Quantitative analysis of the cutaneous *Malassezia* microbiota in 770 healthy Japanese by age and gender using a real-time PCR assay

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Although the lipophilic yeasts of the genus *Malassezia* are part of the cutaneous microbiota in healthy individuals, they are also associated with several skin diseases, such as seborrheic dermatitis. However, the effects of age and gender on the *Malassezia* microbiota have not been completely elucidated. We analyzed the cutaneous *Malassezia* microbiota of 770 healthy Japanese using the highly accurate real-time PCR with a TaqMan probe to investigate the effects of age and gender on the *Malassezia* population. The numbers of *Malassezia* cells increased in males up to 16–18 years of age and in females to 10–12 years old, and subsequently decreased gradually in both genders until senescence. *Malassezia restricta* overwhelmingly predominated at ages over 16–18 years in males and 23–29 years in females. *M. globosa* and *M. restricta* together accounted for more than 70% of *Malassezia* spp. recovered regardless of gender. The total colonization of *Malassezia* and the ratio of the two major species change with age and gender in humans.

**Keywords** *Malassezia* microbiota, age, healthy subject, quantitative analysis, real-time polymerase chain reaction

Introduction

The members of the basidiomycetous yeast genus *Malassezia* are part of the cutaneous microbiota in healthy individuals. At present, the genus contains 13 species, i.e., *M. dermatis*, *M. furfur*, *M. globosa*, *M. japonica*, *M. obtuse*, *M. restricta*, *M. slooffiae*, *M. sympodialis*, *M. yamatoensis*, *M. equina*, *M. caprea*, *M. nana*, and *M. pachydermatis* [1–6]. The last four species listed are primarily associated with animals other than humans. During the past decade, there has been progress in our understanding of the taxonomy of *Malassezia* spp., e.g., *M. furfur*, which was thought to be a single pathogenic species responsible for skin diseases, was reclassified into five different species [1].

One reason for the extensive studies of *Malassezia* is that although part of the normal microbiota, members of the genus are associated with several skin diseases. They are the causative agents of pityriasis versicolor, seborrheic dermatitis, *Malassezia* folliculitis, and exacerbate atopic dermatitis [7–9]. In recent years, a relationship between *Malassezia* and psoriasis has also been suggested [10,11]. Previously, *M. furfur* was thought to be the causative agent or exacerbating factor of *Malassezia*-related skin diseases. However, molecular epidemiological investigations were introduced after Guého et al. [1] elucidated the heterogeneity of *M. furfur sensu lato* in 1996.

Between 1996 and 2002, ‘culture-dependent methods’ methods were used to identify *Malassezia* spp. for
epidemiologic studies. In many of such investigations, the detection ratio of *M. restricta* was generally very low, whereas that of *M. sympodialis* was relatively high compared with other species, regardless of the type of skin disease [12, 13]. This may have been the result of the fact that there are significant differences in the growth rate of *Malassezia* spp. in culture, i.e., *M. restricta* and *M. obtusa* grow relatively slowly, and *M. furfur* and *M. sympodialis* grow rapidly. Consequently, a slow-growing strain may be missed. In addition, results based on culture-dependent methods are not reproducible due to the fact that the recovery is dependent on sampling methods and isolation media. In addition, the isolation of a pure, single colony of a *Malassezia* species is sometimes difficult. To resolve these problems, Sugita *et al.* [14] developed a polymerase chain reaction (PCR)-based, culture-independent method for analyzing the distribution of *Malassezia* that is not influenced by the isolation medium. This is the most reliable method available at present.

Among the skin diseases caused by *Malassezia* spp. in humans, seborrheic dermatitis is most common in infants 2 to 12 weeks after birth and in adolescents and adults from puberty to 40 years of age. Pityriasis versicolor is found mainly from adolescence to middle age. Thus, analyzing the cutaneous *Malassezia* microbiota of healthy individuals at different ages would enhance our understanding of the components of the microbiota in these diseases.

This study used a culture-independent, real-time PCR assay with high accuracy to examine the relationship between the *Malassezia* microbiota and the age and gender of 770 healthy Japanese citizens.

**Materials and Methods**

**Subjects and sample collection**

The subjects were 770 dermatologically healthy Japanese who were free of skin diseases and had not received any anti-microbiological agents for 4 weeks before the initiation of the study. They included 405 males and 365 females, from 0 to 82 years old. The subjects were divided into the following 13 age groups of at least 30 subjects each (Table 1); 0–3, 4–6, 7–9, 10–12, 13–15, 16–18, 19–22, 23–29, 30–39, 40–49, 50–59, 60–69, and over 70 years old. Members of the 7–9, 10–12, 13–15, 16–18, and 19–22 years old age groups were all students in the lower and higher grades of elementary school, junior high school, high school, and university, respectively.

The study protocol was approved by the institutional review board, and informed consent was obtained from each subject. Cutaneous microorganisms were collected by applying a 5 × 7 cm transparent OpSite dressing (Smith & Nephew Medical, Hull, UK) from the right and left cheeks of each subject in the morning, before they washed their faces [14]. The use of cosmetics before going to bed was not prohibited and all individuals were asked whether they used makeup on a daily basis. While none of the men wore makeup on their faces, almost all of the females between 19 and 49 years of age indicated that they did so (Table 1).

**Analysis of the *Malassezia* microbiota**

The collected OpSite transparent dressings were cut into pieces measuring 3 × 3 cm, and *Malassezia* DNA was extracted directly from them by using the method of Sugita *et al.* [14] and stored at –20°C until used in the present studies. Total *Malassezia* DNA and that of two major species, *M. globosa* and *M. restricta*, were quantified using universal or species-specific primers and TaqMan probes according to the method of Sugita *et al.* [15]. Amplification and detection were performed with an ABI PRISM 7500 sequence detection system (Applied Biosystems, Foster City, CA, USA). The assay was repeated in triplicate for the samples from each cheek.

**Results**

**Total amount of *Malassezia* DNA**

In males, the total amount of *Malassezia* DNA remained essentially the same in individuals from ages 0–9 years of age and then increased markedly in those up to 16–18 years old (Fig. 1). In females, the amount of DNA increased in subjects until 10–12 years of age, decreased in those 19–22 years old, and then increased again in individuals 30–39 years of age before finally decreasing gradually with age. There was more total *Malassezia* DNA in males than in females. The ratio of the total *Malassezia* colonization between males and females ranged from 0.7–3.4 until 15 years of age, after which it increased rapidly to 34 in subjects 19–22 years of age (Fig. 1).
Amounts of the major microbiota: *M. globosa* and *M. restricta*

*Malassezia globosa* and *M. restricta* accounted for more than approximately 70% of the total *Malassezia* DNA in the microbiota of both males and females at all ages. In males, *M. restricta* predominated at all ages and was overwhelmingly the most common species in subjects after 16 years of age (Fig. 2). In females, *M. restricta* overwhelmingly predominated over *M. globosa* in individuals 23 years of age or older, whereas *M. globosa* was the dominant species in subjects between 10 and 18 years of age (Fig. 2).

**Discussion**

This study is the first to accurately demonstrate that the level of *Malassezia* in the skin of healthy subjects is different between the sexes and changes with age in an analysis of 770 subjects. Although previous studies have analyzed the cutaneous *Malassezia* microbiota in healthy individuals, they used culture-dependent methods, as noted previously. The largest of these studies was conducted by Gupta et al. [16], who examined 245 healthy individuals. Culture-dependent methods revealed that in individuals from 0 to 3 years of age, *M. furfur* and *M. globosa* were the predominant species, accounting for 36.4 and 54.5%, respectively, of the cutaneous *Malassezia* microbiota. In subjects from 4 to 14 years of age, *M. globosa* (59.7%) was the predominant species, while the recoverable levels of *M. furfur* (5.4%) decreased and those of *M. sympodialis* (31.6%) increased. With individuals over 15 years of age, the *Malassezia* microbiota remained relatively constant with both *M. globosa* and *M. sympodialis* as the predominant species, accounting for 23.6 to 31.8% and 56.1 to 67.7%, respectively, of the cutaneous *Malassezia* microbiota. *Malassezia restricta* was rarely detected at any age (0 to 2.1%).

As *Malassezia* are lipophilic yeasts, it can be predicted that the extent of *Malassezia* colonization of the skin would correlate with sebaceous gland activity. Sebaceous secretions are low in children and begin to increase in mid- to late childhood under the influence of androgens. This rise continues until 16 to 19 years of age, after which no further significant change takes place until advanced age [17]. Sebaceous secretions are higher in males than in females at all ages. The distribution of sebaceous secretions at different ages resembles that of cutaneous *Malassezia* colonization. However, cutaneous *Malassezia* colonization decreased in 19- to 29-year-old Japanese females, even though sebaceous secretions are active in this age group. This may be explained by the use of cosmetics.

In Japan, school regulations generally prohibit the use of make-up until after high school (i.e., until 18 years of age), and young women begin to wear makeup at about 19 years of age. In fact, the rate of cosmetics use was 18.8% for females between 16 and 18 years of age, and 94.7% for those between 19 and 22 years of age. Almost all females up to 49 years of age used cosmetics (96–100%; Table 1). Cosmetics normally contain chemical preservatives, such as a paraben, to prevent contamination by environmental saprophytes. This compound also inhibits the growth of *Malassezia in vitro* (unpublished data) and therefore, may affect the growth of cutaneous *Malassezia*.

Among females 50 years and older, those in their 50s, 60s, and 70s who did not wear make-up had 3.7, 6.7, and 2.0 times the level of *Malassezia* present in their microbiota than those of the same respective ages who did wear makeup, although the numbers examined were limited.

The major components of the cutaneous *Malassezia* microbiota differed markedly with age. The ratio of *M. globosa* to *M. restricta* changed noticeably after 16 years of age in males and after 23 years of age in females. Both these microorganisms are major components of the microbiota in healthy subjects, as well as in those with *Malassezia*-associated skin diseases such as seborrheic dermatitis, pityriasis versicolor, and atopic dermatitis. *Malassezia globosa* predominates over *M. restricta* in pityriasis versicolor, but the relationship is reversed in seborrheic dermatitis [18,19].

It is thought that the *Malassezia* microbiota changes with the composition of sebum, given that *Malassezia* species require lipids for growth. Sebum consists of ceramides, fatty acids, cholesterol, cholesterol esters, squalene, triglycerides, and wax esters. Lipase secreted by cutaneous microorganisms hydrolyze the triglycerides into glycerin and free fatty acids, which become nutrients for the
microorganisms. Yamamoto et al. [20] investigated the relationship between aging and the composition of saturated and unsaturated fatty acids from C\textsubscript{14} to C\textsubscript{18}, including straight and branched chains, in sebum. The proportion of C\textsubscript{16} iso-branched fatty acids decreased markedly from infancy through the 20s and then increased until senescence. The C\textsubscript{18:1} straight-chain component decreased in the 20s and did not change significantly thereafter. The percentages of C\textsubscript{15:1} and C\textsubscript{16:1} straight-chain components increased with age from infancy through the 20s and then decreased until the 50s. By contrast, the proportion of C\textsubscript{16:1} iso-branched fatty acids decreased from infancy through maturity, with a nadir in the 20s, and then increased until the 50s. Overall, the proportion of each fatty acid changed significantly in the 20s, as did the amount of sebum.

Thus, the age-related changes in the amount of Malassezia colonization and in the composition of the Malassezia microbiota are closely associated with the age-related changes in sebaceous gland activity and in the fatty-acid composition of sebum.

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References


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