Human external ear canal as the specific reservoir of Malassezia slooffiae

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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 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 56.3 g 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.

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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). 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.

<table>
<thead>
<tr>
<th>No. of subjects</th>
<th>Sampling method (culture media)</th>
<th>Presence on (% of detected subjects)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forehead</td>
<td><em>M. sympodialis</em> (33.3%)</td>
<td><em>M. sympodialis</em> (44.4%)</td>
</tr>
<tr>
<td></td>
<td><em>M. slooffiae</em> (22.2%)</td>
<td><em>M. slooffiae</em> (22.2%)</td>
</tr>
<tr>
<td></td>
<td><em>M. furfur</em> (11.1%)</td>
<td><em>M. furfur</em> (11.1%)</td>
</tr>
<tr>
<td></td>
<td><em>C. albicans</em> (11.1%)</td>
<td><em>C. albicans</em> (11.1%)</td>
</tr>
<tr>
<td></td>
<td>Culture negative (44.4%)</td>
<td>Culture negative (44.4%)</td>
</tr>
<tr>
<td>External ear</td>
<td><em>M. sympodialis</em> (33.3%)</td>
<td><em>M. sympodialis</em> (44.4%)</td>
</tr>
<tr>
<td></td>
<td><em>M. slooffiae</em> (22.2%)</td>
<td><em>M. slooffiae</em> (22.2%)</td>
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<tr>
<td></td>
<td><em>M. furfur</em> (11.1%)</td>
<td><em>M. furfur</em> (11.1%)</td>
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<tr>
<td></td>
<td><em>C. albicans</em> (11.1%)</td>
<td><em>C. albicans</em> (11.1%)</td>
</tr>
<tr>
<td></td>
<td>Culture negative (44.4%)</td>
<td>Culture negative (44.4%)</td>
</tr>
<tr>
<td>Nose wing</td>
<td><em>M. sympodialis</em> (55.5%)</td>
<td><em>M. sympodialis</em> (55.5%)</td>
</tr>
<tr>
<td>Trunk</td>
<td><em>M. sympodialis</em> (44.4%)</td>
<td><em>M. sympodialis</em> (44.4%)</td>
</tr>
<tr>
<td>Armpit</td>
<td><em>M. sympodialis</em> (44.4%)</td>
<td><em>M. sympodialis</em> (44.4%)</td>
</tr>
<tr>
<td>Cubital fossa</td>
<td><em>M. sympodialis</em> (11.1%)</td>
<td><em>M. sympodialis</em> (11.1%)</td>
</tr>
<tr>
<td></td>
<td><em>C. albicans</em> (11.1%)</td>
<td><em>C. albicans</em> (11.1%)</td>
</tr>
<tr>
<td></td>
<td>Culture negative (22.2%)</td>
<td>Culture negative (22.2%)</td>
</tr>
</tbody>
</table>

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

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Kaneko et al. 

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. 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.

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