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Physiological and Molecular Characterization of *Malassezia pachydermatis* Reveals No Differences between Canines and Their Owners

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Abstract

Introduction: The genus *Malassezia* comprises 17 species of commensal and pathogenic yeasts of homeotherms animal skin. The most common species are *M. furfur*, *M. globosa*, and *M. sympodialis* in humans and *M. pachydermatis* in animals. However, some publications have reported potentially serious human infections by *M. pachydermatis* in individuals with risk factors and the isolation of human species from domestic animals. Given the scarcity of information about their capacity for transmission between hosts and zoonotic potential, the aim of the present study was to physiologically and molecularly characterize *Malassezia* spp. isolates obtained from canines and their human owners. **Materials and Methods:** An experimental study was conducted at the Veterinary Clinic of Universidad de Ciencias Aplicadas y Ambientales of Bogotá (Colombia) from July 2015 to December 2016. Phenotypic identification and molecular characterization via the amplification of the 5.8S rDNA-ITS2 and 26S rDNA gene regions, nucleic acid sequencing, and phylogenetic analyses were performed on isolates originating from canines with otitis externa and from the skin of healthy owners compatible with *Malassezia* spp. **Results:** Eighty samples were cultured, of which 32 (40%) were suggestive of *Malassezia* spp. A total of 29 out of 46 (63%) isolates in canines and 3 out of 34 (9%) isolates in humans corresponded entirely with *M. pachydermatis*. Isolates from the canines and their owners presented similar behavior in biochemical and phospholipase activity tests, 100% molecular sequence identities, and close proximity in the phylogenetic trees. **Conclusion:** The isolation of *M. pachydermatis* from humans and their dogs with identity based on biochemical, physiological, molecular, and phylogenetic perspectives indicate the

ability of this species to adapt to new hosts and its potential for zoonotic transmission. These findings contribute to knowledge of the ecology of this important fungus in human and veterinary medicine.

Keywords

Malassezia, Transmission, Animals, Humans, Zoonosis

1. Introduction

The genus *Malassezia* comprises lipophilic, lipid-dependent, and non-lipid-dependent yeasts that are commensal on the skin of humans and animals. These yeasts may become pathogenic in the presence of predisposing factors, such as changes in the cutaneous microenvironment or alterations of host defense mechanisms [1] [2].

The identification of different species within the genus has traditionally been based on phenotypic tests [3]. More recently, identification has depended on molecular biology techniques based on the polymerase chain reaction (PCR) and the sequencing of different genes, such as genes of the ribosomal DNA complex (rDNA), including the 26S and 5.8S subunits [1] [4] [5]. These molecular studies have contributed to our understanding of the biodiversity of the genus and clarified some aspects of its taxonomy and phylogenetic relationships [6] [7] [8], which has led to the determination of new species. Currently, 17 species are recognized [9]-[17].

Species such as *M. furfur*, *M. globose*, and *M. sympodialis* are part of the human microbiota and have the ability to produce dermatological syndromes, such as pityriasis versicolor, and to exacerbate diseases, such as seborrheic dermatitis, atopic dermatitis, and psoriasis, as well as systemic syndromes in immunocompromised patients [2] [18]. However, these species have occasionally been reported in domestic animals [19]-[25].

M. pachydermatis is the species isolated most often from the skin and mucosa of mammals (mainly canines), in which it can produce otitis externa and dermatitis [26]. However, this species has been reported in potentially serious human infections of individuals with associated risk factors, including some health personnel with contact with dogs [25] [27] [28] [29] [30].

Whether an ecological relationship exists between the presence of this fungus in domestic animals and their owners is not completely clear because few studies have evaluated its capacity for transmission between hosts and the zoonotic potential [31] [32] [33] [34]. Therefore, the objective of the present study was to physiologically and molecularly characterize *Malassezia* spp. isolates obtained from canines and their human owners.

2. Materials and Methods

1) Isolation of *Malassezia* spp.:

Skin or external auditory canal swabs were performed on canines with pathologies such as dermatitis or otitis externa and on the skin of healthy owners who attended the Veterinary Clinic of Universidad de Ciencias Aplicadas y Ambientales of Bogota (Colombia) between October 2015 and September 2016. All samples were immediately transported to the microbiology laboratory in a sterile tube containing distilled water and 0.05% Tween 40 [19] [35] at room temperature. Each sample was inoculated onto modified Dixon agar medium [3] and incubated at 32˚C for 5 days.

2) Phenotypic identification:

A smear and Gram staining were performed from the inoculations in which colonies compatible with *Malassezia* spp. were observed to evaluate the microscopic morphology. Additionally, a new isolation was conducted to obtain pure colonies and to perform phenotypic identification at the species level using the following biochemical tests: urease [36], catalase, β-glucosidase [37], Cremophor-EL assimilation [38], Tween assimilation, growth on Sabouraud agar [38], 39], and growth on Dixon agar at 37˚C and 40˚C [38] [40]. The physiological test for phospholipase activity on Sabouraud agar supplemented with egg yolk was also performed with determination of Pz index [41].

3) Quality control and maintenance of strains:

The following reference strains from the Central Bureau voor Schimmelcultures (CBS) were used: *M. furfur* CBS 7019, *M. pachydermatis* CBS 1879, *M. sympodialis* CBS 7222, and *M. slooffiae* CBS 7956. These strains and the obtained isolates were conserved in skim milk medium at −20˚C.

4) Extraction of genomic DNA:

The reference strains and clinical isolates were cultured on modified Dixon agar for 72 hours at 32˚C. Subsequently, DNA extraction was performed using a Fungi/Yeast Genomic DNA Isolation Kit (Norgen®) according to the manufacturer's instructions. The DNA was stored at −20˚C prior to use [42].

5) Amplification of ribosomal genes:

The primers used to amplify the 5.8S rDNA-ITS2 target sequence were ITS3 (5'-GCATCGATGAAGAACGCAGC-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') [39] [43]. The amplification was performed by PCR in a 25-µL reaction volume containing 10X buffer, 2 mM $MgCl_2$, 1 µmol of each primer, 0.2 mM dNTPs (Thermo Scientific®), 1 U of Taq polymerase (Bioline®), and 1 µL of genomic DNA under the following conditions: an initial denaturation cycle at 95˚C for 5 minutes, 25 cycles at 95˚C for 1 minute, 55.4˚C for 30 seconds, and 72˚C for 1 minute, and a final extension cycle at 72˚C for 5 minutes. The 5'-TAACAAGGATTCCCCTAGTA-3' and 5'-ATTACGCCAGCATCCTAAG-3' primers were used to amplify the 26S rDNA target sequence [4] [39]. The amplification was performed by PCR in a 25-µL reaction volume containing 10X buffer, 1.5 mM $MgCl_2$, 1 µmol of each primer, 0.2 mM dNTPs (Thermo Scientific®), 1 U of Taq polymerase (Bioline®), and 1 µL of genomic DNA under the following conditions: an initial denatura-

tion cycle at 95˚C for 5 minutes, 25 cycles of 95˚C for 1 minute, 51.7˚C for 30 seconds, and 72˚C for 1 minute, and one final extension cycle at 72˚C for 5 minutes. The amplification products were examined by electrophoresis on 1.5% agarose gels with 1X TAE buffer and stained with EZ-Vision®.

6) Sequencing and phylogenetic analysis:

The amplification products corresponding to the 5.8S rDNA-ITS2 and 26S rDNA regions were sent to Macrogen®, Inc. (Korea) for purification and sequencing. The DNA sequences were edited and assembled manually using Geneious v7.0.6 software (created by Biomatters, available at http://www.geneious.com) and subsequently compared with GenBank sequences using the National Center for Biotechnology Information (NCBI) BLASTn tool for identification at the species level. Each data set was aligned using the MUSCLE v3.8.31 algorithm [44] using default parameters, and conserved blocks were selected using Gblocks v0.91b [45]. Phylogenetic reconstruction from the alignments of the conserved blocks was performed using MEGA v7.0.21 software [46]. First, we determined that the evolutionary models that best explained the alignments were T92+G+I and K2. The maximum likelihood methodology was used for the reconstruction of trees under the selected models, and statistical robustness was achieved with 2000 bootstrap replicates. The trees were edited using the iTOL v3. tool [47].

3. Results

A total of 80 samples were collected, including 46 samples of canine origin and 34 of human origin. The average age was 5 years for the canines and 33 years for the humans. A total of 37% of the canines were females and 63% were males, whereas 38% of the humans were females and 62% were males. The demographic and clinical characteristics of the canines and humans included in the study are shown in **Table 1**.

Thirty-two positive cultures were obtained for *Malassezia* spp., of which 29 were of canine origin and 3 were of human origin. Of these isolates, 90.6% were identified as *M. pachydermatis* by phenotypic tests. The remaining isolates were not conclusive for identification at the species level. The phospholipase activity of the studied isolates was high in 87.5% of the cases, very high in 6.25% of the cases, and null in 6.25% of the cases as shown by Pz indexes (**Table 2**).

Amplification of the 5.8S rDNA-ITS2 region was performed for the molecular characterization of the isolates. A band of approximately 500-bp was obtained for all isolates of both canine and human origin, at the same height as the reference strain *M. pachydermatis* CBS 1879 (**Figure 1(a)**). A band of approximately 550-bp was obtained for the 26S rDNA region for all isolates of both canine and human origin, at the same height as the reference strain *M. pachydermatis* CBS 1879 (**Figure 1(b)**). Note that the molecular size of the reference strain *M. furfur* CBS 7019 is slightly higher. These findings allowed to suspect the molecular identification of *M. pachydermatis.*

Table 1. Demographic and clinical characterization of the study population.

	Canines	Humans
Cases	46 (57.5%)	34 (42.5%)
Age (years)	5.1 (0.3 - 15)	33.4 (17 - 57)
Sex		
Female	17 (37%)	13 (38.2%)
Male	29 (63%)	21 (61.8%)
Breed		
Mixed breed	13 (28.3%)	-
Bulldog	7 (15.2%)	-
Golden retriever	6 (13%)	-
Pitbull	4 (8.7%)	-
Other	16 (34.8%)	-
Type of ear		
Drop	39 (84.8%)	-
Erect	7 (15.2%)	-
Type of hair		
Short	24 (52.2%)	-
Long	22 (47.8%)	-
Lesion site		
Skin	6 (13%)	4 (11.8%)
Ear	44 (95.6%)	-
None	0	30 (88.2%)
Signs and symptoms		
Erythema	15 (32.6%)	1 (2.9%)
Desquamation	5 (10.9%)	2 (5.9%)
Excoriations	3 (6.5%)	0
Scratching	12 (26.1%)	2 (5.9%)
Head movements	8 (17.4%)	-
Exudate	23 (50%)	0
Fetid odor	12 (26.1%)	-
Pruritus	-	4 (11.8%)
Alterations in cutaneous pigmentation	-	2 (5.9%)
None	12 (26.1%)	28 (82.3%)
Previous otitis		
Yes	17 (37%)	-
No	29 (63%)	-
Previous dermatitis		
Yes	10 (21.7%)	5 (14.7%)
No	36 (78.3%)	29 (85.3%)
Pathological antecedents		
Endocrine	2 (4.3%)	0
Dermatological	1 (2.2%)	2 (5.9%)
Other	8 (17.4%)	3 (8.8%)

Continued

None	36 (78.3%)	29 (85.3%)
Previous treatments		
Antimicrobials	7 (15.2%)	0
Immunosuppressants	5 (10.9%)	1 (2.9%)
Other	11 (23.9%)	3 (8.8%)
None	33 (71.7%)	31 (91.2%)

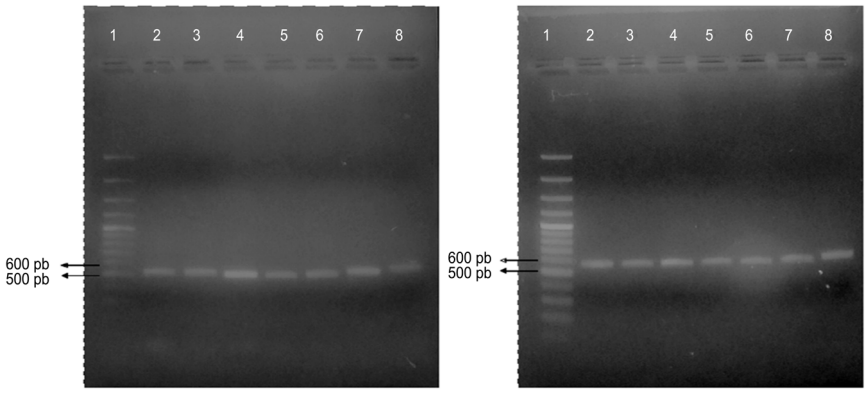
Table 2. Results of biochemical tests for phenotypic identification.

Isolates	Origin	Morphology	SDA	TDT				Cremophor	Catalase	Urease	β-glucosidase	Growth on Dixon			Pz index	Identification
				20	40	60	80					32˚C	37˚C	40˚C		
M. pachydermatis CBS 1879	R	E	±	+	+	+	+	+	+	+	+	+	+	+	0.76	-
M. furfur CBS 7019	R	E	-	+	+	+	+	+	+	+	±	+	+	+	0.69	-
M. sympodialis CBS 7222	R	E	-	±	+	+	+	+	+	+	+	+	+	+	0.60	-
M. slooffiae CBS 7956	R	C	-	+	+	+	-	-	+	+	-	+	+	+	1.00	-
001C	C	E	+	+	+	+	+	+	±	+	-	+	+	+	1.00	*M. pachydermatis*
003C	C	E	±	+	+	+	+	+	±	+	-	+	+	+	0.71	*M. pachydermatis*
003H	H	E	±	+	+	+	+	+	±	+	-	+	+	+	0.76	*M. pachydermatis*
004C	C	E	+	-	+	+	-	+	±	+	-	+	+	±	0.75	*M. pachydermatis*
005C	C	E	±	+	+	+	+	+	±	±	±	+	+	+	0.76	*M. pachydermatis*
008C	C	E	±	+	+	+	+	+	±	+	+	+	+	±	0.69	*M. pachydermatis*
009C	C	E	+	-	+	+	+	+	±	+	+	+	+	+	0.85	*M. pachydermatis*
012C	C	E	±	+	+	+	+	+	±	+	+	+	+	+	0.69	*M. pachydermatis*
013C	C	E	+	-	+	+	+	+	±	+	+	+	+	+	0.79	*M. pachydermatis*
015C	C	E	+	±	+	+	+	+	±	+	+	+	+	+	0.69	*M. pachydermatis*
016C	C	E	±	+	+	+	+	+	±	+	+	+	+	±	0.64	*M. pachydermatis*
018C	C	E	+	±	+	+	+	±	±	+	+	+	+	+	0.77	*M. pachydermatis*
019C	C	E	+	+	+	+	+	+	±	±	+	+	+	+	0.87	*M. pachydermatis*
020C	C	E	+	±	+	+	±	+	±	+	-	+	+	+	0.85	*M. pachydermatis*
021C	C	E	±	+	+	+	+	+	±	+	-	+	+	+	0.86	*M. pachydermatis*
022C	C	E	+	+	+	+	+	+	±	+	+	+	+	+	0.86	*M. pachydermatis*

Continued

025C	C	E	+	-	±	+	+	+	±	+	+	+	+	±	0.77	*M. pachydermatis*
026C	C	E	+	-	±	+	+	+	±	+	+	+	+	±	0.77	*M. pachydermatis*
029C	C	E	+	-	+	+	+	±	±	+	+	+	+	±	0.63	*M. pachydermatis*
031C	C	E	+	-	+	+	+	+	±	+	+	+	+	+	0.73	*M. pachydermatis*
036C	C	E	+	-	-	+	+	-	±	+	+	+	+	+	0.86	*Malassezia* sp.
037C	C	E	+	-	+	+	+	+	±	+	±	+	+	+	0.81	*M. pachydermatis*
038C	C	E	+	-	+	+	+	-	±	+	+	+	+	+	0.76	*Malassezia* sp.
039C	C	E	+	-	+	+	+	+	±	+	+	+	+	+	0.82	*M. pachydermatis*
040C	C	E	+	-	+	+	+	+	±	+	+	+	+	+	0.76	*M. pachydermatis*
041H	H	E	+	-	+	+	+	+	+	+	-	+	+	+	1.00	*M. pachydermatis*
042C	C	E	+	-	+	+	+	+	±	+	-	+	+	+	0.76	*M. pachydermatis*
043H	H	E	+	+	+	+	+	+	+	+	-	+	+	+	0.59	*M. pachydermatis*
044C	C	E	+	±	+	+	+	+	±	+	-	+	+	+	0.76	*M. pachydermatis*
046C	C	E	+	+	+	+	+	±	±	+	-	+	-	-	0.69	*Malassezia* sp.
047C	C	E	+	±	+	+	+	+	±	+	-	+	+	±	0.84	*M. pachydermatis*
048C	C	E	+	±	+	+	±	+	±	+	-	+	+	+	1.00	*M. pachydermatis*

Reference strain (R); canine (C); human (H); ellipsoidal (E); cylindrical (C); Sabouraud dextrose agar (SDA); weakly positive (±); Tween diffusion test (TDT).



(a) (b)

Figure 1. Amplification of ribosomal genes. (a) Amplification of the 5.8S ADNr-ITS2 region: lane 1, HyperLadder II molecular size marker; lanes 2 - 4, canine isolates; lanes 5 - 6: human isolates; lane 7, *M. pachydermatis* CBS 1879; lane 8: *M. furfur* CBS 7019; (b) Amplification of the 26S rDNA region: lane 1, HyperLadder II molecular size marker; lanes 2-4, canine isolates; lanes 5-6: human isolates; lane 7, *M. pachydermatis* CBS 1879; lane 8: *M. furfur* CBS 7019.

However, the consensus sequences of the 5.8S rDNA-ITS2 and 26S rDNA regions were obtained, a BLASTn analysis was performed, and these sequences were compared with the sequences deposited in the databases. One hundred percent (100%) identification of the isolates (*i.e.*, *M. pachydermatis*) was achieved using the two genetic markers, with identity percentages of 95.6% - 100% and E-values of 0.0. Thus, the occurrence of *M. pachydermatis* was confirmed in 63% of the canines and 9% of the humans included in the study.

Phylogenetic analyses were performed with the two genetic regions (Figure 2 and Figure 3). All isolates of both canine and human origin clustered together in the same clade with sequences corresponding to *M. pachydermatis* identified with the GenBank accession numbers KY272204 to KY272250 and KU757185 to KU757234 and the reference strain *M. pachydermatis* CBS 1879. The reference strains of other species of the genus *Malassezia* and *Cryptococcus neoformans* CBS 132 were used as outgroups in addition to the sequences of three isolates identified as *Cryptococcus* spp. In these trees, we can observe the phylogenetic proximity of the isolates of canine and human origin.

Specifically, the 003H isolate of human origin and the 003C isolate of the canine owned by this individual showed almost identical biochemical and phospholipase activity profiles (Figure 4), as well as 100% sequence identity and a close proximity in the phylogenetic reconstructions (Figure 2 and Figure 3).

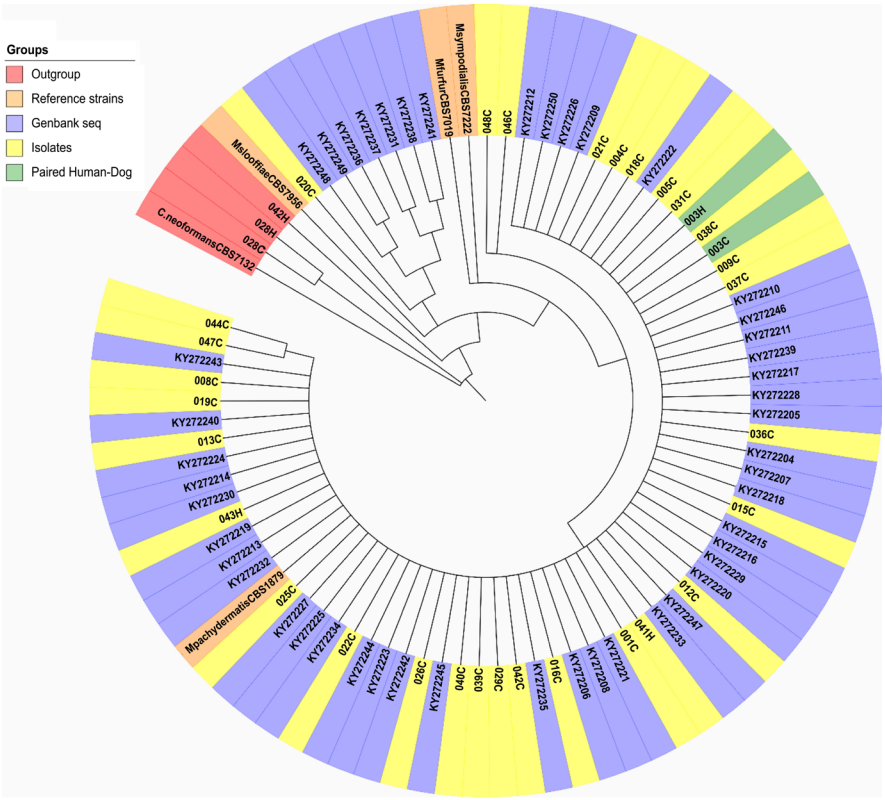


Figure 2. Phylogenetic analysis by maximum likelihood for the 5.8S rDNA-ITS2 region. Human isolates have the letter H and canine isolates have the letter C.

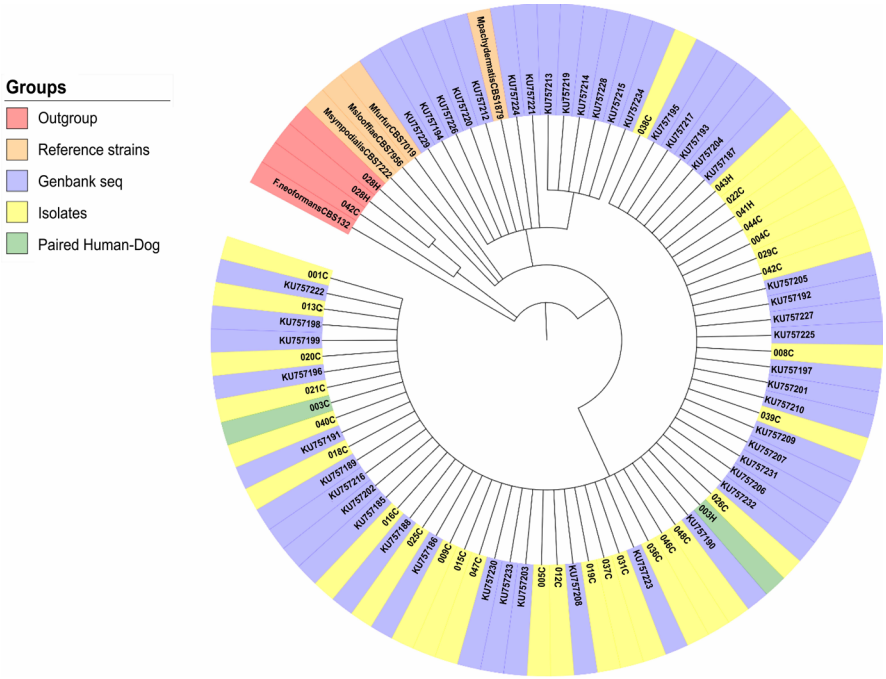


Figure 3. Phylogenetic analysis by maximum likelihood for the 26S rDNA region. Human isolates have the letter H and canine isolates have the letter C.

4. Discussion

In the present study, *M. pachydermatis* was isolated from 63% of the canine subjects with otitis externa or dermatitis, which coincided with the results reported by several authors who claimed that this yeast was most commonly associated with otic and dermatologic pathologies in canines [19] [20] [23] [48] [49] [50] [51] [52]. *M. pachydermatis* was isolated from animals of all ages, ranging from 3-month-old puppies to older dogs (15 years). Similar results were found in a Colombian study in which no significant differences were found in the ages of canine patients with otitis caused by *Malassezia* [19].

When analyzing the canine breeds from which this yeast was isolated, we observed that less than 30% of the canines corresponded to mixed-breed dogs. This finding agreed with a previous publication that showed that pure breeds were more susceptible to suffering pathologies caused by *M. pachydermatis* [53]. Moreover, the majority of dogs in which the fungus was isolated belonged to breeds with drop ears, which seemed to be a predisposing factor for otitis caused by *Malassezia* [26]. The main clinical manifestations of the affected canines were scratching, erythema, exudate, and a fetid odor; this description coincides with reports by other researchers [50] [53] and is the result of the inflammatory response triggered by the fungus and associated bacteria, such as *Staphylococcus* spp. [54].

According to the literature, the canine dermopathies produced by *M. pachydermatis* are generally secondary to other skin conditions, such as allergies, pyoderma, defective keratinization and endocrinopathies [20] [48] [50] [55], or

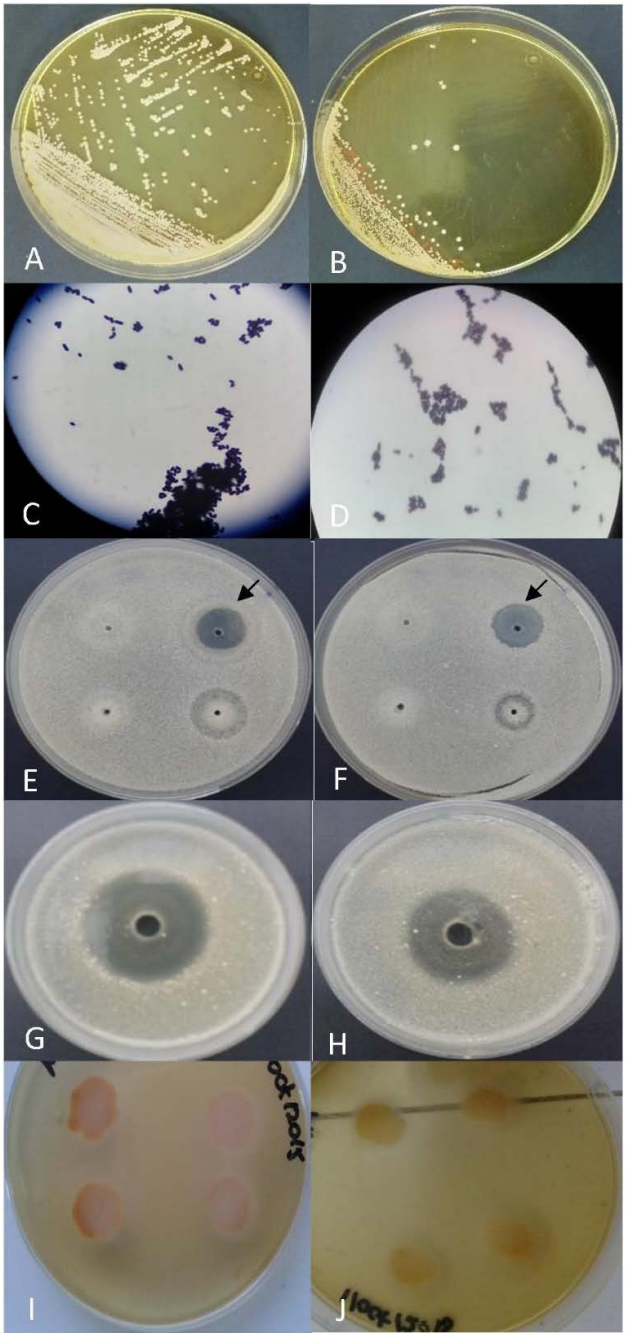


Figure 4. Phenotypic characterization of an isolate of canine origin (column on the left) and an isolate from its owner (column of the right). A and B: Culture on modified Dixon agar in which cream-colored colonies are observed; C and D: Gram staining in which oval levaduriform cells and blastoconidia are observed, 100X; E and F: Diffusion test with Tween 20 (arrow), 40, 60, and 80 (clockwise) in which assimilation of the four lipids can be observed; G and H: Diffusion test with Cremophor-EL (castor oil) in which assimilation of this lipid can be observed; I and J: High phospholipase activity can be observed withPz index of 0.71 and 0.76, respectively. The results of the enzymatic activity are expressed as a ratio (Pz) which is determined by measuring the diameter of the colony and the diameter of the colony plus the hydrolysis halo that was generated around it. Ranks have been established to classify the phospholipase activity, as follows: PZ < 0.64: very high, Pz ≥ 0.64 and <1: high and Pz = 1: null [41].

to the use of immunosuppressive and antimicrobial drugs [53]. However, most of the animals that participated in the study did not have a history of dermatitis or other conditions that could favor the growth and pathogenicity of this opportunistic yeast. Therefore, the majority of canines presented a primary dermatomycosis. This observation suggests that there may be companion animals with dermatomycosis, but without underlying diseases. This possibility may facilitate transmission to their owners because the probability of a timely diagnosis and adequate treatment and prevention measures decreases in the absence of significant signs of illness.

M. pachydermatis was isolated from the skin of 9% of the healthy humans participating in this study. The first publications describing the presence of *M. pachydermatis* on human skin dated back to the 1970s in patients with chronic cutaneous diseases, in which this yeast was isolated in 16% of the cases [56] and in one patient with a primary immunodeficiency [57]. Later, the isolation of *M. pachydermatis* from skin and other anatomical sites was reported in 47% of preterm infants and in adults with various syndromes, although whether this yeast actually caused the infections or was a colonizer was unclear [58]. This species was also found in 6% of healthy patients from cultures of skin smears and conventional biochemical tests, indicating that in most cases, the presence of *M. pachydermatis* on human skin was rare and transient [59].

An additional finding suggesting the exogenous origin of *M. pachydermatis* in human infections is that cutaneous colonization by *Malassezia* species in healthy full-term infants begins at birth and increases in the first weeks of life for *M. sympodialis* and *M. globosa*, whereas *M. pachydermatis* has not been isolated from the skin of newborns or their mothers [60]. In more recent studies, *M. pachydermatis* was identified in less than 1% and up to 5% of patients with dermatological lesions and healthy controls, respectively, with the isolation of *M. globosa*, *M. sympodialis*, and *M. furfur* being more common [30] [35] [61]. However, in the present work, these species were probably not isolated due to their more demanding nutritional requirements.

Using molecular techniques, *M. pachydermatis* was found in only 0.4% of patients with pityriasis versicolor [62] and in 0.7% of patients with seborrheic dermatitis [63]. Studies attempting to determine the species of the genus most common in patients with pityriasis versicolor noted that *M. pachydermatis* has only been found in one person with contact with domestic animals [64]. These data support the hypothesis that *M. pachydermatis* is not a member of the normal human microbiota and that its presence on the skin indicates transmission from an external source, such as contact with companion animals.

No predisposing conditions for infection by this fungus were present in any of the three cases of *M. pachydermatis* isolation in humans apart from contact with canines. The risk factors for *M. pachydermatis* infection have not been well studied. Initially, the most important predisposing factor for systemic *M. pachydermatis* infection was thought to be the same as the case of fungemia by *M.*

furfur. Infection with this fungus was associated with the lipid-rich parenteral nutrition in immunocompromised patients due to the lipophilicity shared by all species within the genus and their opportunistic behavior because most cases were isolated from the blood and other body fluids of preterm infants in neonatal intensive care units (NICUs) [65]. Thus, *M. pachydermatis* was found to colonize several anatomical sites in hospitalized patients [66].

The use of molecular biology tests and genetic typing of clinical isolates clearly demonstrated the nosocomial nature of the epidemics caused by *M. pachydermatis* in the NICUs because all isolates recovered from both the patients and the incubator surfaces were genetically comparable and persisted for several months despite regular cleaning [67]. Moreover, identical genetic profiles were generated among strains of *M. pachydermatis* isolated from hospitalized neonates [68]. The data from these studies supported the theory that *M. pachydermatis* could cause outbreaks of nosocomial fungemia and that children with low birth weights, who were severely ill, and who had arterial catheters in place for several days might be at greater risk. Furthermore, nosocomial dissemination from health personnel to patients was thought to be facilitated by inappropriate aseptic and antiseptic techniques of surfaces, and improper hand washing. Thus, meticulous measures of personal hygiene should be implemented for the doctors and nurses who handle newborns, and modifications in cleaning procedures should be implemented [32] [69].

The characterization of isolates of canine and human origin recovered in the present work suggests genetic identity and a possible route of zoonotic transmission. We perform these phenotypic tests because they have been used traditionally in the identification of the species of the genus [1] and to observe if the yeasts recovered from dogs and their owners had similar biochemical and physiological behaviors. The zoonotic association of pets with *M. pachydermatis* infections was speculated in an outbreak described in a NICU whose origin apparently was from the hands of a nurse who had dogs at home. This conclusion was reached by molecular typing because a common strain colonized the dogs, the health worker, and the newborns [32] [70]. The carrier status of *M. pachydermatis* in dog owners was shown from cultures. This species was found in 38.7% of canine owners with dermatitis or otitis attributed to *Malassezia* and in only 6% of healthy dog owners. Conversely, the diagnostic yield of the PCR-based tests was much higher (up to 94%) [33].

In the literature, the case of a woman with a facial granuloma caused by *M. pachydermatis* was reported; this strain was also found in skin and cerumen scrapings from the dog she owned using microbiological and histopathological techniques [34]. All of the above findings validate the possible zoonotic transmission of *M. pachydermatis* to humans, specifically from close contact with canines. Healthy patients are likely to behave as asymptomatic carriers or reservoirs of the fungus, whereas patients with predisposing factors, either local or systemic, will be at risk for cutaneous or disseminated infection by *M. pachydermatis.*

In this study, molecular and phylogenetic characterizations of *M. pachydermatis* isolates from humans and canines were performed. Two genetic markers have been described for the systematic analysis of yeasts of the genus *Malassezia*: the 5.8S rDNA-ITS2 region, which includes the internal transcribed spacers (ITS), whose length differs between species and is usually identical between strains of the same species, and the 26S rDNA region, which includes the D1/D2 domains, whose lengths are identical in all species of the genus and the similarity of the sequences within the same species is greater than 99% [71]. The amplification of these regions showed similar results to the literature, such as a 483-bp product using the 5.8S rDNA-ITS2 region for *M. pachydermatis* isolates from the lesions of canines with otitis [43] and a 550-bp product for eight *Malassezia* species using the 26S rDNA region [12]. Sequencing of these products confirmed that all of the isolates corresponded to *M. pachydermatis*.

Phylogenetic trees constructed with both markers showed the inclusion of isolates of human and canine origin within the same clade. A study in the late twentieth century classified isolates recovered from humans in the sequevar with the most common type sequences of strains isolated from dogs, which suggested the capacity of this canine-specific yeast to adapt to new hosts [31]. Subsequently, the molecular characterization of lipid-dependent isolates from different species of domestic animals was performed. Although that study did not include human strains, four independent clusters as a function of the origin of the yeast were obtained. Thus, whether the value of the genetic differences observed was important for the definition of species or for the process of adaptation to specific hosts was unclear [72].

The observation of genetic variants of *M. pachydermatis* from canine skin and their relationship with corporal distribution and phospholipase activity has also been evaluated. Higher activity is associated with isolates recovered from cutaneous lesions compared to healthy skin, suggesting that the presence of particular genotypes of *M. pachydermatis* on the skin of canines is related to the affinity of the yeast for the host and specific cutaneous sites [73]. In the present work, the relationship between phospholipase activity and anatomical sites was not studied. However, the activity indexes were similar between isolates of canine and human origin, which indicated similar virulence patterns in both host types. The characterization of atypical *M. furfur* isolates from skin lesions of one canine showed the presence of genetic and physiological polymorphisms that could be the result of the adaptation process of this human-specific species to a new host [21]. However, in the present study, the lipid-dependent species specific to humans were not isolated from the canines.

5. Conclusions

This study is the first to perform a phenotypic, physiological, molecular, and phylogenetic characterization of *M. pachydermatis* isolates from dogs and their owners. The association between this species and canine otitis externa is well

known, but few reports of human infections by *M. pachydermatis* are available, and the risk factors have not been well studied. Currently, this yeast is considered rare and transient on human skin and is not part of the normal microbiota, so its isolation suggests an exogenous origin.

Although more robust molecular epidemiology studies are required to confirm the transmission of this canine microorganism to its owners and catalog human infections caused by *M. pachydermatis* as a true emerging zoonosis, finding the yeast in people living with dogs with otitis or dermatitis suggests its capacity to adapt to new hosts. These new hosts will behave as asymptomatic carriers in the case of healthy patients or become ill if they have predisposing factors. All of these findings will contribute to knowledge of the ecology and epidemiology of this fungus of importance in human and veterinary medicine.

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Author's Disclosure Statement

There are no financial interests.

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