida*

infections (reviewed in Butler et al. 2009). Other species, including *C. krusei*, *C. lusitaniae*, and *C. guilliermondii*, account for less than 5% of invasive candidiasis (reviewed in Butler et al. 2009).

C. utilis is generally not considered as an etiological agent of infection in humans, but it has occasionally been isolated from clinical samples. Based on epidemiological surveys conducted worldwide and on reported cases, the clinical isolation of *C. utilis* is rare (Lyon et al. 2010; Montagna et al. 2014; Oberoi et al. 2012; Odds et al. 2007; Paulitsch et al. 2006; Pfaller et al. 2011; Presterl et al. 2007; Tortorano et al. 2004). *C. utilis* has been recovered from patients' blood, alimentary tract, urine, mouth, feces and cervix (Alsina et al. 1988; Dorko et al. 2000; Hazen et al. 1999; Lyon et al. 2010; Song et al. 2009).

The incidence of *C. utilis* infection is low. It has been linked to a few cases of candidemia in neonates, and individuals who have undergone invasive medical procedures or with existing health conditions (González et al. 2008; Lukic-Grlić et al. 2011; Luzzati et al. 2013; Montagna et al. 2014; Pfaller et al. 2012; Pfaller et al. 2011; Presterl et al. 2007; Tortorano et al. 2004),including keratitis (Alkatan et al. 2012; Shih et al. 1999), urinary tract infection (Dorko and Pilipčinec, 2002; Hazen et al. 1999), vaginitis (Al Akeel et al. 2013), dental thrush (Song et al. 2009), and fungaemia (Dekeyser et al. 2003). Hospital-acquired *C. utilis* fungemia, attributed to contamination of indwelling medical devices, has also been reported (Alsina et al. 1988), while a rare case of fungemia in an immunocompetent (otherwise healthy) individual was also documented (Bougnoux et al. 1993).

No outbreaks related to *C. utilis* have been reported. *C. utilis* infections have rarely resulted in mortality. Deaths are often directly attributed to significant co-morbidities, rather than to the presence of *C. utilis* in the clinical material.

No cases of allergic reaction have been reported specifically for *C. utilis* ATCC 9950; however other strains of *C. utilis* have been linked to sensitization amongst atopic individuals (up to 35% of this sub-population) (Koivikko et al. 1988).

- *C. utilis* shares common antigens with *C. albicans* and multiple concurrent sensitization to extracts of several *Candida sp.* including *C. utilis,* has been reported in atopic patients, suggesting the possible presence of one or more common skin reactive allergens (Koivikko et al. 1988). The theoretical implication is that persons previously sensitized to *C. albicans* are likely to experience elicitation reactions when exposed to *C. utilis* antigens; and
- Sensitization to feed dusts containing *C. utilis* antigens was reported among swine workers with five to ten years' experience in swine production in Finland (Katila et al. 1981). Respiratory symptoms include coughing and dyspnoea.

1.4 Hazard severity

A combination of morphological, biochemical, and molecular traits allows *C. utilis* to be reliably discriminated from other *Candida* species, especially closely-related

pathogens such as *C. albicans*.

Information from the scientific literature suggests that neither *C. utilis*, nor its teleomorph *Pichia jadinii*, is a frank pathogen towards environmental species. There is no evidence to suggest that *C. utilis* has adversely affected terrestrial or aquatic invertebrates, plants or vertebrates. *C. utilis* has an established history of safe use as a feed supplement, either in live or inactive form, in aquaculture, swine, poultry, and livestock diets. Results from pathogenicity testing on mice also indicate that *C. utilis* does not cause adverse effects. Only two incidents of secondary infections (not experimentally-induced) were attributed to *C. utilis* and, in both cases, the animals had pre-existing conditions and the infections were effectively treated with antifungals. Thus, the environmental hazard severity for *C. utilis* ATCC 9950 is estimated to be low.

No human infections have been specifically attributed to the DSL strain *C. utilis* ATCC 9950 in the scientific literature. Despite its long history of use in food production worldwide, there have only been a few cases of infection associated with exposure to *C. utilis*. These include reports of secondary infection in individuals with predisposing factors such as compromised immunity. In most cases, the infections were effectively treated with antifungals. The human hazard severity for *C. utilis* ATCC 9950 is therefore estimated to be low.

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

Exposure Assessment

2.1 Sources of exposure

The focus of this assessment is to characterize the exposure to *C. utilis* ATCC 9950 from its deliberate addition to consumer or commercial products or its use in industrial processes in Canada.

C. utilis ATCC 9950 was nominated to the DSL in 1997 for its use in the production of food products. Responses to a 2007 voluntary questionnaire sent to a subset of key biotechnology companies in Canada, combined with information obtained from other federal government regulatory and non-regulatory programs, did not indicate that *C. utilis* ATCC 9950 was imported into Canada in the 2006 calendar year.

[4] A determination of whether one or more of the criteria of section 64 of CEPA 1999 are met is based upon an assessment of potential risks to the environment and/or to human health associated with exposure in the general environment. For humans, this includes, but is not limited to, exposure from air, water and the use of products containing the substances. A conclusion under CEPA 1999 on *C. utilis* ATCC 9950 is not relevant to, nor does it preclude, an assessment against the hazard criteria for WHMIS that are specified in the *Controlled Products Regulations* for products intended for workplace use.

The Government conducted a mandatory information-gathering survey under section 71 of CEPA 1999 (hereafter referred to as the section 71 Notice), as published in the *Canada Gazette* Part I on October 3, 2009. The section 71 Notice applied to any persons who, during the 2008 calendar year, manufactured or imported *C. utilis* ATCC 9950, whether alone, in a mixture, or in a product. Responses to the section 71 Notice indicate that approximately 80 metric tonnes of *C. utilis* ATCC 9950 (viability, formulation and concentration unknown) were imported into Canada for the production and processing of food during the 2008 reporting year. *C. utilis* used as a flavour enhancer or dietary supplement is commonly in inactive form (non-living). Although the section 71 Notice was intended to gather information about living organisms, it seems likely (given the uses and quantities reported) that the respondents may have included inactive *C. utilis* ATCC 9950 in their responses to the survey. The exposure assessment will only consider exposure to living *C. utilis* ATCC 9950.

C. utilis ATCC 9950 is available for purchase from the ATCC. As it is on the DSL, and so can be used in Canada without prior notification, it could be an attractive choice for commercialization. As mentioned in sections 1.2.4 and 1.3.1, *C. utilis* has properties that allow it to act as a potential biocontrol agent, to be used in fish and animal feed, and as a growth promoter in animals (i.e. probiotic, supplement). Similarly, *C. utilis* ATCC 9950 and other naturally-occurring strains of *C. utilis* have been widely used in the food industry since the mid-1940s, particularly as a flavouring agent in processed foods (inactive form) and as probiotics (Kurtzman et al. 1979). These products are commonly marketed as Dried Torula Yeast, Torula Yeast, or Yeast Torula. A few representative examples are listed below:

- autolyzed yeast or inactive dried yeast for processing and production (emulsification, texture and flavour enhancement) of beverages and food (Product Sheets, 2014a – d)
- inactive dried yeast as a component of human dietary supplements (Product Sheets, 2014e, g-h).
- active yeast in probiotics (Product Sheets, 2014f).

A search of the public domain indicates that other naturally-occurring strains of *C. utilis* are mainly used as production organisms for the following:

- industrial biochemicals such as 4-hydroxybutyrate, 1,4-butanediol, L-phenylacetylcarbinol, and invertase (Belcarz et al. 2002; Khan and Daugulis, 2010; Pharkya et al. 2014)
- cosmetic or pharmaceutical ingredients such as glutathione (Product Sheet, 2014i), lactic acid (Ikushima et al. 2010) and glucomannan (Ruszova et al. 2008)
- bioethanol (Domenech et al. 1999).

Other potential uses identified from the literature and from patent submissions include, but are not limited to:

- reduction of biological oxygen demand and lactic acid in waste effluents from food processing, alcoholic distillation, and pulp and paper processing (Gold et al. 1981; Hang, 1980; Lemmel et al. 1979; Oliva and Hang, 1979; Razif et al. 2006; SivaRaman et al. 1984; Stevenson et al. 1979)
- wood processing and sugar production (Chesonis and Horton, 2014)
- bioremediation of aflatoxins (El-Shiekh et al. 2007)
- removal of gaseous ethanol in biofilters (Christen et al. 2002)
- degradation of crude petroleum (Nwachukwu, 2000).

According to the Material Safety Data Sheets for some of the aforementioned uses, *C. utilis* is packaged as fine powder, pellets, flakes or pills.

2.2 Exposure characterization

2.2.1 Environment

The environmental exposure for *C. utilis* ATCC 9950 is estimated to be low to medium based on responses to the section 71 Notice in which reported uses were limited to use in human food production and processing.

C. utilis is metabolically versatile and is expected to readily adapt to various environments. It has been isolated from soil, water, air, and waste and wastewater under different conditions. As demonstrated in the persistence study commissioned by Environment Canada, *C. utilis* ATCC 9950 in microcosm soil can be detected for up to 42 days.

Exposure of terrestrial and aquatic invertebrates and vertebrates to viable *C. utilis* ATCC 9950 is expected to be greatest through the consumption of livestock probiotics used in agriculture and aquaculture. Residual *C. utilis* ATCC 9950 in soil and other surfaces from feed preparation could also result in dermal exposure to terrestrial vertebrates. Given that the livestock and aquaculture feeds are generally prepared using the inactive form of the yeast, exposure to live *C. utilis* ATCC 9950 through these sources is considered low.

Indirect exposure of environmental species to *C. utilis* ATCC 9950 through disposal of food wastes in municipal landfills or sewers is considered relatively insignificant given that the majority of foods containing *C. utilis* ATCC 9950 will likely contain the inactive form of the yeast.

The following exposure scenarios are based on potential uses of other *C. utilis* strains as described in Section 2.1 Sources of exposure. Uses, such as bioremediation and disposal of solid wastes from manufacturing facilities, are likely to introduce *C. utilis* ATCC 9950 to terrestrial ecosystems. Terrestrial invertebrates living in the soils at the site of application or disposal and plants growing in treated soils are likely to be the most directly exposed. Vertebrates could ingest *C. utilis* ATCC 9950 while feeding on plants or invertebrates growing in treated or contaminated soils.

Aquatic species may come into contact with *C. utilis* ATCC 9950 from runoff subsequent to terrestrial application or disposal of wastewater from facilities that use the organism for production of enzymes and biochemicals. Nonetheless, the release of *C. utilis* ATCC 9950 from these facilities is expected to be limited by the application of good manufacturing practices, in which measures should be taken to minimize the release of production micro-organisms. Also, the extent of exposure will depend on the mass or volume released, on the receiving environment, and on the proximity of environmental species to the sites of application or disposal.

2.2.2 Human

Based on commercial activity in Canada according to the section 71 Notice, and the considerations outlined below, the overall human exposure estimation for *C. utilis* ATCC 9950 is low.

Direct human exposure to live *C. utilis* ATCC 9950 is greatest through the consumption of dietary supplements or probiotics containing viable yeasts. Handling of food products containing *C. utilis* ATCC 9950 could result in skin and inhalation exposures; however, given that these food products are generally prepared using the inactive form of the yeast, exposure to viable *C. utilis* ATCC 9950 through these routes is considered relatively low.

Should other potential uses of *C. utilis* ATCC 9950 be realized in Canada, humans could be more exposed to *C. utilis* ATCC 9950 in the environment subsequent to its use in wastewater treatment, bioremediation, or from disposal of waste generated during the production of enzymes and biochemicals. The extent of this exposure would depend on the mode of use, the mass or volume applied, and proximity to the application or disposal site. Such exposures may be temporally distant from the time of release and are expected to be significantly lower relative to exposure to live yeasts in dietary supplements and probiotics. Furthermore, uses for enzyme and biochemical production in manufacturing facilities that do not release wastes into the environment should not result in human exposure.

In the event that the organism enters municipal drinking water treatment systems (through release from potential uses and from wastewater), treatment processes which include coagulation, flocculation, ozonation, filtration and chlorination are expected to effectively eliminate *C. utilis* ATCC 9950 from drinking water.

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 *C. utilis* ATCC 9950 to be low for both the environment and for human health. Based on responses to the section 71 Notice, the exposure to living *C. utilis* ATCC 9950 from its use in industrial processes, and

consumer or commercial applications in Canada is expected to be low to medium for both the environment and human health.

Owing to the low potential for hazard, 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).

C. utilis ATCC 9950 has useful properties that make it of interest for use in bioremediation, and for the production of biochemicals. These uses could increase environmental and human exposure of this strain in the future. The risk from reasonably foreseeable uses of *C. utilis* ATCC 9950 remains low given that there is no evidence of adverse effects to human health or of adverse ecological effects at the population level for environmental species. This conclusion is also supported by the long history of safe use of *C. utilis* in industrial, environmental, and commercial settings.

Conclusion

Based on the information presented in this screening assessment, it is concluded that *C. utilis* ATCC 9950 is not entering the environment in a quantity or concentration or under conditions that:

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

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

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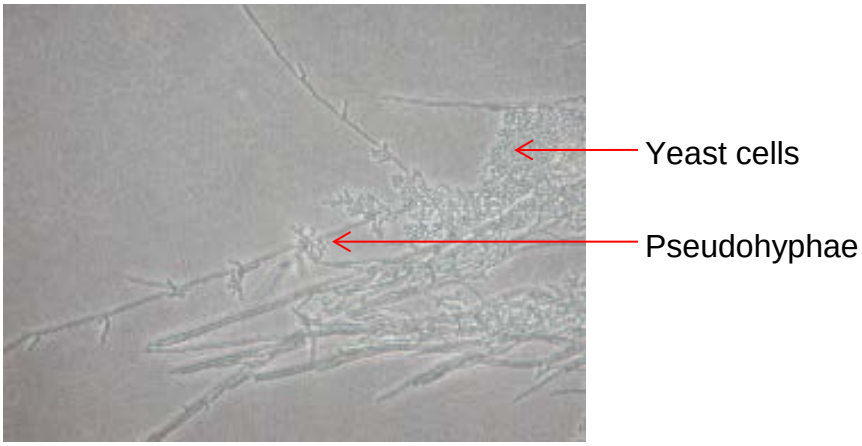
Appendices

Appendix A: Characterization *C. utilis* ATCC 9950

a) *C. utilis* ATCC 9950[a]



b) *C. albicans* SC5314[a]

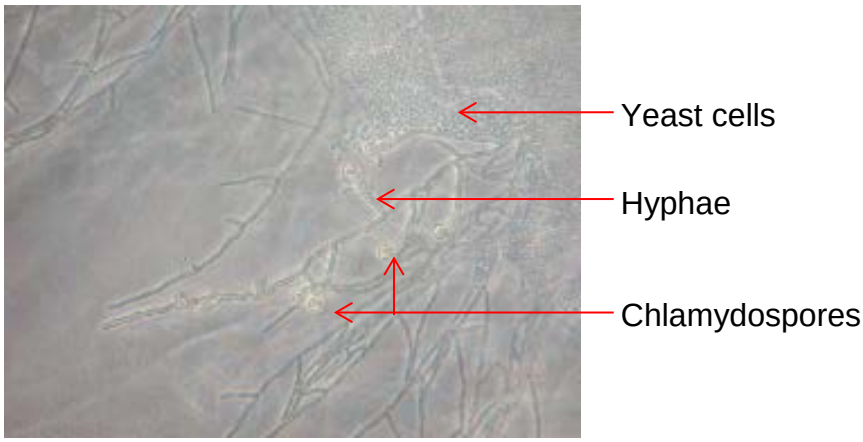


Figure 0-1: Photomicrographs showing differential growth of a) *C. utilis* ATCC 9950 and b) *C. albicans* SC5314 on Corn Meal-Tween 80 Agar at 37°C for 72 h

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

Table 0-1: Growth of C. utilis ATCC 9950 on various media and temperatures

Medium	28°C	32°C	37°C	42°C
Sabouraud	+	+	+	-
Fetal Bovine Serum	+	+	~	-
Dulbecco's Modified Eagles Medium (with FBS, glucose, glutamine)	~	~	-	-

+ Growth - No growth ~ Low level of growth

Data generated by Health Canada's Healthy Environments and Consumer Safety Branch. Growth of *C. utilis* ATCC 9950 in various media was measured by increase in absorbance at 500 nm over a range of temperatures. Concentration of bacteria at time zero was 1×10^6 CFU/mL.