

ORIGINAL ARTICLE

***Malassezia globosa* tends to grow actively in summer conditions more than other cutaneous *Malassezia* species**

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ABSTRACT

Malassezia globosa is a major pathogen of *Malassezia* folliculitis (MF) and the predominant species on human skin. The aim of this study was to clarify the differences between *M. globosa* and other cutaneous *Malassezia* species, *M. restricta*, *M. dermatis*, *M. sympodialis* and *M. furfur*. The optimum growth temperature, effects of compounds of sweat and free fatty acids on growth, and lipase activities of five cutaneous *Malassezia* species were determined. The growth of *M. globosa* was promoted strongly by the compounds of sweat and high temperature unlike that of other cutaneous *Malassezia* species. This result clarified that *M. globosa* tended to grow actively in summer conditions more than other cutaneous *Malassezia* species. Furthermore, *M. globosa* showed high lipase activity. We consider these characteristics of *M. globosa* to relate to the pathogenesis of MF.

Key words: fatty acid, lipase, *Malassezia globosa*, sweat, temperature.

INTRODUCTION

Malassezia are basidiomycetous yeasts. The taxonomy of the genus *Malassezia* was revised in 1996,¹ and new species were recently proposed.^{2–7} This genus now consists of 14 species. *Malassezia* yeasts are part of the cutaneous microbiota. *M. globosa*, *M. restricta*, *M. dermatis*, *M. sympodialis* and *M. furfur* are detected mainly on healthy human skin.^{8,9}

Malassezia species are also associated with cutaneous disorders such as *Malassezia* folliculitis (MF), pityriasis versicolor, seborrheic dermatitis, dandruff, atopic dermatitis and psoriasis.¹⁰ MF is caused by the invasion of hair follicles by large numbers of *Malassezia* cells.¹¹ This results in the development of asymptomatic or pruritic erythematous papules and sometimes pustules on the back, chest and upper aspects of the arms.¹² MF tends to be more prevalent in the summer months and in tropical locations than in temperate regions.¹³ Our quantitative studies clarified that MF is caused by *Malassezia* species that are part of the cutaneous microbiota and not by exogenous species.¹⁴ *M. globosa* was a predominant species recovered from MF lesions. MF tends to be more prevalent in the summer months and in tropical locations than in temperate regions.¹³ It is considered a possibility that *M. globosa* has more suitable properties for increasing on human skin in summer than other cutaneous *Malassezia* species.

The aim of this study was to clarify the differences between *M. globosa* and other cutaneous *Malassezia* species, *M. restricta*, *M. dermatis*, *M. sympodialis* and *M. furfur*, at optimum growth

temperature, effects of compounds of sweat and free fatty acids on growth, and lipase activities.

METHODS

Strains

Malassezia globosa (Centraalbureau voor Schimmelcultures [CBS] 7966), *M. restricta* (CBS 7877), *M. dermatis* (Japan Collection of Microorganisms [JCM] 11348), *M. sympodialis* (CBS 7222), *M. furfur* (NITE Biological Resource Center [NBRC] 0656), and 17 clinical strains of *Malassezia* isolated from *Malassezia* folliculitis patients (five strains of *M. globosa*, and three strains each of *M. restricta*, *M. dermatis*, *M. sympodialis* and *M. furfur*) were used. The clinical isolates were identified as described previously.¹⁴ *Malassezia* strains were harvested in phosphate-buffered saline (PBS; pH 7.0) with an optical density of 0.1 after being maintained on Leeming & Notman agar medium (LNA)¹⁵ at 32°C for 2 days (*M. sympodialis* and *M. furfur*) or 7 days (*M. globosa*, *M. restricta* and *M. dermatis*).

Effects of temperature on growth

As a quantitative analysis of growth, we used a modification of the method by Ushijima *et al.*^{16,17} *Malassezia* suspensions were smeared in an area 1.5 cm in diameter on LNA and cultivated at 25°C, 32°C and 37°C. The slow growth species, *M. globosa*, *M. restricta* and *M. dermatis*, were analyzed after 7 days, and the fast growth species, *M. sympodialis* and *M. furfur*, after 2 days. The colonies that developed were scratched with a cotton swab, and

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harvested in 10 mL of PBS. The suspensions were homogenized with an electronic homogenizer to break clumps, and optical density was measured to compare growth rates.

Effects of compounds of sweat and free fatty acids on growth

Malassezia suspensions were smeared over an area 1.5 cm in diameter on LNA supplemented with sodium chloride, sodium lactate or urea, the main compounds of sweat, at 0.1% or 1%, and sodium laurate (C12), sodium myristate (C14), sodium palmitate (C16), palmitoleic acid (C16:1), sodium stearate (C18), sodium oleate (C18:1), linoleic acid sodium salt (C18:2), or linolenic acid (C18:3), the main free fatty acids on human skin, at 0.01% or 0.1%. These reagents were obtained from Sigma (St Louis, MO, USA). After 2 days (*M. sympodialis* and *M. furfur*) or 7 days (*M. globosa*, *M. restricta* and *M. dermatis*) at 32°C, growth rates were compared as described above.

Comparison of lipase activity

The *Malassezia* strains were harvested in water with an optical density of 0.5 after being maintained on LNA at 32°C for 2 days (*M. sympodialis* and *M. furfur*) or 7 days (*M. globosa*, *M. restricta* and *M. dermatis*). Mixtures of *Malassezia* suspensions (100 µL) and 0.01 mmol/L 4-methylumbelliferyl oleate dissolved in McIlvaine buffer (pH 5.0) (100 µL) were incubated at 37°C for 1 h. The enzymatic reaction was stopped by the addition of hydrochloric acid and sodium citrate. The intensity of fluorescence (excitation, 320 nm; emission, 450 nm) was measured after *Malassezia* cells were removed by centrifugal separation (1000 g, 10 min). For comparison, the lipase activity of *Propionibacterium acnes* (JCM 6425) cultured at 32°C for 2 days with modified GAM broth (Nissui, Tokyo, Japan) under anaerobic conditions was also measured.

RESULTS

Effects of temperature on growth

The growth rates of *Malassezia* species at various temperatures are shown in Figure 1. For all species, the growth rates were significantly lower at 25°C than 32°C. For *M. restricta*, the growth rate was significantly lower at 37°C than 32°C, although for *M. globosa*, it was significantly higher at 37°C than 32°C.

Effects of compounds of sweat and free fatty acids on growth

The effects on growth of sodium chloride, lactic acid and urea are shown in Figure 2. Sodium chloride at 1% increased significantly the growth rate of *M. globosa*, although it decreased that of *M. sympodialis*. Sodium chloride at 0.1% also increased the growth of *M. globosa*. Lactic acid at 1% increased the growth rate of *M. globosa*, but decreased that of *M. sympodialis*. Lactic acid at 0.1% increased growth in *M. globosa* and *M. sympodialis*. Urea decreased the growth of all *Malassezia* species at 1%, and that of *M. globosa*, *M. dermatis* and *M. furfur* at 0.1%.

The effects on growth of lauric acid (C12), myristic acid (C14), palmitic acid (C16), palmitoleic acid (C16:1), stearic acid (C18), oleic

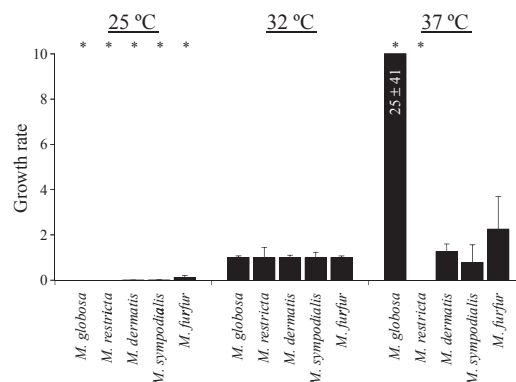


Figure 1. Growth rates of *Malassezia* species at various temperatures. The optical density in the culture at 32°C was taken as 1.0. *M. globosa*, *M. restricta* and *M. dermatis* were analyzed after 7 days, and *M. sympodialis* and *M. furfur* after 2 days. Six strains of *M. globosa*, and four strains each of *M. restricta*, *M. dermatis*, *M. sympodialis* and *M. furfur* were used. Error bars show standard deviation. Asterisks indicate statistically significant differences by Mann-Whitney U-test versus the culture at 32°C ($P < 0.05$).

acid (C18:1), linoleic acid (C18:2) and linolenic acid (C18:3) are shown in Figure 3. Lauric acid reduced the growth rates of all *Malassezia* species except *M. furfur* at 0.1%, and that of *M. sympodialis* at 0.01%. However, the growth of *M. furfur* was increased by lauric acid. Myristic acid decreased growth in all *Malassezia* species except *M. dermatis* at 0.1%, and increased growth in *M. restricta*, *M. dermatis* and *M. sympodialis* at 0.01%. Palmitic acid increased the growth of *M. furfur* at 0.1%, and of *M. globosa*, *M. restricta* and *M. sympodialis* at 0.01%. Palmitoleic acid at 0.1% increased growth in *M. dermatis* and *M. furfur*, although it decreased growth in *M. globosa* and *M. restricta*. Palmitoleic acid at 0.01% increased the growth of *M. dermatis*, although it decreased that of *M. restricta*. Stearic acid at 0.1% decreased growth in *M. globosa* and *M. restricta*, but at 0.01% it did not influence the growth of *Malassezia* species. Oleic acid at 0.1% increased growth in *M. furfur*, but decreased it in *M. globosa* and *M. restricta*. Oleic acid at 0.01% increased the growth rates of all *Malassezia* species except *M. dermatis*. Linoleic acid at 0.1% increased growth in *M. dermatis* and *M. furfur*, but decreased it in *M. globosa*, *M. restricta* and *M. sympodialis*. Linoleic acid at 0.01% increased the growth of *M. sympodialis* and *M. furfur*. Linolenic acid at 0.1% decreased the growth rates of *M. globosa* and *M. restricta*. Linolenic acid at 0.01% increased growth in *M. dermatis*, although it decreased growth in *M. restricta*.

Comparison of lipase activity

The lipase activities of each *Malassezia* species are shown in Figure 4. *M. globosa* and *M. restricta* had higher levels of activity than *M. dermatis*, *M. sympodialis* and *M. furfur*. Moreover, *M. furfur* had less activity than *M. dermatis* and *M. sympodialis*. The lipase activity of *P. acnes* (JCM 6425) was 1.20 ± 0.18 . In addition, there were no significant differences between *M. sympodialis* and *M. furfur* cultured for 2 days and for 7 days (data not shown).

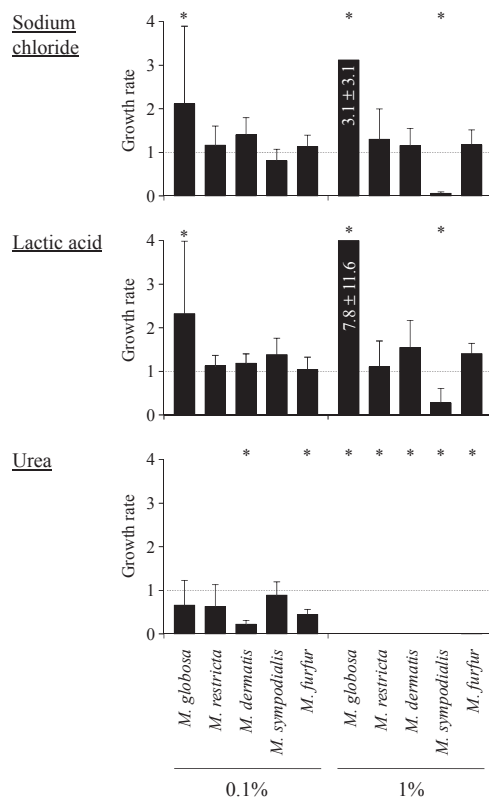


Figure 2. Effects of compounds of sweat on growth rates of *Malassezia* species. The optical density of the negative control (no ingredient) was taken as 1.0. *M. globosa*, *M. restricta* and *M. dermatitis* were analyzed after 7 days, and *M. sympodialis* and *M. furfur* after 2 days. Six strains of *M. globosa*, and four strains each of *M. restricta*, *M. dermatitis*, *M. sympodialis* and *M. furfur* were used. Error bars show standard deviation. Asterisks indicate statistically significant differences by Mann–Whitney *U*-test versus negative controls (no ingredient; dotted lines) ($P < 0.05$).

DISCUSSION

In this study, we referred to the method by Ushijima *et al.*^{16,17} as a quantitative analysis of growth; however, we used LNA, a superior medium in its ability to multiply *M. globosa* and *M. restricta*, because the medium used in their study did not have the ability to multiply some strains (data not shown).

The growth rate for *M. globosa* was significantly higher at 37°C than 32°C. Guého *et al.*, however, reported that *M. globosa* did not show good growth at 37°C unlike other *Malassezia* species,¹ which is totally different from our result. This difference may depend on the culture medium. Our study used LNA for *Malassezia* cultures, although Guého *et al.* used modified Dixon agar medium (mDixon A). We confirmed in a preliminary experiment that *M. globosa* showing growth in LNA did not always show growth in mDixon A, because its ability to grow on LNA was superior to that on mDixon A (data not shown). We consider that *M. globosa* shows good growth at 37°C under the appropriate conditions.

The main compounds of human sweat are sodium chloride (0.648–0.987%), urea (0.086–0.173%) and lactic acid (0.034–

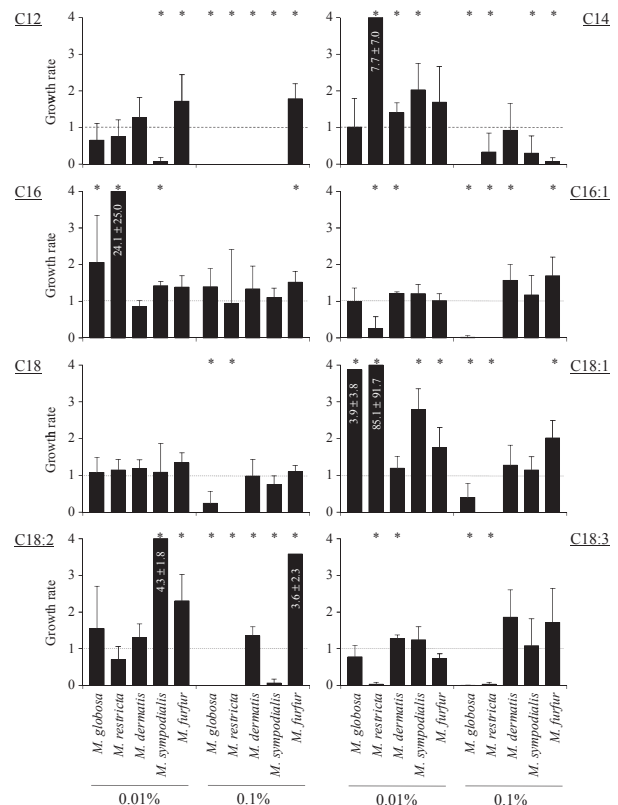


Figure 3. Effects of fatty acids on growth rates of *Malassezia* species. The optical density of the negative control (no ingredient) was taken as 1.0. *M. globosa*, *M. restricta* and *M. dermatitis* were analyzed after 7 days, and *M. sympodialis* and *M. furfur* after 2 days. Six strains of *M. globosa*, and four strains each of *M. restricta*, *M. dermatitis*, *M. sympodialis* and *M. furfur* were used. C12, lauric acid; C14, myristic acid; C16, palmitic acid; C16:1, palmitoleic acid; C18, stearic acid; C18:1, oleic acid; C18:2, linoleic acid; C18:3, linolenic acid. Error bars show standard deviation. Asterisks indicate statistically significant differences by Mann–Whitney *U*-test versus negative controls (no ingredient; dotted lines) ($P < 0.05$).

0.107%).¹⁸ In our study, sodium chloride at 1% and lactic acid at 0.1% significantly increased the growth rate of *M. globosa*, although urea at 0.1% did not. This result indicates that sweat strongly promotes the growth of *M. globosa*. In other words, *M. globosa* growth tends to increase under summer conditions when temperatures are high and people sweat. MF tends to be more prevalent in the summer months and in tropical locations than in temperate regions.¹³ Moreover, we confirmed that the number of *M. globosa* counted by the culture method in healthy subjects tended to increase in summer.⁹ This characteristic of *M. globosa* may relate to the pathogenesis of MF.

The influence that each kind of fatty acid has on the growth of *Malassezia* differs among species. In our experiments with LNA as the base medium, all fatty acids at 0.1% except C16 suppressed the growth of *M. globosa* and *M. restricta*. The sebum on human skin contains an average of 16.4% (7.9–39.0%) free fatty acid.¹⁹ In Japanese, this value is 12.6–27.4%.²⁰ The fatty acids in sebum may

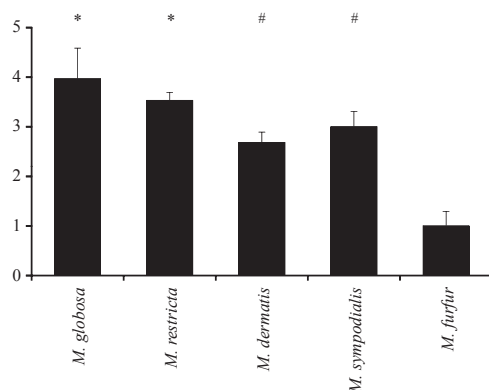


Figure 4. Lipase activity of *Malassezia* species. The lipase activity is relative to that of *M. furfur*. Six strains of *M. globosa*, and four strains each of *M. restricta*, *M. dermatitis*, *M. sympodialis* and *M. furfur* were used. Error bars show standard deviation. Statistically significant differences by Student's *t*-test are indicated; *, versus *M. dermatitis*, *M. sympodialis* and *M. furfur*; #, versus *M. furfur* ($P < 0.05$).

have inhibitory effects generally on the growth of *M. globosa* and *M. restricta*, the predominant species in human skin. However, the influence on the growth of *Malassezia* species by fatty acids differed with the species of *Malassezia* or kind and dose of fatty acid. The cutaneous *Malassezia* microbiota differed by sex and body part. Differences in the kinds and amount of fatty acids in human sebum may control the cutaneous *Malassezia* microbiota. This would also be an interesting topic for further research.

Before the classification of *Malassezia* was established, this yeast was called *Pityrosporum* and consisted of three species: *P. ovale*, *P. orbiculare* and *P. pachydermatis*.²¹ There are also a few comparative studies about differences in the growth characteristics of *Pityrosporum* spp.^{16,17} For example, lactic acid promotes an increase in the growth of *P. orbiculare*. It is considered that much of *P. orbiculare* refers to *M. globosa*,¹⁰ and the present result supports this hypothesis. However, the influence on the growth of *M. globosa* by fatty acids did not relate to that of *P. orbiculare*.^{16,17} It was reported that the growth of *P. orbiculare* was promoted by palmitic acid and stearic acid at 0.2%. Our study used LNA, although the previous reports used a medium with a totally different composition. We consider the difference to depend on the nutrients in the medium.

Malassezia lipase is associated with insoluble fractions, and its optimal pH was 5.0.²² Our experiment to compare the lipase activity of each *Malassezia* species was designed taking into consideration these characteristics. Most of the *Malassezia* species showed higher lipase activity than *P. acnes* (JCM 6425), a representative microorganism having lipase. In particular, *M. globosa* and *M. restricta* showed high lipase activity among the cutaneous *Malassezia* species. One of the reasons why *M. globosa* is predominant on human skin may be high lipase activity.

In conclusion, this study clarified that *M. globosa*, the most predominant species on human skin and a major pathogen of MF, tended to grow actively in summer conditions, when temperatures were high and people sweat more, than other cutaneous *Malassezia*

species. Furthermore, *M. globosa* showed high lipase activity. We consider that these characteristics of *M. globosa* relate to the pathogenesis of MF.

REFERENCES

- Guého E, Midgley G, Guillot J. The genus *Malassezia* with description of four new species. *Antonie Van Leeuwenhoek* 1996; **69**: 337–355.
- Sugita T, Takashima M, Shinoda T *et al.* New species, *Malassezia dermatitis*, isolated from patients with atopic dermatitis. *J Clin Microbiol* 2002; **40**: 1363–1367.
- Nell A, James SA, Bond CJ *et al.* Identification and distribution of a novel *Malassezia* yeast species on normal equine skin. *Vet Rec* 2002; **150**: 395–398.
- Sugita T, Takashima M, Kodama M, Tsuboi R, Nishikawa A. Description of a new yeast species, *Malassezia japonica*, and its detection in patients with atopic dermatitis and healthy subjects. *J Clin Microbiol* 2003; **41**: 4695–4699.
- Sugita T, Tajima M, Takashima M *et al.* A new yeast, *Malassezia yamatoensis*, isolated from a patient with seborrheic dermatitis, and its distribution in patients and healthy subjects. *Microbiol Immunol* 2004; **48**: 579–583.
- Hirai A, Kano R, Makimura K *et al.* *Malassezia nana* sp. Nov., a novel lipid-dependent yeast species isolated from animals. *Int J Syst Evol Microbiol* 2004; **54**: 623–627.
- Cabañes FJ, Theelen B, Castellá G, Boekhout T. Two new lipid-dependent *Malassezia* species from domestic animals. *FEMS Yeast Res* 2007; **7**: 1064–1076.
- Lee YW, Yim SM, Lim SH, Choe YB, Ahn KJ. Quantitative investigation on the distribution of *Malassezia* species on healthy human skin in Korea. *Mycoses* 2006; **49**: 405–410.
- Akaza N, Akamatsu H, Sasaki Y *et al.* Cutaneous *Malassezia* microbiota of healthy subjects differ by gender, body part, season, and analytical method. *J Dermatol* 2010; **37**: 786–792.
- Batra R, Boekhout T, Guého E *et al.* *Malassezia* Baillon, emerging clinical yeasts. *FEMS Yeast Res* 2005; **5**: 1101–1113.
- Back O, Faergemann J, Hornqvist R. Pityrosporum folliculitis: a common disease of the young and middle-ages. *J Am Acad Dermatol* 1985; **12**: 56–61.
- Faergemann J, Johansson S, Back O, Scheynius A. An immunologic and cultural study of Pityrosporum folliculitis. *J Am Acad Dermatol* 1986; **14**: 429–433.
- Gupta AK, Batra R, Bluhm R, Boekhout T, Dawson TL. Skin disease associated with *Malassezia* species. *J Am Acad Dermatol* 2004; **51**: 785–798.
- Akaza N, Akamatsu H, Sasaki Y *et al.* *Malassezia* folliculitis is caused by cutaneous resident *Malassezia* species. *Med Mycol* 2009; **47**: 618–624.
- Leeming JP, Notman FH. Improved methods for isolation and enumeration of *Malassezia furfur* from human skin. *J Clin Microbiol* 1987; **25**: 2017–2019.
- Ushijima T, Takahashi M, Ozaki Y. Selective and differential media for isolation and tentative identification of each species of *Pityrosporum* residing on normal or diseased human skin. *Microbiol Immunol* 1981; **25**: 1109–1118.
- Takahashi M, Ushijima T, Ozaki Y. Comparative studies on biochemical and serological characteristics of each species of *Pityrosporum*. *Jpn J Med Mycol* 1981; **22**: 314–321 [in Japanese].
- Mitsui T. Sweat glands and perspiration. In: Mitsui T, ed. *New Cosmetic Science*. Amsterdam: Elsevier, 1997; 18–19.
- Downing DT, Strauss JS, Pochi PE. Variability in the chemical composition of human skin surface lipids. *J Invest Dermatol* 1969; **53**: 322–327.
- Matsunaga K, Hayakawa R. Skin surface lipids and serum lipids in acne vulgaris: a comparative study of young women with and without acne. *Nishi Nihon Hifuka* 1985; **47**: 1080–1087 [in Japanese].
- Slooff WC. *Malassezia* Baillon. In: Lodder J ed. *The Yeasts, A Taxonomic Study*, 2nd edn. Amsterdam: Elsevier, 1970; 1167–1189.
- Ran Y, Yoshiike T, Ogawa H. Lipase of *Malassezia furfur*: some properties and their relationship to cell growth. *J Med Vet Mycol* 1993; **31**: 77–85.