Phenotypic Characterization of *Malassezia spp* isolated from Healthy Individuals

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Abstract
Malassezia spp is a normal inhabitant of human skin. In certain predisposing conditions, they proliferate and grow in hyphal forms without inflicting inflammation as in pityriasis versicolor and may involve in the pathogenesis of disease with noticeable inflammation such as psoriasis, seborrheic dermatitis and atopic dermatitis. Malassezia spp accounted approximately 60-90% of the total cutaneous fungal microflora. A clear understanding about the distribution of Malassezia spp in healthy individuals is necessary to gain the knowledge in the involvement of Malassezia as pathogen from commensals. A total of 120 healthy men and 120 healthy women without any skin disease or who were not treated for Malassezia associated skin disease were selected for study. The specimen of skin scrapings was collected from forehead, chest, neck and back from each subject totaling 880 sites. Phenotypically the samples were processed.

The isolation rate of Malassezia from 240 healthy individuals was 41% (N=99). The positive culture rate in healthy individuals was high in the age group of 21-30 (32%). In healthy individuals, M. globosa was the most common species with 154 isolates (53%) followed by M. furfur 82 (28%), M. pachydermatis 33(11.3%), M. sympodialis 16(5.4%), M. slooffiae 4(1.3%), M. restricta 2(0.6%) and M obtusa 1(0.3%). The overall colonization rate of Malassezia spp in healthy individuals found in this study was 41%. M. globose was identified as the most common species (53%).

Keywords: *Malassezia*, commensals, healthy individuals, skin microbiome, pathogen.

Introduction

Human skin comprises of seven layers where top most is stratum corneum. Skin microbiome comprises of beneficial microorganism like bacteria, fungi and viruses. The most common organisms present in healthy skin are *Staphylococcus*, *Corynebacterium*, *Propionibacterium* and *Malassezia* in case of fungi. The yeast *Malassezia* is in high proportion in the skin microbiome. These microorganisms serve as a physical barrier to prevent invasion of pathogens. The presence of these microorganism is solely depending on physiology of skin[1,2,13]. *Malassezia* is lipophilic yeast known to be a part of normal flora of human skin. It is lipid dependent yeast found in an area rich in sebaceous gland such as face, chest and back.

In healthy skin, Malassezia exploits the host to get necessary nutrients for their growth without causing disease[12]. Due to certain predisposing factors, they proliferate and grow in hyphal forms without inflicting inflammation as in pityriasis versicolor and may involve in the pathogenesis of disease with noticeable inflammation such as psoriasis, seborrheic dermatitis and atopic dermatitis[21]. Still now there is no clinical presentation on *Malassezia* associated disease and its pathogenic pathway. The mechanism behind the switching of commensal to pathogen of *Malassezia* is open to question.

As the *Malassezia* is the predominant organism in the skin microbiome, a clear understanding about the distribution of *Malassezia* spp in healthy individuals is necessary to gain the knowledge in the involvement of *Malassezia* as pathogen from commensals. Hence, aim of this study was to access the *Malassezia* flora in the skin of healthy individuals.

Material and Methods

Study design: A prospective study was carried out in the Department of Microbiology, Aarupadai Veedu Medical College and Hospital, Puducherry for a period of two years after getting approval from the institutional ethics committee.

Sample size and Study population: A total of 120 healthy men and 120 healthy women without any skin disease or who were not treated for *Malassezia* associated skin disease were selected for study. They were grouped according to their ages as (0-10), (11-20), (21-30), (31-40), (41-50), (Above 51) where each group consisted of 40 subjects (20 men and 20 women). The specimens consisted of scrapings collected from forehead, chest, back and neck from each subject totaling 880 samples.

Data collection: The selected subjects were interviewed and information was recorded in the specially designed questionnaire. A detailed history of the subjects, demographic characteristics like age, sex, occupation and predisposing factors like contact with pets, dandruff, sweating were evaluated and recorded in the pre-designed proforma.

Samples Processing: All samples were cultured on to Modified Dixon's agar (mDixons agar-Twin pack) Hi-media. The cultures were incubated at 32°C for seven days.

Once the growth occurred, gram's staining was performed and colonies were subjected to speciation by biochemical tests.

Biochemicals tests[5,11,12]:

A. Urease test: A loopful of yeast cells from 4-5 days old cultures was inoculated on Christensen's urea agar slant and incubated at 37°C for 24hrs. Changing of pink colour of media indicates positive reaction. This test was not used to differentiate *Malassezia* species but it was done to exclude cultures that are contaminated by bacteria or yeasts of the class ascomycetes such as *Candida* spp. which are commonly found on the skin. All *Malassezia* spp show urease positive.

B. Catalase Reaction: Catalase activity of *Malassezia* yeasts was determined by adding a drop of 10%-20% hydrogen peroxide onto a culture smear on a glass slide or in a small glass tube. The enzymes act as a catalyst in the decomposition reaction of peroxides formed during oxidation reactions. A positive result was indicated by effervescence caused by the liberation of free oxygen. Except *M. restricta*, all *Malassezia* spp are catalase positive

C. β-Glucosidase activity using the esculin medium: Certain *Malassezia* species possess a β-glucosidase that is capable of hydrolyzing the glucosidic bond of esculin, resulting in release of glucose and aesculetin. The phenol functional group reacts with the iron to give a black color. The ß-glucosidase activity of the fungus was assayed by using esculin agar slant. A loop of fresh (2-3 day old) yeasts was deeply inoculated into the esculin agar by stabbing and incubated for 5 days at 32°C. The darkening of the medium indicates splitting of esculin into esculetin and glucose with formation of soluble ferric salt incorporated in the medium. This test is used to distinguish *M. furfur*, *M. slooffiae* and *M. sympodialis* from other *Malassezia* species.

D. Glycine assimilation: *M furfur* is the only species which assimilates glycine. Isolates were inoculated in modified Dixon glycine media (with 7mMol/liter glycine) and incubated at 37°C for 3 days. Growth within 2 to 3 days indicates positive for glycine assimilation. Negative reporting will be done after 7 days.

E. Tween (20,40,60,80) and Cremaphor EL assimilation test: The lipophilic and lipid dependent *Malassezia* yeasts require culture media incorporated with lipids. This particular physiological property was found to be useful to differentiate *Malassezia* species. Strains were tested for their capacity to grow on Sabouraud's dextrose agar (SDA) with 0.05% chloramphenicol and 0.05% cycloheximide, supplemented separately with tweens 20, 40, 60, 80 and Cremaphor EL (CrEL) or castor oil as lipid sources. These five water soluble lipid compounds were tested together using their capacity to diffuse into a solid basic medium.

Method: Two loopful of a 4-5day old Malassezia culture were suspended in 3 ml sterile distilled water and poured into culture plate containing molten SDA with 0.05% chloramphenicol and 0.05% cycloheximide maintained at 50°C. Then the inoculum was spread evenly. After solidification of the plates, five holes were made by means of a 2 mm diameter punch and filled with 5 µl of tween 20, 40, 60, 80 and Cremaphor EL respectively. The plates were incubated for 7-10 days at 32°C -34°C in humid chamber. Utilization of tweens was assessed by the degree of yeasts around the wells.

F. Growth on Sabouraud's dextrose agar: Among Malassezia species, only *M pachydermatis* is able to grow on Sabouraud's agar without lipid supplement, it is not an obligatory lipid dependent species.

Results

The isolation rate of *Malassezia* from 240 healthy individuals was 41% (N=99). Among the 880 samples from the different sites, 292 were found to be positive. Male showed higher isolation rate than female in all age groups as shown in figure 1 whereas in the 21-30 age group, male (22%) was dominant as shown in table 1. The positive culture rate in healthy individuals was high in the age group of 21-30 (32%) followed by 11-20 (20%), 31-40 (16%) and 41-50 (15%). The age group less than ten years showed the least isolation rate.

According to different sites sample, the positive rate of isolation of *Malassezia* spp in culture was highest in forehead 98(33.5%) followed by chest 84(28.7%), back 64(21.9%) and scalp 46(15.7).

Table 1
Isolation rate of *Malassezia* spp according to age group

S.N.	Age group	Male	Female	
1	Less than 10	3	3	6
2	11-20	6	14	20
3	21-30	26	6	32
4	31-40	12	4	16
5	41-50	13	2	15
6	Above 50	9	1	10
Total		69	30	99

Table 2
Distribution of *Malassezia* spp based on the body site

Species	Forehead	Chest	Back	Scalp
M globosa	61	27	38	28
M furfur	14	38	17	13
M pachydermatis	18	11	4	-
M sympodialis	3	7	4	2
M slooffiae	1	-	1	2
M restricta	1	1	-	-
M obtusa	-	-	-	1

In healthy individuals, among the different *Malassezia* spp isolated in six different age groups, *M globosa* was the most common species with 154 isolates (53%) followed by *M. furfur* 82 (28%), *M. pachydermatis* 33(11.3%), *M. sympodialis* 16(5.4%), *M. slooffiae* 4(1.3%), *M. restricta* 2(0.6%) and *M. obtusa* 1(0.3%).

M. globosa was the predominant species isolated from all age groups followed by *M. furfur* and *M. pachydermatis*. The results from each body site (forehead, chest, back and scalp) showed that the *M globosa* (n=61) is the most predominant species isolated from forehead. Maximum number of *M. furfur* (n=38) was isolated from chest region. Of the 33 isolates of *M. pachydermatis*, 18 are isolated from forehead. *M. sympodialis* does not show any site-specific variation. 3, 7, 4 and 2 were isolated from forehead, chest, back, scalp respectively. Out of four *M. slooffiae*, 2 was isolated from scalp region. Two isolates of *M. restricta* were from same individuals of different sites forehead and chest. One isolate of *M. obtusa* was obtained from scalp as shown in the table 2.

Discussion

Malassezia spp are most predominant normal skin flora of humans. The distribution of *Malassezia* spp on our body site is influenced by the presence of sebaceous glands. The sites rich in sebaceous glands are face, scalp, back and upper trunk. In our study, the colonization rate of *Malassezia* spp in healthy individuals is 41% (N=99) which is less when compared to the similar study conducted by Remya et al[18] in northern part of Kerala. They reported 62.78% in healthy individuals. A study conducted in Punjab by Kaur et al[11] was in accordance with our results, as they found that the overall rate of *Malassezia* spp from healthy individuals is 46.66%.

A study from North-East India shows that the isolation rate of *Malassezia* spp from healthy subjects was 51%. Even colder region of the country shows the similar results[22]. As implies the temperature does not have any role in colonization of *Malassezia* in normal skin of healthy individuals.

As in gender wise comparison 68% males showed positive rate of isolation. Prohic et al[17] did not find any gender wise difference in healthy individuals. Most of studies showed no sex difference contrary to our results. Studies show that Pityriasis is more common in male than women. This may be due to extra attention of women to beauty and skin hygiene. The maximum number of *Malassezia* was isolated from the age group of 21-30 (n=32) which is followed by 11-20 (n=20). This is in agreement with the study done in Kerala with healthy individuals[18]. This result has been coinciding with the other study conducted in Assam[22].

Prevalence of pityriasis versicolor is most commonly seen in the age group of 21-30 years. The formation of colony starts immediately after the birth and significantly increases with age in neonates. It has been reported that skin colonization by *Malassezia* yeasts was 5% at the first week and 30% at 2-4 weeks of life[8]. In our study, we could not collect any samples from neonates and infants. In the age group less than 10 years, we isolated 10 *Malassezia* spp. A study conducted in Northern India reported 38% and 52% of hospitalized neonates and infants respectively colonized with *Malassezia* on their skin[8]. Colonization by *Malassezia* species in infants and neonates in a hospital is common and can be a potential source of nosocomial infections[27].

The carriage of *Malassezia* is the highest during puberty because of increase in sebaceous gland activity[26]. Our study found that female at puberty age group (11-20) shows the highest colonization rate on their skin. A similar result has been found by Tarazooie et al[24] where the dissimilarity is due to lower maturity age in female as compared with male. As the age increased, the *Malassezia* colonization rate decreased. In our study we isolated 10 *Malassezia* spp from persons aged above 50 years, contrary to the above statement. In older age, the density of *Malassezia* spp on the skin decreased due to reduction in the level of lipid on the skin[7]. So, few people might have high lipid level on their skin at older ages.

Malassezia is a lipid depended yeast, commonly found to be distributed in areas rich in sebaceous glands such as the chest, back, neck, forehead and shoulders. They reside in stratum corneum and utilize sebum for their growth and maintenance.

The triglycerides and esters present in the sebum are broken down in diglycerides, monoglycerides and free fatty acids which provided nourishment for them[3]. On basis of site specific sample our study found that highest number of 98

positives out of 99 *Malassezia* was isolated from forehead which was followed by chest (n=84), Back(n=64) and scalp(n=46).

The colonization density of *Malassezia* was significantly higher in the forehead (99%) compared with other areas (P 0.05) reflecting the difference in skin lipid levels in different body areas. Some study shows that from chest and neck, the maximum number of *Malassezia* was isolated[11,18,22]. In Pityriasis versicolor mostly affects the trunk predominantly chest and back[11,19]. The area which is uncovered by cloths favors the rapid development of lesions and blockage of glands plays a role in disease[1]. Gupta et al[7] found 47.8% of *Malassezia* species isolated from forehead area which supports our study.

On speciation, the highest number of species was isolated is *M. globosa* (53%) followed by *M. furfur* (28%), *M. pachydermatis* (11.3%), *M. sympodialis* (5.4%), *M. slooffiae* (1.3%), *M. restricta* (0.6%) and *M. obtusa* (0.3%). Remya et al[18] found that *M. globosa* was predominant followed by *M. furfur*, *M. restricta*, *M. sympodialis*, *M. slooffiae*, *M. obtusa* and *M. pachydermatis*. *M. pachydermatis* is the only lipid dependent species of this genus which colonizes the skin and mucosal sites of healthy dogs and cats[10]. However, many healthy individuals are regularly exposed to pet animals as it might be the reason for high number of *M. pachydermatis*.

Sugita et al[23] found that *M. globosa* (22%) was predominantly isolated followed by *M. sympodialis* (10%) and *M. furfur* (3%) in healthy individuals. A study showed contrast report as *M. sympodialis* is predominant followed by *M. restricta* in healthy individuals. In Puducherry, Saranya et al[20] found that *M. globose* (57.62%) was predominant from pityriasis versicolor patients followed by *M. furfur* (20.33%) and *M. sympodialis* (18.33%). This difference in variation of species may be due to skin of individuals in different countries and different sampling methods[15].

According to the distribution of *Malassezia* species based on body sites, *M globosa* (n=61) was maximum isolated from forehead. From chest area n=38 of *M. furfur* was isolated. In back region, highest was *M. globosa* (n=38) followed by *M furfur* (n=17) and one *M. slooffiae* was isolated. From scalp, *M. globosa* and *M. furfur* were the outnumbered and one *M. restricta* was isolated. Kaur et al[11] found that *M. sympodialis* (47.6%) was major isolate from upper back followed by *M. obtusa* and *M. globosa* made up of 19.4% and 14.2% respectively. According to study conducted by Nakabayashi et al[14], *M. globose* (21%) was predominant in scalp. In this study, they isolated two *M. restricta*.

Conclusion

The presence of *Malasesszia* in healthy individuals is a concern as it may lead to opportunistic infection as there is possibility of switching over from commensals to pathogen. Recently the infection due *Malassezia* has increased tremendously but still the pathological role remains unambiguous.

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