Rhodotorula psychrophila sp. nov., *Rhodotorula psychrophenolica* sp. nov. and *Rhodotorula glacialis* sp. nov., novel psychrophilic basidiomycetous yeast species isolated from alpine environments

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Three novel psychrophilic species of the genus *Rhodotorula* are described. *Rhodotorula psychrophila* sp. nov. (type strain PB19^T=CBS 10440^T=DSM 18768^T), *Rhodotorula psychrophenolica* sp. nov. (type strain AG21^T=CBS 10438^T=DSM 18767^T) and *Rhodotorula glacialis* sp. nov. (type strain A19^T=CBS 10436^T=DSM 18766^T) were isolated from soil collected from an alpine railway area, from mud in the thawing zone of a glacier foot and from glacier cryoconite, respectively. All three species have been assigned to the genus *Rhodotorula* on the basis of molecular sequence data and physiological and morphological properties. *Rhodotorula psychrophila* is not able to grow at temperatures above 15 °C. *Rhodotorula psychrophenolica* and *Rhodotorula glacialis* degrade high concentrations of phenol (up to 12.5 and 5 mM, respectively) as the sole carbon source at 10 °C. Sequence analyses of the 26S rDNA D1/D2 regions indicated that the novel species are phylogenetically related and belong to a clade that includes other psychrophilic yeasts.

INTRODUCTION

Members of the genus *Rhodotorula* are known for their ability to degrade phenolic compounds (Sampaio, 1999; Fell *et al.*, 2001). They also colonize extreme environments (Sampaio, 2004). We have previously investigated low-temperature degradation of phenol by cold-adapted basidiomycetous yeasts isolated from cold alpine environments (Margesin *et al.*, 2003, 2005; Bergauer *et al.*, 2005). According to the new classification of basidiomycetes provided by Bauer *et al.* (2006), the majority of the strains belonged to the class Microbotryomycetes of the subphylum Pucciniomycotina. Representatives of the genus *Rhodotorula* were characterized by a high metabolic versatility towards the utilization of a number of phenol-related monoaromatic compounds as the sole carbon

source at 10 °C and were able to grow in the presence of high concentrations of these compounds (Bergauer *et al.*, 2005). A group of strains from these previous studies remained unidentified to the species level. They showed no growth above 15 °C or 20 °C and could thus be classified as true psychrophiles. Morphological and physiological properties, as well as 26S D1/D2 and internal transcribed spacer (ITS) 5.8S rDNA sequences, indicate that these strains belong to hitherto unknown species. In this study, we describe three novel species of the genus *Rhodotorula*: *Rhodotorula psychrophila* sp. nov., *Rhodotorula psychrophenolica* sp. nov. and *Rhodotorula glacialis* sp. nov.

METHODS

Sample collection and isolation. Samples were collected from the following Alpine habitats: alpine glacier cryoconite from the Stubai glacier near Innsbruck in Tyrol, Austria (altitude 2900 m above sea level; 46° 59' 12" N 11° 06' 53" E); mud in the thawing zone of the glacier foot (glacier mud) collected from the Stubai glacier, Austria, and the Etendard glacier near Grenoble, France (altitude 2900 m

Abbreviations: ITS, internal transcribed spacer; MYP, malt-yeast-peptone.

The GenBank/EMBL/DDBJ accession numbers for the 26S rDNA D1/D2 and ITS sequences determined in this study are given in Table 1.

above sea level; 45° 09' 45" N 06° 10' 00" E); soil and sediment samples from an oil-shale mine in Seefeld, Austria, and a railway area at the Brenner pass, at the border of Austria and Italy (Table 1). Yeasts were isolated as described previously (Margesin *et al.*, 2002; Bergauer *et al.*, 2005) and maintained on R2A agar (Difco) plates. Long-term storage was performed in 10% (w/v) skimmed milk at -80 °C.

Physiological and biochemical characterization. Morphological, physiological and biochemical properties were determined according to Barnett *et al.* (2000). Additional assimilation tests using aromatic compounds were performed as described (Margesin *et al.*, 2003; Bergauer *et al.*, 2005). Assimilation of carbon and nitrogen compounds and growth requirements were tested at 15 °C. The effect of temperature was examined at 1–30 °C (at 5 °C intervals) in liquid culture and on agar plates. Phenol degradation was determined in fed-batch cultures at 10 °C as previously described (Margesin *et al.*, 2003, 2005).

Phylogenetic analysis. For DNA isolation, cells were harvested from 5-day-old subcultures and lyophilized. DNA was isolated by the CTAB (hexadecyltrimethylammonium bromide) method (O'Donnell *et al.*, 1997). PCR products including ITS1, 5.8S and ITS2 were obtained by utilizing the forward primer 5'-GTCGCTACTACCCG-ATTGAATGGCT-3' and the reverse primer 5'-CCTCCGCTTATTG-ATATGCTTAAG-3'. PCR products including the D1/D2 domain were obtained by utilizing the forward primer 5'-GCATA-TCAATAAGCGGAGGAAAAG-3' and the reverse primer 5'-CGCCAGTTCTGCTTACC-3'. A DyeTerminator Quick Start kit (CEQ8000; Beckman) was used for the sequencing reactions in a total volume of 20 µl (1 µl purified PCR product, 1 µl of the forward or reverse primers used for PCR, 4 µl Beckman CEQ DTCS Quick Start mix, 14 µl MilliQ water) and samples were placed in a cycler for 35 cycles of 96 °C for 20 s, 50 °C for 20 s and 60 °C for 4 min. Sequences were obtained with an automatic sequencer (Beckman CEQ8000; Beckman-Coulter, Inc.) and aligned with SeqMan (DNASTAR). The heuristic maximum-parsimony analysis was employed (100 rounds of heuristic search with TBR branch swapping, starting from trees obtained by random addition of sequences, MulTrees option on, deepest descent option off) and was validated using 1000 rounds of bootstrap analysis (Felsenstein, 1985).

Maximum-parsimony and bootstrap calculations used PAUP software (Swofford, 2001).

RESULTS AND DISCUSSION

Phenotypic properties

Eleven strains isolated from alpine glacier materials, soil or sediment samples, belonged to the genus *Rhodotorula*. Attempts to induce the sexual stage by incubating single or mixed cultures of each of the three novel species on cornmeal agar (CMA) at 15 °C for two months were always negative. The strains were characterized by the following properties (Barnett *et al.*, 2000; Fell *et al.*, 2001; Sampaio *et al.* 2003): absence of pigments (creamy-white colonies), vegetative reproduction by polar budding, no ballistocoonidia, no fermentation, no *myo*-inositol assimilation but assimilation of D-glucuronate, no production of starch-like compounds, utilization of nitrate as a nitrogen source and utilization of aromatic compounds as sole carbon source (Table 2).

All strains investigated in this study could be classified as true psychrophiles (Morita, 1975). Members of *Rhodotorula psychrophila* sp. nov. were not able to grow at temperatures above 15 °C, whereas representatives of the other two novel species could grow at temperatures of up to 20 °C (Table 2). In addition to variations in the maximum growth temperature, the three novel species differed in their abilities to utilize high concentrations of phenol as the sole carbon source (Table 2). The assimilation patterns of carbon and nitrogen compounds were almost identical. D-Melezitose was well assimilated by members of *R. psychrophila* sp. nov. and was not assimilated by members of *R. psychrophilica* sp. nov.

Table 1. *Rhodotorula* strains examined by ITS 5.8S and D1/D2 rDNA regions

T_{max}, Maximum growth temperature; A, Austria; F, France.

Strain	Source	GenBank accession numbers		T _{max} (°C)
		ITS 5.8S	D1/D2	
<i>Rhodotorula psychrophila</i> sp. nov.				
PB19 ^T (=CBS 10440 ^T =DSM 18768 ^T)	Soil (railway area) (A)	EF151243	EF151252	15
PB03	Sediment (oil-shale mine) (A)	EF151244	EF151253	15
PB15 (=CBS 10439)	Sediment (oil-shale mine) (A)	EF151245	EF151254	15
<i>Rhodotorula psychrophilica</i> sp. nov.				
AG21 ^T (=CBS 10438 ^T =DSM 18767 ^T)	Mud at glacier foot (F)	EF151246	EF151255	20
A12	Glacier cryoconite (A)	EF151247	EF151256	20
AG15	Mud at glacier foot (A)	EF151248	EF151257	20
<i>Rhodotorula glacialis</i> sp. nov.				
A19 ^T (=CBS 10436 ^T =DSM 18766 ^T)	Glacier cryoconite (A)	EF151249	EF151258	20
A10	Glacier cryoconite (A)	—	EF151259	20
A11	Glacier cryoconite (A)	AJ853457	EF151260	20
A43 (=CBS 10437)	Glacier cryoconite (A)	EF151250	EF151261	20
AG18	Mud at glacier foot (A)	EF151251	EF151262	20

Table 2. Phenotypic characteristics that differentiate *Rhodotorula psychrophila* sp. nov., *Rhodotorula psychrophilica* sp. nov. and *Rhodotorula glacialis* sp. nov.

Strains: 1, strain PB19^T (=CBS 10440^T); 2, strain PB03; 3, strain PB15; 4, strain AG21^T (=CBS 10438^T); 5, strain A12; 6, strain AG15; 7, strain A19^T (=CBS 10436^T); 8, strain A10; 9, strain A11; 10, strain A43; 11, strain AG18. All strains are positive for the following features: aerobic growth; vegetative reproduction by polar budding; round and convex colonies with entire margins; diazonium blue B reaction; growth in presence of ampicillin (50 µg ml⁻¹); assimilation of D-glucose, sucrose, N-acetylglucosamine, D-raffinose, potassium-2-ketogluconate, D-mannitol, D-sorbitol, sodium glucuronate, potassium gluconate, nitrate, ethylamide, creatinine and D-tryptophan; urea hydrolysis; β-glucosidase activity and utilization of phenol, catechol, resorcinol, hydroquinone and benzoate (200 µg ml⁻¹) as sole carbon source at 10 °C. All strains are negative for the following features: sexual reproduction; formation of pseudomycelium or true hyphae; pigment formation and slime formation on CMA, MYP agar and R2A agar; production of starch-like compounds; growth in the presence of cycloheximide (200–400 µg ml⁻¹), 1 % acetic acid, 50 % D-glucose and 10 % NaCl; activities of β-galactosidase and protease; presence of glucose fermentation; assimilation of D-galactose, D-lactose, lactic acid, D-arabinose, D-cellobiose, maltose, methyl α-D-glucopyranoside, inositol, dulcitol/galactitol, citrate, methanol, ethanol, 2-propanol, D-ribose, glycerol, palatinose, erythritol, levulinic acid, L-sorbose, glucosamine and nitrite and utilization of guaiacol and o-cresol (200 µg ml⁻¹) as sole carbon source at 10 °C. ++, Very good assimilation; +, positive; –, negative; ND, not determined; [Phe]_{max}, maximum phenol concentration fully degraded at 10 °C.

Characteristic	<i>R. psychrophila</i> sp. nov.			<i>R. psychrophilica</i> sp. nov.			<i>R. glacialis</i> sp. nov.				
	1	2	3	4	5	6	7	8	9	10	11
Growth temp. range (°C)	1–15	1–15	1–15	1–20	1–20	1–20	1–20	1–20	1–20	1–20	1–20
Carbon assimilation:											
L-Arabinose	–	–	–	–	–	–	+	+	+	+	–
D-Melezitose	++	++	++	–	–	–	–	–	+	–	+
D-Melibiose	–	–	–	–	–	–	–	–	–	–	+
L-Rhamnose	–	–	–	+	+	+	+	+	+	–	–
Salicylate	+	–	++	–	++	–	++	+	–	+	–
Trehalose	–	–	–	+	–	–	–	–	–	–	–
[Phe] _{max} (mM)	10	15	10	12.5	ND	12.5	5	5	5	5	2.5

Representatives of *R. psychrophila* sp. nov., as well as of *R. psychrophilica* sp. nov., were found in geographically distant alpine glacier sites.

Phylogenetic placement

The phylogenetic tree based on D1/D2 sequence data indicated that two of the novel species, *R. glacialis* sp. nov. and *R. psychrophila* sp. nov., are phylogenetically related (Fig. 1). Moreover they form a well-supported clade with four unidentified isolates of the genus *Rhodotorula* that show some resemblance to our strains. To our knowledge, these isolates are not described in any publication but, according to the information associated with their sequences at the GenBank database, they were isolated in the Darjeeling Himalaya in India and are psychrophilic. The sister relatives of this entire clade could not be determined with exactitude in our analysis. It seems, however, that other cold-adapted yeasts such as *Leucosporidium antarcticum*, other as yet undescribed yeasts isolated from the Antarctic and *R. psychrophilica* sp. nov., the third novel species described in this report, are relatively close to this group.

Intraspecific variability as revealed by sequence data of the D1/D2 region (Fig. 1) and complete ITS region (data not shown) was very low for *R. psychrophila* sp. nov. (one mismatch for strain PB03 in the ITS region) and *R. glacialis*

sp. nov. (one mismatch for strain A10 in the D1/D2 region and two variable positions in the ITS sequence). Intraspecific sequence polymorphisms were more evident for *R. psychrophilica* sp. nov., with four variable positions in the D1/D2 region and five variable positions in the ITS sequence.

Latin diagnosis of *Rhodotorula psychrophila* Margesin et Sampaio sp. nov.

In agar MYP post 5 dies ad 15 °C, cellulae ovoideae vel ellipsoidae (4–8 × 8–15 µm), interdum elongatae simile hyphae, binae aut catenae breves (Fig. 2). Flosculi sunt polares. Post 15 dies ad 10–15 °C in agar MYP, colonias creameas, rotundas et margine toto. Pseudohyphae et hyphae non formantur. Fermentatio (glucosum) nulla. Sucrosum, N-acetylglucosaminum, D-raffinose, 2-ketogluconicum, D-mannitol, D-sorbitol, sodium glucuronatum, D-melezitose, gluconatum et D-glucosum assimilantur at non D-galactosum, acidum lacticum, L-arabinosum, D-arabinosum, D-cellobiosum, maltosum, trehalosum, D-xylosum, D-ribosum, glycerolum, L-rhamnosum, palatinosum, erythritolum, D-melibiosum, L-sorbose, acidum levulinicum, glucosaminum, methyl α-D-glucopyranosidum, D-lactosum, inositol, dulcitol, citratum, methanol, ethanolum nec 2-propanolum. Nitratum, ethylamidum, kreatininum et D-tryptophanum assimilantur; urea finditur. Non crescit in 1 % acido acetico, 50 % D-glucoso, 10 % NaCl aut cycloheximido

Fig. 1. Phylogenetic tree of *Rhodotorula glacialis* sp. nov., *Rhodotorula psychrophila* sp. nov. and *Rhodotorula psychrophilica* sp. nov. and related taxa of the Microbotryomycetes. Maximum-parsimony analysis (consensus tree) of an alignment of the D1/D2 region of the 26S rDNA. The topology was rooted with *Occultifur externus* and *Rhodotorula minuta* (Cystobasidiomycetes). Numbers on the branches are bootstrap values (1000 replicates; values below 50% are not shown). GenBank accession numbers of the sequences are indicated after strain numbers. Bar, 1 change.

(200–400 µg ml⁻¹); *crescit in ampicillino* (50 µg ml⁻¹). *Diazonium caeruleum positivum*. *Materia amyloidea iodo-phila non formantur*. *Maxima temperatura crescentiae*: 15 °C, *incrementum ad 20 °C non respondet*. *Assimilat phenolum, catecholum, resorcinolum, hydroquinonum vel benzoatum* (200 mg l⁻¹) *ad 10 °C*.

Typus stirps PB19^T *isolatus ex terra*, Brenner pass, *finis Austria/Italia*, *depositus in collectione zymotica* Centraalbureau voor Schimmelcultures, Utrecht, *Nederlandia*, CBS 10440^T (=DSM 18768^T).

Description of *Rhodotorula psychrophila* Margesin & Sampaio sp. nov.

Rhodotorula psychrophila [psy.chro'phi.la. Gr. adj. *psychros* relating to cold; Gr. adj. *philos* relating to loving; N.L. fem. adj. *psychrophila* referring to cold-loving (psychrophilic) growth].

After 5 days growth on malt-yeast-peptone (MYP) agar at 15 °C, cells are ovoidal to ellipsoidal (4–8 × 8–15 µm), sometimes with hypha-like elongations, and occur in pairs or in small chains (Fig. 2). Budding is polar. After 15 days

Fig. 2. Line drawings of yeast cells grown on MYP agar for 5 days at 15 °C. (a), *Rhodotorula psychrophila* sp. nov. CBS 10440^T; (b), *Rhodotorula psychrophilica* sp. nov. CBS 10438^T; (c), *Rhodotorula glacialis* sp. nov. CBS 10436^T. Bar, 10 µm.

at 10–15 °C, colonies are creamy-white on MYP agar. Colonies are round, convex, with entire margins. No pseudomycelium or true hyphae are formed. Fermentation ability (glucose) is negative. The following carbon compounds are assimilated: sucrose, *N*-acetylglucosamine, *D*-raffinose, potassium-2-ketogluconate, *D*-mannitol, *D*-sorbitol, sodium glucuronate, *D*-melezitose, potassium gluconate and *D*-glucose. No growth occurs on *D*-galactose, lactic acid, *L*-arabinose, *D*-arabinose, *D*-cellobiose, maltose, trehalose, *D*-xylose, *D*-ribose, glycerol, *L*-rhamnose, palatinose, erythritol, *D*-melibiose, *L*-sorbitose, levulinic acid, glucosamine, methyl α -*D*-glucopyranoside, *D*-lactose, inositol, dulcitol, citrate, methanol, ethanol or 2-propanol. Assimilation of the nitrogen compounds nitrate, ethylamide, creatinine and *D*-tryptophan is positive. Urea hydrolysis is positive; nitrite is not utilized. No growth occurs in the presence of 1 % acetic acid, 50 % *D*-glucose, 10 % NaCl or cycloheximide (200–400 $\mu\text{g ml}^{-1}$). Growth occurs in the presence of ampicillin (50 $\mu\text{g ml}^{-1}$). Diazonium blue B reaction is positive. No starch-like substances are produced. Growth occurs at 15 °C, but not at 20 °C. Phenol, catechol, resorcinol, hydroquinone or benzoate (200 mg l^{-1}) are utilized as sole carbon sources at 10 °C. Phenol is fully degraded (up to 10 mM) as the sole carbon source at 10 °C.

The type strain, PB19^T (=DSM 18768^T=CBS 10440^T) was isolated from the soil of a railway area at the Brenner pass, on the Austria/Italy border. Other strains, PB03 and PB15, were isolated from the sediment of an oil-shale mine in Seefeld, Austria.

Latin diagnosis of *Rhodotorula psychrophenolica* Margesin et Sampaio sp. nov.

In agaro MYP post 5 dies ad 15 °C, cellulae ovoideae vel ellipsoidae (3–4 × 9–15 μm), singulae vel binae vel catenae breves (Fig. 2). Flosculi sunt polares. Post 15 dies ad 10–15 °C in agaro MYP, colonias creameas, rotundas et margine toto. Pseudohyphae et hyphae non formantur. Fermentatio (glucosum) nulla. Sucrosum, N-acetylglucosaminem, D-raffinose, 2-ketogluconatum, D-mannitolum, D-sorbitolum, L-rhamnosum, sodium glucuronatum, potassium gluconatum et D-glucosum assimilantur at non D-galactosum, acidum lacticum, L-arabinosum, D-arabinosum, D-cellobiosum, maltosum, methyl α -D-glucopyranosidum, D-lactosum, inositolum, dulcitolum, citratum, methanolum, ethanolum, 2-propanolum, D-ribosum, glycerolum, palatinosum, erythritolum, D-melibiosum, D-melezitosum, L-sorbitosum, acidum levulinicum nec glucosaminum. Nitratum, ethylamidum, kreatininum et D-tryptophanum assimilantur; urea finditur. Non crescit in 1 % acido acetico, 50 % D-glucoso, 10 % NaCl aut cycloheximido (200–400 $\mu\text{g ml}^{-1}$); crescit in ampicillino (50 $\mu\text{g ml}^{-1}$). Diazonium caeruleum positivum. Materia amyloidea iodophila non formantur. Maxima temperatura crescentiae: 20 °C, incrementum ad 25 °C non respondet. Assimilat phenolum (12.5 mM), catecholum, resorcinolum, hydroquinonum vel benzoatum (200 mg l^{-1}) ad 10 °C.

Typus stirps AG21^T *isolatus ex caeno glacialis*, Etendard Glacier, France, *depositus in collectione zymotica* Centraalbureau voor Schimmelcultures, Utrecht, *Nederlandia*, CBS 10438^T (=DSM 18767^T).

Description of *Rhodotorula psychrophenolica* Margesin & Sampaio sp. nov.

Rhodotorula psychrophenolica (psy.chro.phen.o'li.ca. Gr. adj. *psychros* relating to cold; N.L. fem. adj. *phenolica* relating to phenol degradation; N.L. fem. adj. *psychrophenolica* relating to cold-adapted phenol-degrading yeast).

After 5 days growth on MYP agar at 15 °C, cells are ellipsoidal (3–4 × 9–15 μm) and occur singly, in pairs (mainly) or in small chains (Fig. 2). Budding is polar. After 15 days at 10–15 °C, colonies are creamy-white on MYP agar. Colonies are round, convex, with entire margins. No pseudomycelium or true hyphae are formed. Fermentation ability (glucose) is negative. The following carbon compounds are assimilated: sucrose, *N*-acetylglucosamine, *D*-raffinose, potassium-2-ketogluconate, *D*-mannitol, *D*-sorbitol, *L*-rhamnose, sodium glucuronate, potassium gluconate and *D*-glucose. No growth occurs on *D*-galactose, lactic acid, *L*-arabinose, *D*-arabinose, *D*-cellobiose, maltose, methyl α -*D*-glucopyranoside, *D*-lactose, inositol, dulcitol, citrate, methanol, ethanol, 2-propanol, *D*-ribose, glycerol, palatinose, erythritol, *D*-melibiose, *D*-melezitose, *L*-sorbitose, levulinic acid or glucosamine. Assimilation of the nitrogen compounds nitrate, ethylamide, creatinine and *D*-tryptophan is positive. Urea hydrolysis is positive. Nitrite is not utilized. No growth occurs in the presence of 1 % acetic acid, 50 % *D*-glucose, 10 % NaCl or cycloheximide (200–400 $\mu\text{g ml}^{-1}$). Growth occurs in the presence of ampicillin (50 $\mu\text{g ml}^{-1}$). Diazonium blue B reaction is positive. No starch-like substances are produced. Growth occurs at 20 °C, but not at 25 °C. Phenol, catechol, resorcinol, hydroquinone or benzoate (200 mg l^{-1}) are utilized as the sole carbon source at 10 °C. Phenol is fully degraded (up to 12.5 mM) as the sole carbon source at 10 °C.

The type strain, AG21^T (DSM 18767^T=CBS 10438^T) was isolated from mud in the thawing zone at the glacier foot of the Etendard Glacier, France. Additional strains were isolated from mud in the thawing zone of the Stubai Glacier, Austria (AG15) and from glacier cryoconite collected from the Stubai Glacier, Austria (A12).

Latin diagnosis of *Rhodotorula glacialis* Margesin et Sampaio sp. nov.

In agaro MYP post 5 dies ad 15 °C, cellulae ellipsoidae (3–5 × 8–15 μm), singulae vel binae vel catenae breves (Fig. 2). Flosculi sunt polares. Post 15 dies ad 10–15 °C in agaro MYP, colonias creameas, rotundas et margine toto. Pseudohyphae et hyphae non formantur. Fermentatio (glucosum) nulla. Sucrosum, N-acetylglucosaminum, D-raffinose, 2-ketogluconatum, D-mannitolum, D-sorbitolum, sodium

glucuronatum, potassium gluconatum et D-glucosum assimulantur at non D-galactosum, acidum lacticum, D-arabinosum, D-cellobiosum, maltosum, trehalosum, methyl α -D-glucopyranosidum, D-lactosum, inositolum, dulcitolum, citratum, methanolum, ethanolum, 2-propanolum, D-ribosum, glycerolum, palatinosum, erythritolum, L-sorbosum, acidum levulinicum nec glucosaminum. Nitratum, ethylamidum, kreatininum et D-tryptophanum assimilantur; urea finditur. Non crescit in 1 % acido acetico, 50 % D-glucoso, 10 % NaCl aut cycloheximido (200–400 $\mu\text{g ml}^{-1}$); crescit in ampicillino (50 $\mu\text{g ml}^{-1}$). Diazonium caeruleum positivum. Materia amyloidea iodophila non formantur. Maxima temperatura crescentiae: 20 °C, incrementum ad 25 °C non respondet. Assimilat phenolum (5 mM), catecholum, resorcinolum, hydroquinonum vel benzoatum (200 mg l^{-1}) ad 10 °C.

Typus stirps A19^T isolatus ex cryoconito glacialis, Stubai Glacier, Austria, depositus in collectione zymotica Centraalbureau voor Schimmelcultures, Utrecht, Nederlandia, CBS 10436^T (=DSM 18766^T).

Description of *Rhodotorula glacialis* Margesin & Sampaio sp. nov.

Rhodotorula glacialis (gla.cia'lis. L. fem. adj. *glacialis* referring to the frozen, icy environment from which the strains were isolated).

After 5 days growth on MYP agar at 15 °C, cells are ellipsoidal (3–5 × 8–15 μm) and occur singly, in pairs (mainly) or in small chains (Fig. 2). Budding is polar. After 15 days at 10–15 °C, colonies are creamy-white on MYP agar. Colonies are round, convex, with entire margins. No pseudomycelium or true hyphae are formed. Fermentation ability (glucose) is negative. The following carbon compounds are assimilated: sucrose, *N*-acetylglucosamine, D-raffinose, potassium-2-ketogluconate, D-mannitol, D-sorbitol, sodium glucuronate, potassium gluconate and D-glucose. No growth occurs on D-galactose, lactic acid, D-arabinose, D-cellobiose, maltose, trehalose, methyl α -D-glucopyranoside, D-lactose, inositol, dulcitol, citrate, methanol, ethanol, 2-propanol, D-ribose, glycerol, palatinose, erythritol, L-sorbose, levulinic acid or glucosamine. Assimilation of the nitrogen compounds nitrate, ethylamide, creatinine and D-tryptophan is positive. Urea hydrolysis is positive. Nitrite is not utilized. No growth occurs in the presence of 1 % acetic acid, 50 % D-glucose, 10 % NaCl or cycloheximide (200–400 $\mu\text{g ml}^{-1}$). Growth occurs in the presence of ampicillin (50 $\mu\text{g ml}^{-1}$). Diazonium blue B reaction is positive. No starch-like substances are produced. Growth occurs at 20 °C, but not at 25 °C. Phenol, catechol, resorcinol, hydroquinone or benzoate (200 mg l^{-1}) are utilized as the sole carbon source at 10 °C. Phenol is fully degraded (up to 5 mM) as the sole carbon source at 10 °C.

The type strain, A19^T (=DSM 18766^T=CBS 10436^T), was isolated from glacier cryoconite collected on the Stubai Glacier, Austria. Additional strains were isolated from glacier cryoconite (A10, A11, A43) or from mud in the thawing zone at the glacier foot collected on the Stubai Glacier, Austria (AG18).

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