Mycopathologia
DOI 10.1007/s11046-012-9587-y

Molecular Epidemiology of *Malassezia globosa* and *Malassezia restricta* in Sudanese Patients with Pityriasis Versicolor

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Received: 24 April 2012 / Accepted: 21 September 2012

Abstract Pityriasis versicolor is a superficial infection of the stratum corneum caused by *Malassezia* yeasts. The cutaneous *Malassezia globosa* and *Malassezia restricta* in Sudanese patients with pityriasis versicolor were elucidated using a molecular-based, culture-independent method and compared with that in healthy individuals. Scale samples were collected by applying an Opsite™ transparent dressing to lesional and non-lesional sites on 29 Sudanese patients with pityriasis versicolor and 54 healthy individuals. *Malassezia* DNA was extracted directly from the samples. The overall level of colonization by *Malassezia globosa* and *Malassezia restricta* was analyzed by real-time PCR using a TaqMan probe. The overall level of colonization by *Malassezia* at the lesional sites was higher than that at the non-lesional sites for all body sites, including the face, neck, cheeks, and trunk (2.7- to 6.0-fold increase). Both *M. globosa* and *M. restricta* were detected in patients and healthy individuals. However, *M. globosa* predominated at lesional sites, whereas the level of colonization by both species was similar in healthy individuals.

Keywords Malassezia globosa · Malassezia restricta · Pityriasis versicolor · Sudan

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Introduction

Malassezia species are members of the fungal skin microbiota in healthy humans. They colonize lipid-rich areas such as the scalp, face, back, and chest because they require a lipid source for growth. The level of colonization by *Malassezia* in healthy subjects is approximately 50–80 % of all fungi [1, 2]. Nevertheless, these microorganisms are associated with several human diseases, including pityriasis versicolor, seborrheic dermatitis, *Malassezia* folliculitis, and atopic dermatitis [1, 3]. Pityriasis versicolor is a superficial infection of the stratum corneum caused by *Malassezia*. It is commonly found on the trunk of people in their twenties and thirties during the summer season, but not during winter [4, 5].

To date, 14 members of the genus *Malassezia* have been identified. Of these, both *Malassezia globosa* and *Malassezia restricta* have been detected in scale samples from the skin of persons affected by various skin diseases, suggesting that they cause or can exacerbate skin diseases, including pityriasis versicolor [6–12].

Published online: 04 October 2012

Although there have been numerous research data about the *Malassezia* microbiota of the skin in patients with *Malassezia*-related diseases, little known about epidemiology of *Malassezia* species in the African populations. Sudan is a large country with mixed African and non-African populations. In Sudan, pityriasis versicolor is common especially during Summer-time. The exact prevalence of pityriasis versicolor in Sudanese population is not documented, but according to our personal observation clinical lesions can be seen in more than 20 % of the general population.

The aim of the current study is to analyze the colonization of the skin of Sudanese patients with pityriasis versicolor as well as healthy individuals with *Malassezia globosa* and *Malassezia restricta* by using a molecular-based, culture-independent method.

Materials and Methods

Subjects

Twenty-nine outpatients with pityriasis versicolor in Khartoum and Gedarif States of Sudan were included in this study. All patients were male, and their ages were ranging between 20 and 50 years old (average, 28.7 ± 7.9). An additional 28 males (20–65 years old; average, 36.8 ± 13.0) and 26 females (20–63 years old; average, 35.1 ± 12.9) were recruited as healthy subjects for comparison with the patients. Written informed consent was obtained from each participant. The study protocol was approved by our Research Review Board, at Sudan University of Science and Technology.

Sample Collection

Scale samples were collected from patients with typical pityriasis versicolor lesions (i.e., lesions on face, neck, chest, and trunk) and from the face and trunk of healthy individuals by applying an Opsite™ transparent dressing (Smith and Nephew Medical, Hull, UK), according to the method of Sugita et al. [6]. Scales were obtained from the patients at both lesional and non-lesional sites in each area. All specimens were collected in Sudan, while extraction and molecular analysis was done in Japan. To preserve the quality of DNA detection, the specimens were kept in refrigerator for few weeks before shipping to its final destination for processing.

DNA Extraction

Fungal DNA was extracted from the scale samples as described by Sugita et al. [6]. Briefly, the collected Opsite™ dressing was placed in a 1.5-ml Eppendorf tube containing 1 ml of lysis solution [100 mM Tris–HCl (pH 8.0), 30 mM EDTA (pH 8.0), and 0.5 % sodium dodecyl sulfate] and incubated for 15 min at 100 °C. The suspension was transferred to a new tube and extracted with phenol–chloroform–isoamyl alcohol (25:24:1, vol/vol/vol). Subsequently, the suspension was extracted with chloroform–isoamyl alcohol (24:1, vol/vol), and the DNA was precipitated using 2.5 volumes of ethanol with Etachinmate (Nippon Gene, Toyama, Japan). The DNA pellet was suspended in 30 µl of TE [10 mM Tris–HCl (pH 8.0) and 1 mM EDTA (pH 8.0)] and stored at −20 °C until use.

Quantitative Analysis of *Malassezia* DNA Using Real-Time PCR

The level of colonization by *Malassezia* in the scale samples was determined by a real-time PCR assay as described by Sugita et al. [8]. The amounts of DNA belonging to all *Malassezia* 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. [8]. Amplification and detection were performed using an ABI Prism 7,500 sequence detection system (Applied Biosystems, Foster City, CA, USA). The assay was performed in triplicate for the samples from each site.

Results

The Level of Colonization by *Malassezia*

The levels of colonization (%) by all *Malassezia* species at the lesional and non-lesional sites from 29 patients on the face, neck, chest, and trunk are shown in Fig. 1. The level of colonization at the lesional sites was higher than that at the non-lesional sites for all body areas, including the face, neck, cheeks, and trunk (2.7- to 6.0-fold increase). The levels of *Malassezia* colonization were also determined in scale samples

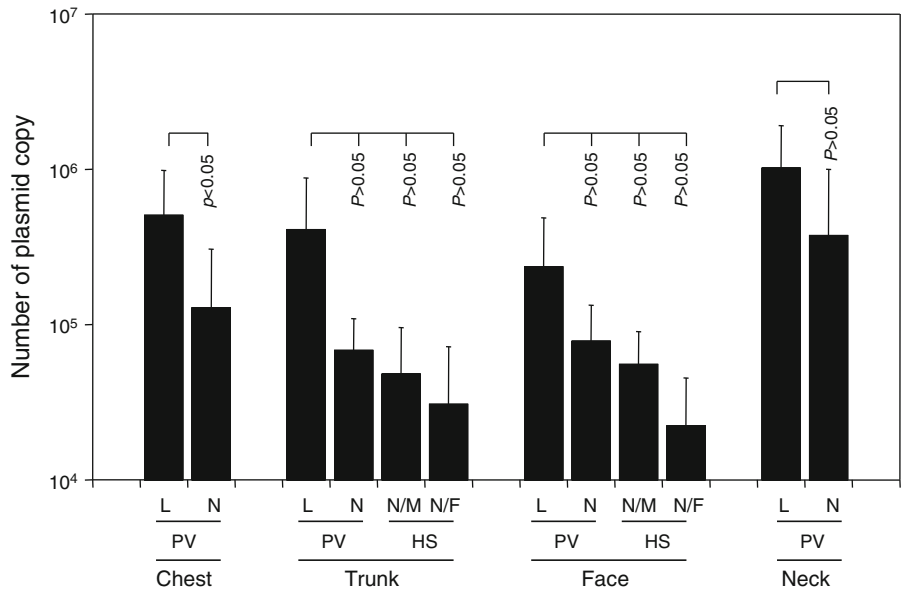

Fig. 1 The level of colonization by all *Malassezia* species in scale samples collected from Sudanese patients with pityriasis versicolor and healthy individuals. *L*, lesional site; *N*, non-lesional site; *M*, male; *F*, female

from the face and trunk of healthy individuals. The level of *Malassezia* colonization in the healthy male subjects was similar to that at the non-lesional sites of the patients, all of whom were male. Among the healthy subjects, *Malassezia* colonization was more extensive in males than in females.

Colonization Ratio of *M. globosa* to *M. restricta*

The levels of colonization by the two major components of the *Malassezia* microbiota, *M. globosa* and *M. restricta*, were further analyzed by real-time PCR. In the patients, these two species were predominant at all body sites, including the lesional and non-lesional sites and accounted for over 70 % of all *Malassezia* species. However, the colonization ratio was significantly different between lesional and non-lesional sites. In the non-lesional sites of the patients, *M. globosa* accounted for 58.1 ± 19.1, 59.7 ± 22.4, 53.7 ± 12.0, and 22.3 ± 16.6 % on the chest, trunk, neck, and face, respectively, whereas the ratios were increased to 81.3 ± 12.4, 80.9 ± 15.7, 84.3 ± 10.0, and 49.2 ± 47.7 %, respectively, at the lesional sites. In contrast, the ratios of *M. restricta* at non-lesional sites were decreased compared with those at lesional sites: from 38.5 ± 19.0 to 15.1 ± 15.0 % on the chest, from 30.0 ± 20.4 to 13.0 ± 12.2 % on the trunk, from 37.7 ± 16.2 to 10.7 ± 8.0 % on the neck, and from 46.5 ± 9.1 to 35.6 ± 30.4 % on the face. Thus, *M. globosa* predominated at lesional sites, while *M. restricta* predominated at non-lesional sites for all tested body sites. By comparison, both *M. globosa* and *M. restricta* were predominant in the healthy subjects and accounted for >95 % of the overall *Malassezia* colonization level. With the exception of the colonization ratio on the face in males, the ratio of *M. globosa* to *M. restricta* in the healthy subjects was similar between the sexes, at all tested body sites Fig. 2.

Discussion

Studies of the cutaneous *Malassezia* microbiota in patients with pityriasis versicolor have been conducted in numerous countries (14–25). The previously reported colonization frequencies of *Malassezia* species in patients with pityriasis versicolor are summarized in Table 1. These data come from studies conducted in North and South America, Europe, Asia, the Middle East, and Africa, without Oceania. Of the 15 studies reviewed, 12 were conducted by a culture-dependent method. Scales were collected from patients by swabbing or washing and were

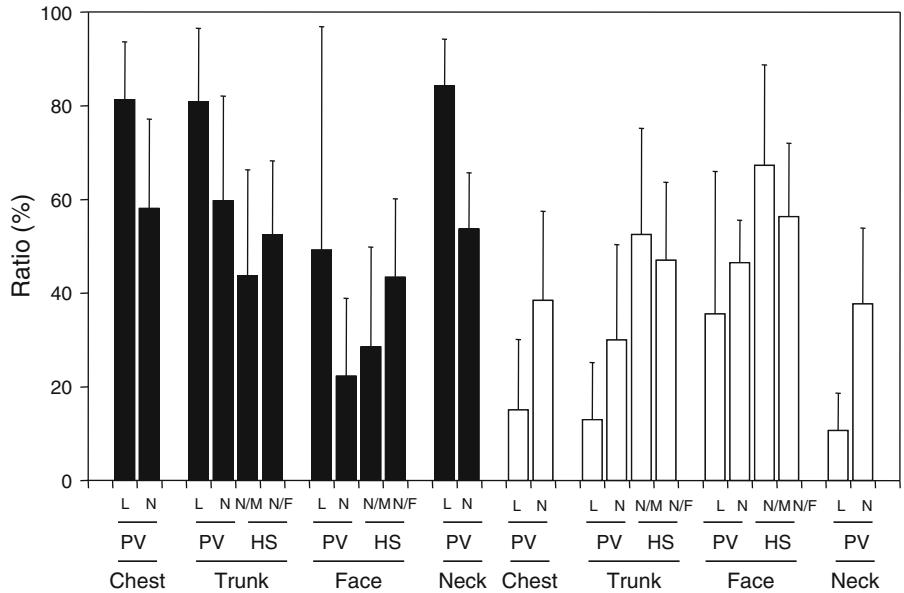

Fig. 2 Proportions of the predominant species *Malassezia globosa* and *Malassezia restricta* in scale samples collected from Sudanese patients with pityriasis versicolor and healthy individuals. *L*, lesional site; *N*, non-lesional site; *M*, male; *F*, female. *Black square*, *M. globosa*; *white square*, *M. restricta*

subsequently transferred to Dixon agar or Leeming and Notman agar. Strains recovered from the media were identified based on their morphological or biochemical characteristics, or by using PCR. The recovery rate of *Malassezia* species by culture method varies significantly between different studies, possibly because of differences in the sampling method or amount of scales included in the samples. However, there were no great differences in the predominant species among these studies (14–25). In each case, the major species were *M. globosa*, *Malassezia sympodialis*, and *Malassezia furfur.* Interestingly, *M. restricta* was not consistently detected (16, 19–23).

In 2001, Sugita et al. [6] developed a molecular-based, culture-independent method for analyzing the cutaneous *Malassezia* microbiota in patients and healthy individuals. Since the introduction of this analytical method, the detection sensitivity has increased dramatically. Remarkable differences in the detection frequency of *M. restricta* have been found between culture-dependent and culture-independent methods. The former detected *M. restricta* at a frequency of 0–8.0 % compared with a frequency of 100 % for the latter (12, 14). As the growth rate of *M. restricta* is relatively very slow compared with that of the other species, the efficiency of culturing of *M. restricta* on medium is poor. Considering these facts, a molecular-based, culture-independent method is the most reliable and appropriate method for elucidating the cutaneous *Malassezia* microbiota. The extent of colonization by *M. globosa* and *M. restricta* was almost identical in the healthy Sudanese subjects, with the exception of the face microbiota in males. Considering that the level of colonization by *M. globosa* was increased at lesional sites, while that of *M. restricta* was increased at non-lesional sites, *M. globosa* may play a significant role in the development of pityriasis versicolor. Indeed, hyphae are frequently observed in the scales of patients with pityriasis versicolor, suggesting that *M. globosa* is the causative agent of pityriasis versicolor, as *M. restricta* does not form hyphae [13]. In general, sebaceous gland activity or the composition of sebum is influenced by sex, age, race, and environment. These conditions can affect the amount of lipids available for growth, and thus, the *Malassezia* microbiota can vary. Quantitative information related to the *Malassezia* microbiota in patients with pityriasis versicolor was formerly available only for Japanese patients. The quantitative data produced in the present study on the *Malassezia* microbiota in Sudanese patients were identical to those in Japanese patients, despite differences in race, climate, and residential environment between the subjects. Our

Table 1 Detection frequency of *Malassezia* species in scale samples of patients with pityriasis versicolor

Country	Authors and year (Reference)	Number of patients	Detection frequency (%)[a]									Medium used for isolation or analytical method
			M. globosa	M. restricta	M. sympodialis	M. dermatis	M. furfur	M. obtusa	M. japonica	M. slooffiae	M. yamatoensis	
Sudan	This study	29	100	100								Real-time PCR
Japan	Sugita et al. 2010 [12]	49	100	100								Real-time PCR
Japan	Morishita et al. 2006 [7]	49	93.9	93.9	34.6	24.4	10.2	8.1	6.1	4.1	4.1	Nested PCR
Brazil	Petry et al. 2011 [14]	87	###	8.0	###		###	8.0		1.5		Dixon
Tunisia	Trabelsi et al. 2010 [15]	58	76.2	1.4	4.8		9.6			4.8		Sabouraud agar overlaid with olive oil
Argentina	Giusiano et al. 2010 [16]	218	40.8		41.3	0.5	23.4			1.8		Modified Dixon
India	Chaudhary et al. 2010 [17]	90	57.5	3.4	17.2		6.9	6.9				Modified Dixon
Iran	Shokohi et al. 2010 [18]	61	###	1.6	8.2		###					Modified LNA
Turkey	Karakaş et al. 2009 [19]	97	###				###			7.2		Modified Dixon
Indonesia	Krisanty et al. 2008 [20]	98	###		###		###					LNA
Bosnia and Herzegovina	Prohic A and Ozegovic L., 2007 [21]	90	###		###		###	7.8		4.4		Modified Dixon
Greece	Gaitanis et al. 2006 [22]	76	72.42[b]									Modified Dixon
Iran	Tarazooie B et al. 2004 [23]	94	42.6		7.4		20.2	6.4		3.2		Dixon
Canada	Gupta AK et al. 2001 [24]	111	25.2	0.9	59.4		10.8	0.9		2.7		LNA
Spain	Crespo Erchiga V et al. 2000 [25]	96	96.9		32.3					7.3		Modified Dixon

[a] Number indicates the percentage of the total number of patients and the samples were collected from lesional site

[b] Percentage refers to co-isolation with *M. globosa*

results provide useful information that can be used to elucidate the relationship between the *Malassezia* microbiota and ethnicity in patients with pityriasis versicolor. To our knowledge, this is the first report to analyze an African population with high accuracy using a culture-independent method.

Acknowledgments This study was supported in part by a Grant-in-Aid from the Japan Society for the Promotion of Science (JSPS), a research grant from the Asian and African Science Platform Program from JSPS, and the "High-Tech Research Center Project" from the Ministry of Education, Culture, Sports, Science, and Technology, Japan (TS).

Conflict of interest There were no conflicts of interest.

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