

## New Yeast Species, *Malassezia dermatis*, Isolated from Patients with Atopic Dermatitis

Takashi Sugita,<sup>1\*</sup> Masako Takashima,<sup>2</sup> Takako Shinoda,<sup>1</sup> Hajime Suto,<sup>3</sup>  
Tetsushi Unno,<sup>3</sup> Ryoji Tsuboi,<sup>3</sup> Hideoki Ogawa,<sup>3</sup>  
and Akemi Nishikawa<sup>4</sup>

Department of Microbiology<sup>1</sup> and Department of Immunobiology,<sup>4</sup> Meiji Pharmaceutical University, Kiyose, and Department of Dermatology, School of Medicine, Juntendo University, Bunkyo-ku,<sup>3</sup> Tokyo, and Japan Collection of Microorganisms, RIKEN (The Institute of Physical and Chemical Research), Wako, Saitama,<sup>2</sup> Japan

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***Malassezia* species are considered to be one of the exacerbating factors in atopic dermatitis (AD). During examination of the cutaneous colonization of *Malassezia* species in AD patients, we found a new species on the surface of the patients' skin. Analysis of ribosomal DNA sequences suggested that the isolates belonged to the genus *Malassezia*. They did not grow in Sabouraud dextrose agar but utilized specific concentrations of Tween 20, 40, 60, and 80 as a lipid source. Thus, we concluded that our isolates were new members of the genus *Malassezia* and propose the name *Malassezia dermatis* sp. nov. for these isolates.**

*Malassezia* species are known causative factors in pityriasis versicolor, seborrheic dermatitis (SD), and atopic dermatitis (AD) (3). In the last decade, research has focused primarily on isolating *Malassezia* strains and detecting specific immunoglobulin E (IgE) antibodies from patients (9, 13, 14, 26). A comparison of the isolation rates of *Malassezia* species from the skin of AD patients and healthy control subjects detected a significantly higher rate for patients than for healthy subjects (8). AD patients had specific IgE antibodies against *Malassezia*, while healthy subjects did not. In recent years, studies have increasingly been directed towards analyzing how the cutaneous microflora at the species level are related to each disease type (pityriasis versicolor, SD, and AD) (1, 6, 7, 12, 16). We previously compared the distribution of *Malassezia* species in skin lesions of AD patients and in healthy subjects using a nonculture method (nested PCR) that is not affected by the isolating medium (21). Of the seven members of the genus *Malassezia*, *Malassezia globosa* and *M. restricta* were the species most commonly associated with AD, while *M. obtusa* and *M. pachydermatis* were not detected in AD. In our survey of cutaneous *Malassezia* microflora, we isolated new *Malassezia* species from several patients with AD. In this paper, we propose a new species, *M. dermatis*, for these isolates.

### MATERIALS AND METHODS

**Malassezia isolates.** Nineteen AD outpatients at Juntendo University Hospital were included in the study. To obtain samples, OpSite transparent dressings (3 by 7 cm; Smith and Nephew Medical Ltd., Hull, United Kingdom) were applied to skin lesions (erosive, erythematous, and lichenoid) on the scalp, back, and nape of the neck of AD patients. Samples were then transferred onto Leeming and Notman agar (LNA) (10.0 g of polypeptone, 5.0 g of glucose, 0.1 g of yeast extract, 8.0 g of ox gall, 1.0 mg of glycerol, 0.5 g of glycerol stearate, 0.5 mg of Tween 60, 10 ml of cow's milk [whole fat], and 12.0 g of agar per liter) plates

containing 50  $\mu$ l of chloramphenicol (Sankyo, Tokyo, Japan) and incubated at 32°C for 2 weeks.

**Direct DNA sequencing of rRNA genes of the isolates.** Yeast isolates recovered from LNA medium were identified by analysis of rRNA gene sequences. Nuclear DNA of the isolates was extracted by the method of Makimura et al. (15). The D1 and D2 regions of 26S ribosomal DNA (rDNA) and internal transcribed spacer (ITS) regions in the rRNA gene were sequenced directly from PCR products using the primer pairs NL-1 (forward; GCATATCAATAAGCGGAG GAAAAG) and NL-4 (reverse; GGTCCGTGTTTCAAGACGG) (11) and pITS-F (forward; GTCGTAACAAGGTTAACCTGCGG) and pITS-R (reverse; TCCTCCGCTTATTGATATGC) (22), respectively. The PCR products were sequenced using an ABI 310 DNA sequencer and an ABI PRISM BigDye Terminator Cycle Sequencing kit (Perkin-Elmer Applied Biosystems, Foster, Calif.) according to the manufacturer's instructions. Strains with 99% or more similarity of the D1 and D2 regions of 26S rDNA and the overall ITS sequences were defined as conspecific (18, 22). The sequence data were searched using the BLAST system (<http://www.ncbi.nlm.nih.gov/BLAST/>) at the National Center for Biotechnology Information, Bethesda, Md.

**Molecular phylogenetic analysis.** The sequences were aligned using ClustalW software (25). For neighbor-joining analysis (20), the distances between sequences were calculated using Kimura's two-parameter model (10). A bootstrap analysis was conducted with 100 replications (2).

**Morphological, physiological, and chemotaxonomic characteristics.** Morphology was examined on LNA after incubation at 32°C for 7 days. Tween 20, 40, 60, and 80 utilization, catalase reactions, and diazonium blue B reactions were performed as described by Guého et al. (4). Identification of ubiquinone was carried out using methods described by Nakase and Suzuki (17). The nuclear DNA base composition (moles percent G+C) was determined by high-pressure liquid chromatography after enzymatic digestion of DNA into deoxyribonucleosides (23, 24). The DNA-GC kit (Yamasa Shouyu, Chiba, Japan) was used according to the manufacturer's instructions.

**Nucleotide sequence accession numbers.** The nucleotide sequences determined in this study have been deposited with DDBJ (DNA Data Bank of Japan) under the accession numbers shown in Fig. 1.

### RESULTS

**Molecular phylogenetic analysis.** All of the yeast isolates obtained from the 19 AD patients were identified by analysis of rRNA gene sequences (D1 and D2 regions of 26S rDNA and ITS). Of these, the DNA sequences of five isolates did not match sequences in the DNA sequence database. These isolates were designated M 9927, M 9928, M 9929, M 9930, and M

\* Corresponding author. Mailing address: Department of Microbiology, Meiji Pharmaceutical University, 2-522-1 Noshio, Kiyose, Tokyo 204-8588, Japan. Phone: 81-424-95-8762. Fax: 81-424-95-8762. E-mail: sugita@my-pharm.ac.jp.

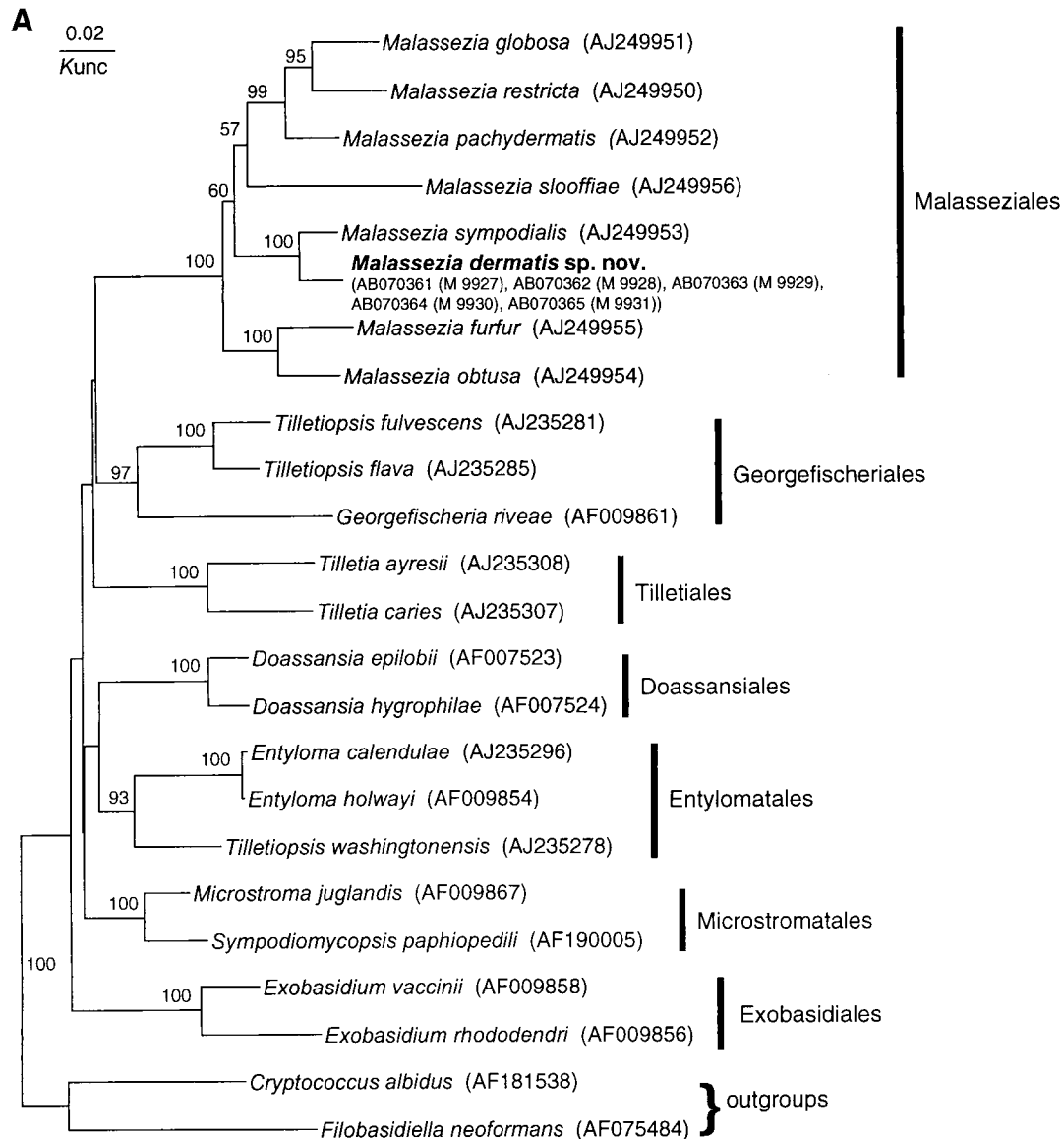


FIG. 1. Molecular phylogenetic trees constructed using the sequences of D1 and D2 26S rDNA of *M. dermatis* sp. nov. and related *Ustilaginomycetes* species (A) and the ITS1 region of *M. dermatis* sp. nov. and other member of the genus *Malassezia* (B). DDBJ/GenBank accession numbers are indicated in parentheses. The numerals represent the confidence levels from 100 replicate bootstrap samplings (frequencies of less than 50% are not indicated). T, type strain. Kunc, Kimura's parameter (10).

9931. The first three strains were isolated from a single patient, while the last two strains were isolated from one patient each. Sequence analysis of their 26S rDNA indicated that these isolates belonged to the genus *Malassezia* (Fig. 1A). The sequences of five isolates were completely identical in both the 26S rDNA and ITS regions and clustered with *M. sympodialis* with high bootstrap values (100%) (Fig. 1). Dissimilarities between these isolates and the *M. sympodialis* strain in their D1 and D2 regions of 26S, ITS1, and ITS2 were 1.2% (7 of 578), 10.5% (17 of 162), and 10.3% (24 of 233), respectively. The overall dissimilarity of ITS regions was 10.4% (41 of 395).

**Taxonomic characteristics.** The characteristics that differentiate the new species, *M. dermatis*, from the other seven known

*Malassezia* species are summarized in Table 1. The physiological characteristics of *M. dermatis* are identical to those of *M. furfur*. However, the moles percent G+C of *M. dermatis* nuclear DNA is 60.4% while that of *M. furfur* is 66.0 to 66.7%. A difference of more than 1 mol% G+C between two strains is considered taxonomically significant (19, 24).

**Latin description of *Malassezia dermatis* Sugita, Takashima, Nishikawa et Shinoda sp. nov.** In LNA, post dies 7 ad 32°C, cellulae vegetativae sphaericae, ovoideae vel ellipsoideae (2–8) × (2–10) μm. Cultura xanthoalba, semi-nitida aut hebetata, butyracea et margo glabra aut lobulata. In agaro glucoso-peptonico Tween 20, 40, 60, 80 (0.1–1%) addito crecit. H<sub>2</sub>O<sub>2</sub> hydrolysat. Commutatio coloris per diazonium caeruleum B positiva. Proportio molaris guanini+cytosini in acido deoxyri-

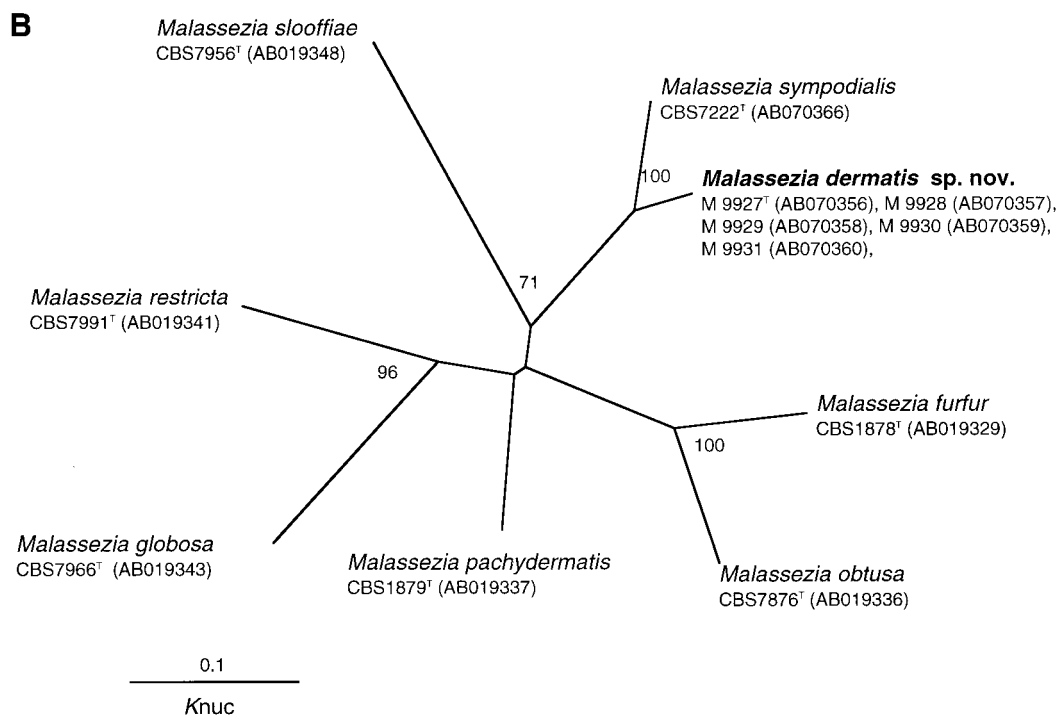


FIG. 1—Continued.

bonucleico: 60.4 mol%. Ubiquinonum majus: Q-9. Teleomorphus ignota. Typus: JCM 11348<sup>T</sup>, ex cute morbosa, Tokyo, Japonia, 30.9.1999, T. Sugita (originaliter ut M 9927), conservatur in collectionibus culturarum quas 'Japan Collection of Microorganisms,' Saitama, Japan sustentat.

**Description of *M. dermatis* Sugita, Takashima, Nishikawa et Shinoda sp. nov.** After 7 days on LNA at 32°C, the vegetative cells were spherical, oval, or ellipsoidal (2 to 8 by 2 to 10 μm), and they reproduced by budding (Fig. 2). The colony was yellowish white, semishiny to dull, convex, and butyrous and had an entire or lobed margin. Filaments sometimes formed at the area of the origin of the bud. Growth was seen on glucose-peptone agar with either 0.1, 0.5, 1.0, 5.0, or 10% Tween 20, 40, 60, or 80 as sole source of lipid. Catalase reaction was positive. The diazonium blue B reaction was positive. The G+C content of nuclear DNA was 60.4 mol%, and the major ubiquinone was Q-9. Teleomorph is unknown. JCM 11348<sup>T</sup> (originally M 9927) isolated from skin lesions of an AD patient in Tokyo, Japan, by T. Sugita on 30 September 1999 is maintained in the Japan Collection of Microorganisms (JCM), Saitama, Japan. The other strains, M 9929 and M 9931 have also been deposited in the JCM, as JCM 11469 and JCM 11470, respectively. Etymology is from the Latin name for skin, from which this strain of the species was obtained.

## DISCUSSION

The genus *Malassezia* is phylogenetically monophyletic with a high bootstrap value (100%) and is positioned in the class *Ustilaginomycetes* (Fig. 1A). Our isolates clustered with *M. sympodialis* on the tree. We did not perform a nuclear

DNA-DNA hybridization study between *M. sympodialis* and our isolates, but sequence analysis of rDNA (26S and ITS) strongly suggests that our five isolates represent a new species in the genus *Malassezia*. At present, D1 and D2 26S rDNA sequences are used from almost all yeasts for species identification or phylogenetic analysis. Peterson and Kurtzman (18) correlated the biological species concept with the phylogenetic species concept by the extent of nucleotide substitutions in 26S rDNA sequences. Their study demonstrated that strains of a single biological species show less than 1% substitution in this region. Subsequently, Sugita et al. (22) also found that conspecific strains have less than 1% dissimilarity in the ITS1 and -2 regions after comparing the nuclear DNA-DNA hybridization values and similarity in the sequences of the entire ITS1 and -2 regions. According to the species concept used in the two reports cited above, the divergence between *M. sympodialis* and our isolates is sufficient to resolve them as individual species. In addition, the approximately 1 mol% difference in the G+C content of nuclear DNA that was found between these two species also has taxonomic significance (19, 24). Guillot et al. (5) described a practical approach (combination of Tween utilization and catalase tests) for easy and simple identification of *Malassezia* species. Unfortunately, *M. dermatis* cannot be identified by this system, despite having similar characteristics to *M. furfur*. At present, sequence analysis of the D1 and D2 regions of the 26S rDNA or the ITS regions is the most reliable and simplest method for *M. dermatis* identification.

We previously demonstrated cutaneous *Malassezia* colonization in 32 AD patients by a nonculture method (nested

TABLE 1. Taxonomic characteristics of *M. dermatis* and other *Malassezia* species

Characteristic	<i>M. dermatis</i> sp. nov.	<i>M. furfur</i> <sup>a</sup>	<i>M. pachydermatis</i> <sup>a</sup>	<i>M. sympodialis</i> <sup>a</sup>	<i>M. globosa</i> <sup>a</sup>	<i>M. obtusa</i> <sup>a</sup>	<i>M. restricta</i> <sup>a</sup>	<i>M. slooffiae</i> <sup>a</sup>
Morphological characteristics								
Colony morphology	Convex, butyrous, entire or lobed margin	Mat, dull, smooth, umbonate or slightly folded with convex elevation	Mat, convex, umbonate (sometimes)	Glistening, smooth, flat or with a slight central elevation	Raised, folded, rough	Smooth, flat	Dull, smooth to rough at the edges	Rough but usually with fine grooves
Cell shape (size [μm])	Spherical, oval, ellipsoidal (2.0–8.0 by 2.0–10.0)	Oval, cylindrical (1.5–3.0 by 2.5–8.0), spherical (2.5–5.0)	Oval (2.0–2.5 by 4.0–5.0)	Oval, globosol (1.5–2.5 by 2.5–6.0)	Spherical (2.5–8.0)	Cylindrical (1.5–2.0 by 4.0–6.0)	Spherical, oval (1.5–2.0 by 2.5–4.0)	Short cylindrical (1.0–2.0 by 1.5–4.0)
Physiological characteristics <sup>b</sup>								
Growth on Sa at 32°C	–	–	+	–	–	–	–	–
Growth on mDixon	+	+	+	+	+	+	+	+
32°C	+	+	+	+	± or –	± or +	+	+
37°C	+	+	+	+	+	+	+	+
40°C	+	+	± or +	+	+	+	+	+
Catalase reaction	+	+	–	–	–	–	–	± or +
Utilization of:								
Tween 20	+	+	+	+	–	–	–	+
Tween 40	+	+	+	+	–	–	–	+
Tween 60	+	+	+	+	–	–	–	+
Tween 80	+	+	+	+	–	–	–	+
Mol% G+C	60.4	66.0–66.7	55.5–56.0	61.9–62.5	53.5–53.7	60.5–60.7	59.8–60.0	68.5–69.0

<sup>a</sup> Data are from the work of Guého et al. (4).

<sup>b</sup> Sa, Sabouraud dextrose agar; mDixon, modified Dixon agar; +, positive; –, negative; ±, weakly positive.

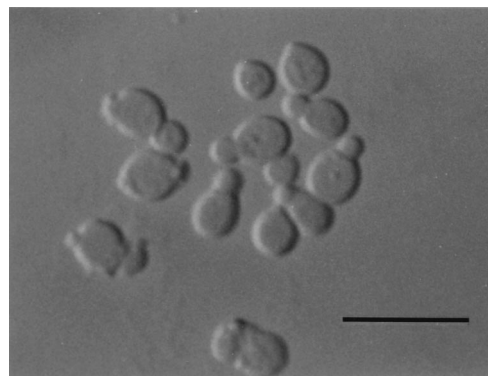


FIG. 2. Vegetative cells of *M. dermatis* M 9927 (JCM 11348) grown in LNA for 7 days at 32°C. Bar, 10 μm.

PCR) and compared anti-*Malassezia* antibody levels with those in healthy subjects. The detection of *M. globosa* and *M. restricta* in more than 90% of AD patients suggested that these two species play an important role in AD. Since we were not aware of the existence of *M. dermatis* at that time, we did not detect the DNA of this species in our previous study. Although the present study examined a limited number of patients, *M. dermatis* was found in 3 of 19 patients. However, the degree of *M. dermatis* colonization of the skin surface in the AD patients was not as great as that of *M. globosa* and *M. restricta*.

In conclusion, we described a novel species, *M. dermatis*, isolated from skin lesions of AD patients. Further studies should examine whether this microorganism is responsible for AD or other skin diseases and whether it is specific to Japan.

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