수의학석사학위논문

Genotype analysis of *Malassezia pachydermatis* isolated from canine skin and ear in Korea

한국에서 개의 귀와 피부에서 분리한
Malassezia pachydermatis 의 유전자형 분석

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이 논문을 수의학 석사 학위 논문으로 제출함.

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Abstract

Malassezia species include commensal fungi of dog skin that are primarily localized to moist and sebum-rich regions of the body, such as the external ear canal. One hundred and thirty four samples from four groups (diseased ear, diseased skin, healthy ear, and healthy skin) of dogs were obtained and direct sequencing of the 26s ribosomal DNA regions, intergenic spacer 1 (IGS-1) and

internal transcribed spacer 1 (ITS-1) DNA regions was done to investigate the *Malassezia* species and/or genotypes were colonized on dogs comparing ear canal and other sites of the body (anatomical sites) or skin-diseased and healthy (health status). Analysis of the DNA regions confirmed *Malassezia pachydermatis* and yielded three main sequence genotypes (A, B, C). Genotype C was more frequently isolated from the ear canal (36 – 55%) than other body regions (5 – 13%) regardless of the presence of lesions; hence, the body region may be more influential to *M. pachydermatis* colonization than health status. *M. pachydermatis* genotype C is considered to have affinity to colonize sebum-rich and humid environments, and it is suggested that *M. pachydermatis* has heterogenic subtypes that have different pathological and physiological features. The present findings provide prospects for further discovery of diversity in *M. pachydermatis* along with its pathogenic nature.

Keywords : *Malassezia pachydermatis*, intergenic spacer 1 (IGS-1), internal transcribed spacer 1 (ITS-1)

***Student Number* : 2010-21635**

CONTENTS

Introduction

The lipophilic, non-mycelial, unipolar budding yeasts of the genus *Malassezia* populate the resident skin flora of warm-blooded vertebrates and occasionally are associated with skin disease [1, 2]. *Malassezia* are primarily categorized into lipid-dependent or non-lipid-dependent species [3]. Lipid-dependent *Malassezia* species originally were thought to occur only on human skin [4]. However, lipid-dependent species, such as *M. furfur*, *M. globosa*, and *M. sympodialis* have since been isolated from healthy and diseased skin regions and ear canals of dogs and cats [5-8]. The non-lipid dependent *M. pachydermatis* is the major pathogen of domestic animal skin and is detected particularly on dogs with seborrhic dermatitis, atopic dermatitis, or otitis externa [9, 10]. Although zoophilic, *M. pachydermatis* also is a causative agent of nosocomial infections in high-risk infants [11, 12] and is detected as a commensal microorganism on the skin of dog owners [13].

Several genetic variants of *M. pachydermatis* have been isolated from dog skin and genotyped by sequencing nuclear ribosomal DNA regions, such as the large subunit (LSU), the first internal transcribed spacer (ITS-1) of ribosomal RNA (rRNA), the chitin synthase 2 gene (chs-2), and the first intergenic spacer (IGS-1) [14-18]. DNA sequencing of ITS-1, which is located between the 18S and 5.8S rRNA genes, is rapid and specific for genotyping *Malassezia* species and strains. This technique facilitates phylogenetic analyses of closely related isolates [19]. The IGS-1 region is thought to exhibit more intraspecific diversity than the ITS-1

region [18]. IGS-1 has been used to differentiate *Cryptococcus neoformans* and *C. gatti* [20], *Trichosporon* species [21], *M. globosa* [22], *M. restricta* [23], and *M. pachydermatis* [18].

Malassezia species may become pathogenic under the influence of predisposing factors, such as high humidity or a sebum-rich environment [4, 24]. Summer in Korea is very hot and humid, which could influence the pathogenesis of the yeast. The frequencies and population sizes of *M. pachydermatis* vary across anatomical sites in dogs. Cafarchia *et al.* [25] reported that certain *M. pachydermatis* subgenotypes are localized to particular body regions. The external ear canal is regarded as more sebum-rich and humid than other parts of the body, which may influence pathogenicity of *Malassezia* species. Hypothesis was that sebum-rich and humid ear canal may be associated with a different *Malassezia* strain distribution than other sites of the body.

In this study, *Malassezia* species harvested from the ear and other parts of the skin of dogs (*e.g.*, nasal folds, axillary and inguinal areas, interdigital areas, and perianal region) was investigated to evaluate the prevalence of lipid-dependent species and non-lipid-dependent in Korea. Furthermore, research on genotypes and subgenotypes by analyzing internal transcribed spacer-1 (ITS-1) and intergenic spacer 1 (IGS-1) DNA sequences, and investigations of the epidemiology of *M. pachydermatis* genotypes/ subgenotypes according to sebum-rich and humid ear versus other parts of the skin was done.

Materials and Methods

1. Strains and Animals

One hundred and thirty four *Malassezia* isolates were obtained from dogs referred to the Seoul National University Hospital for Animals (Korea) in 2010 and 2011. Sixty-one swab samples were obtained from ear canals, and 73 swab samples were obtained from other skin sites. The study population included 21 intact male dogs, 28 neutered males, 27 intact females, and 22 spayed females (total of 98 dogs). The ages of the dogs varied from 3 months to 20 years (mean age: 8.7 years). Shih tzu (n = 25) and Cocker spaniel (n = 24) were the most common breeds; other breeds included Beagle (n = 10), Maltese (n = 9), Yorkshire terrier (n = 7), Poodle (n = 4), and Miniature Schnauzer (n = 3). The remaining 16 dogs consisted of several other breeds. Animals that had diseased skin or ear lesions were put on an anti-fungal therapy for 4 weeks. Antifungal agents were oral medication of ketoconazole 5 mg/kg BID or itraconazole 10 mg/kg SID. Only animals responding within 4 weeks were included as the diseased group. Animals that did not benefit were ruled out.

2. Sampling procedures and culture

Samples were divided into diseased or healthy groups depending on health status clinically evaluated by dermatologist on the original swabbed site. Diseased ear group (Group 1) were taken from ear lesions that had inflammation, ear discharge, and thickening of the ear canal, and diseased skin group (Group 2) were taken from skin lesions that had such as pruritus, erythema, malodor, alopecia, seborrhea, lichenification, and hyperpigmentation. Healthy ear (Group 3) and skin (Group 4) showed clinically normal appearance and no dermatologic history for the prior 5 months. Each sample were taken from ear canals, ventral neck, interdigital region, nasal fold, perianal region, axillary region, internal elbow, and inguinal region of each dog using sterile cotton swabs moistened with sterile saline (0.9% NaCl) solution [26]. Another cotton swab was used for cytological examination; each swab was enrolled on a glass slide and stained with Diff-quick® staining solution. More than 10 *Malassezia* yeast visible in 15 random oil-immersion microscopic fields (1000X) were considered as *Malassezia* dermatitis [27]. Swabbed samples was directly smeared to Sabouraud dextrose agar (SDA; BD, Le Pont de Claix, France) and Leeming and Notman agar [28] (provided by laboratory) and incubated for 7 days at 37°C.

3. Fungal DNA extraction and Polymerase chain reaction (PCR)

Freshly scraped colonies were homogenized using TissueLyser (Qiagen, Hilden, Germany) with 5 mm-diameter stainless steel beads, and genomic DNA was isolated using the DNeasy Plant Mini Kit (Qiagen), according to the manufacturer's instructions. Genomic DNA was eluted into 50 μl aliquots and stored at -20°C until PCR amplification.

To identify the strains of *Malassezia*, 26S ribosomal RNA gene fragment was amplified using the following primers: forward 5' -TAA CAA GGA TTC CCC TAG TA- 3', reverse 5' -ATT ACG CCA GCA TCC TAA G -3'[29]. The PCR protocol used was denaturation at 94°C for 7 min; 30 cycles of template denaturation at 94°C for 45 sec, primer annealing at 55°C for 45 sec, and polymerization at 72°C for 1 min; and a final extension at 72°C for 7 min. PCR products were electrophoresed through a 1.5% agarose gel and were stained with ethidium bromide. Fragments were identified at approximately 580 bp. PCR products were then purified using the MEGAquick-spin PCR & Agarose Gel DNA Extraction System (Intron Biotechnology, Seoul, Korea) for direct sequencing. ITS-1 and IGS-1 regions were amplified with primer 18SF1 (5' -AGG TTT CCG TAG GTG AAC CT -3') and 5.8SR1 (5'- TTC GCT GCG TTC TTC ATC GA -3') [19] and with primer 26S-F (5'- ATC CTT TGC AGA CGA CTT GA- 3') and Mala-R (5'- TGC TTA ACT TCG CAG ATC GG -3') [30]. PCR mixtures were predenatured at 94°C for 5 min followed by 30 – 35 cycles of 94°C for 1 min, 60°C for 30 sec, and 72°C for 30 sec for ITS-1, and by 30 – 35 cycles of 94°C for 45 sec,

57°C for 45 sec, and 72°C for 1 min for IGS-1. Both protocols included a final extension at 72°C for 7 min. Amplicons were purified using MEGAquick-spin PCR & Agarose Gel DNA Extraction System (Intron Biotechnology).

4. Cloning and direct sequencing

Purified amplicons were sequenced directly using Abi Prism BigDye Terminator v3.1 (Applied Biosystems, Foster City, CA, USA) and were analyzed on a 3730XL automatic sequencer (Applied Biosystems). For confirmation, a randomly chosen sample from each group was cloned into the pGEM-T Easy Vector using the cloning system supplied by the manufacturer (Promega, Madison, WI, USA). Identification of *M. pachydermatis* was done by direct sequencing of the D1/D2 domains of the rDNA and compared with sequences available in GenBank using the BLAST program (www.ncbi.nlm.nih.gov/blast). Nucleotide sequences for ITS-1 and IGS-1 were deposited in GenBank (http://www.ncbi.nlm.nih.gov/Genbank/index.html). GenBank accession numbers for the ITS region were: A1, JQ619042; A2, JQ619044; A3, JQ619043; B1, JQ619045; C1, JQ619047; C2, JQ619046; C3, JQ619048, and Gen Bank accession numbers for the IGS regions were: 1a, JQ619029; 1b, JQ619030; 1c, JQ619031; 1d, JQ619032; 1e, JQ619033; 2a, JQ619034; 3a, JQ619035; 3b, JQ619036; 3c, JQ619037; 3d, JQ 619038; 3e, JQ619039; 3f, JQ619040; 3g, JQ619041.

5. Phylogenetic analysis

Sequences were aligned using ClustalW software [31] and phylogenetic analyses were conducted using the MEGA v.4.0 program [32]. A bootstrap analysis was performed with 1,000 replications. Neighbor-joining [33] then was carried out according to the Kimura two-parameter model [34].

6. Statistical analysis

The difference between positive rate from diseased and healthy sites, and frequency of genotype A and C, and recovery rate of genotype A and C from each group were evaluated using two-proportion test when comparing. Statistical analysis involved the Minitab® 16 program. $P < 0.05$ was considered statistically significant.

Results

1. Prevalence of Malassezia species

One hundred and forty one positive cultures of *Malassezia* species were obtained from 228 samples of diseased skin and ear sites from dogs (61.8% positive rate). Twenty four samples were gathered from 55 samples of healthy skin and ear (43.6% positive rate). Among the 141 samples from diseased site, 110 samples were genotyped by ITS-1 and IGS-1 region. Fifty two samples corresponded to ear canal (Group 1) and 58 were derived from other skin regions (Group 2). All 24 samples were genotyped from normal sites; 9 were from ear canal (Group 3) and 15 were from other skin sites (Group 4). (See Table 1)

2. Confirmation of M. pachydermatis

BLAST analysis of the sequenced 26s ribosomal RNA gene fragment revealed homologies to several *M. pachydermatis* 26s ribosomal RNA gene fragment including DQ 915502, AB118938, AB118937, DQ915500, AY743605, AY745724, AB118941, AJ249952, and AF063215, showing 100% query coverage and 100% maximum identity.

Table 1. Number and percentage of three major *Malassezia pachydermatis* sequence types for each region (ITS-1, IGS-1) isolated from healthy or diseased canine skin. Recovery rate of Genotype C is higher in either healthy or diseased ear.

Classification	Groups	Location	Sequence types							
			Number of isolates	ITS-1			Number of isolates	IGS-1		
				A	B	C		1	2	3
Skin lesions	Group 1	ear	52	32 (61.5%)	1 (1.9%)	19 (36.5%[a])	47	28 (59.9%)	1 (2.1%)	18 (38.2%*)
	Group 2	non-ear	58	54 (93.1%)	1 (1.7%)	3 (5.1%[b])	51	47 (92.1%)	1 (1.9%)	3 (5.8%**)
Normal skin	Group 3	ear	9	4 (44.4%)	0	5 (55.5%[a])	9	4 (44.4%)	0	5 (55.5%*)
	Group 4	non-ear	15	13 (86.6%)	0	2 (13.3%[b])	15	13 (86.6%)	0	2 (13.3%**)

[a, b] : Statistically significant differences by two-proportions test ($P<0.05$)

3. ITS-1 and IGS-1 sequence analysis

The length of the ITS-1 region was approximately 250 bp (246 – 253 bp); the alignment for this region (Fig. 1) and phylogenetic analysis identified the following seven subgenotypes: A1, A2, A3, B1, C1, C2, and C3 (Fig. 3). The IGS-1 region ranged from 546 bp – 939 bp in length, and the alignment of each group is shown in Fig. 2. The IGS-1 region was more variable than the ITS-1 region. Alignment and phylogenetic analysis of IGS-1 identified 13 subgenotypes: 1a, 1b, 1c, 1d, 1e, 2a, 3a, 3b, 3c, 3d, 3e, 3f, and 3g (Fig. 3). Sequences affiliated with Group 3 exhibited a conserved region characterized by a short repeat sequence $(CAGCA)_n$. Groups were classified based on their CAGCA repeat number. Sequenced amplicons could all be categorized into one of three major sequence types based on their levels of sequence similarity in ITS-1 (93.7% – 99.6%) and IGS-1 (41.6% – 99.0%). Genotypic grouping results (ITS-1 = IGS-1; A = 1, B = 2, C = 3) were concordant between each locus and were considered to be identical to previously described genotypes A, B, and C [17]. In most cases (92.6%), same IGS-1 subgenotype belonged to same specific ITS-1 subgenotype, on the other hand, 9 isolates were contradicted.

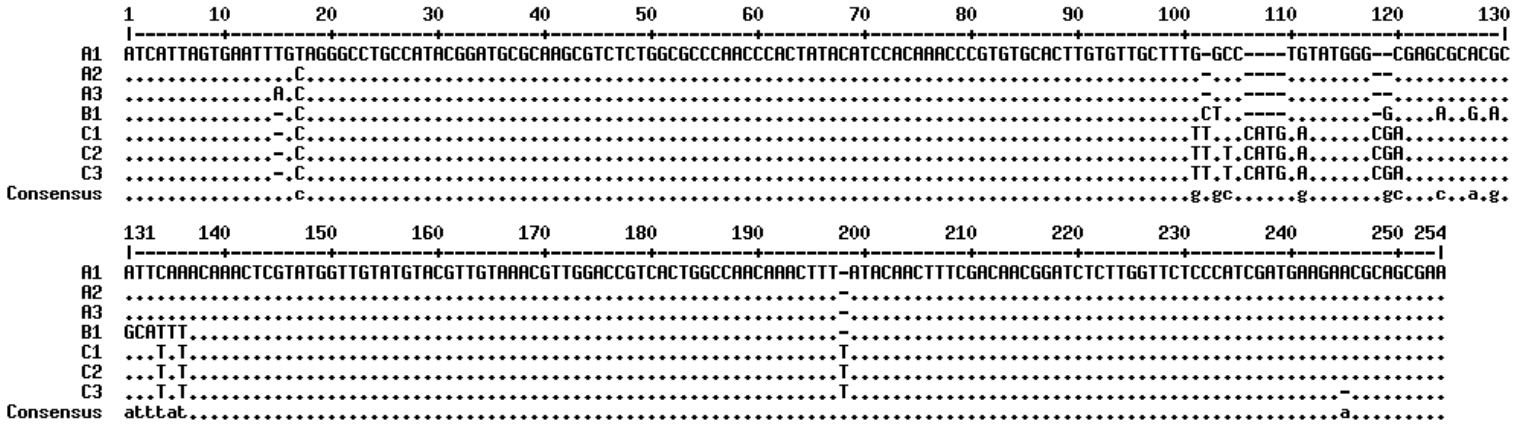


Figure 1. Alignment of ITS-1 sequence groups representing all *Malassezia pachydermatis* genotypes and/or subgenotypes. Dots(·) represent consensus regions, and dashes(-) represent deletions necessary for alignment.

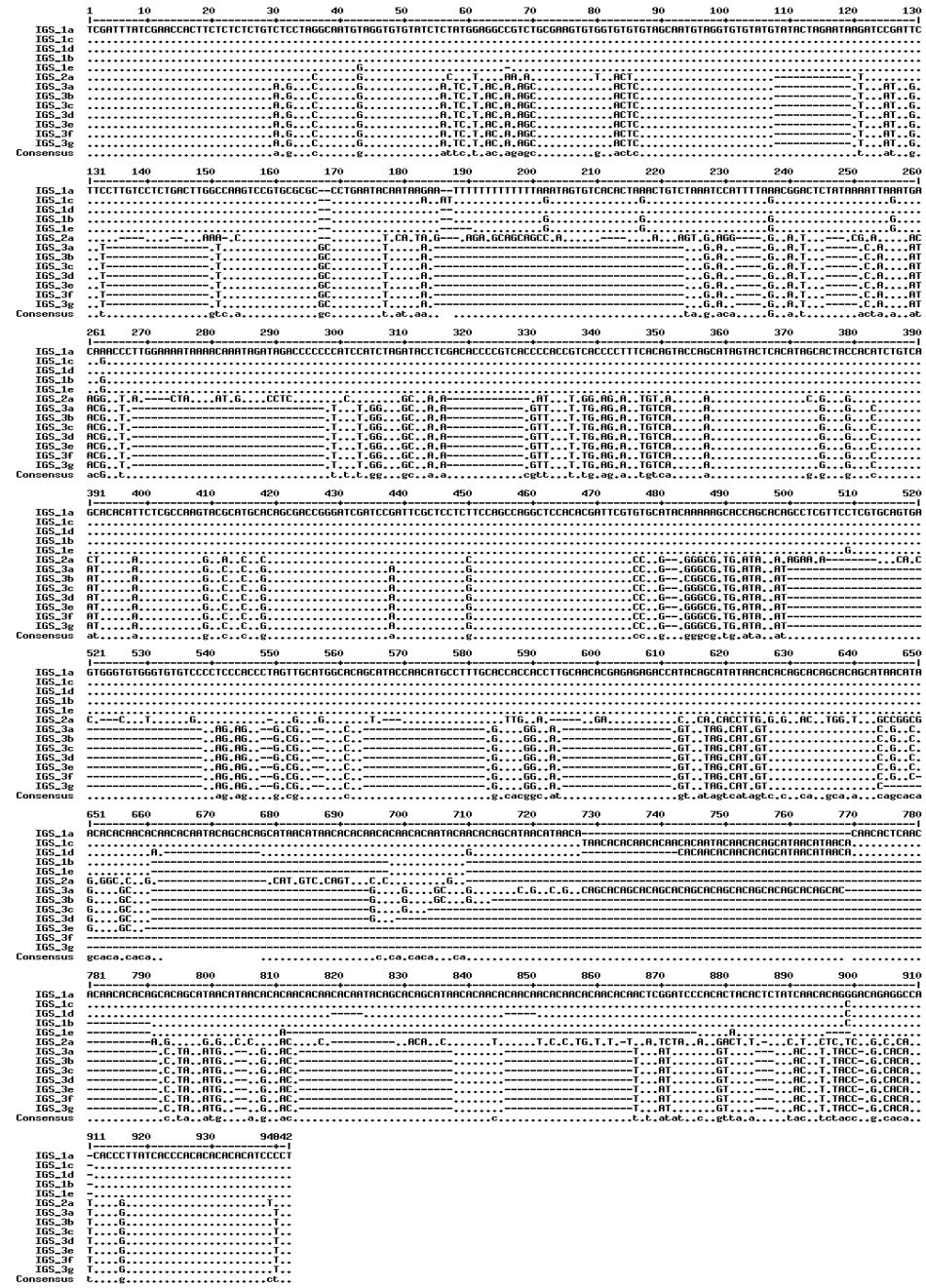
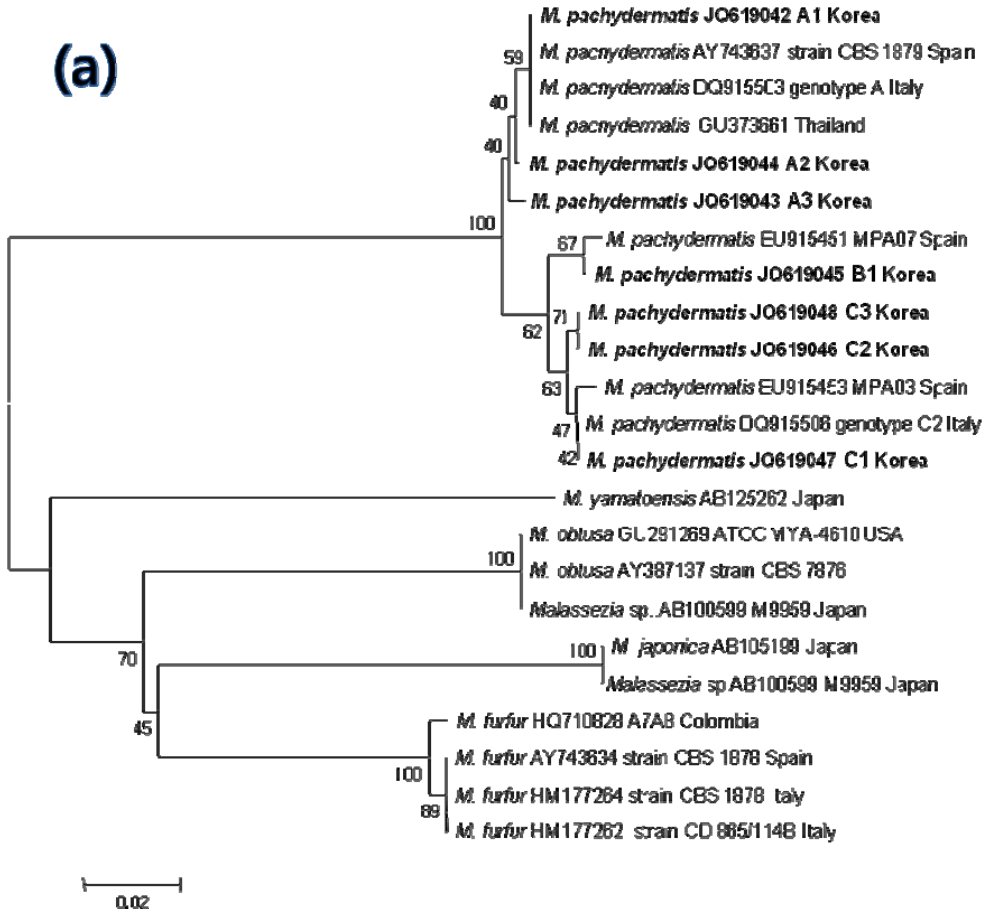


Figure 2. Alignment of IGS-1 region sequence groups representing all *Malassezia pachydermatis* genotypes and/or subgenotypes.


(a)
M. pachydermatis JQ619042 A1 Korea
M. pachydermatis AY743637 strain CBS 1879 Spain
M. pachydermatis DQ915503 genotype A Italy
M. pachydermatis GU373661 Thailand
M. pachydermatis JQ619044 A2 Korea
M. pachydermatis JQ619043 A3 Korea
M. pachydermatis EU915451 MPA07 Spain
M. pachydermatis JQ619045 B1 Korea
M. pachydermatis JQ619048 C3 Korea
M. pachydermatis JQ619046 C2 Korea
M. pachydermatis EU915453 MPA03 Spain
M. pachydermatis DQ915508 genotype C2 Italy
M. pachydermatis JQ619047 C1 Korea
M. yamatoensis AB125262 Japan
M. obtusa GU291269 ATCC MYA-4610 USA
M. obtusa AY387137 strain CBS 7876
Malassezia sp. AB100599 M9959 Japan
M. japonica AB105199 Japan
Malassezia sp AB100599 M9959 Japan
M. furfur HQ710828 A7A8 Colombia
M. furfur AY743634 strain CBS 1878 Spain
M. furfur HM177264 strain CBS 1878 Italy
M. furfur HM177262 strain CD 865/114B Italy
59
40
40
100
67
71
62
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70
100
45
100
89
0.02

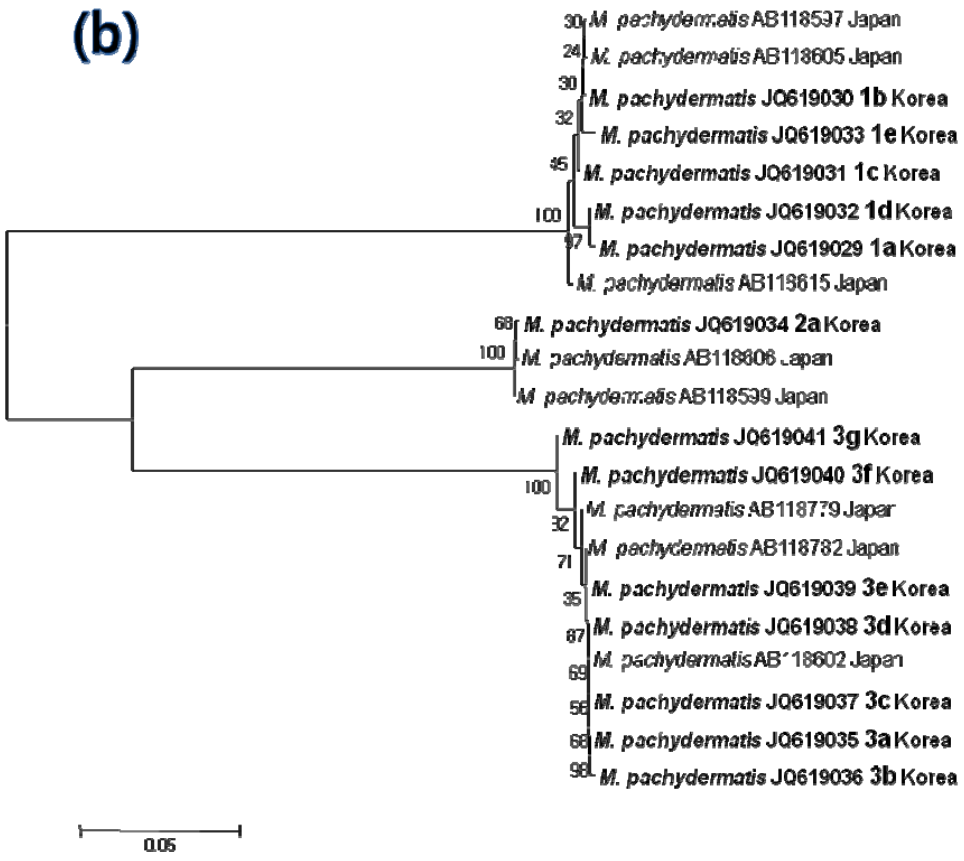

Figure 3. Phylogenetic analysis of ITS-1(a) and IGS-1(b) sequence data for *Malassezia pachydermatis* isolates using Neighbor-Joining method (Kimura-2 parameter). *Malassezia pachydermatis* strains(a, b) and other *Malassezia species* (*Malassezia furfur, Malassezia obtusa, Malassezia yamatoensis, Malassezia japonica*) (a) from GenBank™ (http://www.ncbi.nlm.nih.gov/Genbank/index.html) were employed as the out-group.

4. Identification of M. pachydermatis genotypes/subgenotypes

Based on analyses of the ITS-1 and IGS-1 regions, the frequency distributions of individual genotypes/subgenotypes were determined (Table 1). Six ITS-1 genotypes/subgenotypes were identified in Groups 1 and 2; three ITS-1 genotypes/subgenotypes were identified in Group 3, and four ITS-1 genotypes/subgenotypes were identified in Group 4. In Groups 1 and 2, 12 IGS-1 genotypes/subgenotypes were defined, whereas Groups 3 and 4 were associated with 4 different IGS-1 genotypes/subgenotypes each. The *M. pachydermatis* genotypes identified in ear canal samples were more variable than those detected at other sites. Overall, subgenotype A1 of ITS-1 and subgenotype 1a of IGS-1 were the most frequently detected. Genotype A was most prevalent in Groups 1, 2, and 4 ($P < 0.05$), but in Group 3 (healthy ear) there was no statistic difference between genotype A and C. Subgenotype B1 (2a) was the only sequence type in genotype B that was characterized in both the diseased ear and the interdigital area. Genotype C also was found on other skin sites but was more frequently detected from ear canal samples (36.5% – 55.5% vs. 5.1% – 13.3% at other sites, $P < 0.05$), regardless of healthy or diseased status.

Discussion

In total, 141 *Malassezia* isolates were obtained. Yeast was more frequently isolated from diseased sites than from healthy sites ($P < 0.05$), in accordance with previous studies [9, 10, 35]. The results may be affected by various factors including sampling methods. For example, studies using a sterile swab for sampling procedure, including this study, showed higher prevalence of *Malassezia* from skin-diseased specimen [10, 36]. On the other hand, direct cytological examination showed no difference between healthy ear and ear with otitis from the same study [36]. Breed and age might influence prevalence in *Malassezia* isolation [36, 37]. In addition, overpopulation seems to play an important role in inducing skin disease because a large inoculum of *M. pachydermatis* applied to the healthy ear resulted in otitis externa in dogs [38].

Presently, further genotyping of *Malassezia* species was done because phenotype identification is no longer accurate in *Malassezia* species identification [17]. Furthermore, the genetic distinction between *M. pachydermatis* isolates from dogs by diverse molecular methodology has been applied as an epidemiological tool. A greater genotypic diversity in *M. pachydermatis* has been reported with host [18], body distribution [25], healthy skin condition [39], and geographic origin [35]. The ITS-1 and IGS-1 regions were chosen in this study because these regions are associated with more substantial intraspecies diversity than the LSU, chs-2, or 26S rRNA gene fragment. The intraspecific variation of ITS-1 ranged from 0.4% – 6.4%, which suggests that this genomic region carries greater variability than general yeasts (<1%) [18, 40]. In the IGS-1 region, *M.*

pachydermatis sequences exhibited 41.6% – 99.0% similarity; in other *Malassezia* species, this value has been estimated to exceed 85% [18]. This suggests that the IGS-1 region of *M. pachydermatis* may be more diverse than in other *Malassezia* species, thus supporting its utility for strain characterization.

The ITS-1 and IGS-1 loci of *M. pachydermatis* isolated from dogs were in concordance with regard to grouping into three distinct genotypes (*i.e.*, A, B, and C) in agreement with a previous study [17]; this was further supported by phylogenetic analysis. The numbers and frequencies of each genotype according to dog groups are summarized in Table 2. The *M. pachydermatis* isolates were further categorized into subgenotypes on the basis of ITS-1 and IGS-1; ITS-1 regions had four sequences identical to those deposited previously (GenBank accession numbers: A1, DQ915503; A2, EU158826; B1, DQ915504; C2, DQ915505), and three novel sequences were identified as shown above. IGS-1 region had one sequence matched a sequence already deposited in GenBank (3d, AB118602); 12 sequences were novel Three new ITS-1 sequences and 12 new IGS-1 sequences were characterized in this study, respectively. But both loci were too variable to allow for estimation of relationships between subgenotypes and anatomical sites of isolation.

Table 2. Positive number of *Malassezia pachydermatis* genotypes/subgenotypes for ITS-1(A,B,C) and IGS-1 (1,2,3) from anatomical sites of collection.

Status	Body Parts	ITS-1								IGS-1													
		A1	A2	A3	B1	C1	C2	C3	total	1a	1b	1c	1d	1e	2a	3a	3b	3c	3d	3e	3f	3g	total
Normal skin	ear	4	-	-	-	1	4	-	9	4	-	-	-	-	-	-	1	-	3	-	1	-	9
	other skin	12	-	1	-	1	-	1	15	9	4	-	-	-	-	-	-	-	1	-	-	1	15
Skin lesions	ear	26	4	2	1	4	15	-	52	19	2	1	1	5	1	2	1	2	3	2	8	-	47
	other skin	52	1	1	1	1	2	-	58	39	3	4	1	-	1	1	-	1	1	-	-	-	56

Genotype A was detected on most of the sampled body parts, indicating its common distribution. This is in agreement with a previous report that examined both healthy and diseased skin sites of dogs [35]. Others have reported that genotype B was localized exclusively to healthy skin [25], and that it was present in Japan and Europe [17, 41] but not Brazil [35]. In this study, two isolates obtained from an ear exhibiting otitis externa and from an interdigital area with dermatitis were classified as genotype B, thus implying that genotype B is present in Korea and occurs on diseased skin. The present study indicates that genotype C was found on various skin samples but was more likely to be identified in ear samples than on other skin sites. The number of samples categorized into healthy groups was smaller than the numbers in the diseased groups, but genotype C also was predominantly found in the healthy ear compared to other healthy body regions as with diseased groups. The external ear canal is associated with unique physiological features, including lipid-rich cerumen (earwax) and high humidity due to its unique anatomical peculiarity [42]. The cerumen of canine external ears may facilitate the growth of *M. pachydermatis* [43], whereas human cerumen exhibits antifungal properties [44]. Additional factors of the skin microenvironment, including bacterial microbiota, pH, salts, the immune response, and biochemical and physiological features are considered to play an important role in yeast colonization. [45] Pathogenicity of the yeast could be determined on its adaptation on skin with different microenvironment, such as lipid content or humidity. The unique properties of the external ear canal may explain why *M. pachydermatis* genotype C was more frequently represented at this site. Accordingly, .

pachydermatis genotype C might have affinity to colonize sebum-rich and humid environments.

Heterogeneity of *M. pachydermatis* and different character of each group were addressed in a previous study. Kiss et al. [46] reported that *M. pachydermatis* is a heterogeneous species and can be divided into two groups by comparing morphological, cultural, and biochemical features. In this study, *M. pachydermatis* was categorized in three genotypes by genetic features; genotype C showed unique pathologic and physiologic natures. Based on these results, one can suggest that different genotypes have different characteristics, and that there is a possibility that *M. pachydermatis* can be further divided into more detailed classification.

This study clearly shows that *M. pachydermatis* has a variety of genotypes/ subgenotypes, and that the virulence and/or pathogenicity may be linked to this diversity. In particular, genotype C of *M. pachydermatis* was predominantly found from the external ear canal of dogs than from other body sites. The frequent colonization or pathogenesis of genotype C in the ear canal is still not clearly explained, but Machado *et al.* reported that the distribution of particular *M. pachydermatis* genotypes over skin surfaces may vary depending on individual predispositions [35]. Also, different genotypes are associated with varying virulence [17, 47]. The immune responses of the host may play an important role in distribution of yeast colonization. The increased diversity found in this study could not only be due to geographic difference, but also because *Malassezia* yeast is adapting to various environment such as lipid contents by

genetic polymorphism. However, further study is needed to reveal the physiologic and pathologic features of this particular *M. pachydermatis* genotype. The present findings provide prospects for further discovery of diversity in *M. pachydermatis* along with its pathogenic nature.

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국문 초록

한국에서 개의 귀와 피부에서 분리한 *Malassezia pachydermatis*의 유전자형 분석

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Malassezia 종은 온혈 동물의 피부에서 상재하는 정상 미생물총으로, 환경이나 조건에 따라 병원성을 가지고 병변을 일으키기도 하며 습윤하거나 유분이 많은 환경에서 더 잘 성장한다고 알려져 있다. 개의 외이도는 다른 피부부위보다 더 많은 apocrine gland가 분포하고, 폐쇄적인 해부학적인 구조로 인

하여 습윤하고 유분이 많은 환경이 유지되는 특성이 있다. 본 연구는 이러한 개의 외이도의 환경이 *Malassezia*의 유전자형의 발현에 어떤 영향을 끼치는지, 또한, 병변의 유무에 따라 *Malassezia*의 분포가 어떻게 차이 나는지에 대해서 알아보기 위하여 실시되었다.

2010년과 2011년에 서울대학교 동물병원에 내원한 개 환자를 대상으로 skin swab을 통해서 총 134개의 샘플이 얻어졌으며, 이는 병변이 있는 귀와 피부, 건강한 귀와 피부 즉, 총 4개의 그룹으로 분류되었다. 얻어진 샘플의 종을 확인하기 위해 26s ribosomal RNA gene fragment 부위의 direct sequencing을 하고, 이에 더하여 세분화된 유전형으로 분류하기 위해 Internal transcribed spacer 1 (ITS-1)과 Intergenic spacer 1 (IGS-1) 부위를 direct sequencing을 하여 phylogenetic analysis를 실시하였다. 26s rDNA부위의 분석으로 모든 샘플이 *M.pachydermatis*로 확인되었고, 세분화된 유전형의 분석에서 ITS-1과 IGS-1 두 부위 모두 크게 3개의 유전형 (유전형 A, B, 그리고 C)로 나타났다. 이 중 유전형 C는 귀에서 36-55%, 다른 피부 부위에서 5-13%로 병변의 유무와 상관 없이 유의적으로 더 자주 분리 되었으며, 이런 부위에 대하여 선택적인 *M. pachydermatis*의 유전형 분리는 유분이 많고 습도가 높은 귀의 특징적인 환경이 특정 *Malassezia*의 colony형성에 영향을 미친다는 사실을 보여준다.

주요어: ***Malassezia pachydermatis*, intergenic spacer 1 (IGS-1), internal transcribed spacer 1 (ITS-1)**

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