

Quantitative culture of *Malassezia* species from different body sites of individuals with or without dermatoses

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Quantitative cultures were obtained using contact plates to determine whether the quantity and composition of *Malassezia* species at a given anatomic site in normal individuals differs from that of patients with various cutaneous dermatoses. The sample included 20 clinically healthy individuals (without any dermatosis) and 110 patients with dermatoses (including 31 with atopic dermatitis [AD], 28 with psoriasis [PS], 28 with seborrheic dermatitis [SD] and 23 with pityriasis versicolor [PV]). Contact plates filled with special culture medium were used to obtain a quantitative culture from five body sites (scalp, forehead, arm, trunk and leg) of every individual. The number of cfu were recorded for every plate that grew *Malassezia* yeasts, and 3–5 colonies were isolated for identification to species level using microscopic, physiological and molecular characteristics. The mean cfu counts observed among patients with AD, PS and SD was significantly lower than normal control subjects ($P < 0.05$). The mean cfu counts from PV patients was not different from that of healthy control subjects. Overall, for all conditions considered together, the mean cfu counts in lesional sites were significantly lower than in non-lesional sites ($P < 0.05$). Furthermore, the mean cfu counts from lesional sites in patients with AD and PS were significantly lower than the corresponding value in patients with PV ($P < 0.05$). Six *Malassezia* species were recovered from the different dermatoses. *Malassezia sympodialis* was the most common species associated with AD and PV patients and healthy control subjects, while *M. globosa* was most frequently isolated from PS and SD patients. More than one *Malassezia* species was recovered at any given anatomic site from both controls as well as individuals with dermatoses. *M. globosa* was equally likely to be recovered from scalp, forehead and trunk, but less likely to derive from arms and legs. *M. restricta* and *M. slooffiae* were recovered more frequently from the upper body (scalp and forehead) than from the lower body. Among normal individuals and for patients with AD and PV, *M. sympodialis* was significantly more likely to affect the forehead than the legs.

Keywords contact plates, dermatoses, *Malassezia* species, quantitative culturing

Introduction

The lipophilic yeast *Malassezia furfur* and related species are members of the normal human cutaneous flora of skin and produce clinical disease under conditions that permit massive growth of the fungus. Prior to 1996, the genus *Malassezia* was thought to contain only three

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species, namely, *M. furfur* (Robin) Baillon 1889, *M. pachydermatis* (Weidman) Dodge 1935 and *M. sympodialis* Simmons and Guého 1990. Recently, *Malassezia* was revised to include four new taxa, *M. globosa*, *M. restricta*, *M. obtusa* and *M. slooffiae*, in addition to the three former taxa [1]. The etiological role of *Malassezia* yeasts in tinea (pityriasis) versicolor, *Malassezia* folliculitis and seborrheic dermatitis (SD) has been shown in several reports [2]. Under the current classification system, however, the specific clinical association of each of these species is not clearly understood.

Malassezia yeasts can be cultured from almost all adults. Roberts [3,4] reported that 97% of clinically healthy people carry the fungus on their scalp and 92% carry it on their trunk. Since *Malassezia* species can be cultured from almost all areas of the body, qualitative culture of these organisms has no diagnostic value. However, a quantitative assessment in certain circumstances may correlate with clinical significance [5]. Additionally, such culturing may also be useful in monitoring the effect of antimycotic treatments in SD patients. Quantitative cultures of *M. furfur* complex can be made with the scrub technique [6], a tape method [7] or contact plates [5,8,9]. Bergbrant *et al.* [9] reported the use of contact plates containing a modified medium described by Leeming & Notman [10] for quantitative culturing of *M. furfur*.

Several studies on quantitative culturing of *Malassezia* using different techniques have reported contradictory results. McGinley *et al.* [11], in a study of quantitative microbiology of the scalp, showed that for patients with dandruff, the population densities of *M. furfur* were twice as high on nonaffected scalp. Faergemann [5] used contact plates for quantitative culture of *Pityrosporum orbiculare* (*M. furfur* complex *pro parte*) and reported a much higher number of cfu from patients with SD than clinically healthy individuals. Bergbrant & Faergemann [12], on the other hand, reported no significant difference in the number of *P. ovale* for patients with SD than control subjects. They reasoned that an abnormal reaction in the skin to *P. ovale* may cause the inflammation and that the number of *P. ovale* could be of lesser importance. In addition, SD may be unrelated to the sebum excretion rate and not necessarily associated with seborrhea [13,14]. Ingham & Cunningham [15], in a study on pityriasis versicolor (PV) lesions, reported that population densities of the yeast cells were up to 10^5 cells cm^{-2} in lesions which is two log stages higher than on unaffected skin. Although some of these studies successfully used contact plates for quantitative culturing, there have been no studies to our knowledge where quantitative cultures have been taken with the

objective of identifying *Malassezia* species according to current techniques and determining their possible association with various skin conditions.

This study was undertaken with the following objectives: (i) to quantify cultures of *Malassezia* species from different body sites in humans and (ii) to determine whether the quantity and composition of *Malassezia* species at a given anatomic site in normal individuals differs from that of patients with various cutaneous dermatoses.

Materials and methods

Preparation of contact plates

BBL[®] RODAC[®] (Beckton Dickinson, Cockeysville, MD, USA) contact plates were used for sampling. This 65 × 15 mm-style dish is specially designed to allow a raised convex surface of culture medium. The 10 mm grid on the bottom of the plate facilitates counting and colony location. These plates were filled with a lipid-rich glucose peptone yeast extract medium described by Leeming & Notman [10]. The composition of Leeming–Notman agar (LNA) is as follows: 1 l glass distilled H₂O; 10 g bacteriological peptone (DIFCO, Detroit, MI, USA); 5 g glucose; 0.1 g yeast extract; 4 g Ox-Bile (desiccated; DIFCO); 12 g agar; with lipid supplements as: 1 ml glycerol; 0.5 g glycerol monostearate; 0.5 ml Tween 60, 10 ml homogenized milk (3.25% fat), and the antimicrobial supplements chloramphenicol (50 mg ml⁻¹) 1 ml and cycloheximide (2%) 9.9 ml. The pH of the media was 6.2.

Sample

Twenty clinically healthy individuals and 110 individuals with the following skin conditions: atopic dermatitis (AD; 31 individuals), psoriasis (PS; 28 individuals), SD (28 individuals) and PV (23 individuals), were sampled for quantitative culturing. Healthy control subjects were individuals with normal appearing skin and without any evidence of a dermatosis. We included only those patients who had not used any topical or oral treatment for their dermatoses for the previous 2 and 4 weeks, respectively. There were no known human immunodeficiency virus (HIV)-positive individuals in the study populations. Five body sites, namely scalp, forehead, arm, trunk and leg were sampled for each subject. For patients with skin conditions, a separate record was made for affected and unaffected sites. Because body sites with lesions often have no expanse of unaffected skin sufficiently large to allow exposure of a contact plate, and because inoculum from lesions could easily cross-contaminate adjacent non-lesional areas, patient

body sites were sampled on lesional or non-lesional areas, but not both. Lesional skin was sampled whenever possible. The protocol was reviewed and approved by an Independent Review Board (IRB) an institutional service in Aurora, Ontario. Each participant provided written, informed consent after the nature of the study had been explained to them.

Sampling procedure

To obtain a sample from the scalp, individuals were requested to part hairs and expose an area at the vertex of the scalp. A contact plate was then pressed firmly against the exposed surface for 20 s. Similarly, for each subject four additional contact plates were pressed in succession against flat areas of the forehead, arm, trunk and leg for 20 s each. For each individual all the samples were collected in one sitting. Immediately after the collection of samples, the plates were placed at 32 °C in the dark for 7 days and the number of cfu obtained from different body sites was then recorded. From each contact plate showing multiple colonies, three to five colonies were picked from different locations on the plate; when only one or two colonies grew they were likewise subcultured.

Identification of *Malassezia* species

Malassezia species were identified on the basis of microscopic observation of cells and physiological properties such as presence of catalase and the ability to utilize Tween compounds as a lipid source in a simple medium [16]. For some ambiguous cases molecular criteria for identification were used [17]; these were used mainly to differentiate *M. sympodialis* from *M. furfur* and *M. slooffiae*.

Physiological tests

Catalase reaction

Presence of catalase was determined by application of a drop of hydrogen peroxide (3%, 80% solution) on to a culture smear on a glass slide. Production of gas bubbles was considered a positive reaction. Among all seven species of *Malassezia*, only the recently described *M. restricta* lacks catalase activity [1].

Utilization of Tween compounds

This test was modified from the one reported by Guillot *et al.* [18]. Yeast suspensions (at least 10^7 cfu ml⁻¹) were made in sterilized normal saline (0.85%) and inoculated on Sabouraud peptone-glucose agar. The inoculum was spread evenly. Each plate was divided into four sections and a well was made in the center of each section. Four

drops of a Tween compound, i.e. Tween 20, 40, 60 and 80, were applied in each separate well. These plates were incubated at 32 °C for 7 days and the growth in each section was recorded.

Statistics

Nonparametric statistical analysis was conducted, using either the Kruskal-Wallis chi-square test or Mann-Whitney test, to compare and analyze the data at two different levels: (i) within and between different dermatoses and healthy controls, and (ii) within and between lesional and non-lesional skin of individuals with different dermatoses. These tests were performed using density data, i.e., data recorded as the number of *Malassezia* cfu obtained within the sample categories (see Results, Tables 2 and 3). Two other tests for the microhabitat (body site) specificity of individual *Malassezia* species were carried out using frequency data, i.e., data based on the number of contact plates yielding at least one isolate of the species in question (see Results, Table 4). To determine if the various patient categories differed in the extent to which an individual *Malassezia* species was associated with different anatomic sites, the Cochran nonparametric statistic was used. For a given dermatosis and any particular *Malassezia* species, the McNemar test was used to determine if the species could be recovered more frequently than by chance alone from a given anatomic site.

Results

Recovery rate of *Malassezia* from different body sites of individuals

In at least 67% of individuals from each skin condition, a positive culture was obtained from one or more body sites. The frequency at which contact plates yielded a culture in each patient category, recovery of a positive culture from lesional and non-lesional sites, the number of colonies analyzed for identification and the number of species recovered in each dermatoses category is presented in Table 1.

Occurrence of *Malassezia* on individuals with and without dermatoses

The mean cfu counts for individuals with dermatoses and the healthy controls were more variable than would be expected by chance (chi square, 21.0; degrees of freedom (df), 4; $P < 0.001$) (Table 2). The mean cfu counts from patients with AD, PS and SD were significantly lower than those from normal individuals ($P < 0.05$). On the other hand, the mean cfu counts in patients with PV were similar to those in normal individuals. In a pair wise

Table 1 Recovery rate for *Malassezia* from different sample categories and number of *Malassezia* species recovered among the cultures analyzed for identification

Characteristics	AD	PS	SD	PV	Healthy
Sample size	31	28	28	23	20
No. of patients positive for <i>Malassezia</i>	22	19	23	21	19
Recovery rate (%)	70.9	67.8	82.1	91.3	95
No. of contact plate sampled (No. yielding <i>Malassezia</i>)	155 (49)	140 (44)	140 (55)	115 (61)	100 (62)
Lesional sites*	93 (35)	86 (27)	58 (23)	36 (23)	–
Non-lesional sites†	62 (14)	54 (17)	82 (33)	79 (38)	100 (62)
Number of patients analyzed for culture identification	17	11	17	19	19
Number of cultures analyzed	69	50	80	113	83
Number of species recovered	6	3	4	5	5

*, Numbers in parentheses represent the number of lesional sites sampled that grew *Malassezia* on the contact plates;

†, numbers in parentheses represent the number of non-lesional sites sampled that grew *Malassezia* on the contact plates.

comparison of cfu counts between various skin conditions, the mean cfu counts in patients with PS was significantly lower than the corresponding value for SD.

cfu Counts from lesional and non-lesional sites combined

For each dermatosis, the total number of cfu was recorded at five different anatomic sites, namely the scalp, forehead, arm, trunk and leg (Table 2). In general,

the mean cfu counts on the scalp, forehead and trunk, were significantly higher than on the arms and legs. However, in patients with AD there was no significant difference in the mean cfu counts on the scalp, forehead, trunk and legs. In PS, the mean cfu counts on the scalp, forehead, and trunk were significantly higher than the counts on the arms and legs. In SD, the mean cfu counts were not significantly different on the scalp, forehead, trunk and arms. The counts on the legs were significantly lower than those on the scalp, forehead, arms and trunk.

Table 2 Distribution of cfu by body site and dermatosis

Dermatosis	Number of patients	Average age (years)	Sex (M/F)	Distribution of cfu by body site (lesional and non-lesional sites combined)					Total cfu
				Scalp	Forehead	Arm	Trunk	Leg	
AD	31	41.3	17/14						
Total cfu*				265 (1)	367 (2)	21	49	134 (1)	836
Mean cfu				8.5	11.8	0.7	1.6	4.3	27.0†
PS	28	48.9	12/16						
Total cfu				120	106	9	155	2	392
Mean cfu				4.3	3.8	0.3	5.5	0.07	14
SD	28	57.8	23/5						
Total cfu				328 (1)	130	160	663 (3)	16	1285
Mean cfu				11.7	4.6	5.7	23.7	0.6	45.9
PV	23	32.5	9/14						
Total cfu				449 (2)	952 (2)	129	576 (2)	85	2191
Mean cfu				19.5	41.4	5.6	25	3.7	95.3
Healthy individuals	20	38.3	6/14						
Total cfu				794 (2)	388 (1)	77	88	9	1356
Mean cfu				39.7	19.4	3.9	4.4	0.5	67.8

*, The numbers in parentheses represent the number of patients for whom >100 cfu were recovered from the sampled body site(s);

†, the sum of means within a patient category corresponds to the mean number of colonies isolated per patient at all five body sites combined.

In PV, the cfu counts were not significantly different on the scalp, forehead and trunk, and were significantly higher than those obtained from sites on the arms and the legs. In normal individuals, the cfu counts were significantly higher on the scalp, forehead, arms and trunk, than on the legs ($P < 0.05$).

cfu Comparisons of lesional versus non-lesional sites

Mean values for the cfu counts from lesional and non-lesional sites in all five patient categories are shown in Table 3. For all dermatoses considered together, the cfu counts in lesional sites were significantly lower than the corresponding factor for non-lesional sites (chi-squared test, 4.1; df, 1; $P < 0.05$). This trend appeared to derive mainly from a strong tendency of SD patients to have higher colony counts on non-lesional than on lesional skin. A similar but less pronounced trend was also seen in AD patients (sample numbers were not high enough to test whether the latter apparent trend was significant independently). PS and PV patients actually had slightly higher mean counts on lesional than on non-lesional skin, but the difference was not statistically significant (Table 3).

cfu Comparisons of lesional sites among different patient categories

The cfu counts from lesional sites in patients with AD and PS were significantly lower than in patients with PV (Table 3). No other significant differences were found.

cfu Comparisons of non-lesional sites in different patient categories

The cfu counts from non-lesional sites of patients with AD and PS were significantly lower than those of healthy control subjects and of the remaining two patient groups ($P < 0.05$) (Table 3). No significant difference in cfu counts was observed among non-lesional skin samples from PV and SD patients, and normal individuals.

Table 3 Mean cfu counts for lesional and non-lesional sites in various disease states

Disease state	Mean cfu from all lesional sites	Mean cfu from all non-lesional sites
AD	3.67	7.62
PS	3.09	2.8
SD	4.3	16.05
PV	19.28	18.48
Healthy patients	N/A	13.56

N/A, Not applicable.

Association of *Malassezia* species with different dermatoses

All six known lipophilic *Malassezia* species were recovered (Table 4). In many individuals, more than one *Malassezia* species was recovered from a given anatomic site. The most common species were *M. sympodialis* and *M. globosa*. The remaining species, *M. slooffiae*, *M. furfur*, *M. restricta* and *M. obtusa*, were less frequently isolated.

The distributions of *M. sympodialis* and *M. globosa* were significantly different among body sites in AD and PV patients, as well as in healthy individuals (Table 4). In atopic dermatitis, *M. sympodialis* was recovered significantly more frequently on the forehead than on the arm or leg. In PV and healthy individuals, *M. sympodialis* was observed significantly more frequently on the forehead and trunk than on the legs. For *M. globosa*, in healthy individuals the forehead was colonized significantly more frequently than were the arms and legs. For the remaining *Malassezia* species, the number of observed occurrences at the different anatomic sites within patient categories was too small to permit statistics to be performed.

In overview, however, combining patient categories, the four less common *Malassezia* species appeared to fall into two groups on the basis of their distribution on the upper body (scalp and forehead) versus lower body (arm, trunk and leg). The numbers obtained suggested that additional investigation of these relatively rare organisms might also reveal significant trends in body site preferences. *M. slooffiae* and *M. restricta* were frequently isolated from scalp and forehead. *M. slooffiae* was observed in 10 of 16 records (62.5%) and *M. restricta* was observed in five of six records (83.3%) from these upper body sites. *M. furfur* and *M. obtusa*, on the other hand, were recorded in 12 of 17 positive samples (70.6%) and five of six positive samples (83.3%), respectively, on the lower body (trunk, arms, legs).

There were also some significant differences in *Malassezia* species counts among patient categories when results from all body sites were amalgamated. *M. sympodialis* was the most frequently recorded species in contact plate samples from AD patients (found in 51.3% of samples) and PV patients (62.7% of samples). *M. globosa*, by contrast, was the most frequently recorded species in patients with PS (57.7% of samples) and SD (45.0% of samples).

There was one 17-year-old female PV patient in whom four different species were isolated. The 13 isolates identified from this patient included five *M. sympodialis* isolates from the forehead, two *M. obtusa* from the arm,

Table 4 Distribution of *Malassezia* species by body site in various dermatoses

<i>Malassezia</i> species	Anatomic site	Dermatosis frequency of isolation* (frequency of isolation at lesional site†) [% frequency within disease category‡]					Overall frequency and (% frequency)
		AD (n = 31)	PS (n = 28)	SD (n = 28)	PV (n = 23)	Healthy (n = 20)	
<i>M. sympodialis</i>	Scalp	5 (2)	2 (2)	2	7	4	20
	Forehead	7 (5)	3 (2)	3 (3)	8	10	31
	Arm	1 (1)	1 (1)	2	5 (1)	6	15
	Trunk	6 (6)	1	5 (2)	10 (10)	5	27
	Leg	1 (1)	1 (1)	3	2	0	7
	Species subtotal	20 (15) [51.3%]	8 (6) [30.8%]	15 (5) [37.5%]	32 (11) [62.7%]	25 (N/A) [47.2%]	100 (47.8%)
<i>M. globosa</i>	Scalp	1	4 (3)	4 (2)	5	5	19
	Forehead	4 (3)	4 (1)	5 (4)	3	6	22
	Arm	0	2	4 (1)	0	0	6
	Trunk	2 (2)	4 (4)	5 (4)	1 (1)	4	16
	Leg	0	1	0	0	0	1
	Species subtotal	7 (5) [17.9%]	15 (8) [57.7%]	18 (11) [45.0%]	9 (1) [17.6%]	15 (N/A) [28.3%]	64 (30.6%)
<i>M. furfur</i>	Scalp	1 (1)	0	1	0	1	3
	Forehead	1 (1)	0	0	0	1	2
	Arm	0	0	0	1	1	2
	Trunk	1 (1)	0	2	2 (2)	1	6
	Leg	1 (1)	0	0	1	2	4
	Species subtotal	4 (4) [10.3%]		3 (0) [7.5%]	4 (2) [7.8%]	6 (N/A) [11.3%]	17 (8.1%)
<i>M. slooffiae</i>	Scalp	1 (1)	2 (1)	2	1	1	7
	Forehead	0	0	0	1	2	3
	Arm	0	0	0	0	0	0
	Trunk	0	1	2 (2)	1 (1)	0	4
	Leg	0	0	0	1	1	2
	Species subtotal	1 (1) [2.6%]	3 (1) [11.5%]	4 (2) [10.0%]	4 (1) [7.8%]	4 (N/A) [7.5%]	16 (7.7%)
<i>M. obtusa</i>	Scalp	0	0	0	0	0	0
	Forehead	0	0	0	1	0	1
	Arm	1 (1)	0	0	1 (1)	0	2
	Trunk	1 (1)	0	0	0	0	1
	Leg	2 (2)	0	0	0	0	2
	Species subtotal	4 (4) [10.3%]	0	0	2 (1) [3.9%]	0 (N/A)	6 (2.9%)
<i>M. restricta</i>	Scalp	1	0	0	0	2	3
	Forehead	2 (1)	0	0	0	0	2
	Arm	0	0	0	0	0	0
	Trunk	0	0	0	0	0	0
	Leg	0	0	0	0	1	1
	Species subtotal	3 (1) [7.7%]	0	0	0	3 (N/A) [5.7%]	6 (2.9%)
Totals		39	26	40	51	53	209

*, Species frequency: number of contact plates yielding at least one isolate of the indicated species (including plates from both lesional and non-lesional sites);

†, Number of contact plates from lesional body site yielding species;

‡ Percentage of contact plate samples within individual disease category yielding the indicated species.

N/A, Not applicable.

two *M. furfur* and two *M. slooffiae* from the trunk and one *M. slooffiae* and one *M. sympodialis* from the leg.

Discussion

In this study we have demonstrated that the use of contact plates is a simple and effective method for

quantitative culturing of *Malassezia* yeasts from human skin. The colonies are easy to identify with high and reliable counts. It is not known if all individual species grew in proportion to their true population density (a theoretical problem with all isolation studies); however, it is certain that they were all able to grow to a

significant extent. Although a study with similar design was reported by Faergemann [5] from Europe using a previous taxonomic system now considered insufficiently resolved, this is the first North American study of its kind. A recent Spanish study of the distribution on skin of *Malassezia* species [19] identified only three of the six recognized lipophilic species and sampled only healthy volunteers. Another recent Spanish study identified five of the six recognized species from normal, SD and PV patients sampled by means of a scraping inoculation technique [20].

In the present study, recovery of *Malassezia* species from skin was significantly lower in patients with the inflammatory dermatoses PS and AD than in healthy patients or in patients with the less inflammatory dermatosis PV. PS patients in particular had the lowest densities of *Malassezia* species on both lesional and healthy skin. Non-lesional areas in patients with AD also had a density of *Malassezia* species that was significantly reduced compared to counts from healthy control subjects. The explanation for this phenomenon is not clear. With regard to both lesional and non-lesional skin in these patient categories, it may be significant that patients with certain skin conditions have been shown to excrete skin lipids differing in composition from the lipid mixtures characteristic of normal skin [21,22]. *Malassezia* species may be influenced by differences among these lipid compositions. With particular regard to lesional skin, both PS and AD patients have been shown repeatedly to respond to *Malassezia* species antigens with strong immunological reactions that are not seen in normal control subjects or SD patients [23–28]. In AD, it has been proposed that *P. orbiculare* (an older name for round-celled elements of the lipophilic *Malassezia* species complex, ergo a name suggestive of the modern *M. globosa*) may play a role in maintaining immunoglobulin (Ig)E-mediated skin inflammation [29]. The present study suggests that if such a process does occur, the inflammation may be partially successful in controlling the *Malassezia* populations. It is clearly not impossible that such sensitization may ultimately affect *Malassezia* populations on healthy as well as lesional skin, especially as particular skin areas may change in disease status over the course of time.

There was no significant difference in this study in the density of *Malassezia* species between patients with PV and normal individuals. Nor was there a significant difference in the number of culturable colonies on lesional and non-lesional skin in PV patients; cfu counts were relatively high from both skin categories. In a previous study [17], we found that 56.2% of PV lesions sampled by conventional diagnostic scraping failed to yield a culture even on the highly efficacious

Leeming–Notman medium [10]. At least one previous study has also shown that lesional and non-lesional skin of PV patients yielded equivalent numbers of *Malassezia* colonies, and that these numbers also differed insignificantly from those obtained from control skin and from lesional and non-lesional skin of SD patients [30].

Perhaps surprisingly, our study showed that lesional skin from SD yielded significantly lower colony numbers than non-lesional skin. The existing literature in general appears highly discrepant on the matter of whether or not SD lesions are associated with excess numbers of *Malassezia* cells, perhaps in part because of a lack of standardization in the techniques used. Heng *et al.* [31], using direct microscopy, found that numbers of yeast cells present rose in proportion to the severity of the lesions. At least two culturing studies have supported this finding [5,32], but three independent research groups using cultures, including one group that had earlier associated high culture numbers with lesional SD, have published contradictory results [12,30,33,34]. Our study, which used the most commonly employed culturing technique yielding quantitative results (contact plates with Leeming–Notman agar), is the first to show a significantly lower recovery of cultures from lesional than non-lesional skin in SD.

Our results, as well as those of other studies, may reflect artifactual patient factors rather than differences in sampling techniques. Individuals with dermatoses are more likely than other persons to use home remedies, synthetic detergents or shampoos to control their skin condition. Use of these agents might be associated with reduced colony counts. Detergent soaps and shampoos with additives including climbazole, ketoconazole, ciclopirox olamine, zinc pyrithione, selenium sulfide, or other sulfur-containing substances are known to be quite effective in patients with chronic disease [35], and it is not known how long such treatments must be discontinued before the *Malassezia* populations fully recover. It is possible that the various non-specific hygienic practices and self-treatments used by our SD population were relatively effective in killing or removing *Malassezia* cells from lesional skin.

The results of our study are most comparable to that of a recent study by Nakabayashi *et al.* [36] who used the tape and swab method to isolate and identify *Malassezia* species from Japanese patients with SD, AD, PV and normal subjects. Although lower numbers of positive cultures were recovered from lesional skin than non-lesional skin of patients with AD, no significant differences in the isolation frequency of lesional versus non-lesional skin of patients with SD was found. Moreover, *M. globosa* was the most predominant species to be

isolated from every disease state as well as from the normal subjects. In our study we have found *M. globosa* to be frequently isolated from patients with PS and SD. *M. sympodialis* has by far been the most predominant species to be associated with PV, AD as well as from the normal subjects in our studies. The differences in the results of our study and that of Nakabayashi *et al.* [36] may also reflect the differences in the patient population that was sampled, i.e., North American versus Japanese. This possibility should be explored further.

In the present study, differences were seen in species assemblages detected at the different anatomic sites in healthy and diseased individuals. This finding has been observed previously [19,20,24,25]. However, our study is the first to characterize and report the frequency of each of the species of *Malassezia* in a North American population. The report by Faergemann *et al.* [37] discusses the outmoded taxon *P. orbiculare* only and Bandhaya [38] limited the discussion to two species, *M. furfur* and *M. pachydermatis*, the first of which, as conceived at the time, is now known to be a multi-species complex. Aspiroz *et al.* [19], as mentioned above, identified only three of the six currently recognized species. Only the recent study of Crespo Erchiga *et al.* [20], conducted in Spain, has attempted to identify all recognized species; the data in that study, however, were not subjected to statistical analyses. In the present study, we observed that the *Malassezia* counts were generally higher on the scalp, forehead and trunk than on the arms and legs of healthy controls as well as of subjects in each of the patient categories. Patients with AD and PV, as well as healthy control subjects, grew *M. sympodialis* in approximately half or more of all samples but grew *M. globosa* from less than one-third of samples. By contrast, PS and SD patients grew *M. sympodialis* in only about one-third of samples, but grew *M. globosa* in approximately half of all samples. The *Malassezia* species more likely to grow from the scalp and forehead rather than the lower body were *M. restricta* and *M. slooffiae*. Aspiroz *et al.* [19], studying only healthy individuals, also found *M. restricta* particularly associated with the scalp; they did not identify *M. slooffiae*. In another correspondence with our study, Aspiroz *et al.* [19] found *M. globosa* evenly distributed in all sites sampled. We also found that, even when the more ecologically sensitive frequency data (number of contact plates positive for species) were tested in contrast to the density data (cfu) used by Aspiroz *et al.* [19], our study populations as a group showed no significant difference in the association of *M. globosa* with scalp, forehead and trunk. Only the arm and leg, not investigated by Aspiroz *et al.* [19], showed significantly lower prevalences of this species. Our study found that

M. sympodialis was equally likely to be recovered from the scalp and forehead or the trunk and upper and lower extremities, whereas Aspiroz *et al.* [19] found a significant association with the back, in contrast to the chest and scalp (forehead and leg were not studied). In our study, *M. furfur* and *M. obtusa*, although not common enough to yield definitive results, appeared more likely to be associated with the lower body; again, these species were not recorded by Aspiroz *et al.* [19].

Specifically in lesional sites, Crespo Erchiga *et al.* [20] found *M. globosa* in more than twice the number of PV lesions as *M. sympodialis*, while *M. restricta* and *M. globosa* appeared to be strongly associated with SD lesions. Our contact plate data on PV lesions, consistent with our previous large, independent study on routine clinical PV scrapings [17], showed that Ontario patients grew more *M. sympodialis* (11 samples) than *M. globosa* (one positive sample) from lesions of this disease. In contrast to Crespo Erchiga *et al.* [20], we only obtained *M. globosa*, *M. sympodialis* and *M. slooffiae*, in descending order of frequency, from lesional SD; *M. restricta* was mainly obtained from healthy control subjects and AD patients. Although this species is slow growing and may be overgrown by other species in densely crowded isolation plates, this phenomenon was anticipated in our study, and we did not observe the dense overcrowding on contact plates that might cause a sampling artefact of this nature.

The significance of the trends seen here and elsewhere in *Malassezia* colonization are not clear at present, but may become clearer as future studies clarify the degree of causality of the various species in PV, and possibly also in SD and other diseases. What is of interest here is the clear preliminary confirmation that the *Malassezia* species seen are by no means interchangeable in their ecological relationships: they have varying degrees of niche separation. It is possible that the lipid composition in the stratum corneum plays a role in the growth of particular species of *Malassezia* in certain disease states and at particular anatomic sites [39,40]. In addition, there is a very likely possibility that, as in other fungal dermatoses, there may be significant geographic differences worldwide in species prevalences, both in etiological and commensal *Malassezia* colonizations.

Even when results are discrepant from study to study, however, the enhanced resolution seen when all isolates are identified to species often makes older studies based on previous taxonomies difficult to integrate into current concepts. In general, the identification of individual *Malassezia* species should greatly increase the accuracy and predictiveness of future studies attempting to correlate *Malassezia* distributions with chemical, physical, immunological and biogeographic host factors.

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