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Research Article

Molecular characterization of *Malassezia pachydermatis* isolates obtained from cases of dermatitis and otitis externa in dogs

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Abstract

***Malassezia pachydermatis* is the most common cause of malassezia dermatitis and otitis in dogs. Though this condition has become fairly common in dogs in Kerala, only very few attempts have been made to isolate and characterize this yeast from these cases. In this study, skin and ear swabs from suspected cases were cultured on Sabouraud dextrose agar (SDA) with 0.05 per cent chloramphenicol. The isolates obtained were initially characterized based on colony characteristics, the result of Gram's staining and microscopic morphology. Total DNA was extracted from the pure cultures of the isolates and subjected to confirmation by polymerase chain reaction (PCR) targeting large subunit ribosomal RNA gene followed by nucleic acid sequencing. Phylogenetic analysis was carried out using MEGA X software. In this study, molecular techniques were used to successfully characterize *M. pachydermatis* isolates obtained from skin and ear infection in dogs, and all the isolates were of Type I.**

Key words: Dermatitis, *Malassezia pachydermatis*, Otitis, PCR, Phylogeny

Highlights

- Type I *Malassezia* spp. are prevalent in clinical conditions in dogs in Kerala.
- Molecular techniques can be used successfully to characterize *M. pachydermatis* isolates from skin and ear infection in dogs.

INTRODUCTION

Malassezia yeasts form a unique cluster of lipophilic fungi living almost exclusively on the skin and mucosal sites of humans and warm-blooded vertebrates. *Malassezia pachydermatis* is frequently found on wild and domestic carnivores and has been considered as zoophilic. This yeast is present on the skin and in the external ear canal of healthy dogs. *Malassezia pachydermatis* is the most common cause of Malassezia dermatitis and otitis in dogs. Several typing methods have been used to understand the diversity of *Malassezia pachydermatis*, including techniques like karyotyping and random amplified polymorphic DNA. Nowadays, sequencing has become the most widely used method. In particular, sequencing of the large subunit (LSU), internal transcribed spacer 1 region (ITS-1), the intergenic spacer 1 (IGS-1) of ribosomal RNA and chitin synthase 2 gene (CHS2) has been performed for taxonomic and epidemiological purposes, mainly using strains isolated from dog skin (Guillot *et al.*, 1997). These studies have revealed a considerable genetic diversity among *Malassezia pachydermatis*. In Kerala, only very few attempts have been made to isolate and characterize this yeast from clinical conditions in dogs. The present study reports the characterization of *M. pachydermatis* strains isolated from clinical conditions in dogs.

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MATERIALS AND METHODS

Twelve dogs presented at Teaching Veterinary Clinical Complex, College of Veterinary and Animal Sciences, Pookode, with the clinical signs of alopecia, pruritus, erythema, hyperpigmentation, foul odour and otitis externa were examined. Cytological examination of skin and ear swabs were carried out after Gram's staining of smears made from the swabs. The cultural examination was done by inoculating the samples onto Sabouraud dextrose agar (SDA) with 0.05 per cent chloramphenicol and incubating at 37°C for up to 7 days. Total DNA was extracted from the pure cultures of the isolates using the conventional phenol-chloroform method and was subjected to polymerase chain reaction (PCR) targeting large subunit ribosomal RNA gene as described previously (Guillot *et al*., 2000). PCR reactions were performed in a 25 µL reaction volume containing 12.5 µL of Mastermix [EmeraldAmp GT PCR Master Mix (2X Premix)], 6 µL of NFW, 2 µL of each Forward primer and Reverse primer and 2.5 µL of Template DNA. A no template control was also kept to detect if there was any contamination in the test reagents. PCR reactions were conducted in an automated thermal cycler with a heated lid (Eppendorf, Germany) for 2 hours 10 minutes. The amplification conditions included an initial degeneration step at 95°C for 3 min, followed by 34 cycles of 30 at 94°C for degeneration, 1 min at 55°C for annealing and 1 min at 72°C for extension. A final extension step was carried out at 72°C for 5 min. Representative amplicons obtained were purified and extracted by the PureLink Quick gel extraction kit (Invitrogen). DNA nucleotide sequence determination was carried out by the sequencing service, Agri-genome, Kochi with the same primers that were used for amplification. Automated Sanger dideoxynucleotide sequencing method was used for bidirectional sequencing. Sequences were edited and aligned by using the BioEdit application and EditSeq (DNA Star), and global sequence comparisons were performed by using basic alignment search tool (BLAST) hosted by the National Center for Biotechnology Information (NCBI). The nucleotide sequences obtained were submitted to the GenBank, and accession numbers were obtained (Table 1). Molecular characterization of *Malassezia pachydermatis* isolates was carried out by comparing the obtained sequences with reference LSU ribosomal RNA sequences of various types of *Malassezia pachydermatis* as described by Puig *et al*. (2016) (Table 2). Phylogenetic analysis was carried out using MEGA X software. The evolutionary history was inferred by using Maximum Likelihood method. The bootstrap consensus tree inferred from 100 replicates was taken to represent the

Table 1. GenBank accession number of nucleotide sequences of large subunit region of ribosomal RNA of *Malassezia pachydermatis* isolated in the study

Sl. No.	Isolate	Accession No.
1	MAL 1	MW380597
2	MAL 2	MW380598
3	MAL 3	MW380599
4	MAL 4	MW380600
5	MAL 6	MW380602
6	MAL 7	MW380603
7	MAL 8	MW380604
8	MAL 9	MW380605
9	MAL 10	MW380606
10	MAL 11	MW380607
11	MAL 12	MW380608
12	MAL 13	MW380609

Table 2.GenBank accession number of reference sequences of five types of *Malassezia pachydermatis*

Sl. No.	Reference sequences of *Malassezia pachydermatis* types on the basis of LSU region	Accession No.
1	Type I	AY743605
2	Type II	KU313705
3	Type III	KU313706
4	Type IV	KU313707
5	Type V	KU313708

evolutionary history of the sequences analyzed. The evolutionary distances used to infer the phylogenetic trees were computed using the Kimura 2 parameter method (Kumar *et al*., 2016). Dogs with Malassezia were divided into two treatment groups comprising six animals each. The dogs in the first and second treatment groups were treated with itraconazole and fluconazole, respectively. Both treatments were given @ 5 mg per kg body weight, orally, once daily for 28 days. Necessary supportive therapy including antimicrobials in case of associated pyoderma, antihistaminics and essential fatty acids were given to animals under the treatment groups. Appropriate treatment measures were adopted for animals diagnosed with concurrent underlying disease.

RESULTS

Microscopical examination of smears revealed the presence of Gram-positive budding yeast cells on methylene blue staining (Fig. 1). Twelve isolates suggestive of *Malassezia* spp. were obtained on SDA with 0.05% chloramphenicol. The colonies were raised or high convex and smooth with white or cream colour (Fig. 2). Of these, eight were isolated from skin lesions of dogs and the rest were obtained from cases of otitis externa. All the twelve isolates were positive in the PCR targeting LSU region of *Malassezia* spp. (Fig. 3). On BLAST analysis, all the sequences showed similarity to sequences of *M. pachydermatis* from India. Molecular typing grouped all the 12 isolates into Type I (Fig. 4).

DISCUSSION

Malassezia spp. are commensals of the normal cutaneous microbiota of humans and animals.These yeasts may become opportunistic pathogens under certain conditions and cause dermatitis in dogs. According to Guillot *et al.* (1996), *M. pachydermatis* has been characterized microscopically as small ovoid cells with a very broad base. The yeast was catalase negative or very weak and in the Tween assimilation test, its growth is slightly inhibited by Tween 20. Gueho *et al.* (1996) reported urease production by *M. pachydermatis* strains. The phenotypic identification based on the microscopic and physiological method is difficult and time-consuming. Besides, conventional isolation and characterization techniques, many methods have been used for the characterization of *Malassezia* spp. Previous analysis demonstrated the usefulness of highly variable regions of LSU rRNA for epidemiological purposes within the genus *Malassezia* (Guillot and Gueho, 1995). As per this study, different strains of *Malassezia* spp. isolated from humans and animals were compared using LSU ribosomal RNA sequence similarity and nuclear DNA complementarity. Brito *et al*. (2007) reported that strain characterization of *M. pachydermatis* by phenotypic and molecular analysis gave important information that may be related to pathogenicity, antifungal resistance and epidemiology features. Cafarchia *et al*. (2007) studied molecular characterization of different isolates of *Malassezia* spp. (lipid dependent or

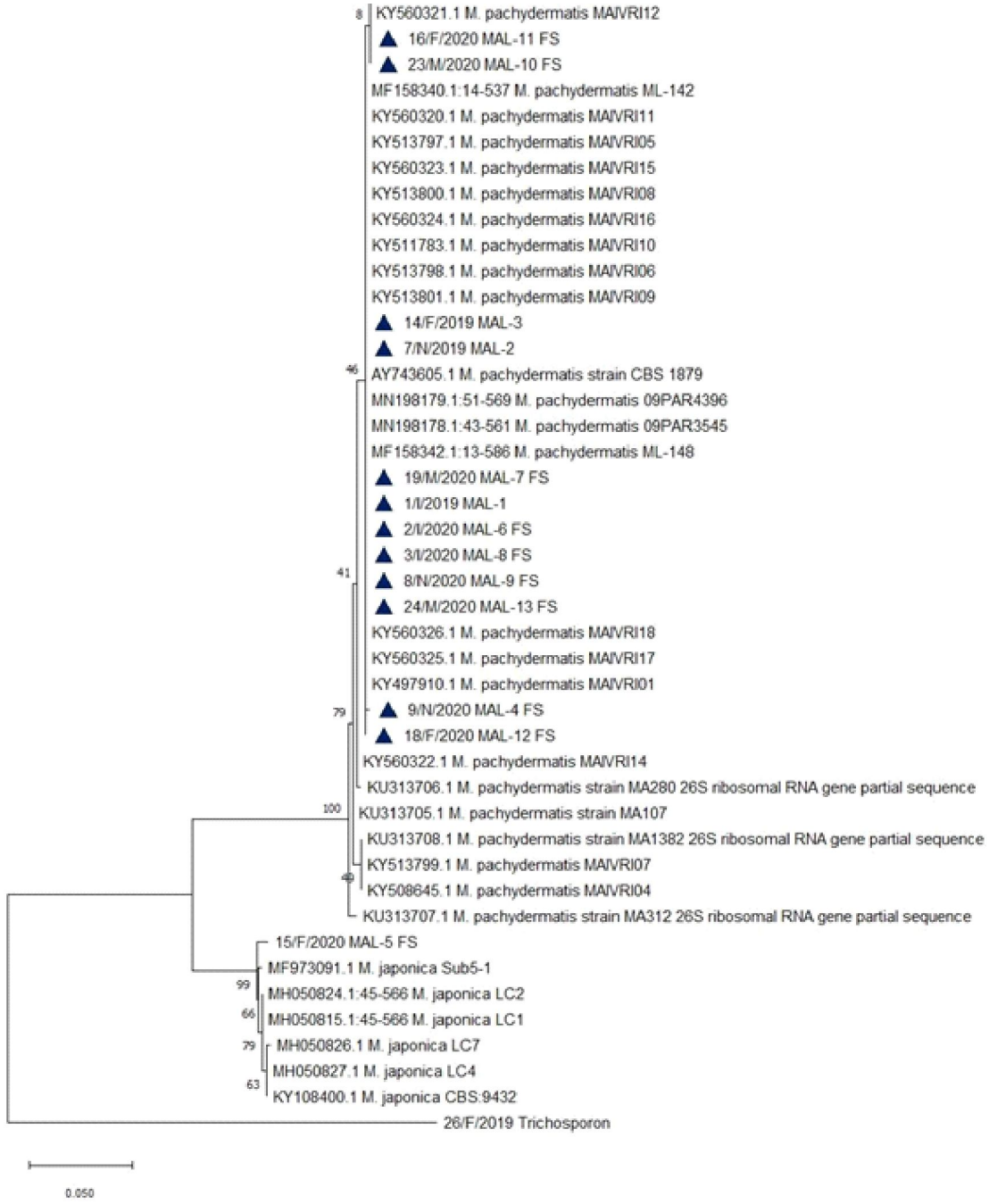

Fig. 4. Phylogenetic tree constructed using the large subunit ribosomal RNA sequences of *Malassezia pachydermatis* isolates (Legend: filled triangles indicate isolates obtained in the study; bracket indicate Type I sequences of *M. pachydermatis*)

non-lipid dependent) obtained from dogs based on their chitin synthase 2 gene (CHS2), the large subunit (LSU) and the first internal transcribed spacer (ITS-1) of nuclear ribosomal DNA sequences. The results were compared genetically with well-defined reference strains of *Malassezia pachydermatis* and heterologous species including *Malassezia furfur* and *Candida albicans.* The application of a simple PCR method provides a sensitive and rapid

identification system for *Malassezia* species, which may be applied in epidemiological surveys and routine practice (Affes *et al*., 2009). Puig *et al*. (2017) studied the characterization of the species *M. pachydermatis* and in the phylogenetic tree of the LSU ribosomal RNA sequences, lipid-dependent strains were grouped and interspersed with the non-lipid dependent *M. pachydermatis* strains.

In the present study, molecular techniques were used to successfully characterize *M. pachydermatis* isolates obtained from skin and ear infection in dogs, and all the isolates were of Type I. This adds to the knowledge of the types of *Malassezia* spp. prevalent in clinical conditions in dogs in Kerala.

Conflict of interest: Authors have no conflict of interest in this study.

Author's contribution: AKD: Data collection, analysis and interpretation; PVD and CR: Supervised the research; SKS: Contributed to the implementation of the research.

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REFERENCES

Affes M, Salah SB, Makni F, Sellami H and Ayadi A, 2009. Molecular identification of *Malassezia* species isolated from dermatitis affections. Mycoses, 52(3): 251-256, doi: 10.1111/j.1439-0507.2008.01589.x

Brito EH, Fontenelle RO, Brilhante RS, Cordeiro RA, Junior FAS *et al*., 2007. Phenotypic characterization and *in vitro* antifungal sensitivity of *Candida* spp. and *Malassezia pachydermatis* strains from dogs. Vet J, 174(1): 147-153, doi: 10.1016/j.tvjl.2006.05.021

Cafarchia C, Latrofa MS, Testini G, Parisi A, Guillot J *et al*., 2007. Molecular characterization of *Malassezia* isolates from dogs using three distinct genetic markers in nuclear DNA. Mol Cell Probes, 21(3): 229-238, doi: 10.1016/j.mcp.2007.01.002

Gueho E, Midgley G and Guillot J, 1996. The genus *Malassezia* with description of four new species. Antonie van Leeuwenhoek, 69(4): 337-355, doi: 10.1007/BF00399623

Guillot J and Gueho E, 1995. The diversity of Malassezia yeasts confirmed by rRNA sequence and nuclear DNA comparisons. Antonie van Leeuwenhoek, 67(3): 297-314, doi: 10.1007/BF00873693

Guillot J, Deville M, Berthelemy M, Provost F and Gueho E, 2000. A single PCR-restriction endonuclease analysis for rapid identification of *Malassezia* species. Lett Appl Microbiol, 31(5): 400-403, doi: 10.1046/j.1472-765x.2000.00839.x

Guillot J, Gueho E, Chevrier G and Chermette R, 1997. Epidemiological analysis of *Malassezia pachydermatis* isolates by partial sequencing of the large subunit ribosomal RNA. Res Vet Sci, 62(1): 22-25, doi: 10.1016/s0034-5288(97)90174-0

Guillot J, Guero E, Lesourd M, Midgley G, Chevrier G *et al*., 1996. Identification of *Malassezia* species. J Mycol Med, 6: 102-110

Kumar S, Stecher G and Tamura K, 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol, 33(7): 1870-1874, doi: 10.1093/molbev/msw054

Puig L, Bragulat MR, Castella G and Cabanes FJ, 2017. Characterization of the species *Malassezia pachydermatis* and re-evaluation of its lipid dependence using a synthetic agar medium. PLoS One, 12(6): e0179148, doi: 10.1371/journal.pone.0179148

Puig L, Castella G and Cabanes FJ, 2016. Cryptic diversity of *Malassezia pachydermatis* from healthy and diseased domestic animals. Mycopathologia, 181(9-10): 681-688, doi: 10.1007/s11046-016-0026-3

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