Medical Mycology September 2010, **48,** 824–827

informa
healthcare

Human external ear canal as the specific reservoir of *Malassezia slooffiae*

TAKAMASA KANEKO*,†, RYOKO SHIOTA‡, SHUICHIROU SHIBUYA§, SHINICHI WATANABE§, YOSHIKO UMEDA†, KIMIKO TAKESHITA‡, MAMI YAMAMOTO#, KEIKO NISHIOKA# & KOICHI MAKIMURA†

**Kanto Chemical Co., Inc., Tokyo, †Teikyo University Institute of Medical Mycology, Tokyo, ‡Department of Microbiology, Takinomiya General Hospital, Kagawa, §Department of Dermatology, Teikyo University School of Medicine, Tokyo, and #Department of Otolaryngology, Takinomiya General Hospital, Kagawa, Japan*

The incidence of *Malassezia* species recovered from the external ear canal was characterized using culture medium optimized for *Malassezia* spp., CHROMagar Malassezia. The results of this study indicated that in healthy individuals *M. slooffiae* was the dominant *Malassezia* species followed by *M. restricta*.

Keywords *Malassezia*, external ear canal, reservoir

Introduction

Malassezia species are members of the normal human cutaneous commensal microbiota and can be isolated from sebaceous-rich areas of the skin, particularly the scalp, forehead, arms, and trunk [1,2]. Many studies have examined the incidences of *Malassezia* species on normal skin in different populations and different age groups [3–5]. An extensive study of the distribution of *Malassezia* species at various sites on adults was carried out by Gupta *et al*. [6], using contact plates containing Leeming and Notman agar (LNA) [7]. While these authors noted the presence of *Malassezia* species over the entire body surface of clinically healthy individuals, they did not include the external ear canal. Furthermore, Sugita *et al*. [8] characterized the cutaneous *Malassezia* microbiota by nested PCR due to the difficulties inherent in culturing these organisms.

The media most widely used for the isolation of *Malassezia* species from clinical specimens is LNA. However, we reported that CHROMagar Malassezia medium (CHROM; CHROMagar, Paris, France) which is optimized for recovery of *Malassezia* spp., is currently available and supports the growth of members of this genus as well as the use of LNA [9].

Since *Malassezia*-related otitis externa was recently reported [10,11] it has become necessary to evaluate the incidence of *Malassezia* species which had been recovered from the external ear canal of healthy individuals. Their isolation would allow the gathering of information with regard to their phenotypic features and antifungal susceptibility, as well as allowing for genotype analysis. Here, we report the results of investigations of the external outer ear canal in which we employed CHROM to evaluate the incidence of *Malassezia* species and age distribution of *Malassezia* carriers.

Material and methods

Culture medium

CHROMagar Malassezia medium (CHROM) was used as the primary isolation medium in this study. CHROM is composed (per liter) of 56.3g of CHROMagar Malassezia basal medium (CHROMagar, Paris, France) and 10 ml of Tween 40 [12].

Subjects and sample collection

A total of 127 clinical specimens from several body sites of healthy adults were obtained from staff of Takinomiya General Hospital (Kagawa, Japan) and Teikyo University Hospital (Tokyo, Japan). Written informed consent was obtained from each individual included in the study. First, samples from the body surface of nine healthy adults were obtained using adhesive tape (10 mm × 10 mm) as reported by Takiwaki *et al*. [13] and then placed on CHROM. Next, samples were taken from the external ear canal of the remaining subjects with swabs and streaked on CHROM.

Received 25 September 2009; Received in final revised form 20 November 2009; 28 November 2009

Correspondence: K. Makimura, Teikyo University Institute of Medical Mycology, 359 Otsuka, Hachioji, Tokyo 192-0395, Japan. Tel: +81 426 78 3256; fax: +81 426 78 3256; E-mail: makimura@main.teikyo-u.ac.jp

DOI: 10.3109/13693780903514880

All isolates observed on CHROM were studied for colony morphology and size after incubation in air at 32°C for 4 to 7 days.

Identification

The strains isolated were gram stained, and pre-identified using a culture-based identification system for *Malassezia* [14]. DNA was then extracted by the procedure of Makimura *et al*. [15] and the internal transcribed spacer 1 (ITS1) region of the ribosomal DNA was sequenced directly from PCR products using the primer pair, 18SF1 and 58SR1 [16]. The PCR products were sequenced with an ABI PRISM 310 Genetic Analyzer according to the manufacturer's instructions (Applied Biosystems, Foster City, CA). Other yeast were identified according to their assimilation patterns using the API ID32C yeast identification panel.

Statistical analysis

Statistical analysis was performed for sex differential using Stat Flex version 5.0 (Artech, Osaka, Japan). $P < 0.05$ was considered significant.

Results

The distribution of *Malassezia* species on the skin of nine healthy adults determined using the taping method is shown in Table 1. The average and median ages of subjects were 33.00 ± 10.82 and 32 years, respectively. The rapidly growing *M. sympodialis* (33.3–44.4%) and *M. furfur* (11.1–33.3%) were isolated on CHROM from areas similar to those reported by Gupta *et al*. [6]. However, *M. slooffiae* (22.2%) was detected with CHROM only in the external ear.

To confirm the results in a relative large study population, we investigated the distribution of *Malassezia* species in 118 external ear canals of healthy adults through the use of swabs (Table 2). The average and median ages of subjects were 40.48 ± 26.25 and 39 years, respectively. The species most commonly isolated from the external ear canal were *M. slooffiae* (44.9%) and *M. restricta* (15.3%), and the incidences of these species were distinct from those found on other areas of the skin. The highest incidence of *Malassezia* species in the external ear canal was observed in healthy adults over 30 years of age (Fig. 1). The *Malassezia*-positive ratios in the external ear canals of healthy adult male and female subjects were 63.8% and 46.7%, respectively, although the difference was not statistically significant.

Discussion

CHROM (Table 1) medium for the recovery of *Malassezia* species described by the authors [9], provides excellent support for the development of members of this genus and is commercially available for use in clinical laboratories.

The *Malassezia* species are part of the normal human cutaneous microbiota. Variations in their concentration and presence at various locations on the skin [1,2,6], age of the host [3–5], skin condition [17–19], and geographical differences [17,20] have been reported. Members of the genus are associated with several skin diseases, such as pityriasis versicolor, *Malassezia* folliculitis, seborrheic dermatitis, and atopic dermatitis [18,19].

In the present study, the incidence of *Malassezia* in the external ear canal was characterized through the use of the culture medium CHROMagar Malassezia [9]. There have been a few case reports of *Pityrosporum ovale* (synonym of *Malassezia* species) in the external ear [21,22], but studies involving a suitable culture medium and incorporating the updated taxonomy of the genus need to be conducted. In our investigation, *M. slooffiae* and *M. restricta* were found at higher levels in the external ear canal than other areas of the body. Furthermore, we found that the incidence of *Malassezia* species

Table 1 Distribution of *Malassezia* on healthy adult human skin.

No. of subjects	Sampling method (culture media)	Presence on (% of detected subjects)					
		Forehead	External ear	Nose wing	Trunk	Armpit	Cubital fossa
9	Taping (CHROM)	*M. sympodialis* (33.3%) *M. furfur* (22.2%) Culture negative (44.4%)	*M. sympodialis* (44.4%) *M. slooffiae* (22.2%) *M. furfur* (11.1%) *C. albidus* (11.1%) Culture negative (11.1%)	*M. sympodialis* (33.3%) *M. furfur* (11.1%) Culture negative (55.5%)	*M. sympodialis* (44.4%) *M. furfur* (11.1%) Culture negative (44.4%)	*M. sympodialis* (44.4%) *M. furfur* (33.3%) *C. uniguttulatus* (11.1) *C. albidus* (11.1%) Culture negative (22.2%)	*M. sympodialis* (44.4%) *M. furfur* (33.3%) Culture negative (22.2%)

GNR; Gram positive rods, GPC; Gram negative coccui.

Table 2 Age distribution of healthy adults evaluated for the incidence of *Malassezia* from the external ear canal and distribution of species recovered.

	Male				Female			
	No. of				No. of			
Age	Subjects	positive subjects (%)	species (%)		subjects	positive subjects (%)	species (%)	
20–29	8	3 (37.5)	*M. slooffiae*	3 (37.5%)	10	3 (30.0)	*M. slooffiae*	3 (30.0%)
			M. restricta	0 (0.0%)			*M. restricta*	1 (10.0%)
30–39	26	16 (61.5)	*M. slooffiae*	12 (46.2%)	20	10 (50.0)	*M. slooffiae*	7 (35.0%)
			M. restricta	6 (23.1%)			*M. restricta*	3 (15.0%)
40–49	10	7 (70.0)	*M. slooffiae*	6 (60.0%)	14	8 (57.1)	*M. slooffiae*	7 (50.0%)
			M. restricta	1 (10.0%)			*M. restricta*	3 (21.4%)
50–59	14	11 (78.6)	*M. slooffiae*	8 (57.1%)	16	7 (43.8)	*M. slooffiae*	7 (43.8%)
			M. restricta	4 (28.6%)			*M. restricta*	0 (0.0%)
Total	58	37 (63.8)	*M. slooffiae*	29 (50.0%)	60	28 (46.7)	*M. slooffiae*	24 (40.0%)
			M. restricta	11 (19.0%)			*M. restricta*	7 (11.7%)

in the external ear canal increased with age, which was probably due to the increasing level of lipid in the external ear canal. Koçer *et al*. reported that the level of free fatty acid in cerumen increased with advancing age [23]. Therefore, subjects from 30–50 years old had higher incidences of *Malassezia* species than the younger age group in our study population.

Yeast and molds can cause otitis externa [24]. Although there have been a few reports of otitis externa caused by *Malassezia* [10,11], our group reported five cases of *Malassezia*-related otitis externa as seborrheic dermatitis (SD) of the ear canal [11]. Accelerated turnover of epidermal cells in the ear canal was suggested and the main symptoms were itching and fullness in the ear, along with observations of redness and erosion. In these cases, *M. slooffiae* was the only member of the genus isolated for the external ear canal and at levels higher than other cases of otitis externa, i.e., 10 cells per field (×400). Several studies have suggested that *Malassezia* species play important roles as aggravating factors in SD [25–29]. *M. slooffiae* seems to be present as part of the normal microbiota in the external ear canal and may be selected under some conditions that can encourage the development of otitis externa.

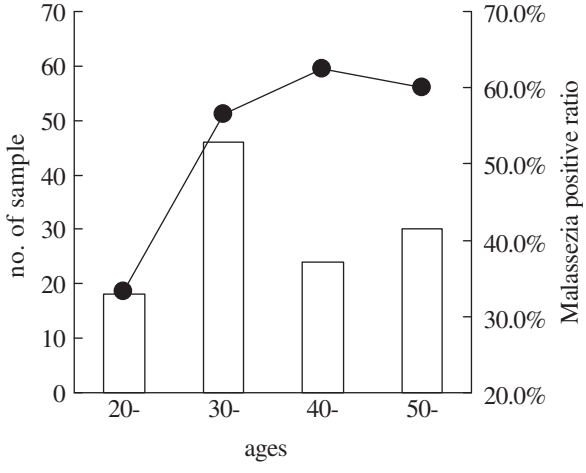

Fig. 1 Positive ratio of *Malassezia* species in the external ear canal from healthy adults at selected ages. Closed circles show the ratio of *Malassezia*-positive samples, and the columns indicate the total number of samples.

Sugita [30] reported that *M. globosa* and *M. restricta* colonize the skin surface in atopic dermatitis patients, suggesting that they play a significant role in exacerbating the condition. These two species have different genotypes relative to the intergenic spacer region of the rRNA gene and the types may correspond to patients and healthy individuals. In addition, the antifungal susceptibilities of these strains to itraconazole and ketoconazole differ. Our group is currently planning to investigate *M. slooffiae* from otitis externa with regard to phenotypic features and antifungal susceptibility, and to perform genotype analysis in comparison to isolates from healthy individuals.

Acknowledgments

This study was supported in part by Health Science Research Grants for Research on Emerging and Re-emerging Infectious Diseases from the Ministry of Health, Labor, and Welfare of Japan (K.M.) H19-shinko-Ippan-008.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

1 Faergemann J, Aly R, Maibach HI. Quantitative variations in distribution of *Pityrosporum orbiculare* on clinically normal skin. *Acta Dermato-Venereol* 1983; **63**: 346–348.

2 Leeming JP, Notman FH, Holland KT. The distribution and ecology of *Malassezia furfur* and cutaneous bacteria in human skin. *J Appl Bacteriol* 1989; **67**: 47–52.
3 Bergbrant IM, Faergemann J. Variations of *Pityrosporum orbiculare* in middle-aged and elderly individuals. *Acta Dermato-Venereol* 1988; **68**: 537–540.
4 Bergbrant IM, Broberg A. *Pityrosporum ovale* culture from the forehead of healthy children. *Acta Dermato-Venereol* 1994; **74**: 260–261.
5 Faergemann J, Fredriksson T. Age incidence of *Pityrosporum orbiculare* on human skin. *Acta Dermato-Venereol* 1980; **60**: 531–533.
6 Gupta, AK, Kohlii Y, Summerbell RC, Faergemann J. Quantitative culture of *Malassezia* species from different body sites of individuals with or without dermatoses. *Med Mycol* 2001; **39**: 243–251.
7 Leeming JP, Notman FH. Improved methods for isolation and enumeration of *Malassezia furfur* from human skin. *J Clin Microbiol* 1987; **25**: 2017–2019.
8 Sugita T, Suto H, Unno T, *et al*. Molecular analysis of *Malassezia* microflora on the skin of atopic dermatitis patients and healthy subjects. *J Clin Microbiol* 2001; **39**: 3486–3490.
9 Kaneko T, Makimura K, Onozaki M, *et al*. Vital growth factors of *Malassezia* species: presumptive identification of *Malassezia* and *Candida* species on modified CHROMagar Candida Agar. *Med Mycol* 2005; **43**: 699–704.
10 Chai FC, Auret K, Christiansen K, Yuen PW, Gardam D. Malignant otitis externa caused by *Malassezia sympodialis*. *Head Neck* 2000; **22**: 87–89.
11 Shiota R, Kaneko T, Yano H, *et al.* A study of otitis externa associated with *Malassezia*. *Jpn J Med Mycol* 2009; **50**: 109–116.
12 Kaneko T, Makimura K, Sugita T, Yamaguchi H. Tween 40-based precipitate production observed on modified chromogenic agar and development of biological identification kit for *Malassezia* species. *Med Mycol* 2006; **44**: 227–231.
13 Takiwaki H, Tsuda H, Arase S, Takeichi H. Differences between intrafollicular microorganism profiles in perioral and seborrhoeic dermatitis. *Clin Exp Dermatol* 2003; **28**: 531–534.
14 Kaneko T, Makimura K, Abe M, *et al*. Revised culture-based system for identification of *Malassezia* species. *J Clin Microbiol* 2007; **45**: 3737–3742.
15 Makimura K, Murayama YS, Yamaguchi H. Detection of a wide range of medically important fungi by the polymerase chain reaction. *J Med Microbiol* 1994; **40**: 358–364.
16 Makimura K, Tamura Y, Kudo M, *et al*. Species identification and strain typing of *Malassezia* species stock strains and clinical isolates based on the DNA sequences of nuclear ribosomal internal transcribed spacer 1 regions. *J Med Microbiol* 2000; **49**: 29–35.
17 Crespo-Erchiga V, Gomez-Moyano E, Crespo M. Pityriasis versicolor and the yeasts of genus *Malassezia. Actas Dermosifiliogr* 2008; **99**: 764–771.
18 Nakabayashi A, Sei Y, Guillot J. Identification of *Malassezia* species isolated from patients with seborrhoeic dermatitis, atopic dermatitis, pityriasis versicolor and normal subjects. *Med Mycol* 2000; **38**: 337–341.
19 Faergemann J. *Pityrosporum* species as a cause of allergy and infection. *Allergy* 1999; **54**: 413–419.
20 Gupta AK, Boekhout T, Theelen B, Summerbell R, Batra R. Identification and typing of *Malassezia* species by amplified fragment length polymorphism and sequence analysis of the internal transcribed spacer and large-subunit regions of ribosomal DNA. *J Clin Microbiol* 2004; **42**: 4253–4260.
21 Randjandiche M. Occurrence of *Pityrosporum ovale* in the human ear. *Dermatologica* 1975; **151**: 100–103.
22 Randjandiche M. Presence of *Pityrosporum ovale* in the ear of newborn infants. *Sabouraudia* 1981; **19**: 143–145.
23 Koçer M, Guldur T, Akarcay M, Miman MC, Beker G. Investigation of age, sex and menstrual stage variation in human cerumen lipid composition by high performance thin layer chromatography. *J Laryngol Otol* 2007; **12**: 1–6.
24 Feinmesser R, Wiesel YM, Argaman M, Gay I. Otitis externa – bacteriological survey. ORL J Otorhinolaryngol Relat Spec. 1982; **44**: 121–125.
25 Ashbee HR, Ingham E, Holland KT, Cunlife WJ. Cell mediated immune responses to *Malassezia furfur* serovars A, B and C in patients with pityriasis versicolor, seborrhoeic dermatitis and controls. *Exp Dermatol* 1994; **3**: 106–112.
26 Bergbrant IM, Faergemann J. The role of *Pityrosporum ovale* in seborrhoeic dermatitis. *Semin Dermatol* 1990; **9**: 262–268.
27 Faergemann J. Lipophilic yeasts in skin disease. *Semin Dermatol* 1985; **4**: 173–184.
28 Faergemann J. Management of seborrheic dermatitis and pityriasis versicolor. *Am J Clin Dermatol* 2000; **1**: 75–80.
29 Faergemann J, Bergbrant IM, Dohse M, Scott A, Westgate G. Seborrhoeic dermatitis and *Pityrosporum* (*Malassezia*) folliculitis – characterization of inflammatory cells and mediators in the skin by immunohistochemistry. *Br J Dermatol* 2001; **144**: 549–556.
30 Sugita T. Genotype analysis of the rRNA gene of *Malassezia* colonizing the skin surface of patients with atopic dermatitis. *Jpn J Med Mycol* 2005; **46**: 147–150.

This paper was first published online on Early Online on 27 January 2010.