Final Screening Assessment of

Saccharomyces cerevisiae strain F53

Environment Canada

Health Canada

January 2017
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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 Saccharomyces cerevisiae strain F53 (S. cerevisiae F53).

S. cerevisiae strain F53 is a yeast that has characteristics in common with other strains of the species S. cerevisiae. S. cerevisiae is known for its fermentative ability and ethanol production. It has been used widely in bakery and brewery industries, and thus has been in close association with humans for centuries. Multiple potential uses of S. cerevisiae in consumer, industrial, commercial and agricultural sectors exist. These include the production of, or the presence in, food, natural health products such as probiotics, feeds, biofuels and biochemicals for the manufacture of cosmetics, perfumes and therapeutic drugs, as well as bioremediation and wastewater treatment.

S. cerevisiae is known to occur in a wide variety of ecological niches, and has a history of safe use through releases into the environment through human activities used in feed and probiotics for animals, and as an agricultural input for plant growth promotion. There are no reports in the literature implicating the Domestic Substances List (DSL) strain S. cerevisiae strain F53 in causing adverse effects on terrestrial or aquatic plants, invertebrates or vertebrates. However, there are few reports of pathogenicity attributed to other strains of S. cerevisiae. These include: one report of infection in a dog with a history of prolonged antibiotic use, and one report of infection in prawns, S. cerevisiae has also been reported to cause some adverse effects on nematodes.

There have been no reported human infections attributed to the DSL strain S. cerevisiae strain F53; however, certain strains of S. cerevisiae and S. cerevisiae var. boulardii can act as opportunistic pathogens in individuals with compromised immunity or pre-existing medical conditions. In most cases, infections are treated effectively with antifungal compounds. Compared with other opportunistic yeast pathogens like Candida albicans, S. cerevisiae is an organism of low virulence, and rarely causes infections among healthy individuals. Based on Health Canada’s in vitro assays, S. cerevisiae strain F53 does not possess putative virulence traits that are generally found in other pathogenic strains; and in vivo pathogenicity testing on 6- to 8-week-old BALB/c mice indicated that S. cerevisiae strain F53 does not cause any adverse effects to healthy animals.

This screening assessment considers the aforementioned characteristics of S. cerevisiae strain F53 with respect to environmental and human health effects associated with consumer and commercial product use and industrial processes subject to CEPA, including releases to the environment through waste streams and incidental human exposure through environmental media. A conclusion under CEPA on S. cerevisiae strain F53 is not relevant to, nor does it preclude,
assessment of products generated by or containing *S. cerevisiae* strain F53 as prescribed under purview of the *Food and Drugs Act*.

To update information about current uses, 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 section 71 Notice indicates that *S. cerevisiae* strain F53 was imported into or manufactured in Canada in 2008 for use in consumer and commercial applications, such as production of foods, feeds and beverages, as well as in research and development.

Based on the information available, it is concluded that *S. cerevisiae* strain F53 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 *S. cerevisiae* strain F53 does not meet the criteria under paragraph 64(c) of CEPA as it is not entering the environment in a quantity or concentration or under conditions that constitute or may constitute a danger in Canada to human life or health.
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Introduction

Pursuant to paragraph 74(b) of the *Canadian Environmental Protection Act, 1999* (CEPA), the Minister of 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 the virtue of section 105 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)*¹*. *Saccharomyces cerevisiae* strain F53 (*S. cerevisiae F53 strain*) was added to the DSL under subsection 105(1) of CEPA because it was manufactured in or imported into Canada between January 1, 1984 and December 31, 1986 and it entered or was released into the environment without being subject to conditions under CEPA or any other federal or provincial legislation.

This screening assessment considers hazard information obtained from the public domain and from unpublished research data generated by Health Canada*²* research scientists, as well as comments from scientific peer reviewers. Exposure information was also 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 “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, *S. cerevisiae* F53, are identified as such. Where strain-specific data were not available, surrogate information from literature searches was used. When applicable, literature searches conducted on the organism included its synonyms, and common and superseded names. Surrogate organisms are identified in each case to the taxonomic level provided by the source. Literature searches were conducted using scientific literature databases (SCOPUS, Google Scholar, CAB Abstracts, and NCBI PubMed), web searches, and key search terms for the identification of human health and environmental hazards. Information identified up to April 2015 was considered for inclusion in this report.

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

² Testing conducted by Health Canada’s Environmental Health Science and Research Bureau
Decisions from Domestic and International Jurisdictions

Domestic

*S. cerevisiae* (as a species) is a Risk Group 1 organism for both humans and terrestrial animals, according to the Public Health Agency of Canada (PHAC) (personal communication, PHAC 2014). Risk Group 1 biological agents are defined as those that may be capable of causing human or animal disease, but are unlikely to do so. These biological agents pose low risk to the health of individuals and/or animals and a low risk to public health, livestock and poultry.

Under the *Food and Drug Regulations* (B.13.021), inactive dried *S. cerevisiae* may be present in bread in an amount not greater than two parts by weight for each 100 parts of flour used (Health Canada, 2014a).

In addition, *S. cerevisiae* is included on the Natural and Non-Prescription Health Products Directorate (NNHPD)’s Probiotics monograph. Products containing ingredients attesting to the monograph as part of a licence application are required to meet specifications outlined in the monograph, which include species identification, strain characterization, demonstration of ingredient stability/viability as well as establishment of safety through genomic assessment of virulence traits. Labelling statements are also required to contraindicate use in immuno-compromised individuals (Health Canada, 2014b).

No pesticides are currently registered under the *Pest Control Products Act* of the Pest Management Regulatory Agency that contain *S. cerevisiae* as an active ingredient [(PMRA, 2014) and personal communication, PMRA 2014].

*S. cerevisiae* is not a regulated plant pest under the *Plant Protection Act* of Canadian Food Inspection agency (CFIA), and non-recombinant *Saccharomyces* spp. do not require a plant protection import permit from the CFIA (CFIA, 2005). Also, there is no plant microbial supplement product containing *S. cerevisiae* currently registered under CFIA’s *Fertilizers Act*.

Brewer’s dried yeast of *S. cerevisiae*, dehydrated yeast and yeast culture are all listed as organic products that can be used in organic livestock husbandry under the Canadian Organic Production Systems Standards, “Animal Health Care Products and Production Aids” of the CFIA (CFIA, 2012).

In addition, active or dehydrated *Saccharomyces* are listed as ingredients that have been evaluated and approved by the CFIA for manufacture, import and sale for use in livestock feed (under Schedules IV and V of the *Feeds Regulations*) in Canada (CFIA, 2012a). By-products of ethanol manufacturing containing *S. cerevisiae* are
also considered acceptable in distillers grain used for feed purposes, provided CFIA-approved strains are used [(CFIA, 2014) and personal communication CFIA, 2014)].

International

The United States Environmental Protection Agency has assessed non-recombinant *S. cerevisiae* and recommended a tiered exemption under the Toxic Substances Control Act (U.S. EPA, 1997).

Several non-recombinant and recombinant *S. cerevisiae* strains have been granted a Generally Recognized as Safe (GRAS) notice under the United States Food and Drugs Administration. These include: GRN 422, for use in the reduction of acrylamide in a variety of food processing; GRN 120 and 350, for use as a starter culture in alcoholic beverage fermentation; and GRN 175, for use in the reduction of ethyl carbamate in fermented beverages (U.S. FDA, 2002-2013).

According to the European Food Safety Authority (EFSA), *S. cerevisiae* is presumed safe for use in animal feed and throughout the food chain with a few qualifications, as explained in their qualified presumption of safety (QPS) publications. For strains capable of growing $\geq 37^\circ$ C, the qualification includes testing for susceptibility to antimycotics of human or veterinary clinical significance. In the case of *S. cerevisiae* var. *boulardii*, the EFSA committee recommends contraindication in patients of fragile health and those with a central venous catheter in place, and that a specific protocol concerning its use as a probiotic be formulated (EFSA, 2007; EFSA, 2010; EFSA, 2011, EFSA, 2012).
1. Hazard Assessment

1.1 Characterization of *Saccharomyces cerevisiae* F53

1.1.1 Taxonomic identification and strain history

**Binomial name:** *Saccharomyces cerevisiae*

**Taxonomic designation:**

- **Kingdom:** Fungi
- **Phylum:** Ascomycota
- **Class:** Hemiascomycetes
- **Order:** Saccharomycetales
- **Family:** Saccharomycetaceae
- **Genus:** Saccharomyces
- **Species:** cerevisiae Hansen, teleomorph
- **Strain:** F53

**Synonym and superseded names:** Reports of use of the name *Saccharomyces cerevisiae* date back to 1883 and an exhaustive list of synonyms is available in the Catalogue of Life and the latest edition of *The Yeasts*, a Taxonomic Study. The currently accepted name and the most commonly used name for this organism is *Saccharomyces cerevisiae* (Vaughan-Martini and Martini, 2011 and Roskov et al. 2014).

**Anamorph:** *Candida robusta* Diddens and Lodder.

**Strain history:** *S. cerevisiae* F53 has been produced by Lesaffre Yeast Corporation (LYC), US, since the mid-1970s and has been part of their culture collection since 1953. There is no information on the source of isolation of *S. cerevisiae* F53.

**Phylogeny of *Saccharomyces* sensu stricto:**

*S. cerevisiae* is one of eight accepted members of the *Saccharomyces sensu stricto* genus (SSSG), along with *S. paradoxus*, *S. mikatae*, *S. arboricolus*, *S. kudriavzevii*, *S. bayanus*, *S. pastorianus* and *S. cariocanus* (Naumov et al. 2000). Each member of the SSSG complex is reproductively isolated from the others, although all the members can be crossed to form viable F1 hybrids that can grow asexually, but are sterile (Hittinger, 2013; Naumov et al. 2000). Several inter-specific sterile hybrids among SSSG members have been reported in natural and industrial settings (Liti and Louis, 2005; Liti et al. 2009; Novo et al. 2009; Sipiczki, 2008). Of particular interest is *Saccharomyces cerevisiae* var. *boulardii*, a variant of *S. cerevisiae* that has been extensively documented for beneficial health effects as well as implicated in clinical infections. *S. cerevisiae* var. *boulardii* was considered a sterile hybrid of *S. cerevisiae × S. paradoxus*; albeit capable of forming fertile hybrids when crossed
Final Screening Assessment  Saccharomyces cerevisiae F53

with haploid or diploid strains of *S. cerevisiae* (Edwards-Ingram et al. 2007; Van Der Aa Kühe and Jespersen, 2003; Vaughan-Martini and Martini, 2011). Based on recent whole genome analyses, however, *S. cerevisiae* var. *boulardii* firmly clusters within the wine/European populations of *S. cerevisiae*, with some introgression of *S. paradoxus* genes (Personal communication, McCusker, 2015). Introgression of *S. paradoxus* genes is common among *S. cerevisiae* strains (Strope et al. 2015).

### 1.1.1.1. Phenotypic and molecular characteristics:

The purpose of this section is to describe methodologies that can be used to confirm the identity of *S. cerevisiae* F53 strain and distinguish it from other *S. cerevisiae* strains, particularly strains of *S. cerevisiae* and *S. cerevisiae* var. *boulardii* that have been associated with human infections. In clinical settings, rapid methods based on biochemical and metabolic endpoints are used. These include API 20C AUX, API 32C, VITEK 2 and Biolog YT Microplate (Denittis et al. 2010; Khambhaty et al. 2013; Loïez et al. 2006; Verweij et al. 1999). However, a polyphasic approach is important in generating a robust taxonomic identification that allows for clear differentiation of *S. cerevisiae* from closely-related pathogenic *Saccharomyces* species (Barnett et al. 2000; Vaughan-Martini and Martini, 2011).

**Morphological Properties:** The morphological features of *S. cerevisiae* F53 are consistent with those of *S. cerevisiae* type strain ATCC 18824, *S. cerevisiae* var. *boulardii* ATCC MYA 796 (reference strain) and *S. cerevisiae* clinical strain, YJM 309 (Table 1-1). All strains of *S. cerevisiae* tested at Health Canada showed butyrous, light colonies, opaque smooth surface occasionally raised or folded, when plated on yeast extract peptone dextrose (YPD) agar. The DSL strain, *S. cerevisiae* F53, grew between 25°C and 40°C, but failed to grow at 42°C (Erreur ! Source du renvoi introuvable.). The ability of *S. cerevisiae* to form pseudohyphal and invasive growth on corn meal agar and low nitrogen SLAD medium were also tested at Health Canada. *S. cerevisiae* F53, *S. cerevisiae* ATCC 18824 and *S. cerevisiae* var. *boulardii* ATCC MYA 796 did not form pseudohyphae or invasive growth on corn meal agar or SLAD medium, whereas the clinical strain YJM 309 produced pseudohyphae and invasive growth on corn meal agar and SLAD medium. In addition, *S. cerevisiae* F53 is found to be susceptible to most of the antifungal agents tested; and its antifungal susceptibility profile was different from *S. cerevisiae* var. *boulardii* ATCC MYA 796 and *S. cerevisiae* clinical strain, YJM 309.

**Table 1-1: Morphological properties of *S. cerevisiae* F53**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th><em>S. cerevisiae</em></th>
<th><em>S. cerevisiae</em> F53</th>
<th><em>S. cerevisiae</em> ATCC 18824</th>
<th><em>S. cerevisiae</em> var. <em>boulardii</em> ATCC MYA 796</th>
<th><em>S. cerevisiae</em> YJM 309</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth c at 25°C</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Growth c at 37°C</td>
<td>v</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Growth c at</td>
<td>n</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

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## Final Screening Assessment

### Saccharomyces cerevisiae F53

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>S. cerevisiae&lt;sup&gt;a&lt;/sup&gt;</th>
<th>S. cerevisiae F53&lt;sup&gt;b&lt;/sup&gt;</th>
<th>S. cerevisiae ATCC 18824&lt;sup&gt;b&lt;/sup&gt;</th>
<th>S. cerevisiae var. boulardii ATCC MYA 796&lt;sup&gt;b&lt;/sup&gt;</th>
<th>S. cerevisiae YJM 309&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>40°C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Growth&lt;sup&gt;c&lt;/sup&gt; at 42°C</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Colony morphology on 5% malt extract agar at 25°C</td>
<td>Butyrous, light cream colonies, opaque, smooth surface, occasionally raised or folded.</td>
<td>Butyrous, light cream colonies, opaque, smooth surface, occasionally raised or folded.</td>
<td>Butyrous, light cream colonies, opaque, smooth surface, occasionally raised or folded.</td>
<td>Butyrous, light cream colonies, opaque, smooth surface, occasionally raised or folded.</td>
<td>Butyrous, light cream colonies, opaque, smooth surface, occasionally raised or folded.</td>
</tr>
<tr>
<td>Growth on Corn meal agar</td>
<td>Pseudo-hyphae not formed or rudimentary</td>
<td>Pseudo-hyphae not observed</td>
<td>Pseudo-hyphae not observed</td>
<td>Pseudo-hyphae not observed</td>
<td>Pseudo-hyphae observed</td>
</tr>
<tr>
<td>Pseudo-hyphal growth on SLAD medium</td>
<td>v</td>
<td>None observed</td>
<td>n</td>
<td>None observed</td>
<td>Observed</td>
</tr>
<tr>
<td>Invasive growth on SLAD medium</td>
<td>v</td>
<td>None observed</td>
<td>n</td>
<td>None observed</td>
<td>Observed</td>
</tr>
<tr>
<td>Antifungal susceptibility profile&lt;sup&gt;d&lt;/sup&gt;</td>
<td>v</td>
<td>Susceptible to 9 (out of 10) antifungal agents; Resistant only to Griseofulvin</td>
<td>Susceptible to all 10 antifungal agents tested</td>
<td>Susceptible to 7 (out of 10) antifungal agents; Resistant to Griseofulvin, Itraconazole and Terbinafine</td>
<td>Susceptible to 5 (out of 10) antifungal agents; Resistant to Amphotericin B, 5-Fluorocytosin, Griseofulvin, Itraconazole and Terbinafine</td>
</tr>
</tbody>
</table>

<sup>a</sup> Results summarized from several S. cerevisiae strains; Adapted from (Barnett et al. 2000; and Vaughan-Martini and Martini, 2011).

<sup>b</sup> Test results conducted by Environmental Health Science and Research Bureau, Health Canada.

<sup>c</sup> Growth on yeast extract peptone dextrose (YPD) culture

<sup>d</sup> Antifungal susceptibility data presented in Table 1-4 of this report.

+ Indicates positive; - indicates negative; v-variable; n-no data
Biochemical Properties: The DSL strain, *S. cerevisiae* F53, also showed comparable biochemical profile (Table 1-2) for fermentation of maltose, melibiose, inulin utilization, growth without vitamins and growth on mannitol and glycerol, with other *S. cerevisiae* strains tested; except for the type strain, ATCC 18824, which did not grow in the absence of vitamins. When compared to other *Saccharomyces* spp. of the SSSG complex, *S. cerevisiae* F53 shared similar inulin utilization property to *S. kudriavzeii*; and its ability to grow without vitamins was similar to *S. bayanus* (Vaughan-Martini and Martini, 2011).

Table 1-2: Biochemical properties of *S. cerevisiae* F53

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Maltose fermentation</th>
<th>Melibiose fermentation</th>
<th>Inulin utilization</th>
<th>Growth w/o vitamins</th>
<th>Mannitol</th>
<th>Glycerol</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. cerevisiae</em> F53&lt;sup&gt;b&lt;/sup&gt;</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>S. cerevisiae</em> ATCC 18824&lt;sup&gt;b&lt;/sup&gt;</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>S. cerevisiae</em> var. boulardii ATCC MYA 796&lt;sup&gt;b&lt;/sup&gt;</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>S. cerevisiae</em> YJM 309&lt;sup&gt;b&lt;/sup&gt;</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>*S. arboricola&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>v</td>
<td>-</td>
</tr>
<tr>
<td><em>S. bayanus</em> var. bayanus&lt;sup&gt;a&lt;/sup&gt;</td>
<td>+</td>
<td>v</td>
<td>-</td>
<td>-</td>
<td>v</td>
<td>+</td>
</tr>
<tr>
<td><em>S. bayanus</em> var. uvarum&lt;sup&gt;a&lt;/sup&gt;</td>
<td>+</td>
<td>v</td>
<td>-</td>
<td>-</td>
<td>v</td>
<td>+</td>
</tr>
<tr>
<td>*S. cariocanus&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>n</td>
<td>-</td>
</tr>
<tr>
<td>*S. cerevisiae&lt;sup&gt;a&lt;/sup&gt;</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>v</td>
</tr>
<tr>
<td><em>S. kudriavzeii</em></td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>v</td>
<td>v</td>
</tr>
<tr>
<td><em>S. mikatae</em></td>
<td>n</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>*S. paradoxus&lt;sup&gt;a&lt;/sup&gt;</td>
<td>v</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>v</td>
</tr>
<tr>
<td>*S. pastorianus&lt;sup&gt;a&lt;/sup&gt;</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>v</td>
</tr>
</tbody>
</table>

+ Indicates positive; - indicates negative; v-variable; n-no data

<sup>a</sup> Results summarized from *The Yeasts, a Taxonomic Study* (Vaughan-Martini and Martini, 2011)

<sup>b</sup> Test results conducted by Environmental Health Science and Research Bureau (EHSRB), Health Canada. Results represent means of duplicate experiments.

Molecular Properties: The *S. cerevisiae* genome was the first fully sequenced eukaryotic genome. Sequencing was primarily of the laboratory strain S288c and its derivatives (Clayton et al. 1997; Goffeau et al. 1996). Key molecular features of *S. cerevisiae* are presented in Table 1-3.
Table 1-3: Molecular properties of \textit{S. cerevisiae} S288C \textsuperscript{a}

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>\textit{S. cerevisiae} S288C and its derivatives</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ploidy</td>
<td>Haploid or Diploid</td>
</tr>
<tr>
<td>Assembled chromosomes</td>
<td>16</td>
</tr>
<tr>
<td>Genome size (haploid)</td>
<td>12,157 Mb</td>
</tr>
<tr>
<td>Total of genes</td>
<td>8068</td>
</tr>
<tr>
<td>Coding genes</td>
<td>6607</td>
</tr>
<tr>
<td>Verified open reading frames</td>
<td>5097</td>
</tr>
<tr>
<td>tRNA genes</td>
<td>299</td>
</tr>
<tr>
<td>rRNA genes</td>
<td>27</td>
</tr>
<tr>
<td>Small nucleolar RNA (snoRNA)</td>
<td>77</td>
</tr>
<tr>
<td>Transposable element genes</td>
<td>90</td>
</tr>
<tr>
<td>Retrotransposon</td>
<td>50</td>
</tr>
<tr>
<td>Genomic Sequence Accession Numbers</td>
<td>NC_001133 to NC_001224</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Adapted from the Saccharomyces Genome Database, 2014 (as of July 11, 2014)

The \textit{S. cerevisiae} ribosomal RNA operon comprises the 18S small subunit (SSU), internal transcribed spacer (ITS) consisting of ITS1, 5.8S ribosomal subunit, and ITS2; followed by the 25S large subunit (LSU) of which the first 600-900 bp comprise the D1/D2/D3 divergent regions. The internal transcribed spacer (ITS1 and ITS2) regions of 18S and 5.8S rRNA gene sequences (Airola et al. 1999; Molina et al. 1992) as well as the D1/D2 region of 26S rRNA gene sequences have been used widely in the identification of \textit{S. cerevisiae} strains (Esteve-Zarzoso et al. 2004; Kurtzman and Robnett, 1998). Primers ITS1 and LR7, used to amplify the ITS and the first 1.5 kb of the LSU, are represented in Figure (White et al. 1990). Health Canada’s Environmental Health Science Research Bureau (EHSRB) used these primers to sequence the ITS 1 and 2, and D1/D2 regions of the 25S LSU, amplifying approximately 2.3 kb of the ribosomal RNA operon of \textit{S. cerevisiae} F53. Other strains sequenced for comparison include: \textit{S. cerevisiae} ATCC 18824 (type strain), \textit{S. cerevisiae} CECT 10431 (food strain), \textit{S. cerevisiae} YJM 309 (clinical strain) and \textit{S. cerevisiae} var. \textit{boulardii} ATCC MYA 796 (reference strain).
Multiple sequence alignment using Microseq® ID fungal D2 LSU library shows that the ITS sequence of *S. cerevisiae* F53 is in consensus with other *S. cerevisiae* strains, with 100% identity with *S. cerevisiae* ATCC 18824 and 99.86% identity with *S. cerevisiae* ATCC 9763 (Table 0-2). Phylogenetic analysis was also conducted by Health Canada, using the determined sequences along with publicly available sequences of *Saccharomyces* spp. from the NCBI entries. The resulting dendrogram (Figure 1-1) demonstrates that *S. cerevisiae* F53 is closely related to other strains of *S. cerevisiae* and *S. cerevisiae* var. *boulardii*. The dendrogram also reveals that sequence analysis of the ITS 1 and 2, and D1/D2 regions of the 25S LSU is not sufficient to differentiate *S. cerevisiae* F53 from *S. cerevisiae* ATCC 18824, *S. cerevisiae* CECT 10431, *S. cerevisiae* YJM 309 or *S. cerevisiae* var. *boulardii* ATCC MYA 796.
Figure 1-1: Phylogenetic tree generated by Health Canada scientists using ITS sequences determined in-house, or identified from literature searches

* indicates sequence data generated by Health Canada’s EHSRB. The phylogenetic tree was constructed first by alignment of the sequences by the MUSCLE method and then analyzed with the Kimura 2-parameter distance model within the MEGA version 5.2 platform (Tamura et al. 2011).

Consistent with Health Canada’s results, DNA-based polymorphism studies have highlighted the difficulty in reliably distinguishing strains of *S. cerevisiae* from *S. cerevisiae var boulardii*, as well as differentiating strains of clinical and non-clinical sources (e.g., food, probiotic or environmental sources). Examples include: analysis of yeast transposons (TY elements and associated delta sequences), pyrosequencing of a hypervariable region of ITS2, restriction fragment polymorphisms of rRNA gene sequences, microarray karyotyping and chromosome length polymorphisms (Borman et al. 2010; Casaregola et al. 2011; Ness et al. 1993; Pannanusorn et al. 2012; Pryce et al. 2006).

These methods also failed to reliably differentiate virulent from avirulent strains of *S. cerevisiae* and *S. cerevisiae var. boulardii* (Büchl et al. 2010; de Llanos et al. 2006b; Diezmann and Dietrich, 2009; Enache-Angoulvant et al. 2010; Hennequin et al. 2001; Klingberg et al. 2008; Muller et al. 2011). For instance, Klingberg et al. (2008) reported that although food, probiotic, environmental and clinical isolates showed an overall clustering pattern within groups, no perfect clustering was observed. Certain probiotic and food strains clustered within larger clusters of clinical strains. Likewise, genome-wide variations for various polymorphic sites in a diverse collection of 63 *S. cerevisiae* strains sampled from different ecological niches (beer, bread, vineyards, immuno-compromised individuals, various fermentations and nature), revealed that clinical isolates are distributed among all subgroups (Schacherer et al. 2009).
Such polymorphism studies also illustrate the genetic diversity of *S. cerevisiae*; in particular, clinical isolates exhibit high levels of genetic diversity compared to environmental isolates. The clinical isolates also share genetic similarity to the laboratory strain S288C, and with commercial baker’s yeast *S. cerevisiae* and *S. cerevisiae* var. *boulardii*, and environmental isolates. This not only suggests the likelihood of common ancestry, but also that commercial and environmental isolates could opportunistically colonize human tissues (Carreto et al. 2008; de Llanos et al. 2004; de Llanos et al. 2006a; Muller and McCusker, 2009; Schacherer et al. 2009).

Similarly, metabolic footprinting using mass spectrometry has also revealed that clinical strains are more diverse than non-clinical strains in their metabolic profiles. In this study, differential metabolite concentrations were reported to discriminate strains of clinical, non-clinical and probiotic groups; however, certain clinical isolates were also metabolically related to baking and probiotic strains (McKenzie et al. 2008).

Although the currently available methods may not permit distinction between *S. cerevisiae* F53 from clinically relevant *S. cerevisiae* and *S. cerevisiae* var. *boulardii* strains, comprehensive whole genome sequencing and analyses of copy number variations and polymorphisms in other regions may reveal genetic differences between *S. cerevisiae* F53 and other strains.

### 1.1.2 Biological and ecological properties

#### 1.1.2.1 Natural occurrence

As a species, *S. cerevisiae* has been isolated from varying ecological niches, including, vineyards, forest soils, natural woodlands, tree barks, insects, fish, and mammals, including humans. However, its distribution is often associated with specific substrates containing high levels of fermentable sugars (e.g., wines, beers, fruits, fruit juices, soft drinks, sugar cane, vinegar); and also where domesticated strains have been released into the environment from human uses (e.g., wineries and fermentation plants) (Cray et al. 2013; Dequin and Casaregola, 2011; Naumov et al. 1996; Raspor et al. 2006; Wang et al. 2012; Sampaio and Goncalves, 2008). *S. cerevisiae* has a history of thousands of years of use in the production of bread and fermented beverages. Throughout its recent history of domestication for applications in baking and brewing (since 1953 by LeSaffre Yeast Corporation), *S. cerevisiae* F53 has been released into the environment.

#### 1.1.2.2 Survival, persistence and dispersal in the environment

It has been suggested that *S. cerevisiae* does not survive or persist in environments that lack sufficient sugar or organic matter; however, it can emerge as a dominant species in habitats with high sugar or nutrients (Cray et al. 2013). For example, damaged grape berries, pears, plums and rotting figs have higher levels of *S. cerevisiae* than intact fruits (Mortimer and Polsinelli, 1999). Likewise, during anaerobic digestion of organic biowastes, the relative abundance of *Saccharomyces* species increased during digestion, indicating that they can proliferate under anoxic
conditions in the reactor with available organic nutrients (Ritari et al. 2012). Similarly, several phylotypes belonging to the family *Saccharomycetaceae* were dominant in the initial mesophilic, low pH phase, as well as the thermophilic phase of a composting process involving municipal, green garden waste and industrial biowastes (Hultman et al. 2010). No species-level characterization was done in either of the above studies, so it is unclear whether *S. cerevisiae* is as capable of tolerating such varying conditions.

In aquatic environments, *S. cerevisiae* was repeatedly isolated from sediments with high organic content, collected from locations where industrial effluents from sugar plants or domestic waste water were released (Sagea et al. 1997). Artificially inoculated *S. cerevisiae* failed to survive in sewage and lake water (Liang et al. 1982), and declined by two orders of magnitude within 40 days in non-sterile water (pH 6.5) (Ando et al. 2005). *S. cerevisiae* did not survive beyond 20 days, in either non-sterile water or waste water (Fujimura et al. 1994).

In soil environments, survival of *S. cerevisiae* depends on multiple factors such as temperature, water saturation and soil type, as well as availability of sugar or organic matter. Artificially inoculated *S. cerevisiae* failed to survive beyond 14 days in sandy loam or 31 days in clay silt; and rates of decline were lower at 10°C than at 20°C (Vahjen et al. 1997). In non-sterile soil, *S. cerevisiae* survived not more than 20 days (6 log reduction) (Fujimura et al. 1994). When commercial *S. cerevisiae* used in wine making was released in large quantities as liquid or solid wine residues into the environment around the winery, it was found to survive for 12 months after release, but was not detected after 2 years (Cordero-Bueso et al. 2011). It is unclear if the variability in survival in aquatic environments and in soil is due to different experimental conditions, including nutrient availability, or whether it represents inherent differences in the survival capacity of different strains of the species.

*S. cerevisiae* released into the environment does not readily disperse. It does not easily become air-borne; however, birds and flying insects are suggested to act as vectors for yeast dispersal (Garijo et al. 2011; Goddard et al. 2010; Mortimer and Polsinelli, 1999; Stefanini et al. 2012). Natural populations of *S. cerevisiae* residing in vineyards (on grape berries, leaves, bark and soil) remained as discrete populations of both vegetative cells and haploid spores; and widespread dispersal is not reported (Cordero-Bueso et al. 2011). In the three year study, indigenous *S. cerevisiae* was isolated from the soil only once; and there is no information to determine if commercial yeast residues had been released into this vineyard soil prior to this study. Commercial *S. cerevisiae* wine strains released into the vineyards of France and Portugal were recovered within 10-200 m of the site of release and migration was largely mediated by water run-off. Introduced strains underwent natural fluctuations of appearance and disappearance like autochthonous strains, but failed to permanently persist in the introduced fields (Valero et al. 2005).
1.1.2.3. Life cycle

*S. cerevisiae* is a saprophytic, homothallic, unicellular yeast with a complex lifecycle. It can exist both in a haploid and diploid state. Where nutrients are not limiting, both haploid and diploid cells proliferate by multi-lateral budding (Herskowitz, 1988); whereas under nutrient depleted conditions, they arrest as stationary phase cells (Dickinson and Schweizer, 2004). Diploid cells can enter meiosis and produce haploid ascospores enclosed within a thick spore wall, enabling them to resist environmental stresses better than haploid cells. The ability to produce ascospores under starvation conditions aids dissemination to new environments and provides protection and survival advantage where conditions are limiting (Brown et al. 2014; Cray et al. 2013; Smits et al. 2001; Van Mulders et al. 2011).

Diploid cells of certain *S. cerevisiae* strains can produce pseudohyphal growth under nitrogen-limited conditions (Gimeno et al. 1992), whereas haploid cells can form filaments (chained cells) that can invade the agar surface (invasive growth phenotype) when grown on nutrient-rich medium for a prolonged period (Roberts and Fink, 1994). Both pseudohyphal growth and invasive filaments allow foraging for nutrients and colonization of new substrates, providing a growth advantage and favoring survival in nature (Dickinson and Schweizer, 2004; Smits et al. 2001; Cullen and Sprague, 2012).

*S. cerevisiae* strains used in fermentative processes exhibit high levels of heterozygosity due to genome duplications and gene copy number variations resulting in aneuploidization and polyploidization (Dunn et al. 2012). Most bread strains of *S. cerevisiae* are tetraploids. The Red Star dry yeast from Universal Foods (LYC, 2001) is a polyploid/aneuploid (Casey et al. 1989). The ploidy level of the DSL strain *S. cerevisiae* F53 is not known and thus, a conservative assumption that it could likely behave like a polyploid has been applied. Polyploidy and heterogeneity contribute to enhanced energy generation and growth rates, resulting in better survival and adaptation to stresses (Cray et al. 2013; Dequin and Casaregola, 2011; Legras et al. 2007).

1.1.2.4 Growth parameters

*S. cerevisiae* is a highly adaptive species compared to other yeasts (Brown et al. 2014; Cray et al. 2013). It can grow over a wide range of temperatures from 0°C to 45°C (Cray et al. 2013). The optimum growth temperature for most strains is 25-30°C; however, *S. cerevisiae* is a thermo-tolerant species, compared to other members of the SSG. Certain *S. cerevisiae* strains are capable of growing at higher temperatures (>37°C), and are viable even up to 58°C (Fietto et al. 2004). *S. cerevisiae* is also capable of tolerating cold temperatures (Schade et al. 2004) and adapting to near-freezing temperatures; thus, it can maintain its viability even at 4°C (Murata et al. 2006).
**S. cerevisiae** can grow over a wide pH range (2.75-5.2) (Serra et al. 2005; Charoenchai et al. 1998; Arroya-Lopez et al. 2009; Belloch et al. 2008), although certain strains are reported as acid tolerant and can maintain viability to 75% at pH 2.0 (Fietto et al. 2004). **S. cerevisiae** can grow over a wide range of salt concentrations between 0% and 5% sodium chloride (NaCl; Hohmann, 2002). NaCl concentrations above 2% decrease its growth rate with increased production of glycerol (Wei et al. 1982).

**S. cerevisiae** can also withstand dehydration. Dehydrated cells are shrunken, and can synthesize and accumulate high concentrations of compatible solutes such as glycerol, trehalose and sterols, to protect membrane integrity during dehydration (Balakumar and Arasaratnam, 2012; Cray et al. 2013).

**S. cerevisiae** is an efficient ethanol producer, and can generally tolerate up to 6-10% ethanol, whereas industrial **S. cerevisiae** hybrids can tolerate even up to 15-20% ethanol (Ghareib et al. 1988; da Silva et al. 2013; Belloch et al. 2008).

**S. cerevisiae** is a facultative anaerobe that can use a range of substrates. It can rapidly switch between respiratory and fermentative metabolism in response to changes in the availability of oxygen and fermentable sugars. When glucose or other hexose sugar levels are high, **S. cerevisiae** quickly initiates fermentation and produces ethanol. When glucose becomes limiting, metabolism shifts to respiration, to allow the use of ethanol and acetate accumulated during the fermentative growth phase (Dickinson and Schweizer, 2004; Smets et al. 2010; van den Brink et al. 2009; Van Urk et al. 1988). The ability of **S. cerevisiae** to rapidly change its nutrient metabolism allows it to monopolize high sugar environments and inhibit growth of other microorganisms through the production of ethanol, thereby outcompeting them. The complex interconnected signal transduction pathways that are involved in regulating its nutrient metabolism also regulate life cycle transitions such as meiosis, sporulation, autophagy and pseudohyphal or invasive growth (Carlson, 1999; Cray et al. 2013; Schneper et al. 2004; Zaman et al. 2008) in response to nutrient availability.

1.1.2.5 **Resistance to metals, chemical agents and antifungal drugs**

**S. cerevisiae** is tolerant to various metals and metalloids, and is used in removal of heavy metals such as lead and cadmium from contaminated soils. Under aerated soil condition, stationary cells can tolerate up to 250 ppm of Pb\(^{2+}\) and 500 ppm of Cd\(^{2+}\) with the biosorption of 67-82% of Pd\(^{2+}\) and 73-79 % of Cd\(^{2+}\) within 30 days (Damodaran et al. 2011).

**S. cerevisiae** is sensitive to chlorine treatment. Sodium hypochlorite (NaClO), at concentrations of 1.0 and 5.0 ppm, caused cell mortality in both stationary and logarithmic **S. cerevisiae** cells. Significant cytotoxic and genotoxic effects were reported at NaClO concentrations ≥ 10 ppm. Also, significant toxic effects were
reported for chlorine (ClO₂) treatment at ≥ 5 ppm, and stationary cells were more sensitive than logarithmic cells, even at 1.0 ppm ClO₂ (Buschini et al. 2004).

Two major classes of antifungal drugs are used to treat *S. cerevisiae* infections with variable efficacy: the polyenes (e.g., amphotericin B, nystatin) and the azoles (e.g., clotrimazole, fluconazole, itraconazole, voriconazole or ketoconazole). Among polyenes, amphotericin B is the treatment of choice for serious *S. cerevisiae* infections, except where underlying conditions preclude its use (Aucott et al. 1990; Papaemmanouil et al. 2011). *S. cerevisiae* is also susceptible to flucytosine, and moderately resistant to nystatin (Swinne et al. 2005; Zerva et al. 1996). In cases where amphotericin B therapy is not advised, prolonged treatment with azoles is effective (Aucott et al. 1990; de Llanos et al. 2006a; Burkhardt et al. 2005; Enache-Angoulvant and Hennequin 2005; Murphy and Kavanagh 1999; Echeverría-Irigoyen et al. 2011). However, there is also evidence suggesting moderate- to high-level resistance of *S. cerevisiae* (particularly those strains involved in vaginitis) to fluconazole, posaconazole and itraconazole B (Papaemmanouil et al. 2011, Echeverria-Irigoyen 2011).

Health Canada’s *in vitro* antifungal susceptibility study showed that the DSL strain, *S. cerevisiae* F53 was susceptible to most antifungal agents tested (Table 1-4). The most effective antifungal agent was micafungin (MIC-0.37 µg/ml), while griseofulvin was relatively ineffective (MIC >24 µg/ml). At the concentrations tested, the clinical strain, YJM-309 showed most resistance (resistant to five antimicrobials), followed by *S. cerevisiae* var. boulardii MYA 796 (resistant to three antifungal agents tested).
Table 1-4: *In vitro* antifungal susceptibility of *S. cerevisiae* F53

<table>
<thead>
<tr>
<th>Antifungal agents</th>
<th><em>S. cerevisiae</em> F53</th>
<th><em>S. cerevisiae</em> ATCC 18824</th>
<th><em>S. cerevisiae</em> var. <em>boulardii</em> ATCC MYA 796</th>
<th><em>S. cerevisiae</em> YJM 309</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphotericin B,</td>
<td>8.0 ± 3.5</td>
<td>14.0 ± 9.2</td>
<td>6</td>
<td>&gt;24</td>
</tr>
<tr>
<td>Amphotericin B + 5-fluorocytosine</td>
<td>16 ± 7</td>
<td>7 ± 4.6</td>
<td>12</td>
<td>24</td>
</tr>
<tr>
<td>5-Fluorocytosine</td>
<td>8 ± 3.5</td>
<td>3.5 ± 2.3</td>
<td>21 ± 6</td>
<td>&gt;24</td>
</tr>
<tr>
<td>Clotrimazole</td>
<td>7 ± 4.6</td>
<td>0.5 ± 0.2</td>
<td>6</td>
<td>15 ± 6</td>
</tr>
<tr>
<td>Griseofulvin</td>
<td>&gt;24</td>
<td>12.0 ± 10.3</td>
<td>&gt;24</td>
<td>&gt;24</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>6 ± 5.2</td>
<td>4 ± 1.7</td>
<td>&gt;24</td>
<td>&gt;24</td>
</tr>
<tr>
<td>Isoconazole</td>
<td>5 ± 1.7</td>
<td>0.5 ± 0.2</td>
<td>1.3 ± 0.4</td>
<td>12</td>
</tr>
<tr>
<td>Micafugin</td>
<td>0.37</td>
<td>0.4</td>
<td>0.37</td>
<td>0.37</td>
</tr>
<tr>
<td>Nystatin</td>
<td>7 ± 4.6</td>
<td>2 ± 0.9</td>
<td>2.6 ± 0.8</td>
<td>5 ± 2</td>
</tr>
<tr>
<td>Terbinafine</td>
<td>20 ± 6.9</td>
<td>12 ± 10.4</td>
<td>&gt;24</td>
<td>&gt;24</td>
</tr>
</tbody>
</table>

*a*- Data from Health Canada’s EHSRB. The study was conducted using Sabouraud-MTT liquid assay method to characterize *S. cerevisiae* F53. The reported values are based on a minimum of 3 independent experiments. Values correspond to the minimal inhibitory concentration (MIC) in µg/mL for *S. cerevisiae* F53 (10⁴ CFU/well) grown in the presence of antibiotic for 48 hrs at 37°C.

1.1.2.6 Pathogenic and toxigenic characteristics

*S. cerevisiae* is considered as a yeast of low virulence compared to *C. albicans* (Yanez et al. 2009). A number of putative virulence factors, however, have been associated with its ability to cause infection or adverse effects.

Growth at high temperatures, particularly at 42°C, has been suggested to be an important virulence factor in clinical isolates of *S. cerevisiae*. The maximum growth temperature of most environmental strains of *S. cerevisiae* is 35°C, whereas virulent strains were capable of growth at 39°C (Llopis et al. 2014) or at 42°C (McCusker et al. 1994b); however, the ability to grow at higher temperatures may not be the sole determinant of pathogenicity. Clemons et al. (1994) reported that all isolates tested in their study (including the least virulent strain) were capable of growing at 37°C, and certain highly virulent strains had poor capacity to grow at 39°C or 42°C. Likewise, Klingberg et al. (2008) could not discriminate between clinical, food or probiotic strains on the basis of temperature since none could grow at 42°C.
Hydrolytic enzymes, such as proteases, phospholipase A and lyso-phospholipase are putative virulence factors in yeasts, and particularly in *C. albicans* and *Cryptococcus neoformans* (Kabir et al. 2012). Probiotic *S. cerevisiae* strains produce higher levels of proteases compared to wine and laboratory strains (Llopis et al. 2014); however, the ability to produce proteases is not always strongly associated with virulence in *S. cerevisiae*, and the total protease activity was not significantly different among clinical and non-clinical isolates (Clemons et al. 1994; de Llanos et al. 2006b; McCusker et al. 1994a; McCusker et al. 1994b; Siccardi et al. 2006). In *S. cerevisiae*, phospholipase activity appears to correlate with virulence. Over 80% of clinical strains tested produced higher levels of phospholipase than food industry strains (Sakamoto et al. 1977); however, *S. cerevisiae* is generally known to have low phospholipase activity compared to *C. albicans* and several other fungi (Barrett-Bee et al. 1985; Pico et al. 1999).

The ability of *S. cerevisiae* to form pseudohyphae has also been proposed as a virulence factor as it could enhance its ability to colonize a host and cause infection. Virulent *S. cerevisiae* strains exhibit pseudohyphal growth under nutrient-limited conditions (Navarro-García et al. 2001). Most clinical and probiotic strains produce pseudohyphae in synthetic low ammonia dextrose media, while the food strains do not display this property (Llopis et al. 2014; Sakamoto et al. 1977). In contrast, Klingberg et al. (2008) reported that 83% of food strains and 100% of probiotic strains were capable of pseudohyphal growth on low ammonia media, whereas none produced pseudohyphae on low carbon media. They concluded that the ability to form pseudohyphae under nitrogen-limiting conditions may be a common trait of *S. cerevisiae* that is not solely associated with more virulent strains.

Under nutrient-starved conditions, haploid cells can form invasive filaments that penetrate agar substrates, and that are resistant to being dislodged by rinsing the surface (Sakamoto et al. 1977; Torres et al. 2008). Invasive filaments are strongly induced at temperatures above 37°C (Zupan and Raspor, 2010), as well as by exogenous indole acetic acid (IAA), a plant hormone. *S. cerevisiae* is also capable of synthesizing metabolites similar to IAA, which is suggested to play a role on plant-fungal pathogenesis (Prusty et al. 2004 and 2010). Although studies have investigated the ability to form invasive filaments as a putative virulence trait, Klingberg et al. (2008) reported that haploid cells of clinical and food strains of *S. cerevisiae* were capable of invasive growth, whereas this property was not found in the laboratory strain S288C and probiotic strains.

The capacity of yeasts to adhere to epithelial cells or to synthetic surfaces such as plastic catheters is an important virulence trait for invasiveness and infections (Vartivarian, 1992). *S. cerevisiae* exhibits detectable epithelial cell adhesion, but only at low levels (5-6% adhesion) compared with *C. albicans* (35-70% adhesion). *In vitro* studies using human epithelial cells have shown that *S. cerevisiae* does not affect the membrane integrity of the intestinal barrier; and a pre-existing breach would likely be required to initiate systemic infections via the gut (Pérez-Torrado et al. 2012; Yanez et al. 2009).
S. cerevisiae can selectively bind to each other and form complex biofilms as an adaptive mechanism to cope with hostile environments (Reynolds and Fink, 2001). For example, it may protect cells from high concentrations of ethanol in wine-making or biofuel production (Stovicek et al. 2011; Sidari et al. 2014 and Hope and Dunham, 2014). Biofilm formation is common among ethanol-tolerant sherry wine and biofuel strains of S. cerevisiae (Zara et al. 2005). A clinical S. cerevisiae strain that displayed several other virulence attributes such as growth at high temperature, pseudohyphal growth and virulence in mice, was also capable of forming biofilms under glucose-limited conditions (Granek and Magwene, 2010).

The ability of S. cerevisiae to evade the immune system is an important consideration in defining its pathogenesis. Resistance of S. cerevisiae to phagocytosis is associated with virulence. Probiotic S. cerevisiae strains are less frequently phagocytosed (19% engulfing rate) than clinical and food strains or even some C. albicans strains (29-39%), suggesting that phagocytosis resistance is higher in probiotic strains (Yanez et al. 2009). In addition, pro-inflammatory cytokine production by immune cells plays an important role in a host’s response to infection and changes in cytokine production is associated with increased virulence in S. cerevisiae (Saegusa et al. 2009 and Wheeler et al. 2003). Furthermore, virulent S. cerevisiae strains are suggested to survive the macrophage oxidative burst attack, allowing them to persist longer and potentially form systemic infections (Llopis et al. 2012).

Certain S. cerevisiae strains secrete virion-encapsidated double-stranded RNA mediated proteinaceous “killer” toxins, which are lethal to other competing S. cerevisiae strains and yeast species (Dabhole and Joishy, 2005; Marquina et al. 2002). Such toxin-producing S. cerevisiae strains occur in nature, and have been used in industrial settings as a means of controlling contamination of fermentation systems by other yeasts (Bussey et al. 1988; Vagnoli et al. 1993). Killer toxins are generally stable at pH 2.8 - 4.8 and are only active around pH 4.7 (Van Vuuren and Wingfield, 1986). These toxins are not expected to have adverse environmental or human effects due to their limited target range and short persistence in soil and water (U.S. EPA, 1997). There are no reports suggesting that strains of S. cerevisiae produce toxins that are active against insects, fish, animals, plants or humans.

In order to determine the putative virulence attributes (pseudohyphal and invasive growth, phospholipase production and activation of pro-inflammatory cytokines in HT29 human colonic epithelial cells and J774A.1 mouse macrophage cells) of the DSL strain S. cerevisiae F53, Health Canada scientists conducted in vitro assays on S. cerevisiae F53, along with S. cerevisiae ATCC 18824, S. cerevisiae var. boulardii ATCC MYA 796 and S. cerevisiae YJM 309. As summarized in Table 0-3, the DSL strain, S. cerevisiae F53 was negative for pseudohyphal and invasive growth when tested on corn meal agar and SLAD medium, and also for phospholipase production. Similar results were observed for S. cerevisiae ATCC 18824 and S. cerevisiae var. boulardii MYA 796. In contrast, the clinical strain YJM 309 was positive for pseudohyphal and invasive growth, but negative for phospholipase production. In addition, S. cerevisiae F53 was found not to be toxic to HT-29 or J774A.1 cells, 4
hours and 24 hours post-exposure. In addition, activation of pro-inflammatory cytokines (G-CSF, GM-CSF, IL-6, KC, RANTES, TNF-a, caused by YJM 309) in HT29 colonic epithelial cells and J774A.1 macrophage cells, caused by S. cerevisiae var. boulardii ATCC MYA 796 and S. cerevisiae YJM 309, were significantly higher than that of S. cerevisiae ATCC 18824 and S. cerevisiae F53 ³.

1.1.3 Effects

1.1.3.1 Environment

Despite its occurrence in nature and widespread domestic and industrial use in producing foods, feeds and alcoholic beverages, there are few reports of pathogenicity or toxicity of naturally-occurring S. cerevisiae towards terrestrial or aquatic plants, vertebrates or invertebrates in the published scientific literature. There are no reports in the literature implicating the DSL strain S. cerevisiae F53 in causing adverse effects on either terrestrial or aquatic plants, invertebrates or vertebrates.

a. Plants

S. cerevisiae is generally considered non-pathogenic or beneficial to terrestrial plants. There are several reports in the literature suggesting its ability to promote plant growth, as well as, control plant pathogenic and food spoilage fungi such as Fusarium spp., Penicillium spp., Botrytis spp., Monilinia spp., Rhizoctonia spp., Macrophomina phaseolina, Rhizoctonia solani and Trichoderma viride (El-Sayed Shalaby and El-Nady, 2008, Nally et al. 2012, Attyia and Youssry, 2001, Suzzi et al. 1995, Oro et al. 2014, Zhou et al. 2008). In addition, S. cerevisiae cell wall glucan reduced the concentration of fusaric acid produced by the plant pathogen F. verticillioides, and protected the plants against its toxic effect (Srobarova et al. 2005).

Certain S. cerevisiae strains with high pectolytic activity and pseudohyphae formation are reported to be pathogenic to the grapevine Vitis vinifera, penetrating the plant, delaying its growth, and causing death (Gognies et al. 2001; Gognies et al. 2006). Nevertheless, considering its ubiquity in vineyards and its use without adverse effect as a plant growth-promoting supplement for different crops (Karajeh, 2013), this parasitic behaviour is not considered ecologically significant.

There are no reports in the literature implicating S. cerevisiae in causing adverse effects to aquatic plants.

³ Testing conducted by Health Canada’s EHSRB (unpublished data)
b. Vertebrate animals

Although there have been occasional reports of infection in animals, *S. cerevisiae* is not known as an animal pathogen. The beneficial effects as a probiotic and immunomodulator are reported in both aquatic and terrestrial vertebrates.

*S. cerevisiae* cell wall extracts containing glucan, mannoprotein and chitin act as immunostimulants, promote growth and provide cellular and humoral defenses against disease in fish (Abu-Elala et al. 2013; Dimitroglou et al. 2008; Misra et al. 2006; Staykov et al. 2007; Whittington et al. 2005). There is no evidence of adverse effects on fish, when live *S. cerevisiae* are included as a feed supplement (AbdelTawwab et al. 2008; Abu-Elala et al. 2013; Kafilzadeh et al. 2013). No other reports of adverse effects in aquatic vertebrates have been reported.

*S. cerevisiae* has also been tested as a probiotic in terrestrial vertebrates. Dietary supplements of *S. cerevisiae* activate the humoral immune response in lambs (Harikrishna et al. 2010). Cows supplemented with *S. cerevisiae* have increased dry matter intake and milk yield, and reached milk production peak faster than non-supplemented cows (Dann et al. 2000; Desnoyers et al. 2009). Feed products containing *S. cerevisiae* are also suggested to increase fibre degradation, reduce the risk of rumen acidosis and provide complex B vitamins, selenium and other micronutrients in cows and lambs; thus, contributing to overall animal performance (Thurne et al. 2009; Guedes et al. 2008; Issakowicz et al. 2013). No adverse effects due to *S. cerevisiae* were reported in any of the above studies. *S. cerevisiae* also has antitoxic potential. It can degrade or adsorb mycotoxins, including trichothecenes, patulin, zearalenone, deoxynivalenol, ochratoxin A, fumonisins B1 and B2, aflatoxin and T-2 toxin, from animal feed (Etienne-Mesmin et al. 2012; Moslehi-Jenabian et al. 2010).

Adverse effects of *S. cerevisiae* in terrestrial vertebrates are exceedingly rare. There has been one report of *S. cerevisiae* chronic diarrhea in a dog (Milner et al. 1997). Although *S. cerevisiae* can be present in milk samples that test positive for mycotic mastitis (1-11% of total yeasts), it has not been implicated in causing mastitis (Türkyılmaz and Kaynarca, 2010; Al-Ameed, 2013; Malinowski et al. 2001). In addition, under experimental conditions, certain *S. cerevisiae* strains have been shown to act as opportunistic pathogens, in both immuno-compromised and immuno-competent mice (summarized in Appendices B-1, B-2.1, B-2.2 and B-2.3). However, based on Health Canada's *in vivo* animal studies, endotracheal instillation of the DSL strain *S. cerevisiae* F53 is found to be non-pathogenic and non-toxic to healthy animals (discussed below under 1.1.3.2 Human health effects).

c. Invertebrate animals

*S. cerevisiae* is not reported as an insect pathogen, in spite of its wide prevalence on insects. It is reported to be non-pathogenic and non-toxic to *Galleria mellonella* (great wax moth) larvae, in contrast to *C. albicans*, which caused mortality within 72
hours at 30°C (Cotter et al. 2000). *S. cerevisiae* is reported to have biocontrol activity against 5 different terrestrial plant-disease-causing root nematode species (*Meloidogyne incognita*, *Rotylenchulus reniformis*, *Meloidogyne javanica*, *Helicotylenchus exallus* and *Pratylenchus zeae*) however, no direct nematicidal activity was demonstrated in field studies (Ismail et al. 2005a; Ismail et al. 2005b; Karajeh, 2013; Karajeh, 2014 and Mokbel and Alharbi, 2014). One study proposed indirect biocontrol mechanisms mediated by *S. cerevisiae*, including induction of plant disease resistance via enhanced production of root phenolics, and promotion of overall plant health (as evidenced by increased root and shoot weight) (Karajeh, 2014). However, experimental tests have shown that *S. cerevisiae* strain BY4741 (a derivative of S288c) introduced as a food, can cause infection and death in the nematode model organism *Caenorhabditis elegans* (Jain et al. 2009). No other adverse effects in terrestrial invertebrates have been reported.

There is one report of *S. cerevisiae* as an opportunistic pathogen (<0.8% of total yeast infections) in freshwater prawns, in which the virulence of the isolated *S. cerevisiae* strain Myr-2 was demonstrated by experimental re-infection of prawns with strain Myr-2 (Chen et al. 2007). However, several other studies of *S. cerevisiae* as a feed supplement found no evidence of adverse effects on shrimp (AbdelTawwab et al. 2008; Abu-Elala et al. 2013; ChinChyuan et al. 2013; Kafilzadeh et al. 2013).

No other adverse effects in aquatic invertebrates were identified in the scientific literature.

**1.1.3.2 Human Health**

*S. cerevisiae* is generally considered an occasional digestive commensal, present transiently in the duodenal mucosa, oral cavities, digestive tract, vagina, skin, and oropharynx of healthy individuals (Aucott et al. 1990, Salonen et al. 2000; Ghannoum, 2010). Oral administration of *S. cerevisiae* in healthy human volunteers did not result in permanent implantation or multiplication of *S. cerevisiae* in the gastrointestinal system; and it was eliminated from the intestines within 5 days of end of treatment (Pecquet et al. 1991).

*S. cerevisiae* and, more particularly, strains of *S. cerevisiae* var. *boulardii* are commonly used as probiotics because of their ability to survive passage to the target organ (the colon) in the presence of gastric acid and bile salts and at human body temperature (37°C), form biofilms, display resistance to proteolysis and antibiotics, and because of their antimicrobial activity against foodborne pathogens (reviewed in Kelesidis and Pothoulakis, 2012 and Perricone et al. 2014). As such, *S. cerevisiae* and *S. cerevisiae* var. *boulardii* are commonly used in the prevention and treatment of intestinal disease caused by *Escherichia coli* and *Salmonella enterica* serovar Typhimurium, *Clostridium difficile*, *Citrobacter rodentium*, *Shigella flexneri* in humans (Moslehi-Jenabian et al. 2010). In healthy individuals, orally administered *S. cerevisiae* and *S. cerevisiae* var. *boulardii* has been documented to activate both
innate and adaptive immunity, with a significant increase in the number of erythrocytes, leucocytes, polymorphs and neutrophils; and activation of different complement system components such as C3, C5, C3d (Kelesidis and Pothoulakis, 2012; Lewis and Freedman, 1998; Machado Caetano et al. 1986). Based on fecal microbiota studies, *S. cerevisiae* var. *boulardii* improves the re-establishment of normal gut flora among patients with chronic idiopathic diarrhea, with no adverse effects in healthy subjects (Swidsinski et al. 2008).

Despite its widespread use in domestic and industrial settings and its occurrence in nature, *S. cerevisiae* infections in healthy individuals are rare. However, under certain circumstances it can proliferate, persist and disseminate in the body, invade different organs, and cause mucosal and systemic infections (Enache-Angoulvant and Hennequin, 2005) and therefore, it is considered as an emerging low virulence opportunistic pathogen (Yanez et al. 2009). Improper use of probiotics and ingestion of certain food strains have been documented as a plausible sources of infection (de Llanos et al. 2006a; Munoz et al. 2005); and *S. cerevisiae* var. *boulardii* is the most frequently reported organism causing *Saccharomyces* infection (Enache-Angoulvant and Hennequin, 2005; and reviewed in Kelesidis and Pothoulakis, 2012).

Based on the ARTEMIS clinical and laboratory sample surveillance program (1997-2007), 9.6 % of non-*Candida* yeast isolates (1080 isolates out of 11,240) were *S. cerevisiae* (Pfaller et al. 2009). According to Pfaller and Diekema (2010), 27% of the clinical non-*Candida*, non-*Cryptococcus* yeasts isolates in North America are *S. cerevisiae*. However, among the reported invasive fungal pathogens in North America, *Saccharomyces* spp. (*S. cerevisiae* and *S. cerevisiae* var. *boulardii*) infections accounted only for 0.35% (21 out of 6,031 cases) of invasive mycoses, compared to 75% of infections caused by *Candida* spp., 12.3% by *Aspergillus* spp., and 4.5% by *Cryptococcus* spp. (Pfaller et al. 2012; Pfaller et al. 2009; Pfaller and Diekema, 2010). *S. cerevisiae* infections in humans are reported predominantly in immuno-compromised individuals and/or those with underlying disease or medical conditions, and mostly in hospital settings (Enache-Angoulvant and Hennequin, 2005). *S. cerevisiae* fungemia is the most commonly reported infection; other reported infections include peritonitis, endocarditis, pneumonia, empyema, urinary tract infection, liver abscess, pyelonephritis, esophagitis and vaginitis (Belet et al. 2005; Chitasombat et al. 2012; de Llanos et al. 2006a; Enache-Angoulvant and Hennequin 2005; Lopes et al. 2006; Munoz et al. 2005). Infections have been reported in individuals with:

- gastrointestinal diseases (Candelli et al. 2003; Munoz et al. 2005);
- cancer (Anaissie et al. 1989; Aucott et al. 1990; Henry et al. 2004; Hovi et al. 1996; Williams et al. 2007);
- AIDS (Doyle et al. 1990; Konecny et al. 1999; Tawfik et al. 1989);
- broad-spectrum antibiotic therapy regime (de Llanos et al. 2006b; de Llanos et al. 2011);
- implantation of prosthetic devices or intravenous catheters (Belet et al. 2005; Cassone et al. 2003; Lherm et al. 2002);
- bone marrow or organ transplants (Cairoli et al. 1995; Olver et al. 2002);
- surgery or significant trauma (Belet et al. 2005; Riquelme et al. 2003);
- chronic kidney disease and diabetes mellitus (Pillai et al. 2014); and
- in newborns (Perapoch et al. 2000).

There is one reported outbreak of \textit{S. cerevisiae} var. \textit{boulardii} fungemia among three intensive care unit patients administered with lyophilized probiotic preparations, and also among those who did not receive the probiotic preparation possibly due to central venous catheter contamination (Cassone et al. 2003).

In a comprehensive review of reported cases of \textit{S. cerevisiae} infection, Enache-Angoulvant and Hennequin (2005) found that the treatment outcome (using antimicrobials or surgical intervention) was favorable in 63% of the patients (58 out of 92 cases), with 30% morbidity (27 deaths out of 92 cases) and 7% (6 out of 92 cases) where no treatment outcome was reported. The morbidity data cannot be attributed specifically to \textit{S. cerevisiae} infection due to concomitant isolation of other organisms in several of the case reports (Enache-Angoulvant and Hennequin, 2005).

The incidence of \textit{S. cerevisiae} infections in immuno-competent individuals is low. Approximately 0.9% to 5.8% of women are naturally colonized with non-pathogenic \textit{S. cerevisiae} strains (Posteraro et al. 1999). Few reports link \textit{S. cerevisiae} with \textit{Candida}-like vaginal infections. It is estimated that less than 1% of vaginal infections are caused by \textit{S. cerevisiae}; however, a higher incidence (5.4%) of \textit{S. cerevisiae} infections has been reported in women of child bearing age (Agatensi et al. 1991; Sobel et al. 1993; Posteraro et al. 1999; Enache-Angoulvant and Hennequin, 2005). When they occur, \textit{Saccharomyces} infections are clinically indistinguishable from invasive candidiasis (Al-Hedaiithy, 2002; McCullough et al. 1998; Paulitsch et al. 2006; Saporiti et al. 2001; Savini et al. 2008; Skovgaard, 2007). Other reported infections in healthy individuals are very rare. \textit{S. cerevisiae} was identified as the causative agent of a lung nodule in a healthy baker, who was successfully treated (Ren et al. 2004). Also, a case of recurrent community-acquired \textit{S. cerevisiae} fungemia over a period of 6 years was reported in a healthy adult woman in Israel, who was also treated successfully with anti-fungal medications (Hamoud et al. 2011).

\textit{In vivo} testing at Health Canada was conducted using four replicates of six- to eight-week-old BALB/c mice exposed to $10^6$ CFU/25µL of \textit{S. cerevisiae} F53 and \textit{S. cerevisiae} var. \textit{boulardii} MYA 796 for 48 hours and 1 week by endotracheal instillation. Animals were monitored for behaviour and necropsied 48 hours or one week following exposure and tissues were processed for clearance from lungs, trachea and esophagus. Lung cytokine expression and pulmonary granulocyte infiltration (inflammation) were also tested. Neither \textit{S. cerevisiae} F53 nor \textit{S. cerevisiae} var. \textit{boulardii} MYA 796...
caused mortality or observable adverse effects in BALB/c mice. Animals were asymptomatic and showed no changes in behaviour or physical appearance. Lung clearance was significant even within 48 h following exposure, although low levels were still detected. At one week, both tested yeast strains were completely cleared from the lungs, trachea and esophagus. Levels of lung pro-inflammatory cytokines, namely IL-1a, IL-1b, IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12(p40), IL-12(p70), IL-13, IL-17, Eotaxin, G-CSF, GM-CSF, IFN-g, KC, MCP-1, MIP-1a, MIP-1b, RANTES and TNF-α, were not significantly elevated at 48 hours or one week post-exposure. To determine the systemic response of the animals upon yeast exposure, the level of an acute phase marker serum amyloid A (SSA) was measured in the blood 48 hours and one week post-exposure. There was no significant elevation of SAA at these time points. Based on these studies, the DSL stain S. cerevisiae F53 is not virulent to the mouse model tested; and healthy animals are able to tolerate relatively high concentrations of S. cerevisiae F53.4

The effects of experimental challenge in murine species using S. cerevisiae have also been reported in the literature. These studies, described below, also indicate that S. cerevisiae is an opportunistic pathogen of low virulence, particularly in comparison with C. albicans. Some studies also highlight the variability in responses to S. cerevisiae challenge in immuno-competent and immuno-compromised hosts, as summarized in Table 0-4, Table 0-5, Table 0-6, and Table 0-7. Although clinical strains are generally virulent in mice, not all clinical strains caused mortality or significant yeast burdens in the organs tested (e.g., brain, liver, kidneys or spleen). In most studies, immuno-competent mice were able to clear administered S. cerevisiae. Although S. cerevisiae virulence has been suggested to be a strain-specific trait, host immune status appears to be the more important factor. In vivo studies comparing the pathogenic potential of commercial biotherapeutic, probiotic, food, clinical or other environmental strains, indicate that certain dietetic, probiotic or environmental strains may be as virulent as clinical strains, depending on the host immune status (Byron et al. 1995; Clemons et al. 1994; de Llanos et al. 2011; Llopis et al. 2014; Yanez et al. 2009). A feral strain, EM93, isolated from decaying fruit, has been reported to be much more virulent than a clinical strain tested in DBA/2 mice, but not in BALB/c mice (Wheeler et al. 2003); and the authors had suggested that environmental or plant isolates could also serve as a source of infection for immuno-compromised patients (Wheeler et al. 2003).

Furthermore, in mice, deficiency of the complement component C5 factor and immuno-suppression induced by treatment with cyclohexamide or betamethasone (along with prolonged antibiotic administration) increased susceptibility to certain dietetic and probiotic S. cerevisiae strains (Byron et al. 1995; Yanez et al. 2009; de Llanos et al. 2011, Llopis et al. 2014).

4 Testing conducted by Health Canada’s EHSRB (unpublished data)
1.1.3.2.1 Allergenicity

A few cases of allergic reaction resulting from either oral, dermal, or inhalation exposure to *S. cerevisiae* have been reported (Airola et al. 2006; Bataille et al. 1995; Belchi-Hernandez et al. 1996; Houba et al. 1998; Ogawa et al. 2004; Pajno et al. 2005). The major allergens from *S. cerevisiae* include enolase, acid protease and mannan (Nitter-Marszalska et al. 2001). Allergic reactions are usually related to repeated and prolonged occupational exposure to *S. cerevisiae*. Yeast allergens in wine and baked products are effectively degraded in the intestinal tract (Kortekangas-Savolainen et al. 1994).

1.2 Hazard severity

A combination of morphological, biochemical, and molecular studies allow *S. cerevisiae* F53 to be reliably identified; however, it is difficult to discriminate the DSL strain from other *Saccharomyces* strains, including its closely-related subspecies, *S. cerevisiae* var. *boulardii*, which has been implicated in clinical infections.

Despite its occurrence in nature and a history of release into the environment as a probiotic food and feed supplement in aquaculture, swine, poultry and livestock diet, as a fertilizer or soil-amending substance and from industrial fermentation facilities, reports of *S. cerevisiae* pathogenicity to wildlife are exceedingly rare. Information from the scientific literature suggests that *S. cerevisiae* is not a frank pathogen towards environmental species. There is one report of *S. cerevisiae* infection in a dog with a history of chronic diarrhea and prolonged antibiotic use; and one report of infections in prawns. There are reports of biocontrol activity of *S. cerevisiae* against five different plant-disease-causing nematodes, and one report of *S. cerevisiae* toxicity to *Caenorhabditis elegans* in feeding experiments. Also, under experimental conditions, some strains of *S. cerevisiae* are pathogenic to certain mouse models. However, pulmonary exposure (via endotracheal instillation) of 6-8 week old BALB/c female mice to *S. cerevisiae* F53 up to 1 week, showed that healthy animals can tolerate a high concentration (10^6 CFU) of *S. cerevisiae* F53 with no observable adverse effects (described in section 1.1.3.2).

*S. cerevisiae* F53 has been in use for several decades in Canada, during which it has been released into the environment, yet there are no reports in the literature implicating the DSL strain *S. cerevisiae* F53 in causing adverse effects on either terrestrial or aquatic plants, invertebrates or vertebrates. Although some adverse effects have been shown for the species towards invertebrates, and there is a lack of specific pathogenicity/toxicity testing for this strain on invertebrates, the overall hazard severity to the environment of it is considered to be low, given the extensive history of safe use and evidence that *S. cerevisiae* F53 does not possess any known virulence characteristics based on Health Canada’s *in vitro* and *in vivo* studies.

*S. cerevisiae* is an organism of low virulence in humans when compared to *C. albicans* and other fungal pathogens and opportunistic pathogens. In spite of a long
history of human exposure associated with domestic and industrial use of *S. cerevisiae* in baking and brewing, and as a probiotic, there are few reports of human infection. When they occur, infections are usually in individuals with predisposing factors such as compromised immunity, trauma, previous surgery or invasive medical procedures, or are hospital-acquired. In most cases, oral ingestion of *S. cerevisiae* var. *boulardii* as a biotherapeutic agent was associated with these infections. *S. cerevisiae* infections have been effectively treated with antifungals in the majority of cases. Few mortalities have been reported, and these cannot be attributed solely to *S. cerevisiae* infection, since several of the infections were polymicrobial and subjects had underlying medical conditions. *S. cerevisiae* rarely causes infection in healthy individuals.

No human infections have been specifically attributed to the DSL strain, *S. cerevisiae* F53, despite its use for several decades as a baking strain. Health Canada studies with murine models have indicated that *S. cerevisiae* F53 is avirulent in healthy animals. The human hazard severity for *S. cerevisiae* F53 is therefore estimated to be low.

Hazards related to microorganisms used in the workplace should be classified under the Workplace Hazardous Materials Information System (WHMIS)\(^5\).

### 2. Exposure Assessment

#### 2.1 Sources of Exposure

This assessment considers exposure to *S. cerevisiae* F53 resulting from its addition to consumer or commercial products and its use in industrial processes in Canada.

*S. cerevisiae* F53 was nominated to the DSL in 2004 for commercial use in Canada. According to the nominator, 10-100 metric tonnes were imported in 1986.

Responses to a voluntary questionnaire sent in 2007 to a subset of key biotechnology companies in Canada, combined with information obtained from other federal government regulatory and non-regulatory programs, indicate that 10-100 metric tonnes of products potentially containing *S. cerevisiae* F53 (formulation and concentration unknown) were imported into or manufactured in Canada in 2006-2007, for various commercial uses.

\(^5\) 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 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 on *S. cerevisiae* F53 is not relevant to, nor does it preclude, an assessment against the hazard criteria for WHMIS that are specified in the *Hazardous Products Regulations* for products intended for workplace use.
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 *Saccharomyces cerevisiae* F53, whether alone, in a mixture, or in a product. Responses to the section 71 Notice indicate that over 10,000 metric tonnes of products of *Saccharomyces* F53 were imported into or manufactured in Canada during the 2008 reporting year, for use in consumer and commercial applications, such as production of foods, feeds and beverages, as well as in, research and development. Although the section 71 Notice was intended to gather information about living organisms, based on the uses reported, some respondents may have included inactive *Saccharomyces* F53 (for example, yeast extract) in their responses to the survey. This assessment will only consider exposure to living *Saccharomyces* F53.

*S. cerevisiae* F53 is advertised as baker's yeast (Product Sheet-1, 2014) and has a long history of use in food industry. While direct human exposure to *Saccharomyces* F53 and other naturally-occurring *Saccharomyces* strains used in products such as novel foods, food additives, pharmaceuticals, human biologics, medical devices, veterinary drugs, cosmetics and natural health products is regulated under the Food and Drugs Act, exposure through environmental media from these uses is subject to CEPA, and will be considered as part of the exposure assessment.

As *Saccharomyces* F53 is on the DSL, and so can be used in Canada without prior notification as a ‘new substance’ under the New Substances Notification Regulations, it could be an attractive choice for further commercialization. A search of the public domain (SDS, literature and patents) revealed the following consumer, commercial and industrial applications of other strains of *Saccharomyces*. These represent possible uses of the DSL strain, as strain *Saccharomyces* F53 is likely to share characteristics (modes of action) with other commercialized *Saccharomyces* strains. The known uses of *Saccharomyces* include:

- home and industrial brewing of alcoholic beverages (Product Sheet-2a, 2013; Product Sheet-2b, 2013; Product Sheet-2c, 2013);
- addition to dairy, beef, horse, pig and water buffalo feed (Product Sheet-3, 2014);
- as a probiotic growth-promoting and immune-modulating dietary substance, in fish, shrimp, pigs and broiler chicken farming (AbdelTawwab et al. 2008; Abu-Elala et al. 2013; ChinChyuan et al. 2013; Kafilzadeh et al. 2013);
- as a nutrient for rearing of insects such as aphids, beetle, fruit flies, mites etc. (Product Sheet-4, 2014);
- production of bioethanol (Product Sheet-5, 2014), UManitoba, 2014);
- production of enzymes and biochemicals (Vakhlu and Kour, 2006; Product sheet-6, 2014);
- bioremediation (Product Sheet-7, 2014);
- as a deodorizer for home use to eliminate foul smells (Product Sheet-8, 2014);
as a cleaning agent for pond and aquarium to improve water quality (Product Sheet-8, 2014);
for agricultural purposes, including use in fungi production, micro-algae and organic farming (Product sheet-9, 2014); and
for research and development purposes, in a wide array of fields, including genetics, genomics and synthetic biology.

Other potential uses include, but are not limited to:

treatment of diabetes and cardiovascular diseases in humans (Hosseinzadeh et al. 2013 and Díaz-Apodaca et al. 2010);
addition to feed to reduce odour from animal waste products (Cheung, 2003);
production of isobutanol (Feldman et al. 2012);
decomposition of agricultural and food processing wastes that are applied to agricultural fields to increase soil microbial activity and improve soil fertility (El-Sayed Shalaby and El-Nady, 2008; Nally et al. 2005);
post-harvest treatment of grapes to reduce food spoilage caused by bacterial infections (Nally et al. 2012);
bio remediation of soils and treatment of waste water containing metals and other contaminants (Wang and Chen, 2006);
treatment of waste water generated from rice noodle industries (Siripattanakul-Ratpukdi, 2012); and
for decolourization of dyes (e.g., methyl red and malachite green) from the textile effluents (Jadhav and Govindwar, 2006)

2.2 Exposure Characterization

2.2.1 Environment

Environmental exposure to S. cerevisiae F53 is estimated to be high based on the response to the voluntary survey and the section 71 Notice, in which reported uses included use in consumer and commercial applications, including production of foods, feeds and beverages. In addition, use in consumer, industrial, commercial and agricultural sectors were also identified, based on known and potential uses of other S. cerevisiae strains, as described in Section 2.1 Sources of exposure. The following environmental exposure scenarios are therefore considered based on the known and potential uses of S. cerevisiae F53, along with the persistence and survival properties of this microorganism.

Terrestrial species, including plants, invertebrates and vertebrates are expected to be exposed to S. cerevisiae F53 through uses such as application to agricultural fields and crops (e.g., for soil conditioning), composting, decomposition of food and agricultural wastes, and bio remediation through disposal of food and feed wastes, and release of biomass from the fermentation industries.
Aquatic species, including plants, invertebrates and vertebrates are expected to be exposed to *S. cerevisiae* F53 through uses such as the treatment of ponds and aquariums, and via the release of effluents from fermentation industries. In addition, aquatic species could be exposed to run-off subsequent to application in soil.

Direct exposure of terrestrial vertebrates to *S. cerevisiae* F53 is expected to be greatest through consumption of livestock probiotics and feed supplements for which the use of active or dehydrated *Saccharomyces* species have been approved under the *Feeds Act* by the CFIA. Feed supplements used in aquaculture could expose aquatic invertebrates and vertebrates to high concentrations of *S. cerevisiae* F53.

The extent of exposure to *S. cerevisiae* F53 will depend on the mass or volume released, on its persistence in the receiving environment, and on the proximity of environmental species to the sites of application or disposal.

*S. cerevisiae* is metabolically versatile and is expected to readily adapt to various environments. It has been isolated from soil, water, plants, animals, wastes and wastewater under different conditions. *S. cerevisiae* does not disperse readily and its survival and persistence in the receiving environment will be dependent on several factors including availability of high sugar or organic matter, temperature, water, and soil type. Artificially-introduced *S. cerevisiae* has been shown to persist in waste water and soil not more than 40 days after release (Fujimura et al. 1994). Large quantities of liquid or solid residues of *S. cerevisiae* released from wine industries into the environment around the winery persisted for 12 months in soil and 18 to 24 months on live plant material (Cordero-Bueso et al. 2011). Although in theory, continuous and repetitive release of commercial yeasts could lead to the maintenance of a population of introduced *S. cerevisiae* in the receiving environment, particularly where its persistence was favoured by the presence of fermentable sugars and organic matter. It is not known to permanently persist or survive in receiving environments, or to out-compete autochthonous strains (Valero et al. 2005).

**2.2.2 Human**

Human exposure to *S. cerevisiae* F53 is estimated to be high based on the response to the voluntary survey and the section 71 Notice, in which reported uses included use in consumer and commercial applications, including production of foods, feeds and beverages. In addition, use in consumer, industrial, commercial and agricultural sectors were also identified, based on known and potential uses of other *S. cerevisiae* strains, as described in Section 2.1 Sources of Exposure. The following human exposure scenarios are therefore considered based on the known and potential uses of *S. cerevisiae* F53.
Direct human exposure to live *S. cerevisiae* is expected to be greatest through the consumption of natural health products such as probiotics containing viable yeasts, considering that *S. cerevisiae* is included on the NNHPD’s Probiotics monograph. Post-harvest treatment of fruits and vegetables with *S. cerevisiae* to prevent food spoilage has not been identified in Canada, but is a potential use that could result in direct exposure from handling and consuming treated foods. Direct human exposure could also occur if *S. cerevisiae* F53 is present in consumer products for the treatment of wastewater (septic tank additives), or ponds and aquaria.

The general population could be exposed as bystanders during the application of *S. cerevisiae* F53 for agricultural uses, composting and pond and aquarium treatment. The route and extent of exposure will depend on the nature of the product, the application method, the concentration of *S. cerevisiae* F53 in the product, the amount of product applied, and the proximity of the bystander to the site of application, but in general is expected to be moderate to low, compared to exposure to live yeasts in dietary supplements and probiotics. The general population could also come into contact with residual *S. cerevisiae* F53 on surfaces treated with commercial products.

Furthermore, indirect human exposure to *S. cerevisiae* F53 in the environment could occur following 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 the proximity 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 from the consumption of natural health products such as probiotics.

In the event that the organism enters municipal drinking water treatment systems through release from known and potential uses, the treatment processes which include coagulation, flocculation, ozonation, filtration and chlorination, are expected to effectively eliminate *S. cerevisiae* F53 from drinking water.

### 3. 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 *S. cerevisiae* F53 to be low for the environment and human health. Based on responses to the section 71 Notice, the exposure to living *S. cerevisiae* F53 from its use in industrial processes or commercial applications in Canada is expected to be high for the environment and for humans. The overall risk
associated with current uses is, nevertheless, estimated to be low to the environment and low for 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).

The potential risk for opportunistic infections particularly among immuno-compromised individuals through probiotic use of *S. cerevisiae* F53, is mitigated through the probiotic licensing process administered by the NNHPD under the *Food and Drugs Act*; wherein, in addition to demonstration of product safety, contraindication of use in immuno-compromised individuals is a requirement of product labels as part of the recommended conditions of use for this species.

*S. cerevisiae* F53 has other useful properties that make it of interest for use as a home deodorizer, and for bioremediation, prevention of food spoilage, and for the production of biochemicals. These uses could increase environmental and human exposure to this strain in the future. Nevertheless, the risk from reasonably foreseeable uses of *S. cerevisiae* F53 is expected to remain low to the environment, and low to human health.

### 4. Conclusion

Based on the information presented in this screening assessment, it is concluded that *S. cerevisiae* F53 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 *S. cerevisiae* F53 does not meet the criteria as set out in section 64 of the CEPA.
5. References


Arlorio, M., Coïsson, J.D., and Martelli, A. (1999). Identification of *Saccharomyces cerevisiae* in bakery products by PCR amplification of the ITS region of ribosomal DNA. European Food Research and Technology 209, 185-191.


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EFSA. (2012). Scientific opinion on the substantiation of health claims related to a combination of Lactobacillus rhamnosus CNCM I-1720, Lactobacillus helveticus CNCM I-1722, Bifidobacterium longum subsp. longum CNCM I-3470 and Saccharomyces cerevisiae var. boulardii CNCM I-1079 and defence against pathogenic gastro-intestinal microorganisms. EFSA Panel on Dietetic Products,Nutrition and Allergies; ID 3017, (further assessment), pursuant to Article 13(1) of Regulation (EC) No 1924/2006.,


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Appendices

Appendix A- Characteristics of *S. cerevisiae* F53

Table 0-1: Growth of *S. cerevisiae* F53 in liquid media at various temperatures

<table>
<thead>
<tr>
<th>Medium</th>
<th>28°C</th>
<th>32°C</th>
<th>37°C</th>
<th>42°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yeast extract peptone dextrose (YPD)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Dulbecco's Modified Eagle's medium (DMEM) With 10% FBS and Glutamine</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fetal Bovine Serum (FBS)</td>
<td>~</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10% FBS</td>
<td>~</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

- no growth, + growth, ~ low or negligible level growth,

Data generated by Health Canada’s Environmental Health Science and Research Bureau (EHSRB). Growth of *S. cerevisiae* F53 in broth culture, as measured by increase in absorbance at 500 nm, in four different growth media and over a range of temperatures: Concentration of the yeast at time zero was $10^4$ CFU/mL.
Table 0-2: Multiple sequence alignment percent identity matrix of *S. cerevisiae* sequences

<table>
<thead>
<tr>
<th><em>S. cerevisiae</em> strain</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>1: <em>S. cerevisiae</em> F53</td>
<td>100.00</td>
<td>99.46</td>
<td>99.63</td>
<td>99.91</td>
<td>99.68</td>
<td>99.91</td>
</tr>
<tr>
<td>2: <em>S. cerevisiae</em> CECT10431</td>
<td>99.46</td>
<td>100.00</td>
<td>99.86</td>
<td>99.91</td>
<td>99.91</td>
<td>99.91</td>
</tr>
<tr>
<td>3: <em>S. cerevisiae var boulardii</em> ATCC MYA-796</td>
<td>99.63</td>
<td>99.86</td>
<td>100.00</td>
<td>99.95</td>
<td>99.95</td>
<td>99.95</td>
</tr>
<tr>
<td>4: <em>S. cerevisiae</em> ATCC 18824</td>
<td>99.91</td>
<td>99.91</td>
<td>99.95</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
<tr>
<td>5: <em>S. cerevisiae</em> ATCC 9763</td>
<td>99.68</td>
<td>99.91</td>
<td>99.95</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
<tr>
<td>6: <em>S. cerevisiae</em> YJM-309</td>
<td>99.91</td>
<td>99.91</td>
<td>99.95</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
</tbody>
</table>

Data generated by Health Canada’s EHSRB. The identity matrix was developed using the ribosomal RNA operon sequences for various *S. cerevisiae* strains, amplified using the primer positions (ITS1 and LR7) used in sequence analysis. The transcriptional unit contains the 18S small subunit (SSU), Internal transcribed spacer (ITS) consisting of ITS1, 5.8S rDNA subunit, and ITS2; followed by the 25S large subunit rDNA (LSU) of which the first 600-900bp comprise the D1/D2/D3 divergent regions.
Table 0-3: In vitro tests to determine putative virulence traits of *S. cerevisiae* F53

<table>
<thead>
<tr>
<th>Characteristics</th>
<th><em>S. cerevisiae</em> F53</th>
<th><em>S. cerevisiae</em> ATCC 18824</th>
<th><em>S. cerevisiae</em> var. boulardii ATCC MYA 796</th>
<th><em>S. cerevisiae</em> YJM 309</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth at 42º C</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Pseudohyphal growth on low nitrogen medium</td>
<td>None observed</td>
<td>None observed</td>
<td>None observed</td>
<td>Observed</td>
</tr>
<tr>
<td>Invasive growth on low nitrogen medium</td>
<td>None observed</td>
<td>None observed</td>
<td>None observed</td>
<td>Observed</td>
</tr>
<tr>
<td>Phospholipase secretion</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Pseudo-hyphal growth on SLAD medium</td>
<td>None observed</td>
<td>None observed</td>
<td>None observed</td>
<td>Observed</td>
</tr>
<tr>
<td>Invasive growth on SLAD medium</td>
<td>None observed</td>
<td>None observed</td>
<td>None observed</td>
<td>Observed</td>
</tr>
</tbody>
</table>

aData generated by Health Canada’s EHSRB.

Figure A-1: Clearance of yeast cells from the lungs of BALB/c mice after exposure to *S. cerevisiae* F53 and *S. cerevisiae* var. boulardii MYA-796

aData generated by Health Canada’s EHSRB.
Figure 0-2: Clearance of yeast cells from the trachea of BALB/c mice after exposure to *S. cerevisiae* F53 and *S. cerevisiae* var. *boulardii* MYA-796a

Data generated by Health Canada's EHSRB.

Figure 0-3: Clearance of yeast cells from the esophagus of BALB/c mice after exposure to *S. cerevisiae* F53 and *S. cerevisiae* var. *boulardii* MYA-796a

Data generated by Health Canada's EHSRB.
Appendix B: Summary of literature on pathogenicity of S. cerevisiae and S. cerevisiae var. boulardii relevant to this screening assessment

Table 0-4: Summary of literature of in vivo studies published between 1990 and 2010

<table>
<thead>
<tr>
<th>Mouse strain and dose</th>
<th>Test strain and dose</th>
<th>Observations</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 week old CD-1</td>
<td>clinical and non-clinical isolates (laboratory strains, industrial strains, fresh isolates from natural fermentations) isolates; Laboratory strain Y55 used as control.</td>
<td>- 7, 14 and 28 day time-points and yeast clearance from tissues over time studied - comparison of temporal yeast burden in brain, spleen, liver, kidney and lungs - no mortalities; clinical strains proliferated more in brain. - the burden of a clinical strain in brain and kidney was significantly higher compared to Y55 on 7 days and 14 days post-inoculation, but eventually cleared off after 28 days post-inoculation. On day 56, 80% of infected mice were free of infection. - the rate of clearance of Y55 from all organs was significantly faster than the clinical strain.</td>
<td>Clemons et al. 1994</td>
</tr>
<tr>
<td>4 week old DBA/2N a; and mice congenic for C5 (B10.D2/oSnJ C+) and (B10.D2/oSnJ C-)</td>
<td>clinical and non-clinical isolates (laboratory strains, industrial strains, fresh isolates from natural fermentations) isolates; Laboratory strains Y55, Y237 and a non-clinical strain YJM 264 were used as controls.</td>
<td>- 14 time-points and yeast clearance from tissues over time studied - comparison of temporal yeast burden in brain, spleen, liver, kidney and lungs In DBA/2N mice: - 90% mortality by a clinical strain YJM 128, 67% mortality by YJM 264 and no mortality by YJM 237 or Y55. Mortality caused by other clinical isolates ranged from 0-100%. - The yeast burden of clinical strains were significantly higher than the laboratory and non-clinical strains. YJM 128 burden was 20 fold higher in the brain and 4 fold higher in kidneys, compared to Y55 accumulation.</td>
<td>Byron et al. 1995</td>
</tr>
<tr>
<td>Mouse strain and dose</td>
<td>Test strain and dose</td>
<td>Observations</td>
<td>References</td>
</tr>
<tr>
<td>-----------------------</td>
<td>----------------------</td>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Clinical strain YJM 128, caused 63% mortality in C⁻ mice, and no mortality in C⁺ mice; The yeast burden in all organs were significantly higher in C⁻ mice compared to C⁺ mice.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-Laboratory strain Y55, caused no mortality in both C⁻ and C⁺ mice; The yeast burden in brain, kidneys and spleen were significantly higher in C⁻ mice compared to C⁺ mice; whereas the burdens recovered from liver and lung were equivalent.</td>
<td></td>
</tr>
<tr>
<td>4 week old DBA/2 and BALB/C</td>
<td>EM93 (environmental isolate from rotting fig; progenitor of S288C and contributes to ~88% of S288C gene pool) and CISC44 (clinical isolate)</td>
<td>14 days study; observation also done up to 4 weeks for surviving mice</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>In DBA/2 mice: EM 93 caused 100% mortality within one week; Large number of live yeasts (≥10⁶ yeasts) in kidneys; whereas CISC44 caused no mortality up to 4 weeks.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>In BALB/C mice: EM93 caused no mortality.</td>
<td></td>
</tr>
<tr>
<td>8-10 weeks old DBA/2 C3H/HeN; and C57BL/6</td>
<td>vaginal isolates and probiotic and dietetic strains and food reference strain (natural wine isolate) and virulent C. albicans strains used as controls</td>
<td>14 day study:</td>
<td>Yanez et al. 2009</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-no mortalities other than a dietetic S. cerevisiae strain (D14) caused 14% death (2 out of 14 animals died) ;</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>- virulent C. albicans at 1x10⁶ CFU/mouse causes mortality in a few days</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-no difference in survival curves of all 3 mouse strains</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-yeast burdens of probiotic, dietetic and clinical strains in brain, kidney and spleen;</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-yeast burden diminished by day 14 for all strains</td>
<td></td>
</tr>
<tr>
<td>6 weeks old Balb/c female mice</td>
<td>probiotic and dietetic strains and food reference strain CECT 10431; and Laboratory strain W303 and virulent strains YJM</td>
<td>- 7, 15 and 30 days post inoculation</td>
<td>(Llopis et al. 2014)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>For IV inoculation: mice inoculated with probiotic strains D2, D4 and D14; and clinical strain YJM 128 showed weight loss, hair frizz and low mobility 3 days post-inoculation; 28% mortality caused only by D14, while the remaining mice from other treatments</td>
<td></td>
</tr>
</tbody>
</table>

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Final Screening Assessment

Saccharomyces cerevisiae F53
<table>
<thead>
<tr>
<th>Mouse strain and dose</th>
<th>Test strain and dose</th>
<th>Observations</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFU/mouse</td>
<td>128 and clinical isolate 102 used as controls</td>
<td>recovered after 7 days.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>- All strains colonized the brains and kidneys, more preferentially in the brains. Probiotic strain D23 and laboratory strain W303 were undetectable in brains and kidneys from day 7 post-infection.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Differential virulence pattern was clearly evident 15 days post-infection; probiotic strains D2, D4 and D14; and clinical strain YJM 128 had significantly higher burden in brain than other strains. D14 showed higher burden levels compared to YJM 128.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Comparatively, D14 is rated as a virulent isolate, whereas D2 and D4 are considered as of low virulence.</td>
<td></td>
</tr>
<tr>
<td>5-6 weeks old Balb/c female mice</td>
<td>- probiotic strains D23 and D14</td>
<td>5, 6 and 7 days post inoculation, and observation of Peyer’s patches, mesenteric lymph nodes (MLNs), liver, kidney and brain.</td>
<td>(Llopis et al. 2014)</td>
</tr>
<tr>
<td>Dose: Oral inoculation of 1 x 10⁹ CFU (via intragastric incubation), followed by 7 x 10⁹ CFU/ml via drinking water for 3 days;</td>
<td></td>
<td>- Indigenous gut microbiota was cleared using high dose of antibiotics prior to the experiment; Antibiotics was continuously administered during the tests to prevent bacterial infections; 3-days post-yeast administration, mice treated with dexamethasone for immunosuppression.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Fecal yeast levels were higher for D14 compared to D23. Significantly higher burden levels were observed for D14 in Peyer’s patches and MLNs (indicators of translocation of yeasts through intestinal barriers and spreading into MLNs), and also in the organs (via dissemination).</td>
<td></td>
</tr>
</tbody>
</table>

a C5 deficient (immune compromised)

b Vallimon et al. 2004 (virulence of C. albicans)
Table 0-5: Summary of results of the BALB/C mouse in vivo study published by de Llanos et al. 2011

<table>
<thead>
<tr>
<th>S. cerevisiae strain</th>
<th>Mortality (%)</th>
<th>Yeast burden in brain $^a$</th>
<th>Yeast burden in kidney $^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biotherapeutic strain (Ultralevure-S. cerevisiae subsp. boulardii)</td>
<td>20% (2 out of 10)</td>
<td>UD</td>
<td>UD</td>
</tr>
<tr>
<td>Bakery strain (cinta roja)</td>
<td>30% (3 out of 10)</td>
<td>UD</td>
<td>UD</td>
</tr>
<tr>
<td>Wine strains (Fermivin crio 7303; T73)</td>
<td>0%</td>
<td>D</td>
<td>UD</td>
</tr>
<tr>
<td>Clinical strain 20</td>
<td>0%</td>
<td>UD</td>
<td>UD</td>
</tr>
<tr>
<td>Clinical strain 60</td>
<td>20% (2 out of 10)</td>
<td>UD</td>
<td>UD</td>
</tr>
<tr>
<td>Clinical strain 75</td>
<td>0%</td>
<td>UD</td>
<td>UD</td>
</tr>
<tr>
<td>Clinical strain 102</td>
<td>20% (2 out of 10)</td>
<td>UD</td>
<td>D</td>
</tr>
<tr>
<td>Food reference strain (CECT 10431)</td>
<td>0%</td>
<td>UD</td>
<td>UD</td>
</tr>
</tbody>
</table>

$^a$ Detectable levels (statistically significant burden level compared to food reference strain); UD-Undetectable

$^a$ 30 days post-infection
Table 0-6: Summary of results of the DBA 2/N\(^{a}\) mouse in vivo study published by de Llanos et al. 2011

<table>
<thead>
<tr>
<th>S. cerevisiae strain</th>
<th>Mortality (%)</th>
<th>Yeast burden in brain (^{b})</th>
<th>Yeast burden in kidney (^{b})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biotherapeutic strain (Ultralevure-S. cerevisiae subsp. boulardii)</td>
<td>0%</td>
<td>UD</td>
<td>UD</td>
</tr>
<tr>
<td>Bakery strain (cinta roja)</td>
<td>0%</td>
<td>UD</td>
<td>UD</td>
</tr>
<tr>
<td>Wine strains (Fermivin crio 7303; T73)</td>
<td>0%</td>
<td>D</td>
<td>UD</td>
</tr>
<tr>
<td>Clinical strain 20</td>
<td>0%</td>
<td>UD</td>
<td>UD</td>
</tr>
<tr>
<td>Clinical strain 60</td>
<td>0%</td>
<td>UD</td>
<td>UD</td>
</tr>
<tr>
<td>Clinical strain 75</td>
<td>20% (2 out of 10)</td>
<td>UD</td>
<td>UD</td>
</tr>
<tr>
<td>Clinical strain 102</td>
<td>50% (5 out of 10)</td>
<td>UD</td>
<td>UD</td>
</tr>
<tr>
<td>Food reference strain (CECT 10431)</td>
<td>0%</td>
<td>UD</td>
<td>UD</td>
</tr>
</tbody>
</table>

\(^{b}\) Detectable levels (statistically significant burden level compared to food reference strain); UD - Undetectable

\(^{a}\) C5 deficient (immune compromised)

\(^{b}\) 30 days post-infection
### Table 0-7: Summary of results of the ICR/Swiss-CY \(^a\) mouse in vivo study published by de Llanos et al. 2011

<table>
<thead>
<tr>
<th>S. cerevisiae strain</th>
<th>Mortality (%)</th>
<th>Yeast burden in brain (^b)</th>
<th>Yeast burden in kidney (^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biotherapeutic strain (Ultralevure-S. cerevisiae subsp. boulardii)</td>
<td>20% (1 out of 5)</td>
<td>D</td>
<td>D</td>
</tr>
<tr>
<td>Bakery strain (cinta roja)</td>
<td>40% (2 out of 5)</td>
<td>D</td>
<td>D</td>
</tr>
<tr>
<td>Wine strains (Fermivin crio 7303; T73)</td>
<td>0%</td>
<td>No data</td>
<td>No data</td>
</tr>
<tr>
<td>Clinical strain 20</td>
<td>0%</td>
<td>No data</td>
<td>No data</td>
</tr>
<tr>
<td>Clinical strain 60</td>
<td>80% (4 out of 5)</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Clinical strain 75</td>
<td>0%</td>
<td>No data</td>
<td>No data</td>
</tr>
<tr>
<td>Clinical strain 102</td>
<td>20% (2 out of 10)</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Food reference strain (CECT 10431)</td>
<td>0%</td>
<td>No data</td>
<td>No data</td>
</tr>
</tbody>
</table>

D - Detectable levels (statistically significant burden level compared to food reference strain); UD - Undetectable

\(^a\) cyclophosphamide treated (immune compromised)

\(^b\) 30 days post-infection