

## Revised Culture-Based System for Identification of *Malassezia* Species<sup>∇</sup>

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**Forty-six strains of *Malassezia* spp. with atypical biochemical features were isolated from 366 fresh clinical isolates from human subjects and dogs. Isolates obtained in this study included 2 (4.7%) lipid-dependent *M. pachydermatis* isolates; 1 (2.4%) precipitate-producing and 6 (14.6%) non-polyethoxylated castor oil (Cremophor EL)-assimilating *M. furfur* isolates; and 37 (34.3%) *M. slooffiae* isolates that were esculin hydrolyzing, 17 (15.7%) that were non-tolerant of growth at 40°C, and 2 (1.9%) that assimilated polyethoxylated castor oil. Although their colony morphologies and sizes were characteristic on CHROMagar *Malassezia* medium (CHROM), all strains of *M. furfur* developed large pale pink and wrinkled colonies, and all strains of *M. slooffiae* developed small (<1 mm) pale pink colonies on CHROM. These atypical strains were distinguishable by the appearance of their colonies grown on CHROM. Three clinically important *Malassezia* species, *M. globosa*, *M. restricta*, and *M. furfur*, were correctly identified by their biochemical characteristics and colony morphologies. The results presented here indicate that our proposed identification system will be useful as a routine tool for the identification of clinically important *Malassezia* species in clinical laboratories.**

Members of the genus *Malassezia* are among the microbiological flora of the skin of homiothermic animals. Most species of this genus are lipid-dependent yeasts which colonize the seborrheic part of the skin, and they have been reported to be associated with pityriasis versicolor, seborrheic dermatitis, *Malassezia* folliculitis, and atopic dermatitis (1, 6, 19, 20, 24, 29). Although *M. furfur* was previously thought to be the causative agent or trigger factor in all of these skin disorders, Guého et al. (9) reclassified this genus into five species in 1996. *Malassezia* has since been reclassified into seven species based on molecular biological analysis of nuclear ribosomal DNA/RNA (9, 10), and the results agreed with those of mitochondrial ribosomal DNA analyses (30). As members of the genus *Malassezia* share similar morphological and biochemical characteristics, it was thought that differentiating between them based on phenotypic features would be difficult. While molecular biological techniques are the most reliable for the identification of *Malassezia*, they are not available in most clinical laboratories. Therefore, culture methods for the identification of *Malassezia* species are required. Some of these identification

or differentiation methods have been reported previously. Guillot et al. reported a method of identification based on lipid usage pattern, catalase reaction, growth temperature, and cell shape (11). Hammer and Riley reported the production of a precipitate by some *Malassezia* strains on Dixon's agar (12); for example, *M. furfur*, *M. obtusa*, and *M. slooffiae* were precipitate-negative strains, while *M. sympodialis* and *M. globosa* were precipitate-positive strains. Mayser et al. reported that some *Malassezia* species hydrolyzed esculin and assimilated polyethoxylated castor oil (Cremophor EL; Sigma, St. Louis, MO) (18), and these properties could be used to differentiate among species (8). However, it is necessary to develop an updated phenotype-based identification method applicable to new *Malassezia* species (25, 26). We reported previously that CHROMagar *Candida* medium with vital growth factors for *Malassezia*, CHROMagar *Malassezia* medium (CHROM), could be used for isolating and differentiating between *Malassezia* and *Candida* spp. simultaneously (14) and that a biological feature-based identification method was developed for nine species of *Malassezia* (15). However, we found that some strains showed atypical features in fresh clinical isolates. Here, we (i) report the incidence of atypical biochemical features in *Malassezia* species and (ii) propose a culture-based identification system for three clinically important *Malassezia* species, *M. furfur*, *M. globosa*, and *M. restricta*.

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TABLE 1. Origin and distribution of *Malassezia* strains

Species	No. of total strains	No. of stock strains	No. of fresh isolates	Origin	Comments
<i>M. pachydermatis</i>	43	1	30	CBS 1879	External otitis
			12	Dog, external ear	Healthy dogs
<i>M. sympodialis</i>	84	1	80	CBS 7222	Healthy adults
			1	Human	Psoriasis vulgaris
			1	Human	Atopic dermatitis
			1	Human, external ear	External otitis
<i>M. globosa</i>	14	1	10	CBS 7966	Pityriasis versicolor
			3	Human	Seborrheic dermatitis
<i>M. dermatis</i>	5	2	2	JCM11348 and JCM11470	Healthy adults
			1	Human	Seborrheic dermatitis
<i>M. furfur</i>	41	1	38	CBS 1878	Healthy adults
			1	Human	Psoriasis vulgaris
<i>M. slooffiae</i>	108	1	1	Human, external ear	External otitis
			95	CBS 7956	Healthy adults
			8	Human, external ear	External otitis
			3	Human	Seborrheic dermatitis
<i>M. obtusa</i>	4	1	1	Human	Psoriasis vulgaris
			3	CBS 7876	Healthy adults
<i>M. restricta</i>	71	1	31	CBS 7877	Healthy adults
			35	Human, external ear	Seborrheic dermatitis
			2	Human	Psoriasis vulgaris
			2	Human	Pityriasis versicolor
<i>M. japonica</i>	2	2	0	M9966, M9967	
<i>M. yamatoensis</i>	5	0	2	Human	Healthy adults
			2	Human	Psoriasis vulgaris
			1	Human	Seborrheic dermatitis
Total	377	11	366		

#### MATERIALS AND METHODS

**Organisms.** Three hundred sixty-six fresh clinical isolates of *Malassezia* (42 *M. pachydermatis*, 83 *M. sympodialis*, 13 *M. globosa*, 40 *M. furfur*, 107 *M. slooffiae*, 3 *M. obtusa*, 70 *M. restricta*, 3 *M. dermatis*, and 5 *M. yamatoensis* isolates) obtained from human subjects or dogs, as described below, as well as type and standard strains of *Malassezia* (Table 1), were used in this study. All strains were identified by molecular biological analysis (16, 17).

**Culture media.** Strains of *Malassezia* were maintained on modified Leeming and Notman agar composed of (per liter) 10 g of peptone (Oxoid, Basingstoke, United Kingdom), 10 g of glucose, 2 g of yeast extract (Oxoid), 8 g of ox bile (Oxoid), 10 ml of glycerol, 0.5 g of glycerol monostearate, 5 ml of Tween 60, 20 ml of olive oil, and 15 g of agar (Oxoid) and sterilized by autoclaving.

The following specific media were used in this study. CHROMagar *Malassezia* medium (CHROM) was composed (per liter) of 56.3 g of CHROMagar *Malassezia* basal medium (CHROMagar, Paris, France) and 10 ml of Tween 40 (15). Sabouraud's dextrose agar (SDA) was composed (per liter) of 10 g of mycological peptone, 40 g of glucose, and 15 g of agar. Cremophor EL (Sigma, St. Louis, MO) agar (EL slant) was composed (per liter) of 65 g of SDA and 10 ml of Cremophor EL (15). Tween 60-esculin agar (TE slant) was composed (per liter) of 10 g of peptone, 10 g of glucose, 2 g of yeast extract, 5 ml of Tween 60, 0.5 g of ferric ammonium citrate, 1 g of esculin, and 15 g agar (15).

**Clinical specimens.** Two hundred eighteen clinical specimens from the body surface of patients with atopic dermatitis, seborrheic dermatitis, psoriasis vulgaris, and external otitis and from healthy adults were obtained from Teikyo University Hospital (Tokyo, Japan), Kitasato University Hospital (Kanagawa, Japan), and Takinomiya General Hospital (Kagawa, Japan) and from 65 dogs with and without external otitis from Nihon University Veterinary Hospital (Kanagawa, Japan) and veterinary clinics in the Kanto area. Samples from the body surface were taken using adhesive tape (10 mm by 10 mm) as reported by

Padilha-Goncalves (22), which was then placed on CHROM. External ear samples were taken with swabs and then streaked on CHROM. All isolates observed for CHROM were checked by colony morphology and size after incubation in air at 32°C for 4 to 7 days.

**Phenotypic feature testing.** The "typical phenotypic features" of *Malassezia* species were defined as shown in Table 2. All isolates of *Malassezia* were inoculated onto CHROM and specific media (SDA, EL slant, and TE slant) and incubated at 32°C for 4 days before observation. SDA was used to determine the isolates' lipid dependence, EL slants for the isolates' abilities to utilize polyethoxylated castor oil, and TE slants for the isolates' abilities to hydrolyze esculin and utilize Tween 60. Fresh cultures grown on CHROM were subjected to catalase testing with 3% hydrogen peroxide. Colony size on CHROM was determined by measuring well-isolated single colonies and assessed as small (<1 mm), medium (1 to 2 mm), or large (2 to 5 mm). Additional phenotypic characterization of the ability to grow at 40°C was performed if the isolate was thought to be a strain of *M. slooffiae*. All test strains of *Malassezia* species were identified by molecular analysis.

**Molecular analysis.** DNA was extracted by the procedure of Makimura et al. (16). The internal transcribed spacer 1 (ITS1) region was sequenced directly from PCR products by using the primer pair 18SF1 and 58SR1 (17). The PCR products were sequenced on an ABI PRISM 310 genetic analyzer according to the manufacturer's instructions (Applied Biosystems, Foster City, CA).

#### RESULTS

**Phenotypic features of *Malassezia* and incidence of atypical phenotypic features found in *Malassezia* species.** The phenotypic features of nine *Malassezia* species are shown in Table 3.

TABLE 2. Typical phenotypic features in nine species of *Malassezia*<sup>a</sup>

Species	Growth on SDA	Growth on mDixon at			Precipitate production on CHROM	Utilization of				Esculin	Polyethoxylated castor oil	Catalase reaction
		32°C	37°C	40°C		10% Tw 20	0.5% Tw 40	0.5% Tw 60	0.1% Tw 80			
<i>M. pachydermatis</i> <sup>b</sup>	+	+	+	+	+ <sup>f</sup>	+	+	+	+	± <sup>e</sup>	± <sup>e</sup>	+
<i>M. sympodialis</i> <sup>b</sup>	-	+	+	+	+ <sup>f</sup>	-	+	+	+	+ <sup>e</sup>	- <sup>e</sup>	+
<i>M. globosa</i> <sup>b</sup>	-	+	± or -	-	+ <sup>f</sup>	-	-	-	-	- <sup>e</sup>	- <sup>e</sup>	+
<i>M. dermatis</i> <sup>c</sup>	-	+	+	+	+ <sup>f</sup>	+	+	+	+	- <sup>f</sup>	- <sup>f</sup>	+
<i>M. furfur</i> <sup>b</sup>	-	+	+	+	- <sup>f</sup>	+	+	+	+	- <sup>e</sup>	+ <sup>e</sup>	+
<i>M. slooffiae</i> <sup>b</sup>	-	+	+	+	- <sup>f</sup>	± or +	+	+	-	- <sup>e</sup>	- <sup>e</sup>	+
<i>M. obtusa</i> <sup>b</sup>	-	+	± or +	-	- <sup>f</sup>	-	-	-	-	+ <sup>e</sup>	- <sup>e</sup>	+
<i>M. restricta</i> <sup>b</sup>	-	+	+	-	- <sup>f</sup>	-	-	-	-	- <sup>e</sup>	- <sup>e</sup>	-
<i>M. japonica</i> <sup>d</sup>	-	+	+	-	- <sup>f</sup>	-	±	+	-	+ <sup>f</sup>	- <sup>f</sup>	+

<sup>a</sup> mDixon, modified Dixon agar; Tw, Tween; +, positive; -, negative; ±, weakly positive.

<sup>b</sup> Guého et al. (9).

<sup>c</sup> Sugita et al. (25).

<sup>d</sup> Sugita et al. (26).

<sup>e</sup> Maysers et al. (18).

<sup>f</sup> Kaneko et al. (15).

(i) **Precipitate production and colony morphology on CHROM.** All of the type and reference strains and fresh clinical isolates of *M. pachydermatis* (43/43), *M. sympodialis* (84/84), *M. globosa* (14/14), and *M. dermatis* (5/5) produced pre-

cipitates after incubation at 32°C for 4 days on CHROM. Other strains of the species *M. furfur* (40/41), *M. slooffiae* (108/108), *M. obtusa* (4/4), *M. restricta* (71/71), and *M. japonica* (2/2) did not produce such precipitates, with the exception of only

TABLE 3. Biological features and incidence of a typical phenotype of nine species of *Malassezia*

Species identified by molecular biological analysis	No. of strains	Colony characteristic on CHROM (% of incidence) <sup>a</sup>			Growth characteristic (% of incidence) <sup>b</sup>				Catalase reaction
		Size	Color/morphology	Precipitate	SDA	TE slant	EL slant	40°C	
<i>M. pachydermatis</i>	43	Large (100)	Pale pink/smooth (100)	+ (100)	Growth (95.3) <sup>d</sup>	Growth and produced a black zone (95.3) <sup>d</sup>	Growth (95.3) <sup>d</sup>	NT	+ (100)
<i>M. sympodialis</i>	84	Large (100)	Pale pink/smooth (100)	+ (100)	No growth (100)	Growth and produced a black zone (100)	No growth (100)	NT	+ (100)
<i>M. globosa</i>	14	Small (100)	Purple/smooth (100)	+ (100)	No growth (100)	No growth and no change (100)	No growth (100)	NT	+ (100)
<i>M. dermatis</i>	5	Large (100)	Pale pink to purple/smooth (100)	+ (100)	No growth (100)	Growth and no change (100)	No growth (100)	NT	+ (100)
<i>M. furfur</i>	41	Large (100)	Pale pink/wrinkled (100)	- (97.6) <sup>c</sup>	No growth (100)	Growth and produced a black zone (100)	Growth (85.4)	NT	+ (100)
<i>M. slooffiae</i>	108	Small (100)	Pale pink/smooth (100)	- (100)	No growth (100)	Growth and no change (65.7) <sup>e</sup>	No growth (98.1)	Growth (84.3) <sup>e</sup>	+ (100)
<i>M. obtusa</i>	4	Medium (100)	Pink/rough (100)	- (100)	No growth (100)	No growth but produced a black zone (100)	No growth (100)	NT	+ (100)
<i>M. restricta</i>	71	Small (100)	Pink/smooth (100)	- (100)	No growth (100)	No growth and no change (100)	No growth (100)	NT	- (100)
<i>M. japonica</i>	2	Large (100)	Pink/smooth (100)	- (100)	No growth (100)	Growth and produced a black zone (100)	No growth (100)	No growth (100)	+ (100)

<sup>a</sup> Incubated at 32°C for 4 to 7 days.

<sup>b</sup> Incubated at 32°C for 4 days. NT, not tested; +, positive; -, negative.

<sup>c</sup> Only one strain of fresh clinical isolate produced a precipitate.

<sup>d</sup> Only two strains of fresh clinical isolates did not grow on SDA and EL and did not produce a black zone on TE.

<sup>e</sup> Thirty-seven strains of fresh clinical isolates produced a black zone on TE, and 17 strains did not grow on modified Leeming and Notman agar at 40°C for 4 days.

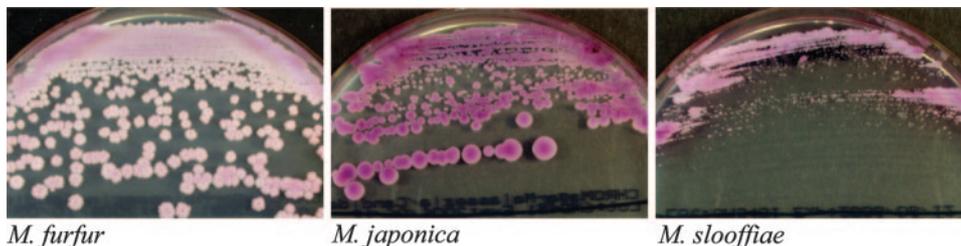


FIG. 1. Colony characteristics of *M. furfur*, *M. japonica*, and *M. slooffiae* on CHROM were observed after incubation at 32°C for 4 days. Colonies of *M. furfur* were large, pale pink, and wrinkled and did not produce precipitates. Colonies of *M. japonica* were larger (2 to 5 mm) than those of *M. slooffiae* (<1 mm). Their sizes were measured in well-isolated single colonies.

one (2.4%) strain of a fresh clinical isolate of *M. furfur*. *Malassezia furfur* colonies, including this one atypical strain, were easily distinguishable from those of other *Malassezia* species on CHROM due to their characteristically large pale pink and wrinkled colonies (Fig. 1). Colony size on CHROM was measured from well-isolated single colonies, and isolates were divided into three groups: small (*M. globosa*, *M. slooffiae*, and *M. restricta*), medium (*M. obtusa*), and large (*M. pachydermatis*, *M. sympodialis*, *M. dermatis*, *M. furfur*, and *M. japonica*).

(ii) **Lipid dependence.** Only *M. pachydermatis* (41/43) grew on the lipid-free culture medium (SDA), and other *Malassezia* species did not grow. This biological feature of *M. pachydermatis* was specific, but two (4.7%) atypical strains of lipid-dependent *M. pachydermatis* were obtained.

(iii) **Utilization of polyethoxylated castor oil.** EL slants were used to determine the ability to utilize polyethoxylated castor oil, and we obtained six (14.6%) atypical strains of *M. furfur*

and two (4.7%) of *M. pachydermatis* that did not grow on EL slants. Two (1.9%) *M. slooffiae* strains grew on EL slants. This biological feature was not acceptable as a key identifying feature of *M. furfur*.

(iv) **Catalase reaction.** *Malassezia restricta* was the only catalase-negative species. This biological feature was acceptable as a key identifying feature of *M. restricta*.

(v) **Hydrolysis of esculin and utilization of Tween 60.** Forty-one (95.3%) isolates of *M. pachydermatis*, 84 (100%) of *M. sympodialis*, 41 (100%) of *M. furfur*, and 2 (100%) of *M. japonica* showed production of a black zone around the colonies due to esculin hydrolysis products and ferrous iron in TE slants. On the other hand, 5 (100%) *M. dermatis* and 71 (65.7%) *M. slooffiae* isolates did not produce such a zone. None of the strains of *M. globosa* or *M. restricta* utilized Tween 60 or grew on TE slants. None of the strains of *M. obtusa* utilized Tween 60, but they hydrolyzed esculin and produced a black zone on TE slants.

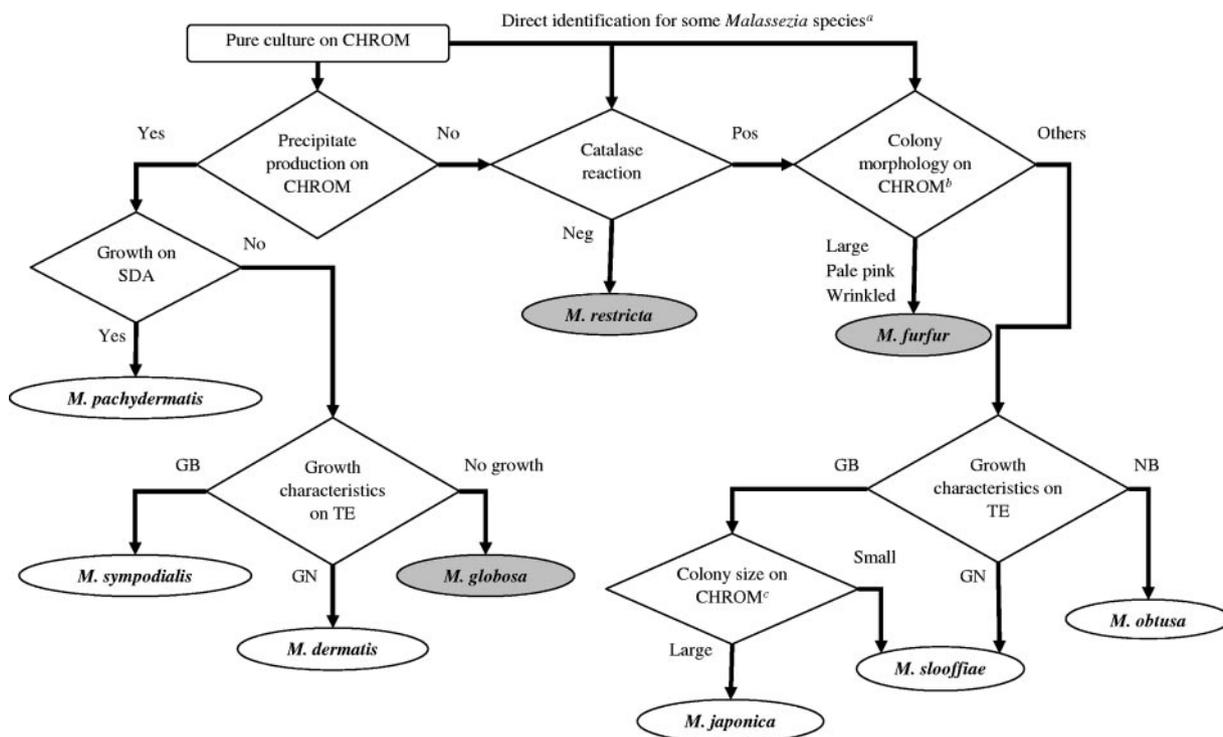


FIG. 2. Proposed identification workflow for nine species of *Malassezia*. Pos, positive; Neg, negative; GB, growth and black zone; GN, growth and no change; NB, no growth and black zone. *a*, Direct identification by catalase reaction and features of colonies on CHROM for *M. restricta* and *M. furfur*. *b*, Colony morphology on CHROM as shown in Fig. 1. *c*, Colony size of *M. japonica* and *M. slooffiae* on CHROM as shown in Fig. 1.

TABLE 4. Identification results and sensitivity and specificity of the proposed identification system

Species identified by molecular biological analysis	No. of test strains	No. of correct results	No. of incorrect results	Sensitivity (%)	Specificity (%)
<i>M. pachydermatis</i>	43	41	0	95.4	100
<i>M. sympodialis</i>	84	84	2 <sup>a</sup>	100	97.7
<i>M. globosa</i>	14	14	0	100	100
<i>M. dermatitis</i>	5	5	2 <sup>b</sup>	100	71.4
<i>M. furfur</i>	41	41	0	100	100
<i>M. slooffiae</i>	108	108	3 <sup>c</sup>	100	97.3
<i>M. obtusa</i>	4	4	0	100	100
<i>M. restricta</i>	71	71	0	100	100
<i>M. japonica</i>	2	2	0	100	100
<i>M. yamatoensis</i>	5	0	0	NA <sup>d</sup>	NA
Total	377	370	7		

<sup>a</sup> Two strains of fresh clinical isolates of *M. yamatoensis* were incorrectly identified as *M. sympodialis* by this modified identification method.

<sup>b</sup> Two strains of fresh clinical isolates of *M. pachydermatis* were incorrectly identified as *M. dermatitis* by this modified identification method.

<sup>c</sup> Three strains of fresh clinical isolates of *M. yamatoensis* were incorrectly identified as *M. dermatitis* by this modified identification method.

<sup>d</sup> NA, this method was not applicable.

(vi) **Tolerance of 40°C.** None of the strains of *M. japonica* and 17 (15.7%) *M. slooffiae* strains did not grow on modified Leeming and Notman agar at 40°C. This biological feature was not acceptable for distinguishing between *M. slooffiae* and *M. japonica* species. Other *Malassezia* species were not tested.

**Proposal for an identification system for *Malassezia* species.** Our proposed identification system for nine species of *Malassezia* using 366 fresh isolates and 11 type and reference strains identified by molecular analysis is shown in Fig. 2. First, some *Malassezia* species could be identified directly. *M. furfur* developed characteristically large pale pink and wrinkled colonies on CHROM and could be differentiated from other *Malassezia* species. *M. restricta* was the only catalase-negative species. Second, only *M. pachydermatis* grew on SDA, but 2 (4.7%) atypical strains were lipid dependent and were incorrectly identified as *M. dermatitis*. Third, with the exception of *M. yamatoensis*, other *Malassezia* species were correctly identified by this system. This system was not applicable for the identification of *M. yamatoensis*. Table 4 shows the numbers of correct and incorrect results and the sensitivity and specificity of our identification system. Three clinically important *Malassezia* species, *M. furfur* (100% [41 of 41]), *M. globosa* (100% [14 of 14]), and *M. restricta* (100% [71 of 71]), were correctly identified by this system, and the rate of concordance of this system with molecular analysis was 98.1% (370/377).

**Cost effectiveness of the proposed identification system.** Cost comparison results were based on the commercial price of each system (Table 5), but the labor costs associated with testing were not included. The cost of identifying *Malassezia* was reduced by about \$9,100 in this study.

## DISCUSSION

The biological tests described above were designed to identify *Malassezia* species in clinical laboratories. We investigated the variation of phenotypic features of 377 *Malassezia* species in this study.

TABLE 5. Cost comparison in U.S. dollars of the molecular biological analysis system and that of the proposed system

Item	Molecular biological analysis		Proposed biological identification system	
	Cost per test	Total cost <sup>a</sup>	Cost per test	Total cost <sup>a</sup>
CHROM	2.00	566	2.00	566
Molecular biological analysis	25.00	9,425	0	0
3% hydrogen peroxide <sup>b</sup>	0	0	0.417	0.0264
SDA slant	0	0	0.417	157.2
TE slant	0	0	0.417	157.2
Total	27.0	9,991	3.251	880

<sup>a</sup> During the present study.

<sup>b</sup> Catalase test.

First, CHROMagar was used as the primary culture medium, and we obtained 1 (2.4%) atypical strain of *M. furfur* that produced precipitates on CHROM. On the other hand, *M. pachydermatis*, *M. sympodialis*, *M. globosa*, and *M. dermatitis* were recognized from their precipitates in the agar, as reported by Kaneko et al. (15). The atypical strain of *M. furfur* developed characteristically large pale pink and wrinkled colonies on CHROM, and all test strains of *M. furfur* were characteristically similar without precipitate production. Therefore, *M. furfur* was identified correctly by colony morphology on CHROM. Although *M. globosa* and *M. obtusa* were similar to each other in terms of their phenotypic features (9), lipid usage patterns, catalase reactions, and growth temperatures, their characteristics with regard to precipitation on CHROM were different. Furthermore, *M. dermatitis* and *M. japonica* are new species reported by Sugita et al. (25, 26), and their biological properties resembled those of *M. slooffiae* and *M. sympodialis*, except for their lipid usage patterns, respectively, but their precipitations on CHROM were different (Tables 2 and 3).

Second, SDA was used to determine the lipid dependence; none of the lipid-dependent species grew on SDA. We obtained two (4.7%) atypical *M. pachydermatis* strains that could not be cultured on lipid-free culture medium (SDA), and the sensitivity and specificity of this culture medium for *M. pachydermatis* were 95.4% and 100%, respectively. The atypical strains were incorrectly identified as *M. dermatitis* based on their biological features. Duarte et al. (5) reported that one lipid-dependent variant strain of *M. pachydermatis* was isolated in an investigation of 964 cattle and 6 dogs, and unambiguous identification required sequencing.

Third, EL slants were used to determine the ability to utilize polyethoxylated castor oil for *M. furfur*. Mayser et al. (18) showed that polyethoxylated castor oil was metabolized only by five strains of *M. furfur* in the agar diffusion test, but we obtained six atypical strains (14.6%) of *M. furfur*, two (4.7%) of *M. pachydermatis*, and two (1.9%) of *M. slooffiae*. Their biological features were not acceptable as key features of *M. furfur*, and this culture medium's sensitivity and specificity for *M. furfur* were 85.4% and 97.3%, respectively. However, all test strains of *M. furfur* were directly identified by colony morphology on CHROM as described above.

Fourth, TE slants were used to determine the ability to hydrolyze esculin and utilize Tween 60 as key features for

differentiation among the precipitate-producing group (*M. sympodialis*, *M. dermatis*, and *M. globosa*) and the nonprecipitate-producing group (*M. japonica*, *M. slooffiae*, and *M. obtusa*). Mayer et al. (18) reported that two tested strains of *M. sympodialis* were able to split esculin with the black zone, while two tested strains of *M. slooffiae* remained negative. We confirmed these results previously with three strains each of *M. sympodialis* and *M. slooffiae* (15). However, 37 atypical strains (34.3%) of *M. slooffiae* hydrolyzed esculin and showed a black zone around the colonies on TE slants. In addition, 17 (15.7%) strains of *M. slooffiae* were not tolerant of growth at 40°C. Therefore, the previously reported biological features (15, 18) were not useful for differentiation between *M. slooffiae* and *M. japonica*. On the other hand, all test strains of *M. slooffiae* developed pale pink colonies that were smaller than those of *M. japonica* on CHROM and could be differentiated based on colony size.

Fifth, catalase reaction was used as a key feature of *M. restricta*, and this reaction allowed correct recognition of all test strains except *M. restricta*.

The results presented here indicated that the clinically important species *M. globosa*, *M. restricta*, and *M. furfur* were identified correctly using our proposed method. Recently, data from several institutions have suggested that *M. globosa* is associated with pityriasis versicolor (19, 20), that *M. restricta* is associated with seborrheic dermatitis (24, 29), and that *M. furfur* infections have been observed in hospitalized neonates with very low birth weight receiving intravenous lipid emulsions (2, 3, 4, 7, 23, 28). In agreement with these reports, we isolated mainly *M. globosa* from pityriasis versicolor and *M. restricta* from seborrheic dermatitis in the present study.

A simple, reliable, and cost-effective identification method is required in most clinical laboratories. CHROM with two specific media (SDA and TE slant) and catalase reactions allowed identification of *Malassezia* species easily, quickly, and at reasonable cost without requiring any expensive or specialized equipment. For example, automated sequencers are very expensive in Japan (about \$84,000). In addition, we estimated that the use of this system will result in cost savings equivalent to about \$9,100 per 377 samples in our laboratory. This biological identification system was used to correctly identify three clinically important *Malassezia* species, and we believe that this system can be adopted easily by most clinical laboratories and thus reduce labor costs and enable rapid reporting of clinically relevant laboratory results. Recently, *M. nana*, *M. yamatoensis*, and *M. equi* were reported as new species of *Malassezia* (13, 21, 27). Although these species are very rare, we hope that a technique for the easy differentiation of these species will be developed in future. The results presented here indicate that this system will be a useful tool for the routine identification of clinically important *Malassezia* species.

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