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Association between Phospholipase Production by *Malassezia pachydermatis* and Skin Lesions

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An evaluation was made of the phospholipase activities of *Malassezia pachydermatis* strains isolated from healthy dogs versus those from dogs with dermatitis and otitis. A high percentage of strains of *M. pachydermatis* obtained from lesion sites (93.9%) produced phospholipase, compared to the strains obtained from healthy skin of the same dog with localized lesions (41.4%) and healthy dogs (10.6%).

Phospholipases are a heterogeneous group of enzymes that hydrolyze one or more ester linkages in glycerophospholipids by cleaving a specific ester bond (17).

In the early 1980s, it was shown that pathogenic fungi produce phospholipases (9) that damage the host cell membranes (1, 10, 18). Among opportunistic yeasts, phospholipases have been detected from *Candida albicans* (4, 10, 12, 16, 18), *Rhodotorula rubra* (12), and *Cryptococcus neoformans* (5).

Malassezia yeasts are lipophilic organisms that belong to the normal cutaneous microflora of most warm-blooded animals and sometimes act as opportunistic pathogens (3, 8).

As regards *Malassezia*, phospholipase activity and protease production have been investigated for *Malassezia furfur* isolated from humans (19) and for several different species of *Malassezia* isolated only from dogs and cats with skin lesions (6, 11).

The aim of this work is to investigate the phospholipase activities of different isolates of *Malassezia pachydermatis* from healthy dogs versus those from dogs with dermatitis and otitis.

From March 2002 to July 2003, 87 dogs were clinically examined for *Malassezia* spp. and grouped as follows. (i) Dogs with skin diseases: 54 pet dogs with pruritic erythematosus dermatitis localized in only one anatomical site (i.e., perioral area, interdigital areas, inguinal area, and external ear canal). (ii) Healthy dogs: 33 pet dogs in good general health with no history of skin or ear diseases in the preceding 5 months. These animals had had no medication during the 16-month period.

All the dogs included in the trial were from the province of Bari (Apulia, southern Italy).

For all the dogs in both groups, samples were collected from seven anatomical sites (i.e., periorbital, perioral, dorsal area of neck, perianal, inguinal, interdigital, and external ear canal). Samples were collected from 25 cm^2 of skin of each region of the body for mycological testing, using sterile cotton swabs moistened with sterile saline solution, as well as from the right external ear canal and all the interdigital webs.

Within 2 h of collection, the samples were inoculated onto modified Dixon's agar (2, 15) and then incubated at 32°C for 7 days. The identification process was performed as previously described (7, 13, 14).

Two hundred fifty-four isolates of *M. pachydermatis* from 44 dogs were examined for phospholipase activity and divided into three groups: group A, 66 isolates (2 for each site) collected directly from lesional skin of 33 dogs with dermatitis localized in one site; group B, 122 isolates (2 for each site) collected from one to two healthy skin sites of the same dog with localized lesions (i.e., group A); group C, 66 isolates (2 for each site) collected from different skin sites of 11 healthy dogs.

Isolates were chosen when at least two colonies were available for each site.

The determination of phospholipase production was performed as previously reported (16) using the semiquantitative egg-yolk plate method (18). The inoculated egg-yolk plates were incubated at 32°C, and readings were taken daily from day 7 to day 12.

The formation of zones of precipitation around the colony was considered indicative of enzyme production.

The production of phospholipase (*Pz*) was expressed as a ratio of colony diameter to total diameter of the colonies and zone of precipitation (18). The phospholipase activity was classified as very high ($Pz < 0.64$), high (*Pz* value of ≥ 0.64 and <1), or null ($Pz = 1$), as previously reported (6). Each strain was tested in duplicate, and the *Pz* value represents an average of the two *Pz* values reported.

The Student *t* test was used for a statistical analysis of the *Pz* value. The chi-square test was used to assess the occurrence of phospholipase-positive strains and compare their occurrences in different groups of dogs; a *P* value of <0.05 was considered significant.

A total of 646 isolates were obtained from the 87 dogs examined, of which 618 (95.7%) were identified as *M. pachydermatis* and 28 (4.3%) were identified as *M. furfur.*

Table 1 indicates the *Pz* values of *M. pachydermatis* strains isolated from different sites of dogs with and without lesions that were positive for phospholipase production.

The Student *t* test showed significantly different *Pz* values among the isolates from the three groups (Table 1). The number of dogs with at least one isolate producing phospholipase activity is also reported in Table 1.

The chi-square test showed significant differences among the

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TABLE 1. Dogs presenting at least one isolate with phospolipase activity (*Pz*) and numbers and percentages of *M. pachydermatis* isolates from different sites

Isolate group[a]	No. of dogs with positive isolates/ no. studied (%)[b]	No. of positive isolates/no. studied (%)[b]	*Pz* range[c]	*Pz* value, mean (SD)[b,c]
A	33/33 (100)a	62/66 (93.9)b	0.46–1	0.72 (0.13)c
B	28/33 (84.8)a	51/122 (41.8)b	0.48–1	0.89 (0.15)c
C	5/11 (45.5)a	7/66 (10.6)b	0.43–1	0.97 (0.10)c
Total		120 (47.2)		

[a] Group A, isolates collected directly from lesional skin; group B, isolates collected from healthy skin sites of the same dog (group A) with localized lesions; group C = isolates collected from skin sites of healthy dogs.

[b] a and b, statistical differences ($P < 0.05$) using chi-square test; c, statistical differences ($P < 0.05$) using Student's *t* test; positive, phospholipase activity detected.

[c] *Pz*, production of phospholipase expressed as a ratio of colony diameter to total diameter of the colonies and zone of precipitation. SD, standard deviation.

dogs presenting at least one isolate with phospholipase activity among the three groups (Table 1).

Table 2 shows the results for phospholipase activity of *M. pachydermatis* classified according to the *Pz* value.

In our study, all the dogs with lesions (group A) presented phospholipase-producing isolates, unlike the dogs in groups B and C. Only five healthy dogs (group C) presented one or more phospholipase-producing isolate. In particular, a very high percentage of *M. pachydermatis* strains from group A (93.9%) produced phospholipase activity, compared to those from group B (41.8%) and group C (10.6%). *M. pachydermatis* isolates from lesional skin produced higher phospholipase activity (mean *Pz* value = 0.72) than those from group B (mean *Pz* value = 0.89) and C (mean *Pz* value = 0.97).

The presence on skin lesions of four (6.0%) *Malassezia* isolates that do not produce phospholipase activity, together with those that do, may be accounted for by the concomitant presence of two separate *Malassezia* populations: presumably, the lesions are in an earlier stage of infection. Conversely, the finding of seven (10.5%) *Malassezia* isolates producing phospholipase from five (45.5%) healthy dogs (group C) may be explained by the fact that the lesions had not yet appeared at the time of sampling.

The finding of phospholipase activity in 41.4% of isolates from 28 (84.8%) dogs in group B confirms our assumption that dogs presenting with one localized lesion harbored different populations of phospholipase-producing and nonproducing *M. pachydermatis* in sites with no detectable skin lesions. This may be due to contamination by the lesional skin or to a changing pattern of pathogenicity in the commensal yeast population. The genotyping of different phospholipase-producing or nonproducing isolates would contribute important insights to better understand the pathogenic role of *Malassezia* spp.

This study suggests that phospholipase activity may play a pathogenic role in the occurrence of skin lesions caused by *Malassezia* spp. yeasts, thus contributing to its virulence. Nevertheless, phospholipases should probably be considered one of many factors involved in the complex interaction between yeast and host leading to the development of skin lesions.

TABLE 2. Phospholipase activity of *M. pachydermatis*, classified according to *Pz* coefficient

Isolate group[a]	No. of isolates in activity category/ total no. in group (%)		
	Very high (Pz < 0.64)	High ($Pz \geq 0.64$ and < 1)	Absent ($Pz = 1$)
A	18/66 (27.3)	44/66 (66.6)	4/66 (6.0)
B	15/122 (12.3)	36/122 (29.5)	71/122 (58.2)
C	2/66 (3.0)	5/66 (7.5)	59/66 (89.4)
Total	34/254 (13.4)	85/254 (33.5)	134/254 (52.8)

[a] Group A, isolates collected directly from lesional skin; group B, isolates collected from healthy skin sites of the same dog (group A) with localized lesions; group C, isolates collected from skin sites of healthy dogs.

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