Rhodotorula benthica sp. nov. and *Rhodotorula calyptogenae* sp. nov., novel yeast species from animals collected from the deep-sea floor, and *Rhodotorula lysiniphila* sp. nov., which is related phylogenetically

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Three novel species of the genus *Rhodotorula* are described. *Rhodotorula benthica* sp. nov. (type strain JCM 10901^T = SY-91^T) and *Rhodotorula calyptogenae* sp. nov. (type strain JCM 10899^T = SY-86^T) were respectively isolated from the tubeworm *Lamellibrachia* sp. and the giant white clam *Calyptogena* sp., collected from the deep-sea floor of the Pacific Ocean off Japan. *Rhodotorula lysiniphila* sp. nov. (type strain JCM 5951^T) is proposed for strains isolated previously in Japan and Pakistan. The three species were placed phylogenetically into a species complex comprising *Rhodotorula laryngis*, *Rhodotorula minuta*, *Rhodotorula pallida* and *Rhodotorula slooffiae*. *R. minuta* and *R. slooffiae* are closely related in both the D1/D2 region of the 26S rDNA and the internal transcribed spacer and 5·8S rDNA regions. *R. benthica* and *R. laryngis* were closer to *R. pallida* based on the D1/D2 region. Other relationships were not clear.

INTRODUCTION

Rhodotorula minuta is isolated frequently from marine sources such as water, plants and animals. An extensive study of the basidiomycetous yeasts based on the D1/D2 region of 26S rDNA sequences confirmed the heterogeneity of the physiological and biochemical properties of this species and the presence of at least five species among strains related to *R. minuta* in the *Erythrobasidium* clade (Fell *et al.*, 2000). Of these five species, only *Rhodotorula marina* was phylogenetically divergent. The other four species, *Rhodotorula laryngis*, *R. minuta*, *Rhodotorula pallida* and *Rhodotorula slooffiae*, formed a phylogenetically coherent group. Some strains belonging this group originated from animal sources, such as humans, fish or other terrestrial or marine animals (Phaff *et al.*, 1952; Reiersøl, 1955; Novák & Vörös-Felkai, 1962; Galgóczy & Novák, 1965; Sugiyama & Goto, 1967; Hagler & Ahearn, 1987). We investigated the distribution of yeasts in deep-sea environments in the north-west Pacific Ocean (Nagahama *et al.*, 2001a) and

found that some strains isolated from benthic animals such as tubeworms or giant white clams were phylogenetically located within the *Erythrobasidium* clade. We found that deep-sea isolates within this clade occurred only in animal sources, although *R. minuta* is frequently found in oceanic regions (Fell, 1976). One of these isolates, related to *Sakaguchia dacryoides*, was described as *Rhodotorula lamellibrachiae* (Nagahama *et al.*, 2001b). Others were part of the *R. minuta* species complex. Phylogenetic analysis of these deep-sea strains and other strains treated as *R. minuta* showed substantial heterogeneity in their internal transcribed spacer (ITS) sequences (Nagahama *et al.*, 2001a).

We sequenced these and additional strains of *R. minuta* and its relatives in the ITS–5·8S rDNA region and D1/D2 of the 26S rDNA to clarify the phylogenetic relationships and species outlines within the *R. minuta* species complex. Three novel *Rhodotorula* species are described in this paper.

METHODS

Sample collection and isolation. Samples were collected from Iheya Ridge and Sagami Bay using both a manned and an unmanned submersible (see for example Takami *et al.*, 1997;

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Abbreviations: ITS, internal transcribed spacer of 18S–26S rDNA; ML, maximum-likelihood.

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

Table 1. *Rhodotorula* strains examined in the ITS-5.8S and D1/D2 regions of the rDNA

Tubeworms sampled were of *Lamellibrachia* sp., and clams sampled were of *Calyptogena* sp. Sampling site 1 was Sagami Bay (35°00'N, 139°14'E) at a depth of 1156 m (unless indicated otherwise); sampling site 2 was Iheya Ridge (27°47'N, 126°53'E) at 1023 m. Representative strains used in analyses are in bold.

Strain	Source	Sampling site	Accession numbers	
			ITS-5.8S	D1/D2
<i>Rhodotorula benthica</i> sp. nov.				
SY-91 ^T (=JCM 10901 ^T =CBS 9124 ^T)	Tubeworm	1	AB026001	AB026001
SY-92	Tubeworm	1	AB026002	—
SY-145	Tubeworm	1	AB078483	—
SY-146	Tubeworm	1	AB078484	—
SY-147	Tubeworm	1	AB078485	—
SY-148	Tubeworm	1	AB078486	—
SY-202	Tubeworm	2	AB078490	—
<i>Rhodotorula calyptogenae</i> sp. nov.				
SY-86 ^T (=JCM 10899 ^T =CBS 9125 ^T)	Clam	1	AB025996	AB025996
SY-87	Clam	1	AB025997	—
<i>Rhodotorula laryngis</i>				
JCM 10953 (=CBS 5695)	—	—	AB078500	AB078500
CBS 2221 ^T	—	—	AF189937	AF190014
CBS 5695	—	—	AF189942	AF190013
<i>Rhodotorula lysiniphila</i> sp. nov.				
JCM 5951 ^T (=CBS 9126 ^T)	—	—	AB078501	AB078501
JCM 8105	—	—	AB038117	AB038117
<i>Rhodotorula minuta</i>				
JCM 3777 ^T (=CBS 319 ^T)	—	—	AB026016	AB026016
JCM 8100 (=CBS 2177)	—	—	AB078496	—
JCM 8101 (=CBS 4407)	—	—	AB078497	—
JCM 8102 (=CBS 4408)	—	—	AB078498	—
JCM 8103	—	—	AB078499	—
CBS 319 ^T	—	—	AF189945	AF190011
CBS 2177	—	—	AF189948	AF190010
CBS 4407	—	—	AF189949	AF190012
SY-149	Tubeworm	2	AB078487	—
SY-150	Tubeworm	2	AB078488	—
SY-201	Tubeworm	1*	AB078489	—
JCM 3781	—	—	AB078493	—
SY-90 (=JCM 10900)	Tubeworm	1	AB026000	AB026000
<i>Rhodotorula pallida</i>				
JCM 3780 ^T (=CBS 320 ^T)	—	—	AB078492	—
CBS 320 ^T	—	—	—	AF189962
<i>Rhodotorula slooffiae</i>				
JCM 10954 ^T (=CBS 5706 ^T)	—	—	AB052212	AB052212
JCM 3779	—	—	AB078491	—
JCM 8099	—	—	AB078495	—
SY-84 (=JCM 10898)	Clam	1	AB025994	AB025994
Outgroup				
<i>Rhodotorula lamellibrachiae</i> JCM 10907 ^T	Tubeworm	1	AB025999	AB025999

*Isolated at 884 m.

Nagahama *et al.*, 2001a). Fourteen strains isolated from a clam and tubeworms and 13 strains of *R. minuta* already in the JCM culture collection were studied (Table 1).

Physiological and biochemical characteristics. Strains were characterized morphologically and physiologically by standard methods with some modifications (Yarrow, 1998). Assimilation of

nitrogen compounds was examined on solid media using a starved inoculum (Nakase & Suzuki, 1986). Vitamin requirements were investigated according to the method of Komagata & Nakase (1967). Ubiquinones were extracted by the method of Yamada & Kondo (1973) with slight modifications and determined by HPLC as described previously (Hamamoto & Nakase, 1995).

Nucleic acid analyses. DNA extraction and purification for analyses of DNA base composition and DNA–DNA hybridization were performed following the procedure described by Hamamoto & Nakase (1995) with slight modifications. The DNA base composition was determined by the HPLC method of Tamaoka & Komagata (1984). DNA–DNA hybridization experiments were performed using fixed-DNA microplates, following the procedures described by Ezaki *et al.* (1989).

Phylogenetic analysis. DNA extraction for PCR was performed using the QIAamp DNeasy tissue kit (Qiagen), with some modifications (Nagahama *et al.*, 2001b). The primers used for amplifying and sequencing the 5.8S rDNA and ITS regions were those described by White *et al.* (1990); the primers for the D1/D2 region of 26S rDNA were those described by Fell *et al.* (2000). PCR products were purified using Suprec-02 (Takara) and sequenced using a LI-COR model 4000L DNA sequencer. All sequences were aligned using CLUSTAL W 1.81 (Thompson *et al.*, 1994) and adjusted manually. Positions where one or more species contained a length mutation or a region aligned ambiguously were not included in the subsequent phylogenetic analysis. Phylogenetic trees were constructed utilizing the maximum-likelihood (ML) method (Felsenstein, 1981) with the optimal T_4/T_1 ratio of the HKY85 model estimated from the neighbour-joining tree in PAUP 4.0b8 (Swofford, 1998). This tree was derived by a heuristic search with random stepwise addition of 100 replicates. The robustness of branches in the tree was evaluated by bootstrap analysis (Felsenstein, 1985) with 100 resamplings.

RESULTS AND DISCUSSION

Phenotypic characteristics of strains isolated from benthic animals

Fourteen strains isolated from benthic animals obviously belonged to the genus *Rhodotorula*. They were characterized

by the presence of carotenoid pigments, no sexual cycle, no fermentation, no ballistospores, no starch-like substance and the absence of inositol utilization. Their assimilation of carbon and nitrogen compounds suggested affinities with *R. minuta*. The isolates contained Q-9 or -10 as the major ubiquinone isoprenologue.

Groupings based on nucleotide sequence similarity in the ITS1 and ITS2 regions of rDNA

ITS sequences were determined in 14 isolates and 13 additional strains preserved in the JCM culture collection and compared with five published sequences of strains related to the *R. minuta* species complex. Strains with identical sequences in the ITS regions and 5.8S rDNA were represented by a single sequence. They are represented by 13 single representatives (Table 1, in bold). Similarity values for ITS1 and ITS2 sequences among these single representatives are summarized in Table 2. In this study, we treated strains with greater than 99 % ITS region similarities as conspecific; these were JCM 3777^T with JCM 3781 and SY-90 and JCM 10954^T with JCM 3779 and JCM 8099. Strain SY-84 had more than 99 % similarity to JCM 10954^T, JCM 3779 and JCM 8099 in ITS1, but not in ITS2. Because similarity values between JCM 5951^T and JCM 8105 were borderline, they were treated separately. *R. pallida* JCM 3780^T showed exceptionally low similarities to others in both ITS regions.

DNA complementarity

We performed DNA–DNA hybridization experiments with representative strains (Table 2). The G+C contents of nuclear DNA in these strains had differences of less than 2 mol%. On the basis of similarities in ITS sequences, the strains were divided into two groups for simplicity and the relative DNA binding values were examined within each group (A and B in Table 2). It should be noted that JCM

Table 2. Similarity of ITS1 and ITS2 regions and DNA complementarity among strains of *R. minuta* and related species

Strain(s)	Other strains*	ITS1 similarity (%)		ITS2 similarity (%)		Most related strain (ITS)	DNA complementarity (%)		
		Within	Others	Within	Others		Group(s)	Within	Others
<i>R. minuta</i> JCM 3777 ^T	JCM 3781, SY-90	100–99.3	97.0–85.1	100–99.5	94.6–82.1	JCM 3779	A	–	49.9–18.1
<i>R. slooffiae</i> JCM 10954 ^T , SY-84†	JCM 3779, JCM 8099	100–99.3	97.0–85.1	99.5–97.5	94.1–79.6	JCM 3777 ^T	A	55.3	49.9–19.4
<i>R. lysiniphila</i> JCM 5951 ^T , JCM 8105		99.2	97.7–86.7	99.0	94.6–85.6	SY-91 ^T	A/B	75.2	36.3–12.1
<i>R. calyptogenae</i> SY-86 ^T		–	95.5–86.9	–	94.0–87.0	JCM 8105	B	–	15.1–10.1
<i>R. laryngis</i> JCM 10953		–	97.7–87.7	–	93.1–87.1	SY-91 ^T	B	–	24.2–15.1
<i>R. benthica</i> SY-91 ^T		–	97.7–88.3	–	94.0–90.1	CBS 2221 ^T	B	–	23.8–10.1
<i>R. pallida</i> JCM 3780 ^T		–	88.3–85.1	–	90.1–79.6	SY-91 ^T	B	–	37.1–14.0

*Other strains with >99 % ITS sequence similarity; see text.

†Strain SY-84 showed >99 % sequence similarity to JCM 10954^T (JCM 3779, JCM 8099) in ITS1, but not in ITS2; see text.

10953 was substituted for CBS 2221^T due to a procedural problem in these experiments.

Strains SY-86^T and SY-91^T were most closely related to *R. laryngis* (approx. 15 % relative binding) and *R. pallida* (approx. 37 % relative binding), respectively. Values greater than 70 % between JCM 5951^T and JCM 8105 indicate that these strains should be recognized as a single species. The three species represented by SY-86^T, SY-91^T and JCM 5951^T were genetically distinct. Therefore, we describe these as novel species below. Strain SY-84 had considerable relatedness to JCM 10954^T, the type strain of *R. slooffiae*. These strains may be distinct, but we deferred the resolution of this problem because the relationship of *R. slooffiae* with SY-84 was less than that of other species.

Phylogenetic relationships among *R. minuta* and related species

The tree of D1/D2 regions, with an outgroup yeast occurring in the tubeworm, *R. lamellibrachiae*, reconstructed by means of the ML method, is shown in Fig. 1. Strain SY-91^T, *R. laryngis* and *R. pallida* were united with quite high statistical support by the bootstrap method, although the branching order within these three species was uncertain. The relationship between *R. minuta* and *R. slooffiae* had somewhat low bootstrap values. This topology contradicted a previously published one (Fell *et al.*, 2000), in which *R. minuta* was primarily united with *R. laryngis*. This relatedness based on our tree was also supported by higher value (near 50 %) of DNA complementarity between the two type strains (Table 2) and the phylogenetic relationship based on the ITS-5-8S rDNA regions with higher statistical support (data not shown).

Distinctive features other than molecular phylogeny among *R. minuta*-related species are few. Assimilation of lactose, maltose and sucrose was used previously as criteria to

Fig. 1. Phylogenetic tree of *R. minuta* and related species on the basis of a total of 644 aligned nucleotide sites in the D1/D2 region of the 26S rDNA. The maximum-likelihood tree on HKY85 model ($T_g/T_v=2.52558$) was constructed as described in the text. Numbers at branches are bootstrap values, derived only for the nodes supported by greater than 50 % (100 replicates). Bar, 0.01 substitutions per site.

differentiate species within this complex (Novák & Vörös-Felkai, 1962; Galgóczy & Novák, 1965; Phaff & Ahearn, 1970), although these sugar assimilations could not entirely distinguish the three novel species and were occasionally unstable between a species and its synonyms (e.g. *R. minuta* and *Rhodotorula texensis*; *R. laryngis* and *Rhodotorula zsoldii*). Strain SY-86^T was characterized by having Q-9 as the major ubiquinone isoprenologue, while the other *R. minuta*-related species contained Q-10. Additional analysis of other molecular species may be required in order to produce a reliable phylogeny among species in the *R. minuta* complex.

Latin diagnosis of *Rhodotorula benthica* sp. nov. Nagahama, Hamamoto, Nakase & Horikoshi

In medio liquido YM post 3 dies ad 25 °C, cellulae ovoideae vel ellipsoidae (2–4 × 3–5 µm), singulae aut binae. Post unum mensem pellicula fragilis et incompleta, et sedimentum formantur. Cultura in agar YM ad 25 °C, subrosea, nitida, mollis vel mucosa et margine glabra. Hyphae et pseudohyphae non formantur. Fermentatio nulla. Glucosum, galactosum (exiguum), L-sorboseum (exiguum), sucrosum, cellobiosum, trehalosum, lactosum, melezitium, D-xylosum, L-arabiosum, ethanolum, glycerolum, ribitolium (exiguum), D-mannitolium (vel exiguum), D-glucitolium (vel exiguum), salicinum (varium), glucono-δ-lactonum, acidum 2-ketogluconicum, acidum 5-ketogluconicum, acidum DL-lacticum (vel exiguum), acidum succinicum et acidum D-glucuronicum (vel exiguum) assimilantur, at non maltosum, melibiosum, raffinoseum, inulinum, amyllum solubile, D-arabiosum (aut exiguum), D-ribosum, L-rhamnosum, erythritolum, galactitolium, methyl-α-D-glucosidum, acidum citricum, inositolium nec acidum D-galacturonicum. Non kalium nitricum, natrium nitrosum, ethylaminum, lysinum nec cadaverinum assimilantur. Maxima temperatura crescentiae: 41–44 °C. Thiaminum necessaria ad crescentiam. G+C acidi deoxyribonucleati 47.2 mol% (per HPLC). Ubiquinonum majus Q-10.

Typus stirps SY-91^T *ex verme tubuloso Lamellibrachia* sp., Sagami Bay, Japonia, *isolata est. In collectionibus culturarum quas* 'Japan Collection of Microorganisms', Wako, Saitama *sustentant, no. JCM 10901^T deposita est.*

Description of *Rhodotorula benthica* sp. nov. Nagahama, Hamamoto, Nakase & Horikoshi

Rhodotorula benthica (ben'thi.ca. N.L. adj. *benthica* of the benthos, the flora and fauna of the deep sea).

In YM broth (Difco) after 3 days of culture at 25 °C, cells are ovoidal to ellipsoidal (2–4 × 3–5 µm) and occur singly or in parent–bud pairs. A sediment and incomplete fragile pellicle are formed after 1 month. After 1 month on YM agar at 25 °C, the streak culture is light-pink in colour, glistening, soft to slimy and has an entire margin. In Dalmau plate cultures on cornmeal agar (Difco), no branching hyphae or pseudohyphae are formed. Fermentation ability is negative. The following carbon compounds are assimilated:

D-glucose, galactose (weak), L-sorbose (weak), sucrose, cellobiose, trehalose, lactose, melezitose, D-xylose, L-arabinose, ethanol, glycerol, ribitol (weak), D-mannitol (or weak), D-glucitol (or weak), salicin (variable), glucono- δ -lactone, 2-ketogluconic acid, 5-ketogluconic acid, DL-lactic acid (or weak), succinic acid and D-glucuronic acid (or weak); no growth occurs on maltose, melibiose, raffinose, inulin, soluble starch, D-arabinose (or weak), D-ribose, L-rhamnose, erythritol, galactitol, methyl α -D-glucoside, citric acid, inositol or D-galacturonic acid. Nitrogen compound assimilation tests are negative for potassium nitrate, sodium nitrite, ethylamine, lysine and cadaverine. The maximum temperature for growth is 41–44 °C. Thiamin is required for growth. No growth occurs on 50 % glucose/yeast extract agar. Growth occurs in the presence of 100 p.p.m. cycloheximide. Growth in the presence of 10 % sodium chloride is weak or negative. No starch-like substances are produced. The Diazonium blue B reaction is positive. Urease activity is positive. The major ubiquinone is Q-10. The G+C content of the nuclear DNA is 47.2 mol% (by HPLC).

The type strain, strain SY-91^T (=JCM 10901^T=CBS 9124^T), was isolated from a tubeworm, *Lamellibrachia* sp., collected from the deep-sea floor in Sagami Bay, Japan.

Latin diagnosis of *Rhodotorula calyptogenae* sp. nov. Nagahama, Hamamoto, Nakase & Horikoshi

In medio liquido YM post 3 dies ad 25 °C, cellulae ovoideae vel ellipsoideae (2–4 × 3–7 µm), singulae aut binae. Post unum mensem annulus tenuis et sedimentum formantur. Cultura in agarō YM ad 25 °C, subaurantiaca, nitida, mollis et margine glabra. Hyphae et pseudohyphae non formantur. Fermentatio nulla. Glucosum, galactosum (exiguum), L-sorbosum (exiguum), sucrosum, cellobiosum, trehalosum, lactosum (exiguum), melezitosum, D-xylosum, L-arabiosum (exiguum vel lente), ethanolum (lente), glycerolum, ribitolium (exiguum vel lente), D-mannitolium (vel exiguum), D-glucitolium (exiguum), salicinum (exiguum vel lente), glucono- δ -lactonum, acidum 2-ketogluconicum, acidum 5-ketogluconicum, acidum succinicum et acidum D-glucuronicum (vel lente) assimilantur, at non maltosum, melibiosum, raffinolum, inulinum, amyllum solubile, D-arabiosum, D-ribosum, L-rhamnosum, erythritolum, galactitolium, methyl- α -D-glucosidum, acidum DL-lacticum, acidum citricum, inositolium nec acidum D-galacturonicum. Non kalium nitricum, natrium nitrosum, ethylaminum, lysinum nec cadaverinum assimilantur. Maxima temperatura crescentiae: 41–44 °C. Thiaminum necessaria ad crescentiam. G+C acidi deoxyribonucleati 48.0 mol% (per HPLC). Ubiquinonum majus Q-9.

Typus stirps SY-86^T ex *Calyptogena* sp., Sagami Bay, Japonia, isolata est. In collectionibus culturarum quas 'Japan Collection of Microorganisms', Wako, Saitama sustentant, no. JCM 10899^T deposita est.

Description of *Rhodotorula calyptogenae* sp. nov. Nagahama, Hamamoto, Nakase & Horikoshi

Rhodotorula calyptogenae (ca.ly'p.to.ge.na.e. N.L. fem. gen. n. *calyptogenae* of *Calyptogena*, a genus of giant white clam, from which the organism was isolated).

In YM broth (Difco) after 3 days of culture at 25 °C, the cells are ovoidal to ellipsoidal (2–4 × 3–7 µm) and occur singly or in parent–bud pairs. A sediment and thin ring are formed after 1 month. On YM agar after 1 month at 25 °C, the streak culture is light-orange in colour, glistening, soft and has an entire margin. In Dalmau plate cultures on cornmeal agar (Difco), no branching hyphae or pseudo-hyphae are formed. Fermentation ability is negative. The following carbon compounds are assimilated: D-glucose, galactose (weak), L-sorbose (weak), sucrose, cellobiose, trehalose, lactose (weak), melezitose, D-xylose, L-arabinose (weak or delayed), ethanol (delayed), glycerol, ribitol (weak or delayed), D-mannitol (or weak), D-glucitol (weak), salicin (weak or delayed), glucono- δ -lactone, 2-ketogluconic acid, 5-ketogluconic acid, succinic acid and D-glucuronic acid (or delayed); no growth occurs on maltose, melibiose, raffinose, inulin, soluble starch, D-arabinose, D-ribose, L-rhamnose, erythritol, galactitol, methyl α -D-glucoside, DL-lactic acid, citric acid, inositol or D-galacturonic acid. No assimilation of the nitrogen compounds potassium nitrate, sodium nitrite, ethylamine, lysine and cadaverine occurs. The maximum temperature for growth is 41–44 °C. Thiamin is required for growth. No growth occurs on 50 % glucose/yeast extract agar. Growth occurs in the presence of 100 p.p.m. cycloheximide. Growth in the presence of 10 % sodium chloride is weak. No starch-like substances are produced. The Diazonium blue B reaction is positive. Urease activity is positive. The major ubiquinone is Q-9. The G+C content of the nuclear DNA is 48.0 mol% (by HPLC).

The type strain, strain SY-86^T (=JCM 10899^T=CBS 9125^T), was isolated from a giant white clam, *Calyptogena* sp., collected from the deep-sea floor in Sagami Bay, Japan.

Latin diagnosis of *Rhodotorula lysiniphila* sp. nov. Nagahama, Hamamoto, Nakase & Horikoshi

In medio liquido YM post 3 dies ad 25 °C, cellulae ovoideae vel ellipsoideae (2–4 × 3–6 µm), singulae aut binae. Post unum mensem annulus tenuis et sedimentum formantur. Cultura in agarō YM ad 25 °C, subrosea vel aurantiaca, nitida, mollis, et margine glabra. Hyphae et pseudohyphae non formantur. Fermentatio nulla. Glucosum, galactosum (exiguum), L-sorbosum (exiguum), sucrosum, cellobiosum, trehalosum, lactosum, melezitosum, D-xylosum, L-arabiosum, D-arabiosum (vel exiguum), ethanolum, glycerolum, ribitolium (exiguum), D-mannitolium, D-glucitolium (exiguum), salicinum (exiguum), glucono- δ -lactonum, acidum 2-ketogluconicum, acidum 5-ketogluconicum, acidum DL-lacticum (exiguum) et acidum succinicum assimilantur, at non maltosum, melibiosum, raffinolum, inulinum, amyllum solubile, D-ribosum, L-rhamnosum,

erythritolum, galactitolum, methyl- α -D-glucosidum, acidum citricum, inositolum, acidum D-glucuronicum nec acidum D-galacturonicum. Lysinum assimilantur at non kalium nitricum, natrium nitrosum, ethylaminum, nec cadaverinum. Maxima temperatura crescentiae: 31–34 °C. Thiaminum necessaria ad crescentiam. G+C acidi deoxyribonucleati 49.1 mol% (per HPLC). Ubiquinonum majus Q-10.

In collectionibus culturarum quas 'Japan Collection of Microorganisms', Wako, Saitama sustentant, no. JCM 5951^T deposita est.

Description of *Rhodotorula lysiniphila* sp. nov. Nagahama, Hamamoto, Nakase & Horikoshi

Rhodotorula lysiniphila (ly'si.ni.phi.la. N.L. n. *lysina* lysine; Gr. adj. *philos* liking, preferring; N.L. adj. *lysiniphila* referring to the utilization of lysine as a nitrogen compound).

In YM broth (Difco) after 3 days at 25 °C, the cells are ovoidal to ellipsoidal (2–4 × 3–6 µm) and occur singly or in parent–bud pairs. A sediment and thin ring are formed after 1 month. On YM agar after 1 month at 25 °C, the streak culture is light-pink to orange in colour, glistening, soft and has an entire margin. In Dalmau plate cultures on cornmeal agar (Difco), no branching hyphae or pseudohyphae are formed. Fermentation ability is negative. The following carbon compounds are assimilated: D-glucose, galactose (weak), L-sorbose (weak), sucrose, cellobiose, trehalose, lactose, melezitose, D-xylose, L-arabinose, D-arabinose (or weak), ethanol, glycerol, ribitol (weak), D-mannitol, D-glucitol (weak), salicin (weak), glucono- δ -lactone, 2-ketogluconic acid, 5-ketogluconic acid, DL-lactic acid (weak) and succinic acid; no growth occurs on maltose, melibiose, raffinose, inulin, soluble starch, D-ribose, L-rhamnose, erythritol, galactitol, methyl α -D-glucoside, citric acid, inositol, D-glucuronic acid or D-galacturonic acid. Lysine is assimilated. No growth occurs on potassium nitrate, sodium nitrite, ethylamine or cadaverine. The maximum temperature for growth is 31–34 °C. Thiamin is required for growth. No growth occurs on 50 % glucose/yeast extract agar. Growth occurs in the presence of 100 p.p.m. cycloheximide. Growth in the presence of 10 % sodium chloride is negative. No starch-like substances are produced. The Diazonium blue B reaction is positive. Urease activity is positive. The major ubiquinone is Q-10. The G+C content of the nuclear DNA is 49.1 mol% (by HPLC).

The type strain is strain JCM 5951^T (=CBS 9126^T).

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