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ORIGINAL PAPER

Rhodotorula himalayensis sp. nov., a novel psychrophilic yeast isolated from Roopkund Lake of the Himalayan mountain ranges, India

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Abstract Twenty-five psychrophilic yeasts were isolated from the soil of Roopkund Lake, Himalayas, India. Two colony morphotypes were identified and representatives of 'morphotype 1' were identified as *Cryptococcus gastricus*. Representatives of 'morphotype 2', namely $3A^T$, 4A, 4B and Rup4B, showed similar phenotypic properties and are identical with respect to the nucleotide sequence of the ITS1-5.8S rRNA gene-ITS2 region and D1/D2 domain of the 26S rRNA gene. The sequence of D1/D2 domain of $3A^T$ shows 97.6–98.8% similarity with *Rhodotorula psychrophila* $CBS10440^T$, *Rhodotorula glacialis* $CBS10437^T$ and *Rhodotorula psychrophenolica* $CBS10438^T$ and in the neighbour-joining phylogenetic tree strains; $3A^T$, 4A, 4B and Rup4B form a cluster with *Rhodotorula glacialis* and *Rhodotorula psychrophila*. Strains $3A^T$, 4A, 4B and Rup4B also differ from their nearest phylogenetic relatives in several biochemical characteristics such as in assimilation of D-galactose, L-sorbose, maltose, citrate, D-glucuronate and creatinine. Thus, based on the phylogenetic analysis and the phenotypic differences $3A^T$, 4A, 4B and Rup 4B are assigned the status of a new species of *Rhodotorula* for which the name *Rhodotorula himalayensis* sp. nov. is proposed with $3A^T$ as the type strain (=$CBS10539^T$ =$MTCC8336^T$).

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GenBank/EMBL accession numbers for (partial) 18SrRNA gene-ITS1-5.8S rRNA gene-ITS2-26S rRNA gene (partial) sequences of *Rhodotorula himalayensis* sp. nov. $3A^T$ is AM410635.

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Introduction

Yeasts, unlike bacteria, are less versatile with respect to their ability to adjust to different environmental temperatures. The mesophilic yeasts grow between 5 and 35°C. In comparison, the psychrophilic yeasts grow at temperatures below 5°C and exhibit no growth above 20°C (Vishniac 1999). In contrast, the thermophilic yeasts grow between 25 and 45°C (Arthur and Watson 1976) and thermophilic bacteria grow above 50°C. As a group, the mesophilic yeasts are the most predominant and constitute the vast majority of the yeasts studied so far compared to the psychrophilic and thermophilic yeasts. However, the psychrophilic and thermophilic groups have attracted considerable attention because of their ability to produce extremozymes with biotechnological potential (Cavicchioli et al. 2002). Studies on psychrophilic and psychrotolerant microorganisms demonstrated degradation of a wide range of hydrocarbons, including phenol, phenol-related compounds and petroleum hydrocarbons in European alpine soils (Margesin 2007 and references therein). Recently, Margesin et al. (2007) described three phenol-degrading novel yeast species, namely *Rhodotorula glacialis, Rhodotorula psychrophila* and *Rhodotorula psychrophenolica* from alpine habitats. In the present study, a new psychrophilic protease producing yeast isolated from the soil of Roopkund Lake in the Himalayan mountain ranges of India is described. Roopkund Lake is popularly known as the "Skeleton Lake" because of the 1942 discovery of a mass grave of skeletons dating back to the ninth century.

Materials and methods

Isolation of the organism, media and maintenance

Soil samples were collected from the bank of Roopkund Lake (30°15′ 43.2″N, 79°43′ 55.2″E), situated at an elevation of 5,029 m above sea level, in the Himalayan mountain ranges, of the state of Uttaranchal, India, in the month of September 2005 and transferred to the laboratory in an ice bucket for analysis. The air temperature was −10°C and the soil pH was 6 at the time of collection. About 1.0 g of the soil sample was suspended in 3 ml of sterile distilled water and vortexed for 2 min. Subsequently 100 µl of the resulting suspension was plated (100 µl/plate) on yeast-malt agar medium (YM) (M424, HiMEDIA, India, containing per litre peptone, 5 g; yeast extract, 3 g; malt extract, 3 g; dextrose, 10 g and agar, 15 g) supplemented with 0.1 g of chloramphenicol per litre. The plates were incubated at 10°C for 15 days. The yeast colonies that appeared on the plates were grouped initially based on their colony morphology, and two to three representatives of each of the morphotypes were purified by repeated streaking on Rose Bengal Chloramphenicol plates (M640, HiMEDIA, India, containing per litre mycological peptone, 5.0 g; dextrose, 10 g; mono-potassium phosphate, 1.0 g; magnesium sulfate, 0.5 g; Rose Bengal, 0.05 g; chloramphenicol, 0.1 g and agar, 15.50 g). For routine sub-culturing and maintenance, the strains were grown on YM agar or broth at 18°C.

Phenotypic characteristics

The methods used to determine the morphological, physiological and biochemical properties were as described by Yarrow (1998). All assimilation tests were performed twice at 18°C, and the results were read after 5 and 21 days of incubation. Assimilation of nitrogen compounds was investigated on liquid media with starved inocula (Nakase and Suzuki 1986). *Kriegeria eriophori* CBS8387^T (teleomorph of *Zymoxenogloea eriophori*) and *Rhodotorula auriculariae* CBS6379^T were used as control strains for assimilation tests. For urea hydrolysis and diazonium blue B (DBB) reaction tests *Rhodotorula* sp. CBS10549 (AM397866) and *Debaryomyces* sp. NRRL Y-48191 (AM397847) were used as positive and negative controls, respectively. Numerous experimental conditions were used (Thomas-Hall et al., 2002) to induce a sexual state in the yeast strains 3A^T, 4A, 4B and Rup 4B. These tests included the plating of each strain and a mixture of all the four strains onto potato dextrose agar, Gorodokowa agar, yeast nitrogen base (YNB) agar, yeast carbon base agar, corn meal agar and V8 juice agar. The cells of these isolates and the mixture of these isolates were also incubated in yeast malt broth and distilled water. The cultures were incubated at 5, 10, 18 and 22°C and were examined up to 4 months at intervals of 15 days using a phase contrast microscope under a 100× oil immersion objective. The inverted-plate method (Thomas-Hall et al. 2002) was also employed to determine whether ballistoconidia were formed by strains 3A^T, 4A, 4B and Rup 4B. Corn meal agar plates were inverted over YEP plates and incubated at 5, 10, 18 and 22°C for 4 months. *Bullera dendrophila* JCM5357^T and laboratory strains *Sporidiobolus* sp. YSNB7 (NRRL Y-48200, AM397856) and *Bullera* sp. YS105 (NRRL Y-48201, AM397857) were used as positive controls for the study.

Coenzyme Q analysis

For determination of coenzyme Q system cells were grown in 250 ml yeast-malt broth for 3–5 days and centrifuged at 5,000 rpm for 8 min. The cell pellet (5 g wet wt.) was washed once with 0.8% saline, suspended into hydrolysis reagent (containing 7 ml water, 2.5 g KOH, 0.7 g pyrogallol and 19 ml methanol) and hydrolyzed for 30 min in a 90°C water bath. Ubiquinone was then extracted with n-hexane (Yamada et al. 1989), purified by preparative thin-layer chromatography and identified using reverse-phase high-performance thin-layer chromatography (Thanh et al. 2003) using Co-Q standards (Sigma-Aldrich). *Rhodotorula auriculariae* CBS6379^T, *Issatchenkia orientalis* NRRL Y-5396^T and *Debaryomyces hansenii* NRRL Y-17914^T were used as reference strains for Coenzyme Q analysis.

Analysis of cell wall sugars

For determination of cell wall carbohydrates approximately 3 g of cell pellet was washed several times using saline and hydrolyzed using H_2SO_4 following techniques described by Dallies et al. (1998). Sugars in the hydrolysate were identified by high performance liquid chromatography (HPLC) using SHODEX SZ5532 and SP0810 (8 mm × 300 mm) HPLC column. The samples were eluted with HPLC grade water at a flow rate of 0.5 ml/min at 80°C and detected with a differential refractometer (WATERS 410). A laboratory strain of *Cryptococcus albidus* YSDN8 and *Rhodotorula auriculariae* CBS6379^T were used as reference strains for the study of cell wall carbohydrates.

Isolation of DNA and determination of mol% G + C content

Total genomic DNA was isolated from stationary phase grown cultures as described earlier (Bhadra et al. 2007;

Rao et al. 2007). DNA was purified following the protocol of Makimura et al. (1994). The mol% G + C content of DNA was determined from the melting point (*Tm*) curves (Sly et al. 1986) obtained using a Lambda 2 UV-vis spectrophotometer (Perkin Elmer, USA) equipped with Templab 2.0 software package (Perkin Elmer).

Amplification and sequencing

The internal transcribed spacer region (ITS) including 5.8S rRNA gene (ITS1-5.8S rRNA gene ITS2 region) and D1/D2 domain of 26S rRNA gene were amplified using the primer pairs ITS1 and NL4, and the amplicon sequenced using the primers NL1, NL2A, NL3A, ITS3 and ITS4 (Lin et al. 1995; Kurtzman and Robnett 1998). Details of amplification and sequencing protocols are described elsewhere (Bhadra et al. 2007; Rao et al. 2007). The amplicons were purified using QIA Quick PCR purification kit (QIAGEN) and were sequenced on an ABI 3730 DNA analyzer (Applied Biosystems, USA) using BIG-Dye terminator kit (Applied Biosystems) according to manufacturer's instruction.

Phylogenetic analysis

Sequences were manually corrected and aligned using CLUSTAL_X (Thompson et al. 1997). A neighbour-joining phylogenetic tree (Saitou and Nei 1987) was constructed using MEGA 3.1 (Kumar et al. 2004) based on evolutionary distance data that was determined with Kimura's two-parameter model (Kimura 1980). Bootstrap analysis (Felsenstein 1985) was performed for 1,000 replications. Reference sequences were retrieved from GenBank under the accession numbers indicated in the trees.

Protease activity assay

Cells of isolate $3A^T$ were grown at 18°C in yeast nitrogen base (HiMedia, India) containing 2% glucose at 200 rpm, and protease activity was measured in the culture supernatant by assaying the absorbance of the liberated amino acids using casein as the substrate (Ray et al. 1992). A standard curve was generated using solutions of 0–50 mg/l tyrosine. One unit (IU) of protease activity was defined as the amount of enzyme that liberated 1 μg of tyrosine in 1 min. The optimum temperature and pH for the activity of the protease were determined in the temperature range of 5–50°C and pH 5.2 to pH 11, respectively. All samples were incubated for 3 h at 37°C. Data presented in the text are mean of three independent experiments.

Results

The total culturable yeast population of the soil samples of the Roopkund Lake ranged between 50 and 70 CFU g^{-1} soil (which appeared to be moist). The yeast colonies that appeared on YM-chloramphenicol plates could be distinguished into two distinct morphotypes, viz., morphotype 1 (white colonies of 1–1.5 mm diameter) and morphotype 2 (light-cream colour colonies of 3–3.5 mm diameter). On Rose Bengal Chloramphenicol plates colonies of morphotype 1 were white in colour, whereas that of morphotype 2 were pink in colour. On YM plates after 7 days at 15°C the cells of morphotype 1 were round (4–5 μm dia), whereas those of morphotype 2 were elongated (6.3–10.5 × 3.3–4.1 μm).

Phylogenetic analysis

On the basis of phylogenetic analysis of D1/D2 domain sequence of 26S rRNA gene and phenotypic characteristics, representatives of morphotype 1 were identified as *Cryptococcus gastricus* (data not shown).

The sequence of ITS1-5.8S rRNA gene-ITS2 region and D1/D2 domain (of 26S rRNA gene) of the strains $3A^T$, 4A, 4B and Rup4B, representatives of morphotype 2, show 100% similarity with each other and have identical phenotypic properties. In the neighbour-joining phylogenetic tree, isolates $3A^T$, 4A, 4B and Rup4B cluster together with a bootstrap support of 96% (Fig. 1). In the pairwise alignment performed using ExPASy Bioinformatics tool (http://www.expasy.ch/tools/sim-prot.html) the sequence of D1/D2 domain of isolate $3A^T$ shows 91.7–98.8% similarity with validly published species of the genus *Rhodotorula*. The nearest phylogenetic neighbours (>97% similarity) were *Rhodotorula psychrophila* CBS10440^T (98.8%), *Rhodotorula glacialis* CBS10437^T (98.7%) and *Rhodotorula psychrophenolica* CBS10438^T (97.6%). It also shows 97.9% with *Kriegeria eriophori* CBS8387^T (AF189905). The ITS1-5.8S rRNA gene-ITS2 region sequence of strain $3A^T$ also exhibited 94.9–99.7% similarity with *Rhodotorula glacialis, Rhodotorula psychrophila* and *Rhodotorula psychrophenolica* (Margesin et al. 2007) and 89.6% with *Kriegeria eriophori* CBS8387^T (AF444602). Similarity with other validly published basidiomycetous yeast species is less than 90%. In the neighbour-joining phylogenetic tree, $3A^T$, 4A, 4B and Rup4B, clustered with *Rhodotorula psychrophila* (EF151252–EF151254) and *Rhodotorula glacialis* (EF151258–EF151262) with 82% bootstrap support (Fig. 1) but did not cluster with *Rhodotorula psychrophenolica* (EF151255–EF151257) and other validly published species of *Rhodotorula* (Fig. 1). They also did not cluster with *Kriegeria eriophori* CBS8387^T (AF189905) and *K. eriophori*

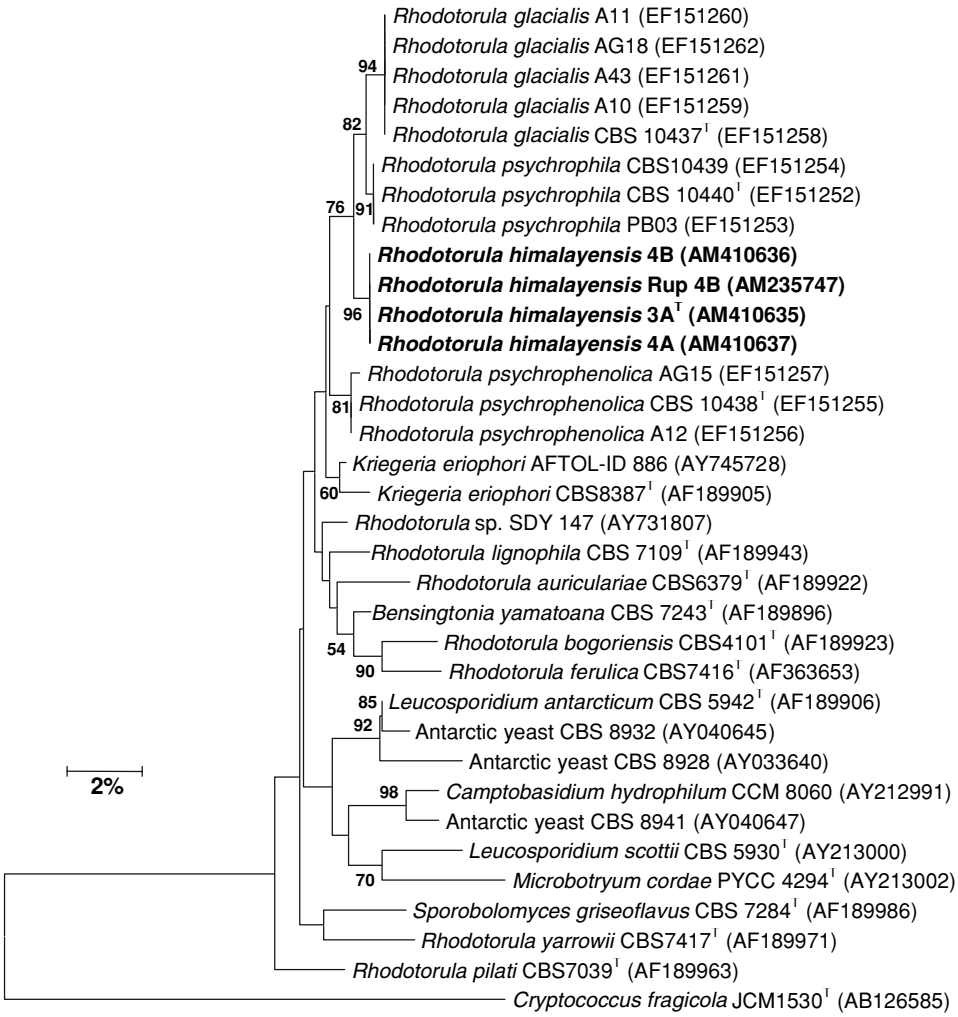

Fig. 1 Phylogenetic tree drawn using neighbour-joining method with Kimura two-parameter model based on the D1/D2 domain sequence of 26S rRNA gene, depicting the relationships of *Rhodotorula himalayensis* sp. nov. with closely related species of the genera *Rhodotorula, Krigeria*, *Sporobolomyces, Bensingtonia* and *Leucosporidium. Cryptococcus fragicola* JCM1530^T was used as the out-group. Bootstrap values calculated from 1,000 replications are indicated in the *branch nodes*. The *bar* represents two substitutions per 100 nucleotides. Reference sequences were retrieved from GenBank under the accession numbers indicated in the *parenthesis*

AFTOL ID 886 (AY745728). Thus, these strains appear to be phylogenetically different from the validly published species of *Rhodotorula* and *Kriegeria eriophori* CBS8387^T, the nearest phylogenetic neighbours.

Phenotypic properties and chemotaxonomy

Strains, 3A^T, 4A, 4B and Rup 4B, have identical phenotypic characteristics (Table 1) but differ from the three nearest phylogenetic neighbours: *Rhodotorula glacialis* CBS10437^T, *R. psychrophila* CBS10440^T and *R. psychrophenolica* CBS10438^T (Table 1). The strains grow between 5 and 22°C but not at 26°C. Optimum temperature for growth is 15–18°C at pH 6.5. On YM medium cells grow between pH 4 and 8, and optimum pH for growth is 6–7. The phenotypic characteristics of isolate 3A^T, 4A, 4B and Rup 4B are detailed in species description. Neither ballistoconidia nor any sexual state was ever observed. Short pseudohyphae (18–36 μm) were observed after 21 days of incubation at 15°C on Corn meal agar and V8 juice agar. Three-week grown culture of the isolates 3A^T, 4A, 4B and Rup 4B showed positive Diazonium Blue B reaction. The psychrophilic yeast 3A^T produces an extracellular protease, and the activity was found to increase with growth (data not shown). Maximum activity was observed at 37°C and at pH 8. At 5°C and 15°C, the protease retained 15% and 46% of the activity, respectively, of the maximum activity. The isolates contained fucose, mannose, glucose, galactose and rhamnose in the cell wall and contain Coenzyme Q9 as the major ubiquinone. Mol% G + C content of DNA of isolate 3A^T is 50 ± 0.4.

Discussion

The D1/D2 domain of the 26S rRNA gene of 3A^T, 4A, 4B and Rup 4B are identical and shows 97.6–98.8% similarity with *Rhodotorula psychrophila* CBS10440^T, *R. glacialis* CBS10437^T and *R. psychrophenolica* CBS10438^T (Margesin et al. 2007). This affiliation between the four strains and the above three species of *Rhodotorula* is further

Table 1 Comparison of phenotypic characteristics of *Rhodotorula himalayensis* sp. nov. 3A^T, 4A, 4B, Rup 4B with *Rhodotorula glacialis*, *Rhodotorula psychrophila* and *Rhodotorula psychrophenolica*

Phenotypic properties	*R. himalayensis* 3A^T, 4A, 4B and Rup 4B	*R. glacialis*[a] (CBS10437^T)	*R. psychrophila*[a] (CBS10440^T)	*R. psychrophenolica*[a] (CBS10438^T)
Growth temperature range (°C)	5–22	1–20	1–15	1–20
Assimilation of carbon source				
D-glucose, sucrose, raffinose, mannitol and D-gluconate	+	+	+	+
D-glucosamine, D-ribose, D-arabinose, lactose, glycerol, DL-lactate, *myo*-Inositol and methanol	–	–	–	–
D-galactose	+	–	–	–
L-sorbose	D	–	–	–
D-xylose	W	NA	–	NA
L-arabinose	+	NA	–	–
Maltose	+	–	–	–
Cellobiose	D	–	–	–
Melibiose	+	NA	–	–
Melezitose	+	NA	+	–
Erythritol	+	–	NA	–
Citrate	+	–	–	–
L-rhamnose	–	NA	–	+
D-glucuronate	–	+	+	+
Assimilation of nitrogen source				
Nitrate, ethylamine and D-tryptophan	+	+	+	+
Nitrite	–	–	–	–
Creatinine	–	+	+	+
Growth in 1% acetic acid or 50% D-glucose	–	–	–	–
Growth in 0.01% cycloheximide	–	–	NA	NA
Diazonium Blue B reaction	+	+	+	+
Urea hydrolysis	D	+	+	+

+ positive; - negative; *W* weak positive; *D* delayed; *NA* data not available

[a] Data from Margesin et al. (2007)

confirmed by the NJ phylogenetic tree (Fig. 1). Further, *R. psychrophila* and *R. glacialis* along with 3A^T, 4A, 4B and Rup4B form a robust clade.

It is also noticed that strains, 3A^T, 4A, 4B and Rup 4B, show 97.9% similarity with a plant parasite *Kriegeria eriophori* CBS8387^T (AF189905) (Doubles and McLaughlin 1992) that produces scattered basidia (Bauer et al. 2006). But, strains 3A^T, 4A, 4B and Rup 4B are asexual, nitrate positive and are not plant parasites and thus cannot be considered as species of *Kriegeria*. Further, 3A^T, 4A, 4B and Rup 4B in the phylogenetic tree (Fig. 1) did not form a clade with species belonging to the genera *Sporobolomyces*, *Bensingtonia*, *Leucosporidium*, although at the 26S rRNA gene (D1/D2 domain) level the similarity between 3A^T and *Bensingtonia yamatoana* CBS7243^T (AF189896), *Sporobolomyces tsugae* (CBS5038^T, AF189998), *S. inositophilus* (CBS7310^T, AF189997), *S. singularis* (CBS5109^T, AF189996), *S. griseoflavus* (CBS7284^T, AF189996) and *Leucosporidium antarcticum* (CBS5942^T, AF189906) ranged from 95.1 to 96.7%. A few of the validly published species of *Rhodotorula*, like *Rhodotorula pilati* (CBS7039^T, AF189963) and *Rhodotorula lignophila* (CBS7109^T, AF189943) formed robust clades with *Sporobolomyces tsugae* (CBS5038^T, AF189998) and *S. singularis* (CBS5109^T, AF189996), respectively, indicating that the genus *Rhodotorula* is polyphyletic in origin as reported earlier (Fell et al. 2000). Thus, strains 3A^T, 4A, 4B and Rup 4B differ phylogenetically from species of the genera *Sporobolomyces*, *Bensingtonia* and *Leucosporidium.* Presence of pseudo and septate hyphae, formation of ballistoconidia and positive results for urease activity are characteristic features of *Sporobolomyces* and *Bensingtonia* (Barnett et al. 2000). These phenotypic features differentiate 3A^T, 4A, 4B and Rup 4B from *Sporobolomyces* and *Bensingtonia. Leucosporidium* unlike 3A^T, 4A, 4B and Rup 4B produces polar buds, pseudo and septate hyphae, teliospores, shows clamp connections and is positive for urea and starch hydrolysis, thus differing from the present strains which do not possess any of these characteristics.

The absence of sexual reproduction and absence of xylose in the cell wall of isolates $3A^T$, 4A, 4B and Rup4B indicate that these isolates are similar to species of *Rhodotorula.* But, unlike *Rhodotorula psychrophila* CBS10440^T, *R. glacialis* CBS10437^T and *R. psychrophenolica* CBS10438^T isolated from alpine soil (Margesin et al. 2007), the present isolates $3A^T$, 4A, 4B and Rup 4B are phenotypically different (Table 1). Thus, it would appear that strains $3A^T$, 4A, 4B and Rup 4B which are phylogenetically and phenotypically related to the asexual yeast *Rhodotorula a*re novel strains *of Rhodotorula*, for which the name *Rhodotorula himalayensis* sp. nov. is proposed with $3A^T$ as the type strain. Earlier studies have reported various species of *Rhodotorula* from diverse cold habitats such as in Antarctica and Arctic (Vishniac 1999, 2006; Butinar et al. 2007; Bergauer et al. 2005). Thus, the occurrence of *Rhodotorula himalayensis* sp. nov around the frozen lake in Himalayas is not surprising.

The protease produced by isolate $3A^T$ exhibits maximum activity at 37°C. This is unusual considering that $3A^T$ is a psychrophilic yeast which grows between 4 and 22°C with optimum growth at 15–18°C. But, from the adaptation point of view it is significant that the enzyme retains >50% of its activity at 18°C. The protease is also stable to several freeze-thaw cycles, a phenomenon quite common in the natural environment of Roopkund Lake.

Description of *Rhodotorula himalayensis* Shivaji, Bhadra & Rao sp. nov.

Rhodotorula himalayaensis sp. nov. (him.a.lay.en'sis. N.L. masc. adj. *himalayensis* pertaining to Himalaya) isolated from soil of Roopkund Lake, Himalayas, India. In liquid media (YM) after 7 days at 18°C, vegetative cells are elongated (6.3–10.5 × 3.3–4.1 μm) and occur singly, in pairs or in groups (Fig. 2). Budding is multilateral. On yeast-malt agar after 12 days at 15–18°C, colonies are smooth, butyrous, glistening and cream coloured with entire margin. Short pseudohyphae (18–36 μm) are formed infrequently after 3 weeks of incubation at 15°C. No sexual state was observed and spores are not formed; grows between 4 and 22°C but not at 26°C; optimum temperature for growth is 15–18°C; produces extracellular protease which is active even at 18°C; does not ferment sugar. Assimilate D-glucose, D-galactose, L-sorbose (delayed), D-xylose (weak), L-arabinose, sucrose, maltose, cellobiose (delayed), melibiose (delayed), raffinose, melezitose, ribitol, xylitol, erythritol (delayed), D-glucitol, mannitol, galactitol, D-Glucono-1,5-lactone, D-gluconate, succinate and citrate; but do not assimilate D-glucosamine, D-ribose, D-arabinose, L-rhamnose, trehalose, salicin, lactose, inulin, starch, glycerol, *myo*-inositol, D-glucuronate, DL-lactate and methanol as sole carbon source. Assimilate nitrate, ethylamine, L-lysine, cadaverine and D-tryptophan; but do not assimilate nitrite, creatinine, D-glucosamine and imidazole as sole nitrogen source; can grow without thiamin, but requires biotin for growth. The strain is sensitive to 0.01% cycloheximide. Cells do not grow in 1% acetic acid and 50% D-glucose. Diazonium Blue B positive and hydrolyzed urea (delayed) contains Coenzyme Q9 as the major ubiquinone and cell wall carbohydrate does not contain xylose but contains fucose, mannose, glucose, galactose and rhamnose. Mol% G + C content of DNA is 50.

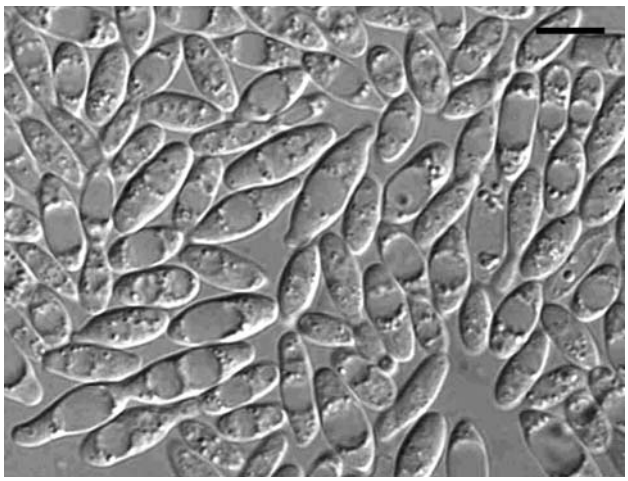

Fig. 2 Differential interference contrast micrograph of *Rhodotorula himalayensis* sp. nov. $3A^T$ grown at 18°C for 5 days on yeast-malt agar. The bar represents 7 μm

Strains $3A^T$, 4A, 4B and Rup 4B are the strains of *Rhodotorula himalayaensis* sp. nov and $3A^T$ is the type strain (=CBS10539^T =MTCC8336^T). The strains were isolated from soil samples of Roopkund Lake, Himalayas, India.

Latin description of *Rhodotorula himalayensis* Shivaji, Bhadra et Rao sp. nov.

In medio liquido YM post dies 7 (15°C)*, cellulae globosae vel oblongum, singulae vel binae sunt* (6.3–10.5 × 3.3–4.1 μm)*; breves pseudohyphae* (18–36 μm) *post dies 21 et 18°C formatae sunt. Per gemmationem multipolarem reproducentes. Status sexualis non apparet; sporae not fiunt. Maxima incrementi temperatio est* 22°C*, optimae crescit in* 15–18°C*, incrementum in* 26°C *non respondet. Fermentatio nulla. Assimilantur D-glucosum, D-galactosum, L-sorbosum (lente), D-xylosum (infirme), L-arabinosum, sucrosum, maltosum, cellobiosum (lente), melibiosum (lente), raffinosum, melezitosum, ribitolum, xylitolum, erythritolum, D-glucitolum, mannitolum, galactitolum, D-glucono-1,5-lactonum, D-gluconatum, succinatum et citratum. Non-assimiluntur D-glucosaminum, D-ribosum, D-arabinosum, L-rhamnosum, trehalosum, salicinum, lactosum, inulinum,*

amylosum, glycerolum, myo-inositolum, D-glucuronatum, DL-lactatum et methanolum. Assimiluntur nitratum, ethylaminum, L-lysinum, cadaverinum et D-tryptophanum; non-assimiluntur nitritum, creatininum, D-glucosaminum et imodazolum. Biotinum externum ad crescentiam necessarium est. Non crescit in medio 0.01% *cycloheximido et medio* 1% *acido acetico addito. Non crescit in medio* 50% *glucosum addito. Ureum hydrolysatur (lente). Diazonium caeruleum B respondens. Xylosum not fiunt in cell wall. Ubiquinone majus Q9.* G + C *acidi deoxyribonucleati* 50 mol%.

Typus: = $3A^T$ (=$CBS10539^T$ =$MTCC8336^T$) *designat stirpem typicum. Isolata ex* soil, Roopkund, Himalayas, India, *depositata in Collectione Culturarum* CBS, The Netherlands.

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