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Distribution and identification of red yeasts in deep-sea environments around the northwest Pacific Ocean

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Abstract

We isolated 99 yeast strains, including 40 red yeasts, from benthic animals and sediments collected from the deep-sea floor in various areas in the northwest Pacific Ocean. Comparing the yeast isolates from animals and sediments collected from shallow locations, the proportion of red yeasts differed considerably, comprising 81.5% and 10.6% of the isolates from animals and sediments, respectively. All of the red yeast isolates belonged to the genera *Rhodotorula* and *Sporobolomyces*. On the basis of morphological and physiological characteristics, the isolates were identified as *R. aurantiaca, R. glutinis*, *R. minuta* and *R. mucilaginosa* of the genus *Rhodotorula*, and *S. salmonicolor* and *S. shibatanus* of the genus *Sporobolomyces*. Only *R. glutinis* and *R. mucilaginosa* were isolated from sediments. All of the others were isolated from animal sources. Phylogenetic analyses based on internal transcribed spacer (ITS) regions and 5.8S rRNA gene sequences allowed us to establish the precise taxonomic placement of each of the isolates and thereby investigate the intraspecific relationships among the isolates. Twenty-two strains identified as members of *R. glutinis*, which showed a wide distribution in the deep-sea, and five isolates identified as *R. minuta*, which were isolated only from benthic animals, showed substantial heterogeneity within the species. The isolates phenotypically identified as *Sporobolomyces* species and *R. mucilaginosa* phylogenetically occupied the placements corresponding to these species. Some strains assigned to known species on the basis of phenotypic features should be regarded as new species as suggested by the results of molecular analysis.

Abbreviations: R. – *Rhodotorula*; Rs. – *Rhodosporidium*; S. – *Sporobolomyces*; Sd. – *Sporidiobolus*

Introduction

Yeasts are known to be present in marine environments in various regions, such as coastal, estuarial and offshore regions (Morris 1968; Fell 1976; Hagler & Ahearn 1987; Spencer & Spencer 1997; Lachance & Starmer 1998). However, there have been relatively few studies on yeasts in aquatic sediments (Hagler & Ahearn 1987). Most oceanic research is limited to relatively shallow regions of the sea due to difficulties in bringing up samples of deep-sea resources (Fell 1976), especially mud or benthic organisms.

Yeast populations are more sparse in marine water than in fresh water, and decrease with increasing depth and increasing distance from land (Hagler & Ahearn 1987). The number of yeast species in marine environments is limited. In aquatic environments, the yeast species prevalent in muds or associated with animals are present often in the water (Hagler & Ahearn 1987). Red yeasts, the predominant yeasts detected in many studies, are primarily members of the genera *Rhodotorula*, *Rhodosporidium* and *Sporobolomyces* (Hagler & Ahearn 1987). *Rhodotorula*, an anamorphic genus of heterobasidiomycetous yeasts

(Boekhout 1998), is characterized by the following distinctive traits: no ballistoconidia, no fermentation ability, no starch-like compounds, and no xylose in whole-cell hydrolyzates (Fell & Statzell-Tallman 1998). The formation of ballistoconidia is a character to distinguish the genus *Sporobolomyces* from the genus *Rhodotorula*. *Rhodosporidium* and *Sporidiobolus* are the sexual states of several species of *Rhodotorula* and *Sporobolomyces*. The genus *Rhodotorula* is thought to be of polyphyletic origin and is a phylogenetically mix-grouping with the genera *Rhodosporidium, Sporidiobolus* and *Sporobolomyces* (Fell et al. 1995; Fell & Statzell-Tallman 1998; Fell et al. 2000). Moreover, identifications of anamorphic (asexual) basidiomycetous yeasts based on phenotypic characters were considerably difficult because of the variable or ambiguous reactions on assimilation tests of the carbon and nitrogen compounds.

Large numbers of microorganisms, including many yeasts, have been isolated from deep-sea samples collected by the manned submersibles Shinkai 2000 and Shinkai 6500, and the unmanned submersible Kaiko (Takami et al. 1997, 1999; Kato et al. 1998). Red yeasts are most common among yeasts isolated from the deep-sea. In this study, we isolated 99 yeast strains from various deep-sea materials and 40 of these strains were red yeasts assigned to the genera *Rhodotorula* and *Sporobolomyces*. The identification of these species and their distribution in the deep-sea are reported here.

Materials and methods

Sample collection

Deep-sea mud samples were collected from Sagami Bay and Suruga Bay by Shinkai 2000, from the Palau-Yap Trench and the Japan Trench by Shinkai 6500, and from the Mariana Trench by Kaiko, without contamination by open water, as previously described (Takami et al. 1997). As benthic organisms, we obtained giant white clams identified as *Calyptogena* sp., a tubeworm identified as *Lamellibrachia* sp. and an unidentified crab from Suruga Bay, and a mussel identified as *Bathymodiolus* sp. from the Iheya Ridge (Figure 1, Table 1). All benthic animals employed as sources for isolation of yeasts were collected from sites at depths of less than 2000 m. The sediment samples from the Palau-Yap Trench were directly spread on agar plates, but the other sediments were each transferred to a 2-ml sterile CryoTube (Nunc) containing 10% glycerol and stored frozen in liquid nitrogen until further treatment of the sample. Animals frozen at −80 °C were pulverized in artificial sea water (3% NaCl, 0.07% KCl, 1.08% $MgCl_2 \cdot 6H_2O$, 0.54% $MgSO_4 \cdot 7H_2O$, 0.1% $CaCl_2 \cdot 2H_2O$) after treatment of their surface with ethanol, and the pulverized specimen was spread on agar plates as described below.

Isolation

Yeasts were isolated from fresh or frozen deep-sea samples on YM agar (Difco), potato-dextrose agar (Difco), nutrient agar (Difco) containing 0.5% glucose, or yeast nitrogen base w/o amino acids (Difco) containing 0.5% glucose and 2% agar, dissolved in artificial sea water supplemented with 0.01% chloramphenicol and 0.002% streptomycin. Agar plates were incubated at a low temperature (5–10 °C) for the first two weeks and then at 20 °C for one month.

Identification of the yeast isolates

Strains were characterized morphologically and physiologically by standard methods (Walt & Yarrow 1984) with slight modifications. Assimilation of nitrogen compounds was examined on solid media using a starved inoculum (Nakase & Suzuki 1986). Vitamin requirements were investigated by the method of Komagata & Nakase (Komagata & Nakase 1967).

Phylogenetic analysis

DNA extraction for PCR was performed as described below. One loop of yeast culture was suspended in extraction buffer (200 mM Tris pH 8.5, 250 mM NaCl, 25 mM EDTA, 0.5% SDS) and the cells were ground by means of a pellet mixer (Tref, Switzerland). The nucleic acid to be employed as a template for PCR was extracted by phenol/chloroform treatment and isopropanol precipitation. The primers used for amplification and sequencing of 5.8S rRNA and ITS regions were those described by White et al. (1990). PCR products were purified using Suprec^{TM}-02 (Takara, Japan) and sequenced using a LI-COR DNA sequencer Model 4000L (LI-COR, NB, USA). All sequences were aligned using CLUSTAL W 1.81 (Thompson et al. 1994), and were adjusted manually. When one or more species had a mutation affecting the length of the sequence and when the sequences were unalignable,

Table 1. Sampling sites, depths and temperatures

Source[1]	Sampling site	Depth	Temp.
S1: Suruga Bay			
Sediment	34°43′ N; 138°36′ E	1,977 m	–
Sediment	34°54′ N; 138°39′ E	1,487 m	–
Sediment	34°54′ N; 138°39′ E	1,487 m	–
Sediment	34°57′ N; 138°38′ E	1,510 m	–
S2: Sagami Bay			
Sediment	35°00′ N; 139°13′ E	1,200 m	–
Sediment	35°00′ N; 139°14′ E	1,127 m	3.1 °C
Giant white clam[2]	35°00′ N; 139°14′ E	1,156 m	3.1 °C
Giant white clam[2]	35°00′ N; 139°14′ E	1,156 m	3.1 °C
Tubeworm[3]	35°00′ N; 139°14′ E	1,156 m	3.1 °C
Sediment	34°59′ N; 139°14′ E	1,241 m	2.6 °C
Sediment	35°00′ N; 139°14′ E	1,143 m	2.8 °C
Sediment	34°59′ N; 139°13′ E	1,166 m	2.8 °C
Crab	35°00′ N; 139°14′ E	1,182 m	2.8 °C
I: Iheya Ridge, Nansei Islands			
Mussel[4]	27°47′ N; 126°54′ E	1,050 m	–
Sediment	27°32′ N; 126°58′ E	1,393 m	3.9 °C
J: Japan Trench			
Sediment	40°06′ N; 144°11′ E	6,367 m	1.7 °C
Sediment	40°09′ N; 144°02′ E	4,505 m	1.5 °C
Sediment	40°07′ N; 144°11′ E	6,455 m	1.7 °C
M: Mariana Trench			
Sediment	11°21′ N; 142°25′ E	10,897 m	2.4 °C
P: Palau – Yap Trench			
Sediment	7°01′ N; 134°47′ E	6,489 m	1.7 °C
Sediment	7°01′ N; 134°45′ E	6,006 m	1.7 °C
Sediment	6°20′ N; 133°56′ E	5,561 m	1.7 °C
Sediment	6°20′ N; 133°56′ E	5,522 m	1.7 °C
Sediment	6°20′ N; 133°55′ E	5,415 m	1.7 °C
Sediment	6°21′ N; 133°55′ E	4,907 m	1.7 °C
Sediment	11°08′ N; 134°45′ E	6,500 m	1.7 °C
Sediment	11°08′ N; 134°45′ E	6,500 m	1.7 °C
Sediment	11°45′ N; 139°06′ E	4,612 m	1.7 °C
Sediment	11°46′ N; 139°06′ E	4,286 m	1.7 °C
Sediment	11°46′ N; 139°07′ E	3,702 m	1.7 °C
Sediment	8°24′ N; 137°43′ E	6,242 m	1.7 °C
Sediment	7°17′ N; 136°30′ E	6,481 m	1.7 °C
Sediment	7°17′ N; 136°30′ E	6,198 m	1.7 °C
Sediment	7°18′ N; 136°30′ E	6,455 m	1.7 °C

[1] S1, S2, I, J, M, P correspond to those in the geographical map.
[2] *Calyptogena* sp.
[3] *Lamellibrachia* sp.
[4] *Bathymodiolus* sp.

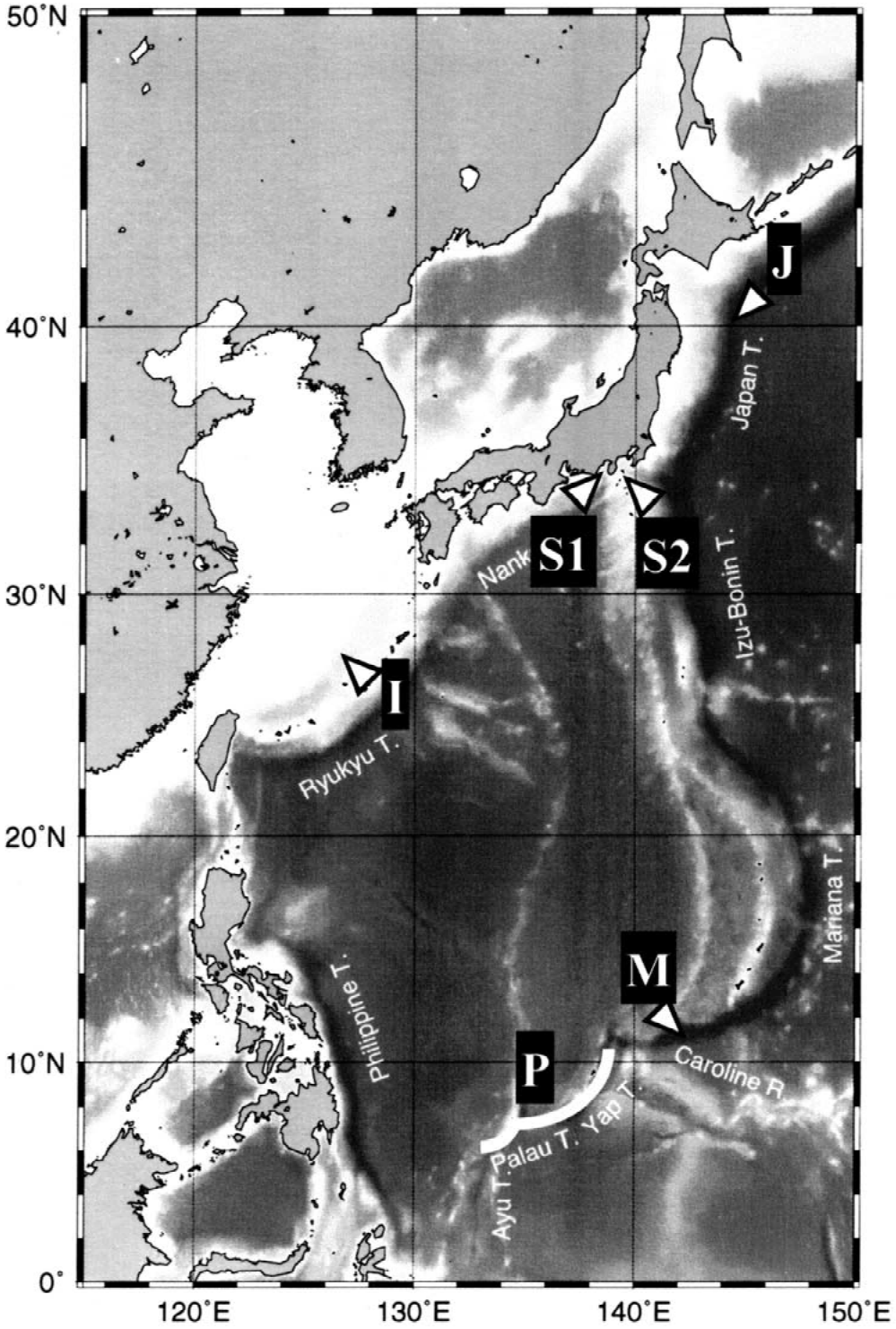

Figure 1. Geographical map of the sampling sites. Details of each site are shown in Table 1.

that sequence was not included in the phylogenetic analysis. A phylogenetic tree was constructed by the neighbor-joining (NJ) method (Saitou & Nei 1987) using NEIGHBOR of PHYLIP 3.57c (Felsenstein 1995), from the evolutionary distances calculated by means of DNADIST with the Kimura two-parameter model. The robustness of branches in the tree was evaluated by bootstrap analysis (Felsenstein 1985) with 1000 replicates. Maximum likelihood (ML) analysis was performed by a local rearrangement search by the RELL method (Kishino et al. 1990) using NUCML in MOLPHY 2.3 (Adachi & Hasegawa 1996), on the basis of the starting tree and the optimal Ts/Tv ratio derived by means of NJDIST.

Nucleotide sequence accession numbers

The ITS and 5.8S rRNA gene sequences determined were deposited in the DDBJ database under the accession numbers shown below: HTY563, AB026014; JCM 1546^{T}, AB038115; JCM 2959^{T}, AB038127; JCM 3770^{T}, AB038067; JCM 3771^{T}, AB026015; JCM 3775^{T}, AB038070; JCM 3776^{T}, AB038116; JCM 3777^{T}, AB026016; JCM 3778, AB038118; JCM 3785, AB038073; JCM 3787^{T}, AB049025; JCM 3795^{T}, AB049027; JCM 8098, AB038119; JCM 8105, AB038117; JCM 8115^{T}, AB026017; JCM 8163, AB038113; JCM 8164, AB038068; JCM 8169, AB038074; JCM 8170, AB038071; JCM 8173, AB038069; JCM 8202^{T}, AB049026; JCM 8208^{T}, AB026018; JCM 8977^{T}, AB038112; JCM 10020, AB049028; SY-30, AB025975; SY-31, AB025976; SY-52, AB025977; SY-53, AB025978; SY-55, AB025979; SY-58, AB025980; SY-71, AB025981; SY-72, AB025982; SY-73, AB025983; SY-74. AB025984; SY-75, AB025985; SY-76, AB025986; SY-77, AB025987; SY-78, AB025988; SY-79, AB025989; SY-80, AB025990; SY-81, AB025991; SY-82, AB025992; SY-83, AB025993; SY-84, AB025994; SY-85, AB025995; SY-86, AB025996; SY-87, AB025997; SY-88, AB025998; SY-89, AB025999; SY-90, AB026000; SY-91, AB026001; SY-92, AB026002; SY-93, AB026003; SY-94, AB026004; SY-95, AB026005; SY-96, AB026006; SY-97, AB026007; SY-98, AB026008; SY-99, AB026009; SY-100, AB026010; SY-101, AB026011; SY-102, AB026012; SY-103, AB026013.

Results and discussion

Ninety-nine strains of yeasts were isolated from 34 sources roughly divided into six regions (Figure 1, Table 1). On the basis of morphological and physiological characteristics, forty-five strains of basidiomycetous yeasts were identified including 40 strains of red yeasts, all of which belonged to the genera *Rhodotorula* and *Sporobolomyces* (Table 2). Red yeasts commonly appeared in samples of substrates from all six regions, but the numbers of other basidiomycetous yeasts were few (Figure 2, Table 2). Yeast isolates having ascomycetous affinities included members of the genera *Candida, Debaryomyces, Kluyveromyces, Saccharomyces* and *Williopsis*.

The proportion of red yeasts among the total yeast isolates from sediments collected at a depth of less than 2000 m was only 10.6% (Figure 2a). There were no basidiomycetous yeasts other than *Rhodotorula* spp. isolated from these sources. The low proportion of red yeasts may have been due to the numerous ascomycetous yeasts in sediments of Sagami Bay and Suruga Bay. Basidiomycetous yeasts were more frequently isolated from sediments from deeper regions (64.0%, Figure 2b) than from sediments collected at depths of less than 2000 m. This may be due to a difference in the amount of organic debris in the sediments or the difference in hydrostatic pressure at the sampling points. Red yeasts comprise a higher proportion of total yeasts in clean water than in polluted water (Hagler & Ahearn 1987; Lachance & Starmer 1998). The other basidiomycetous yeasts isolated from sediments of deeper regions were members of the genus *Cryptococcus*.

The proportions of basidiomycetous yeasts and red yeasts among the total yeast isolates from benthic organisms, two giant white clams, a tubeworm, a crab from Suruga Bay, and a mussel from Iheya Ridge, were 88.5% and 80.8% (Figure 2c), much larger than that in the case of yeasts from sediments. Differences in the proportion of red yeasts recovered from the animals and the sediments collected from almost the same locations and depths suggest that the occurrence of red yeasts in the deep-sea is strongly limited by the nutrient conditions of their habitats. The other basidiomycetous yeasts isolated from the clams were members of the genera *Cryptococcus* and *Pseudozyma*.

Forty isolates of *Rhodotorula* and *Sporobolomyces* were categorized on the basis of morphological and physiological characteristics (Table 2). *R. glu-*

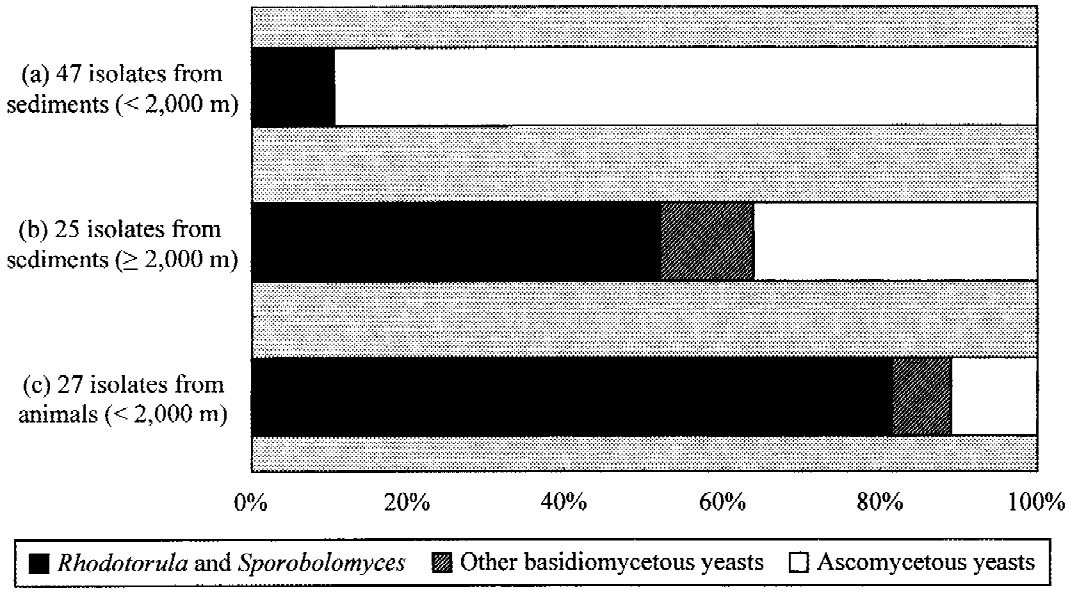

Figure 2. Differences in proportions of red yeasts (*Rhodotorula* and *Sporobolomyces*), the other basidiomycetous yeasts and ascomycetous yeasts among all yeast isolates from each deep-sea location.

tinis and *R. mucilaginosa* are frequently isolated from aquatic environments (Fell 1976; Hagler & Ahearn 1987). These two species and *R. marina* have been detected previously in seawater from the Pacific Ocean (Yamasato et al. 1974).

Twenty-two strains isolated from benthic animals at depths of less than 2000 m were identified as members of five *Rhodotorula* species and two *Sporobolomyces* species, but all 18 strains isolated from sediment collected at various depths were found to be members of only two species, *R. glutinis* and *R. mucilaginosa*. *R. minuta* was not found in the deep-sea sediments examined in this study, although it is commonly isolated from water, sediments and animals in aquatic environments (Hagler & Ahearn 1987). Microbes associated with macroorganisms in deep-sea environments presumably are exposed to favorable conditions with a stable source of nutrients, whereas in deep-sea sediments there is less organic debris available to be utilized by yeasts than in sediments in shallow regions.

We sequenced the ITS regions and the 5.8S rRNA gene of the 40 isolates and strains of related species. Faster substitution rates of ITS regions than 18S rDNA and 26S rDNA were rather useful to examine the genetic variation within a species or the phylogeny among closely related species. On the basis of these sequences, phylogenetic placements were established corresponding to the *Erythrobasidium* clade and the *Sporidiobolus* clade among four major clades suggested from the molecular phylogeny of the urediniomycetous yeasts (Fell et al. 2000). Because branchings of trees derived by the NJ and ML methods (data not shown) were generally consistent, only the NJ trees are considered here. There were some differences between branchings of trees drawn on the basis of the ITS and 5.8S rDNA sequences in this study and the above-mentioned tree drawn on the basis of 26S rDNA sequences (Fell et al. 2000). It is speculated that such discrepancies were due to the difference of molecular species such as 26S rDNA and ITS regions-5.8S rDNA or the difference in methods used in tree construction.

Seven isolates physiologically identified as *R. minuta* and *R. aurantiaca* were placed into the *Erythrobasidium* clade (Figure 3). Six isolates identified as *R. minuta* formed a cluster with the type strain of *R. minuta* although there was substantial heterogeneity within the species. The results of molecular analysis based on 26S rDNA sequences of strains once categorized as *R. minuta* indicated that these strains represent five distinct species, *R. minuta, R. slooffiae, R. pallida, R. laryngis* and *R. marina* (Fell et al. 2000). Isolates SY-86 and SY-87 from clams had identical sequences, as did isolates SY-91 and SY-92 from tubeworms. There was substantial evolutionary distance between the isolates from the clams and those from the tubeworms. Although SY-89 was physiologically identified as *R. aurantiaca*, it was phylogenetically

Table 2. Identification of deep-sea isolates on the basis of the morphological and physiological characteristics

Sources	Isolates			Species of *Rhodotorula* and *Sporobolomyces**					
Location	Total number of yeast isolates	Basidiomycetous yeasts	*Rhodotorula* and *Sporobolomyces*	*R. aur.*	*R. glu.*	*R. min.*	*R. muc.*	*S. sal.*	*S. shi.*
Benthic organisms									
S1: Suruga Bay	26	23	21	1	9	6	2	2	1
I: Iheya Ridge	1	1	1					1	
Total (< 2,000 m)	**27**	**24**	**22**	**1**	**9**	**6**	**2**	**3**	**1**
Sediments									
S1-S2: Suruga–Sagami Bay	47	5	5		4		1		
J: Japan Trench	5	4	1		1				
P: Palau–Yap Trench	19	11	11		7		4		
M: Mariana Trench	1	1	1				1		
Total (< 2,000 m)	**47**	**5**	**5**		**4**		**1**		
Total (≥ 2,000 m)	**25**	**16**	**13**		**8**		**5**		

**R. aur., Rhodotorula aurantiaca; R. glu., Rhodotorula glutinis; R. min., Rhodotorula minuta; R. muc., Rhodotorula mucilaginoca; S. sal., Sporobolomyces salmonicolor; S. shi., Sporobolomyces shibatanus.*

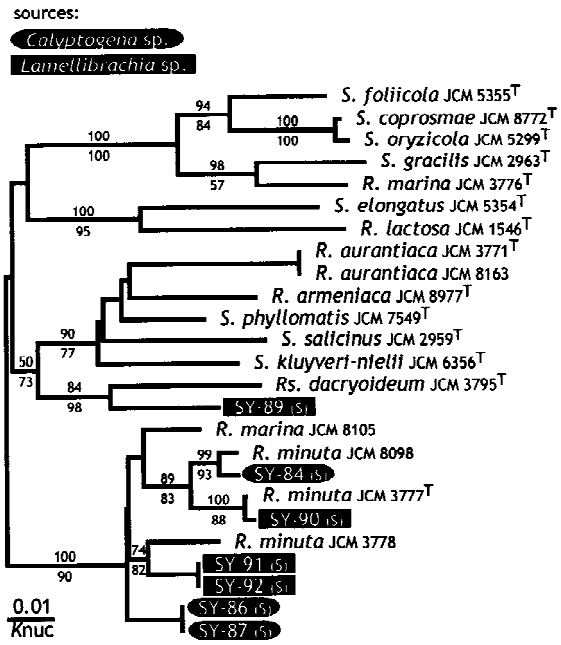

Figure 3. Phylogenetic positions of 7 isolates within the *Erythrobasidium* clade (Fell et al. 2000). The tree was constructed by using the neighbor-joining method, on the basis of a total of 461 aligned nucleotide sites. Bootstrap values obtained by the neighbor-joining method and local bootstrap probability values obtained by maximum likelihood analysis are indicated above and below the branches; only values greater than 50% on branches consistent between both trees are shown. Isolates from animals are indicated by outline letters on a black background. Locations where the sources of the isolates were collected are as follows: (S), Sagami–Suruga Bay.

distinct from JCM 3781^T, the type strain of *R. aurantiaca*. It was barely united to JCM 3795^T, the type strain of *Rs. dacryoideum*. The distance between SY-89 and JCM 3795^T seemed to exceed the permissible range for strains of a single species.

The remaining isolates examined fell into the *Sporidiobolus* clade (Fig. 4). Eighteen strains of *R. glutinis* from geographically dispersed sites differed at only one base in the ITS and the 5.8S rDNA sequences. Twelve of the 18 strains were identical to JCM 3787^T, the type strain of the marine-associated species *Rs. diobovatum*, and were somewhat distant evolutionarily from JCM 8208^T, the type strain of *R. glutinis*. Four strains physiologically identified as *R. glutinis* from the Palau-Yap Trench, SY-96, SY-100, SY-101 and SY-103, clustered with strains of *R. mucilaginosa* on the basis of ITS and 5.8S rDNA sequences. ITS and 5.8S rDNA sequences of SY-103 were identical to those of JCM 8202^T, the type strain of *Rs. sphaerocarpum*, a species prevalent in aquatic environments. SY-96 was found to be distinct from known species examined in this study. SY-100 and SY-101 were identical in ITS and 5.8S rDNA sequences to JCM 8164, which is categorized as *R. glutinis* var. *dairensis* (Fell & Statzell-Tallman 1998) though it is genetically related to *R. mucilaginosa* (Hamamoto et al. 1987). Strains physiologically assigned as *R. mucilaginosa*, including HTY 563 isolated from a site deeper than 10,000 m in the Challenger Deep region of the Mariana Trench were phylogenetically quite homogeneous with the type strain of *R. mucilaginosa* JCM 8115^T, compared to strains assigned as *R. glutinis*. The abil-

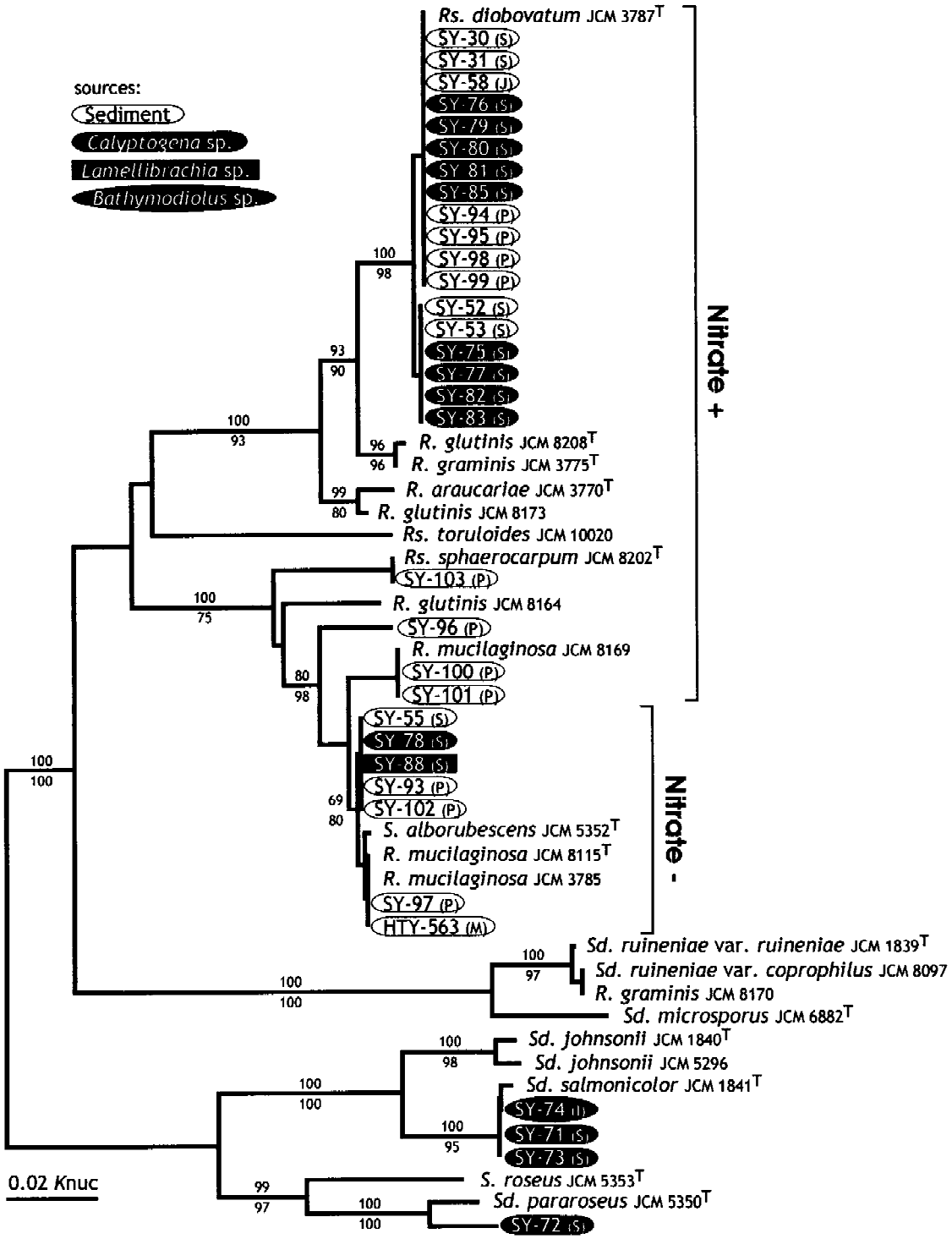

Figure 4. Phylogenetic positions of the 33 isolates within the *Sporidiobolus* clade (Fell et al. 2000). The tree was constructed by the neighbor-joining method, on the basis of a total of 475 aligned nucleotide sites. Bootstrap values obtained by the neighbor-joining method and local bootstrap probability values obtained by maximum likelihood analysis are indicated above and below the branches; only values greater than 50% on branches consistent between both trees are shown. Isolates from animals are indicated by outline letters on a black background. Locations where the sources of these isolates were collected are as follows: (I), Iheya Ridge; (J), Japan Trench; (M), Mariana Trench; (P), Palau–Yap Trench; (S), Sagami–Suruga Bay.

ity to assimilate nitrate is recognized as an important criterion for the distinction between *R. glutinis* and *R. mucilaginosa*. However, identification based on this criterion does not coincide with the species assignments made from the phylogenetic tree based on ITS and 5.8S rDNA sequences (Figure 3). Several species of *Rhodosporidium*, a teleomorphic genus of *Rhodotorula*, were found among *R. glutinis* strains; it is a polytypical species (Fell & Statzell-Tallman 1998) and seems to have sufficient genetic variety to be divided into several species. These problems will be clarified from further investigation at the species level such as the DNA relatedness analysis.

Four strains from clams, SY-71, SY-72, SY-73 and SY-74, fell into a cluster comprised of species of the ballistosporous genera, *Sporidiobolus* and *Sporobolomyces*. SY-71, SY-73 and SY-74, physiologically identified as *S. salmonicolor*, had sequences almost identical to those of JCM 1841^{T}, the type strain of *Sd. salmonicolor*. SY-72 identified as *S. shibatanus* was somewhat distant evolutionarily from JCM 5350^{T}, the type strain of *Sd. pararoseus* as a teleomorph of *S. shibatanus*. Hagler & Ahearn (1987) suggested that ballistosporous yeasts could enter aquatic environments with run-off from foliage. *Sporobolomyces* has been detected in the Pacific Ocean off the west coast of Mexico (Hernandez-Saavedra 1992), but not close to the coastline or at the surface of the ocean.

Previous studies support the premise that most yeasts enter aquatic environments with terrestrial run-off (Hagler & Ahearn 1987). We have described the occurrence of red yeasts in various deep-sea habitats in this paper. Red yeasts isolated from the deep-sea differ in terms of their frequency of occurrence among total yeasts isolated from sources such as sediments and animals, their systematic placement and evolutionary divergence. For unambiguous identification of red yeasts, which comprise the majority of yeasts in deep-sea environments, analysis of phylogenetic relationships on the basis of molecular data is needed. There is no doubt that continued investigation of yeasts from deep-sea sources is important.

Representative strains used in this study have been deposited in the Japan Collection of Microorganisms (JCM), the Institute of Physical and Chemical Research (RIKEN), Wako, Saitama, Japan, as strains JCM 10894 (SY-52), JCM 10895 (SY-58), JCM 10905 (SY-71), JCM 10906 (SY-72), JCM 10896 (SY-75), JCM 10898 (SY-84), JCM 10899 (SY-86), JCM 10907 (SY-89), JCM 10900 (SY-90), JCM 10901 (SY-91), JCM 10902 (SY-93), JCM 10897 (SY-94), JCM 10908 (SY-96), JCM 10903 (SY-100) and JCM 10904 (SY-103).

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