A Comparison Study between Culture Based Technique and Op-site Non-Culture Based Technique for Identifying *Malassezia* Yeasts on Normal Skin

Sang Hee Lim, Yu Ri Kim, Jae Wook Jung, Hyung Jin Hahn, Yang Won Lee, Yong Beom Choe and Kyu Joong Ahn

Department of Dermatology, Konkuk University School of Medicine, Konkuk University Research Institute of Medical Science, Seoul, Korea

= Abstract =

Background: Culture based technique, a traditional method for extraction of DNA from a cultured colony, was complex in culture conditions and was associated with a lower chance of successful culture. Recently, non-culture based technique, which skipped the culture process and directly extracted fungal DNA and differentiated *Malassezia* species, has been introduced.

Objective: Using 26S rDNA PCR-RFLP, the authors identified *Malassezia* yeasts and compared the yield of *Malassezia* DNA by the traditional culture based technique and the non-culture based technique via Op-site adhesive tape.

Methods: DNA of *Malassezia* yeasts were extracted using the culture based technique and the non-culture based technique from normal adults. Comparison was performed in order to clarify the differences between these two techniques.

Results: Use of the culture based technique resulted in a culture rate of 57.8% (78 out of 135 samples). On the other hand, using the non-culture based technique, fungal species were identified from all 135 samples. Using both techniques, *M. globosa* was the most identified species. The identification rate of the non-culture based technique was 100%; however, 7 repeats of PCR were required to reach 100% identification. Among samples from five body sites, those from the thigh required 5.5 repeats of PCR.

Conclusion: The non-culture based technique was better than the culture based technique. However, due to the low amount of DNA extracts from the body sites with low habitation of *Malassezia* yeasts, repeated PCR was required for differentiation of *Malassezia* species.

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Key Words: Culture based technique, *Malassezia* yeasts, Non-culture based technique

INTRODUCTION

Malassezia yeasts are normal flora of the skin, and are harbored by the scalp, face, and trunk, where sebum secretion is abundant[1]. Also, *Malassezia* yeasts are widely recognized as the causative agents of *Malassezia folliculitis*, and

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†Corresponding author: Kyu Joong Ahn, 143-729 Gwangjin Goo, Hwayang Dong, 4-12 Department of Dermatology, Konkuk University Medical Center.
Tel: +82-2-2030-8161, Fax: +82-2-2030-5179, e-mail: kjahn@kuh.ac.kr

considered to be strongly linked to seborrheic dermatitis and atopic dermatitis[2~6]. As the classification system of *Malassezia* has become more detailed, various attempts have been made to establish a link between *Malassezia* yeast-associated skin disorders and certain species of *Malassezia*. However, given the distribution pattern of the normal flora, it is more likely that *Malassezia* skin diseases are related to the resident flora at main lesion, rather than certain specific species of *Malassezia*.

Using the ordinary, culture based technique, yeasts are sampled from normal and diseased skin. The scales or extracts are directly cultured on media, or, alternatively, specimens are obtained by purification with washing solution and the colonies are examined for identification on the basis of their microscopic and physiological characteristics.

A special media containing lipid ingredients designed for growth of lipophilic *Malassezia* is used, and growth requires optimal humidity and an ambient temperature of 34℃[7,8]. Also, a quantitative method, in which the organism is sampled using a washing solution from a given unit area and the number of colonies counted, may also be used.

This conventional method yields a high culture success rate on areas where there is an ample amount of sebum secretion, such as the scalp, face, and trunk; however, the identification process is quite difficult on the extremities because the rate is rather low on these areas. Also, highly-skilled experts are needed for identification of organisms on the basis of gross and microscopic features. Identification by gross examination may be complemented by molecular techniques, which involve extraction of DNA from cultured colonies and amplification of a portion of the DNA base sequence prior to cleavage with restriction endonucleases, and identification of the organisms from bands on electrophoresis agarose gel. However, this method cannot overcome the shortcomings of a low culture success rate.

Recently, Sugita *et al*.[9] introduced a novel method which uses adhesive tape for harvest of *Malassezia* yeasts directly from the skin and extraction of DNA for identification of species using molecular biological techniques. This technique is more convenient than the conventional method; however, since the number of yeasts is very small, even in areas where there is an abundance of *Malassezia* flora, amplification should be repeated several times in order to obtain a distinctive band on electrophoresis.

In this study, the authors used the conventional culture based technique and non-culture based technique using adhesive tape for harvest of *Malassezia* yeasts on subjects who are in their twenties and thirties, in whom there is an abundance of sebum secretion and thus rendering high yield on sampling, and identified the yeasts using a 26S rDNA PCR-RFLP in order to compare the differences in the rate of successful identification among various age groups, body sites, and the precision of matching, and difficulties in the process of identification.

MATERIALS AND METHODS

1. Subjects

Twenty seven healthy adults (12 males, 15 females) between the ages of 20 to 40 years, who had high sebaceous gland activity without skin disease participated in this study. Specimens were gathered from 5 different areas: forehead, cheek, anterior chest, and medial aspect of the upper arms and thighs. Those who have been diagnosed or treated with diseases associated with *Malassezia* yeasts, such as seborrheic dermatitis, were excluded.

2. Methods

1) Culture based technique

(1) Specimen sampling and culture

Specimens were gathered from 5 different body locations (forehead, cheek, anterior chest, and medial aspect of the upper arm and thigh) of healthy adults. They were rubbed thoroughly with a sterile cotton swab measuring 5 cm diameter with detergent (8.7 mM NaH_2PO_4, 12.3 mM Na_2HPO_4, 0.1% Triton X-100 [pH 7.9]) for 10 seconds and then streaked on LNA (Leeming & Notman)[7]. They were cultured for 2 weeks at 34℃.

(2) Extraction of DNA

One of the cultured colonies was transferred to a 1.5 ml e-tube[10], and 200 μl lysis buffer (100 mM Tris-HCl pH 9.5, 1 M KCl, 10 mM EDTA) were added. It was pulverized with the plastic pestle, and treated at 100℃ for 15 minutes, followed by addition of 10 μl Proteinase K (10 mg/ml). After 8~16 hours treatment at 56℃, DNA was extracted. For inactivation of proteinase K, it was treated at 100℃ for 10 minutes, followed by ice for 30 minutes. Thereafter, the entire process was conducted at 4~10℃. Phenol: chloroform: isoamyl alcohol (25:24:1) 200 μl were mixed and centrifuged at 13,000 rpm 4℃ for 20 minutes. Only the supernatant layer was transferred to a new tube. Then, 2.5 times the volume of 100% EtOH and 0.3 M NH_4Oac were added to the tube, followed by treatment at -20℃ for 3~16 hours, and centrifugation at 13,000 rpm 4℃ for 20 minutes. Seventy percent ice-cold EtOH 1 ml was added before centrifugation at 13,000 rpm 4℃ for 5 minutes. It was dried and TE (100 mM Tris-HCl pH 9.5, 1 M KCl, 10 mM EDTA) 30 μl was added to dissolve the DNA. DNA concentration was measured using a spectrophotometer (CM-2600d, MINOLTA, Japan) at a 260/280 ratio of absorbance.

2) Non-culture based technique

(1) Specimen sampling

Specimens were gathered from 5 different areas (forehead, cheek, anterior chest, and medial aspect of the upper arm and thigh) of healthy adults using 3 × 3 cm op-site film (Smith and Nephew Medical Ltd., Hull, United Kingdom). The adhesive surface was applied to the skin and compressed with the tip of the tweezers, so that the entire surface would come in contact with the skin, and it was transferred to a 10-ml tube.

(2) Extraction of DNA

The portion of thin film that came into contact with the skin was placed in a 10 ml tube, and 400 μl-2 ml of DNA lysis buffer (100 mM Tris-HCL pH 8.0, 30 mL EDTA pH 8.0, and 0.5% sodium dodecyl sulfate) was added so that the film would be completely immersed in lysis buffer. It was boiled for 30 minutes so that the specimens would be easily detached from the adhesive surface, and treated for 16 hours at 37℃. It was then treated for ten minutes at 100℃ and ice was placed on it for more than thirty minutes. The film and DNA lysis buffer were isolated and transferred to a new tube. Floating scales were also transferred to DNA lysis buffer. It was centrifuged at 300 rpm for 10 minutes, so that the floating scales would not settle at the bottom and only the lysed portion of the film would be removed. Ten μl of Proteinase K (10 mg/ml) was added to the DNA lysis buffer and treated at 56℃ for 8 to 16 hours for separation of proteins from DNA. For inactivation of proteinase K, it was treated at 100℃ for ten minutes and with ice for 20 minutes. The whole process was performed at 4 to 10℃. An equal amount of Phenol: chloroform: isoamyl alcohol (25:24:1) was added to DNA lysis butter and the mixture was stirred thoroughly, in order to obtain pure DNA. Because there are debris and other contaminant particles when skin is sampled with op-site film, it was treated for one hour at -20℃ in order to fully

adsorbed P:C:I. It was then centrifuged at 13,000 rpm for 20 minutes at 4℃, and only the supernatant was transferred to a new tube. Then, 2.5 times the volume of 100% EtOH and 0.3 M NH_4Oac were added to the tube, followed by treatment at -20℃ for 3 to 16 hours, and centrifugation at 13,000 rpm 4℃ for 20 minutes before DNA extraction. Seventy percent ice-cold EtOH 1 ml was added before centrifugation at 13,000 rpm 4℃ for 5 minutes and dried. TE (100 mM Tris-HCl pH 9.5, 1 M KCl, 10 mM EDTA) 30 μl was added in order to dissolve the DNA. DNA concentration was measured using a spectrophotometer (CM-2600d, MINOLTA, Japan) at a 260/280 ratio of absorbance. Because the amount of genomic DNA is too small, only the amount of colony-genomic DNA was measured.

3) Identification of strains with 26S PCR-RFLP

PCR was performed in order to amplify the 26S rDNA portion of the genomic DNA extracted by the two aforementioned methods[11]. A 20 μl PCR amplification reaction mixture, including genomic DNA, 25 mL dNTP, 10X PCR butter, 0.5 μl primer, 0.4 μm forward primer (5'-TAACAAGGATT-CCCCTAGTA-3'), reverse (5'ATTACGCCAGC-ATCCTAAG-3'), and 1.25 U Taq polymerase was prepared[12]. Veriti (Veriti 96-Well Fast Thermal Cycler, ABI) was used for PCR. The conditions of the reaction were as follows; 10 minutes at 94℃ at pre-denaturation phase, 30 seconds at 94℃ at denaturation phase, 45 seconds at 52.5℃ at annealing phase, one minute at 72℃, and seven minutes at 72℃ at extension phase. The entire process was repeated 35 times. Five μl of the PCR product were added and 50 μl of PCR amplification reaction mixture were prepared under the same conditions. Five μl of the PCR product were electrophoresed at 100 V using 3% Seakem LE agarose gel (Takara Biomedicals, Otsu, Japan), and dyed with ethidium bromide for 15 minutes before washing with DDW for 20 minutes. A roughly 500 bp-long band at the 26S rDNA portion was identified. When the band pattern of the PCR product of the extracted genomic DNA was not well visualized, the PCR process was repeated until it became visible. The PCR product was purified using LaboPass^{TM} gel and a PCR Clean-up kit (Cosmo, Seoul, Korea). 26S rDNA RFLP was performed using two restriction enzymes, *Hha1* (Koschem, Seoul, Korea) and BseGI (fermentas, USA). Twenty μl of reaction mixture were prepared by addition of 10X PCR butter, 10U restriction enzyme, and 26S rDNA. It was treated for 3 to 16 hours at 37℃. Identification of strains was carried out using the previously-mentioned electrophoresis process.

Table 1. The demographics of the subjects

Age	Sex		Total, n
	Male, n	Female, n	
20~29	7	12	19
30~39	4	4	8
Total	11	16	27

RESULTS

1. Subjects of study

Twelve men and 16 women were enrolled in this study. Nineteen of the subjects were in their twenties and 8 in their thirties (Table 1).

2. Identification of species using the culture technique

Successful culture was obtained in 57.9% of the age group 20~29 and 57.5% in the age group 30-39, and there was no significant difference (Table 2). In all sites of sampling, the detection rate of *Malassezia* yeasts was higher in men. In the case of cheek, the rate was only 50% (8/16) in

women, in contrast to 100% (11/11) in men. The overall rate of successful culture was 58.5%, with *Malassezia* yeasts being recovered in 79 cases out of 135 samples collected from the forehead, cheek, anterior chest, medial aspect of the forearm, and medial aspect of the thigh. Yeasts were recovered in all 27 cases of anterior chest, followed by the forehead (21 cases, 77.8%), cheek (19 cases, 70.4%), the medial aspect of the upper arm (6 cases, 22.2%), and the medial aspect of the thigh (6 cases, 22.2%) (Table 3). Among the 79 samples, two species were recovered from each of 8 specimens, and, thus, a total of 86 strains were identified overall. *M. globosa* was the most frequently identified (47 cases, 54.7%), followed by *M. sympodialsis* (16 cases, 18.6%), *M. restricta* (13 cases, 15.1%), *M. dermatis* (4 cases, 4.7%), *M. furfur* (4 cases, 4.7%), and *M. sloofiae* (2 cases, 2.3%) (Table 4, Fig. 1). In all body sites *M. globosa* was most frequently isolated, except in the lateral aspect of the upper arm, in which *M. sympodialis* was most frequently found.

Table 2. The positive culture rate of *Malassezia* species according to the age groups

	Sex		Total, n (%)
	Male, n (%)	Female, n (%)	
20~29	28/35 (80)	27/60 (45)	55/95 (57.9)
30~39	12/20 (60)	11/20 (55)	23/40 (57.5)
Total	40/55 (72.7)	38/80 (47.5)	78/135 (57.8)

3. Identification of *Malassezia* species using the non-culture method

Using the non-culture technique, the rate of identification was 100% (135/135). Three different strains were identified in 2 samples, and two different strains in 9 samples, and, thus, a total of 148 strains were recovered. *M. globosa* was the most frequently identified (74 cases, 50%), fol-

Table 3. The positive culture rate of *Malassezia* species according to the body sites

	Sex		Total, n (%)
	Male, n (%)	Female, n (%)	
Forehead	11/11 (100)	10/16 (62.5)	21/27 (77.8)
Cheek	11/11 (100)	8/16 (50.0)	19/27 (70.4)
Chest	11/11 (100)	16/16 (100)	27/27 (100)
Upper arm	4/11 (36.4)	2/16 (12.5)	6/27 (22.2)
Thigh	3/11 (27.3)	2/16 (12.5)	5/27 (18.5)
Total	40/55 (72.7)	38/80 (47.5)	78/135 (57.8)

*Number of positive culture/total number of sample

Table 4. The identified *Malassezia* species from five body sites-culture based technique (86 species identified from 78 cultured cases)

Malassezia species	Body sites					Total (%)
	Forehead (%)	Cheek (%)	Chest (%)	Upper (%)	Thigh (%)	
M. restricta	7 (25.9)	4 (20)	1 (3.6)	0	0	12 (14.3)
M. globosa	12 (44.4)	12 (60)	17 (60.7)	2 (33.3)	4 (80)	47 (54.7)
M. sympodialis	4 (14.8)	1 (5)	8 (28.6)	3 (50)	1 (20)	17 (19.8)
M. slooffiae	1 (3.7)	0	1 (3.6)	0	0	2 (2.3)
M. furfur	1 (3.7)	2 (10)	0	1 (16.7)	0	4 (4.7)
M. dermatis	2 (7.4)	1 (5)	1 (3.6)	0	0	4 (4.7)
Total	27 (100)	20 (100)	28 (100)	6 (100)	5 (100)	86 (100)

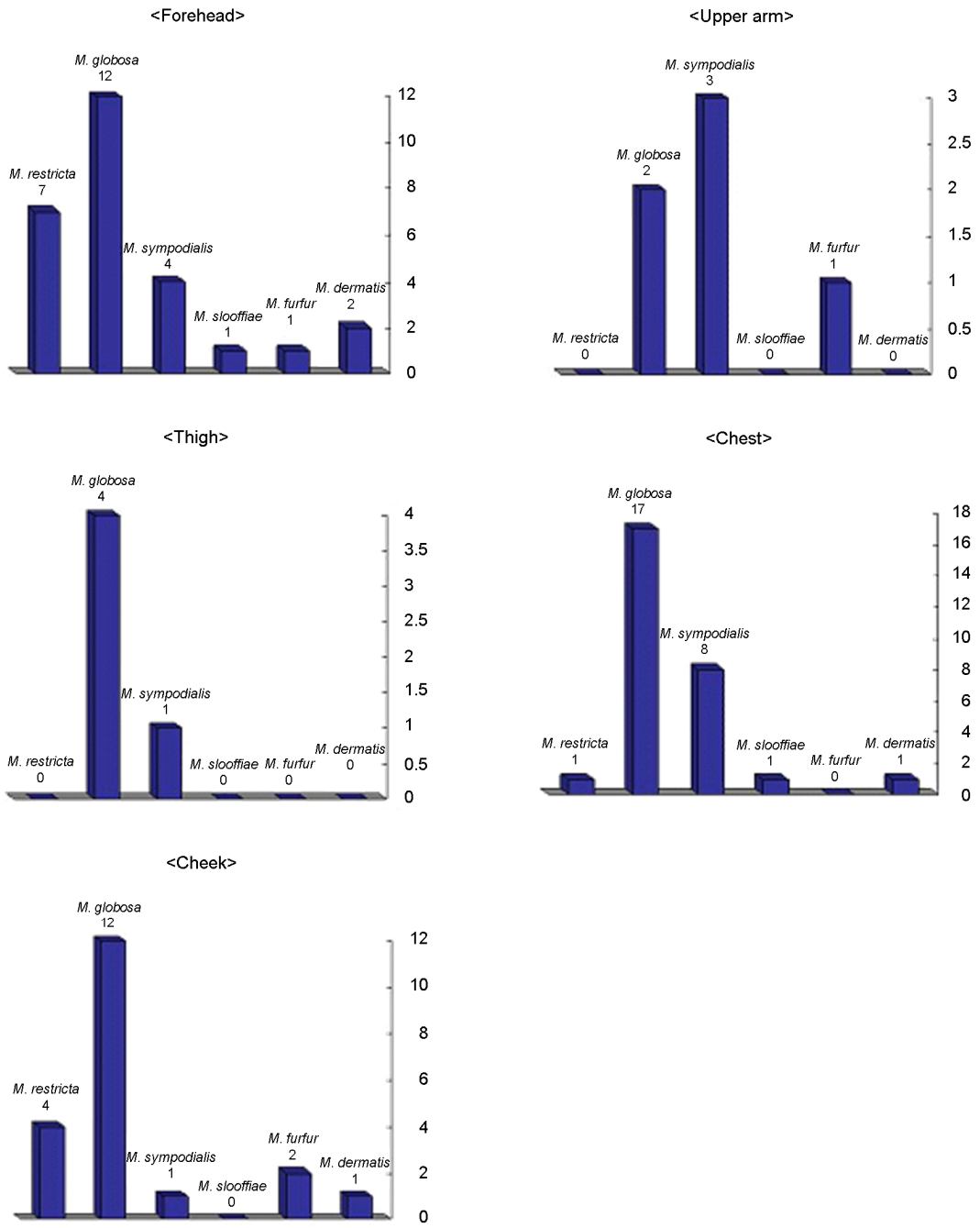

Fig. 1. The identified *Malassezia* species of five body sites: culture based technique

lowed by *M. sympodials* (45 cases, 30.4%), *M. restricta* (17 cases, 11.5%), *M. furfur* (6 cases, 4.1%), *M. dermatis* (4 cases, 2.7%), and *M. slooffiae* (2 cases, 1.4%) (Table 5, Fig. 2). *M. globosa* was most frequently isolated in all body sites, except for the lateral aspect of the thigh, in

Table 5. The identified *Malassezia* species from five body sites-culture based technique (86 species identified from 78 cultured cases)

Malassezia species	Body sites					Total (%)
	Forehead (%)	Cheek (%)	Chest (%)	Upper (%)	Thigh (%)	
M. restricta	9 (25.7)	6 (20.7)	2 (6.9)	0	0	17 (11.5)
M. globosa	15 (42.9)	14 (48.3)	18 (62.1)	14 (51.9)	13 (46.4)	74 (50)
M. sympodialis	6 (17.1)	6 (20.7)	8 (27.6)	11 (40.7)	14 (50.0)	45 (30.4)
M. slooffiae	1 (2.9)	0	0	1 (3.7)	0	2 (1.4)
M. furfur	2 (5.7)	2 (6.9)	0	1 (3.7)	1 (3.6)	6 (4.1)
M. dermatis	2 (5.7)	1 (3.4)	1 (3.4)	0	0	4 (2.7)
Total	35 (100)	29 (100)	29 (100)	27 (100)	28 (100)	148 (100)

Table 6. The cases identified as more than two but same *Malassezia* species

Case No.	Age/Sex	Body sites	*Malassezia* species	
			Op-site	Culture
4	23/F	Forehead	*Mslo*, *MG*	*Mslo*, *MG*
		Cheek	*MF*, *MR*	*MF*, *MR*
13	31/F	Chest	*MS*, *MG*	*MS*, *MG*
15	26/M	Forehead	*MF*, *MS*	*MF*, *MS*
17	30/M	Forehead	*MR*, *MG*	*MR*, *MG*
18	20/M	Forehead	*MD*, *MG*	*MD*, *MG*

MR: *M. Restricta*, *MG*: *M. globosa*, *M. sympodialis*
Mslo: *M. slooffiae*, *MF*: *M. furfur*, *MD*: *M. dermatis*

Table 7. The cases identified as different *Malassezia* species

Case No.	Age/ Sex	Body sites	*Malassezia* species	
			Op-site	Culure
7	29/M	Thigh	*MS*, *MG*	*MG*
10	27/F	Forehead	*MR*, *MS*, *MG*	*MR*
		Cheek	*MR*, *MG*	*MG*
12	29/F	Chest	*MD*, *MG*	*MD*
26	28/F	Forehead	*MR*, *MS*, *MG*	*MR*, *MG*

MR: *M. restricta*, *MG*: *M. globosa*, *MS*: *M. sympodialis*, *MD*: *M. dermatis*

which *M. sympodialis* was most predominant.

4. Comparison of results obtained by culture and non-culture methods

In 23 out of 27 subjects, results obtained by the culture based technique and the op-site non-culture technique were identical (Table 8). One strain was found in 18 of these 23, and 2 strains in 6 samples collected from 5 subjects. Of these 5, 3 were men and 2 women. Three subjects were in their twenties and 2 in their thirties. Samples were separated from the forehead in one case, one in the anterior chest, and both the forehead and cheek in one case. In these body sites, the two strains identified by the culture method were equally recovered by the non-culture method (Table 6).

On the other hand, in four subjects, results obtained by the two different methods were not in consensus with each other (Tables 7 and 8). In these four, the site of sampling was the medial aspect of the upper arm in one subject, anterior chest in one subject, forehead in one subject, and both the forehead and cheek in one subject. Three were women and one man, and all were in their twenties. Strains identified by the non-culture method included all of the strains identified by the culture method, and also included one or more

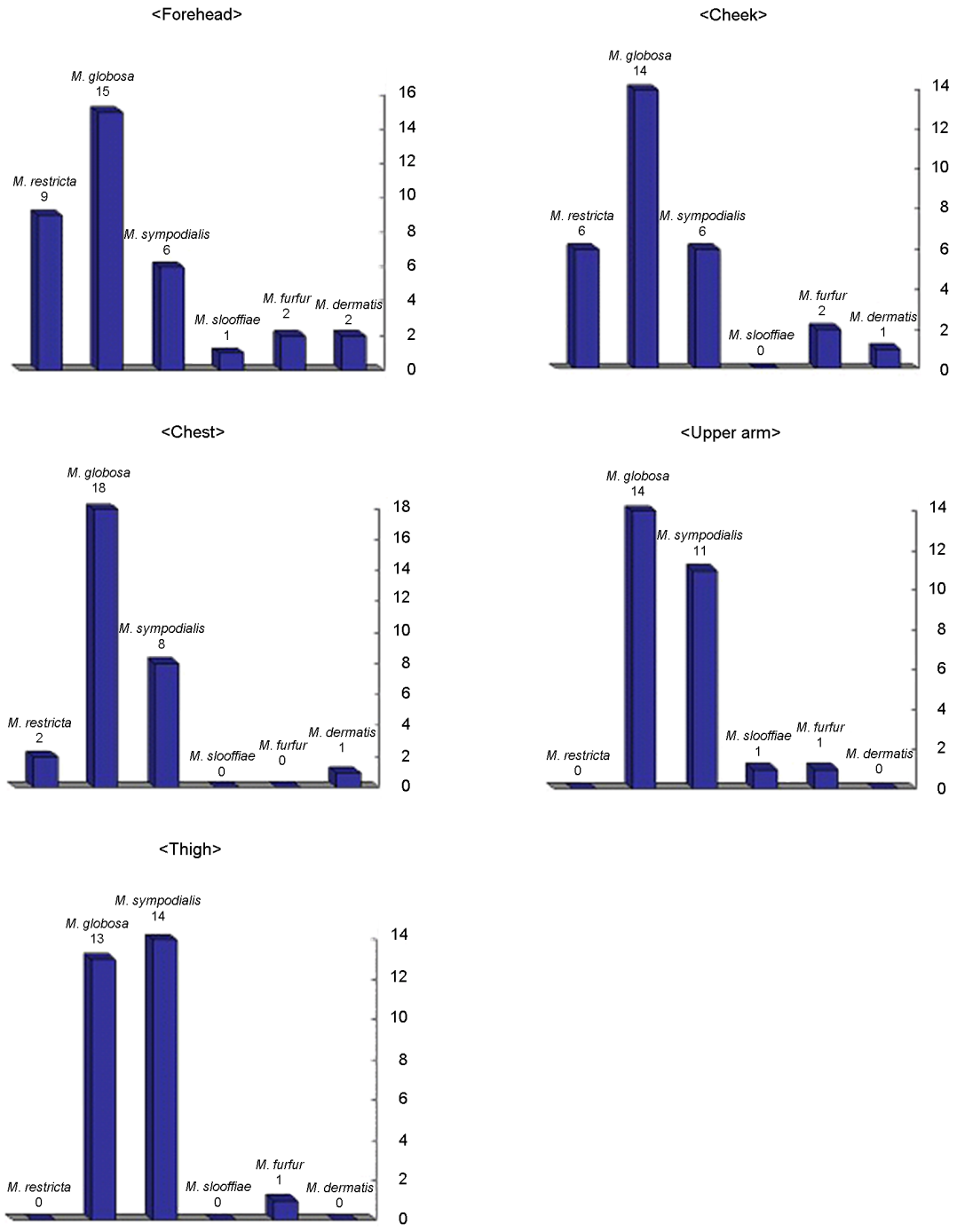

Fig. 2. The identified *Malassezia* species from five body site: non-culture based technique

additional strains. In cases where more than one species was found, the bands on electrophoresis were aligned in order from the darkest to the lightest (Table 7).

Table 8. The number of repeated PCR and identified *Malassezia* species: non-culture based technique

Number of repeated PCR	Number of identified *Malassezia* species	Total cumulative number (%)	Days taken
3	2/135	2/135 (1.5)	4
4	50/135	52/135 (38.5)	6
5	52/135	104/135 (77.0)	8
6	19/135	123/135 (91.1)	10
7	12/135	135/135 (100)	12

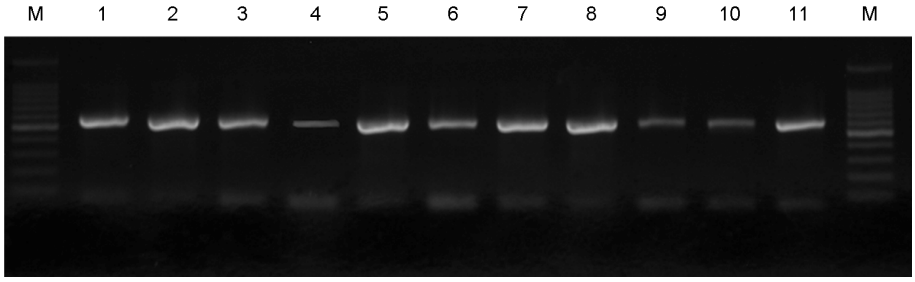

Fig. 3. 26r DNA PCR products of *Malassezia* species. 1: *M. furfur* (CBS 1878), 2: *M. sympodialis* (CBS 7222), 3: *M. globosa* (CBS 7966), 4: *M. restricta* (CBS 7877), 5: *M. slooffiae* (CBS 7956), 6: *M. pachydermatis* (CBS 1879), 7: *M. japonica* (CBS 9432), 8: *M. nana* (JCM 12085), 9: *M. dermatis* (JCM 11348). 10: *M. obtusa* (CBS 7876), 11: *M. yamatoensis* (CBS 9725).

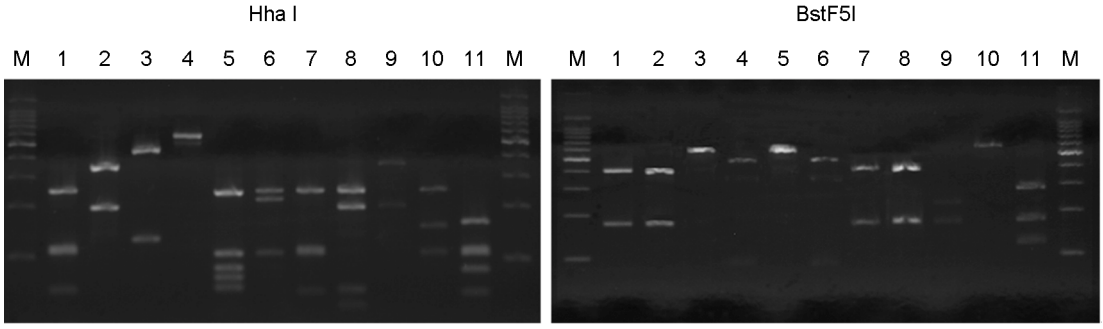

Fig. 4. PCR-RFLP pattern of *Malassezia* species. 1: *M. furfur* (CBS 1878), 2: *M. sympodialis* (CBS 7222), 3: *M. globosa* (CBS 7966), 4: *M. restricta* (CBS 7877), 5: *M. slooffiae* (CBS 7956), 6: *M. pachydermatis* (CBS 1879), 7: *M. japonica* (CBS 9432), 8: *M. nana* (JCM 12085), 9: *M. dermatis* (JCM 11348). 10: *M. obtusa* (CBS 7876), 11: *M. yamatoensis* (CBS 9725).

5. The number of repetitions of PCR required for the non-culture technique

Using the culture based technique, two sets of PCR were performed or identification of strains. However, using the non-culture technique, identification was successful in only 2 of 155 samples (1.3%) when 3 sets were performed (Figs. 3 and 4). Eventually, 7 repetitions were required for identification of all 155 strains. The time required was 12 days (Table 8). The average number of repetitions was 4.8 for the forehead, 4.7 for the

cheek, 4.3 for the anterior chest, 5.3 for the medial aspect of the upper arm, and 5.5 for the medial aspect of the thigh.

DISCUSSION

Malassezia yeasts are classified as normal flora of the skin and are isolated in 75~98% of healthy adults[13,14]. Since its dependence on lipid became known in 1939, culture became possible, and the problem of dimorphism was solved in 1977 with the discovery of the yeast phase and the myceilal phase and was classified into *Malassezia* species[14]. Although they are normal flora of the skin, *Malassezia* yeasts are associated with various common skin disorders, including pityriasis versicolor, *Malassezia folliculitis*, seborrheic dermatitis, and atopic dermatitis. By 1996, seven stains, i.e., *M. pachydermatis*, *M. furfur*, *M. sympodialis*, *M. globosa*, *M. obtusa*, *M. restricta*, and *M. slooffiae* were classified into the *Malassezia* genus[15,16], and recently, four additional species, namely *M. dermatis*[17], *M. yamatoensis*[18], *M. japonica*[19], and *M. nana*[20] have been found. As the genus *Malassezia* has been restructured, not only the clinical significance of each strain, but the sophistication of various molecular techniques that are essential to isolation of the strains, has drawn attention. The frequency of recovery of *Malassezia* yeasts varies according to the age range of subjects and the location of sampling, a finding that may be explained by the extent of sebum secretion. Yeasts are known to be more frequently found in areas where there is an ample amount of sebum secretion, such as the anterior chest, face, and scalp, compared to the upper and lower extremities[1,21,22]. In our study, the detection rate was the highest in the anterior chest at 100%, followed by the forehead, cheek, and the medial aspect of the upper arm, and the medial aspect of the upper thigh. These sites correspond to areas where *Malassezia*-related disorders, such as pityriasis versicolor, seborrheic dermatitis, *Malassezia folliculitis*, commonly occur. The difference according to age is related to the fact that *Malassezia* yeasts are lipophilic and that during puberty sebum secretion is increased under the influence of androgens and the lipid content of the skin changes. However, a report by Kwon *et al*.[23] contrasted from previous literature in that the frequency of detection was high in older age groups compared to the twenties group. This observation may be related to the fact that along with biological factors, such as sebum secretion, lifestyle factors, like hygiene, also play a role in growth of yeasts.

Previous mycological studies focused mainly on morphological characteristics of yeasts, such as size of the colonies, surface contour, color and shape, and biochemical analysis based on observation of enzyme reactions[24]. However, these methods are time-consuming, and are subject to variable interpretation. Also, establishment of objective standards is very difficult, and fails to provide adequate evidence for newly-identified species.

In addition, although the species may have the same morphological and biochemical characteristics, accurate classification is difficult since the strains may differ genetically. This study demonstrates that RFLP using restriction enzymes, which is one of several molecular techniques used for identification and classification of *Malassezia* yeasts, is a relatively quick and accurate method for isolation of organisms from not only cultured colonies but from the scales of the skin. 26S rDNA, which had been set as the target of this study, contains a highly conserved base sequence, which is appropriate for alignment, and possesses adequate variability of base sequence for use as a marker for species-specific restriction enzyme analysis. The 26S rDNA PCR-RFLP method is in agreement with the newly developed morphological classification, and may therefore be applied for identification of the eleven *Malassezia* strains identified

up to this point. Also, the 26S rDNA PCR-RFLP method requires only two restriction enzymes, *Hha1* and *BstF51*, and is technically more accessible, convenient, and accurate, compared to other molecular methods. Several studies are currently underway in search of new molecular methods for identification of new *Malassezia* species and to make up for the limitation of conventional methods. However, no such research studies have been conducted in Korea until this point[25]. In the future, more accurate, reliable, and rapidly-analyzable methods for isolation and identification of strains of *Malassezia* yeasts should be developed, and more sophisticated molecular techniques should be applied in identification, classification, and diagnosis of fungal diseases.

As shown in the results, the detection rate of *Malassezia* yeasts was 100% with the Op-site non-culture based technique; however, there were cases where more than two strains were detected. On the other hand, the culture-based method using a swab showed a detection rate of 57.8%; however, the rate of yeast isolation from the cultured colonies was 100%. In the case of the culture-based technique, the outcome is frequently negative when the condition of culture is fastidious. Also, because the culture medium often contains antibiotics, contaminants may be filtered out during the process of culture. In this study, one representative sample was chosen for identification using colony PCR. Therefore, it is not appropriate to conclude that strains isolated from culture are representative of the entire flora. On the contrary, with the non-culture based technique, a few co-existing strains may also be isolated. However, more studies are needed because elucidation of the way in which various flora may act as pathogens in various skin disorders is difficult.

The non-culture based technique omits the process of culture and extracts DNA directly from scales; therefore, it is quicker and more accurate. However, in this study, the amount of DNA extracted from the scales was scarce, and, therefore, several sets of PCR were required. This could be time-consuming and cost-ineffective. In fact, when successful, culture was complete within 3 to 4 days, and in the case of the culture-based method, only one set of PCR was required because the amount of extracted DNA was large. In fact, during the process of the study, isolation was successful in only two samples out of 135 when three sets of PCR were performed using the non-culture based method. Various types of normal flora were also present during amplification of the DNA, making the analysis difficult. Of the *Malassezia* species, *M. obtusa* and *M. restricta* are difficult to culture; therefore, actual colonies are often not represented. In this case, the non-culture based method could be a more accurate and appropriate measure of identification. In this study, *M. obtusa* was not detected and the detection rate of *M. restricta* was lower than predicted, with 12.6% with the non-culture based technique and 9% with the culture based technique. Specimens were not taken from the scalp, and this could be one of the reasons why the detection rate of *M. restricta* was lower in both the culture based and non-culture based techniques[26,27]. In our study, *M. obtusa*, *M. pachydermatis*, *M. yamatoensis*, *M. japonica*, and *M. nana* were not detected.

This study was carried out in an effort to compare culture based and non-culture based techniques for identification of *Malassezia* yeasts. More studies on not only identification of the yeasts, but also the relationship between each species and various skin disorders are considered necessary.

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