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Yeast diversity in hypersaline habitats

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Abstract

Thus far it has been considered that hypersaline natural brines which are subjected to extreme solar heating, do not contain non-melanized yeast populations. Nevertheless we have isolated yeasts in eight different salterns worldwide, as well as from the Dead Sea, Enriquillo Lake (Dominican Republic) and the Great Salt Lake (Utah). Among the isolates obtained from hypersaline waters, *Pichia guilliermondii, Debaryomyces hansenii, Yarrowia lipolytica* and *Candida parapsilosis* are known contaminants of low water activity food, whereas *Rhodosporidium sphaerocarpum*, *R. babjevae*, *Rhodotorula laryngis*, *Trichosporon mucoides*, and a new species resembling *C. glabrata* were not known for their halotolerance and were identified for the first time in hypersaline habitats. Moreover, the ascomycetous yeast *Metschnikowia bicuspidata*, known to be a parasite of the brine shrimp, was isolated as a free-living form from the Great Salt Lake brine. In water rich in magnesium chloride (bitterns) from the La Trinitat salterns (Spain), two new species provisionally named *C. atmosphaerica* – like and *P. philogaea* – like were discovered.

Keywords: Yeast; Hypersaline water; Biodiversity; Halotolerance; 26S rDNA sequence

1. Introduction

Xerophilic and xerotolerant fungi are known contaminants of low water activity (a_w) foods. Yeasts predominate in liquid products such as syrups, honey and brines. Xerotolerant food borne yeasts include the genera *Candida* (*C. lambica*), *Debaryomyces* (*D. hansenii*), *Pichia* (*P. anomala*, *P. guilliermondii*, *P. ohmeri*), *Rhodotorula* (*R. glutinis*) and *Zygosaccharomyces* (*Z. rouxii*, *Z. bisporus*) [1]. Some yeasts are recognized for their tolerance to high concentrations of sugars (a_w as low as 0.62), while others tolerate high salt concentrations. *Torulopsis famata*, *Rhodotorula rubra*, *Pichia etchelsii*, *Candida parapsilosis* and *Debaryomyces hansenii* are able to grow above 10–15% NaCl [1,2]. Some are more tolerant at higher pH values, while others show better tolerance under more acidic conditions [3].

Osmotolerant yeasts frequently occur on the phylloplane of plants, especially in the Mediterranean area [4]. They were occasionally isolated from moderately

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saline environments such as saline soil [5–8], estuarine water and sediments [9], salt marshes [10] and from deep-sea environments [11,12]. Yeast populations are more sparse in marine water than in fresh water, and decrease with depth and distance from land [13]. Few reports exist on the occurrence and biodiversity of yeasts in natural hypersaline waters. Most strains of the variety *Metschnikowia bicuspidata* var. *bicuspidata* originate in salt lakes and ponds (ca. 10% NaCl), often associated with diseased brine shrimp (*Artemia salina*). Some initially did not grow in the usual media unless they were supplemented with 2% NaCl [14]. However, this feature did not hold when the cultures were maintained in artificial laboratory media. Kritzman in 1973 [15] isolated an osmophilic yeast from the Dead Sea, although no cultures have been preserved. Lahav et al. [16] discovered two yeast species in hypersaline chemical wastewater evaporation ponds. It is generally considered that hypersaline brines that experience extreme solar heating do not harbor any yeast populations [17]. To our knowledge, no further studies on the occurrence of yeast species in this natural extreme habitat were reported. In this study we present the biodiversity and occurrence of non-melanized yeasts in hypersaline waters of different salterns and salt lakes worldwide.

2. Materials and methods

2.1. Sampling sites and determination of environmental parameters

Water samples were taken in 1997 and 1999 from the active Slovenian solar salterns Sečovlje in the northern Adriatic coast, at the border between Slovenia and Croatia. Because of the sub-Mediterranean climate, salt is collected only during the salt production season [18]. Outside the season of salt production the salinity of the water mainly remains below 5% (w/v) NaCl. Four successive evaporitic ponds were selected for fungal isolations, covering salinities from 3% to 30% NaCl.

Sampling within the same salinity range was performed as well in Eilat salterns on the Red Sea coast in Israel, while in the salterns on the Mediterranean coast in Spain (Santa Pola and Ebre delta) and in France (Camargue salterns), on the Atlantic coast in Namibia (Skeleton coast), in the Dominican Republic and in Portugal (Samouco salterns, south of Lisbon) only hypersaline water from the crystallisation ponds was sampled. Hypersaline water samples were also taken from the Dead Sea, the Great Salt Lake (Utah) and the Enriquillo Lake (Dominican Republic).

Salinity (areometer) and water activity (a_w) (CX-1 system, Campbell Scientific Ltd., Loughborough, UK) were determined in all water samples.

2.2. Isolation methods and preservation of isolates

To enable discrimination between xerophilic/xerotolerant and halophilic/halotolerant fungi present in the salterns, selective media were used in which the water activity was lowered by addition of high concentrations of glycerol (18%), NaCl (from 10% to 32%) or sugar (50–70%) [19]. The fungal population dynamics were followed as described by Gunde-Cimerman et al. [19]. In 1997, agar baits in dialysis tubing were incubated in different ponds in the Adriatic salterns, and biofilms covering the brine were spread on low water activity selective media [19].

The isolated and identified strains from all sampling sites are maintained in the Culture Collection of the National Institute of Chemistry (MZKI), Slovenia and in the Portuguese Yeast Culture Collection (PYCC), Portugal.

2.3. Taxonomy

For morphological characterization cultures were grown on cornmeal (Difco), malt [20] and acetate agar [20] at 25 °C and studied with phase-contrast optics.

A selection of physiological tests (urease, Diazonium Blue B colour reaction, fermentation of glucose, assimilation of inositol and D-glucuronate as sole carbon sources; assimilation of nitrate as sole nitrogen source, and production of starch-like compounds) was performed as described by Yarrow [20].

For DNA extraction, two loopfuls of MYP agar grown cultures were suspended in 500 μl lysing buffer (50 mmol l^{-1} Tris; 250 mmol l^{-1} NaCl; 50 mmol l^{-1} EDTA; 0.3% w/v SDS; pH 8) and the equivalent to a volume of 200 μl of 425–600 μm glass beads (Sigma) was added. After vortexing for 3 min, the tubes were incubated for 1 h at 65 °C. The suspensions were then centrifuged for 30 min at 4 °C. Finally, the collected supernatant was diluted 1:750, and 5 μl were directly used in the PCR.

For rDNA sequence analysis DNA was amplified using primers NS7 (5′GAG GCA ATA ACA GGT CTG TGA TGC) or ITS5 (5′GGA AGT AAA AGT CGT AAC AAG G) and LR6 (5′CGC CAG TTC TGC TTA CC). Cycle sequencing of the 600–650 base pair region at the 5′ end of the 26S rDNA D1/D2 domain employed the forward primer NL1 (5′GCA TAT CAA TAA GCG GAG GAA AAG) and reverse primer NL4 (5′TCC TCC GTC TAT TGA TAT GC). Sequences were obtained using either Amersham Pharmacia ALF Express II or ABI 310 (capillary) automated DNA sequencer, following the manufacturer's instructions. For identification, the obtained sequences were compared with those of all known yeast species, available at the GenBank database.