

Biotyping of *Malassezia pachydermatis* strains using the killer system

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Summary

The killer phenomenon has been used as epidemiological marker for *Candida albicans*, where hundreds of biotypes can be obtained. The objective of this study is to observe the behaviour of 30 strains of *Malassezia pachydermatis* isolated from dogs with otitis (15) or dermatitis (15) against 9 killer yeasts, which, when grouped in triplets produced a 3 digit code (biotype). The growth inhibition of the 30 strains of *M. pachydermatis* due to the effect of the killer yeasts used permitted the determination of the following biotypes: 888 (33.3%), 212 (26.7%), 111 (16.7%), 312 (6.7%), 512 (6.7%), 242 (3.3%), 311 (3.3%) and 411 (3.3%). Biotypes 888, 212 and 111 occurred most frequently in both ear canal and skin samples.

Key words

Malassezia pachydermatis, Dogs, Killer system, Otomycosis, Dermatotoxicosis

Biotipificación de cepas de *Malassezia pachydermatis* por el sistema "killer"

Resumen

El fenómeno "killer" ha sido empleado para *Candida albicans* como marcador epidemiológico habiéndose obtenido cientos de biotipos. El objetivo de este trabajo fue estudiar el comportamiento de 30 muestras de *Malassezia pachydermatis*, provenientes de 15 perros con otitis y 15 con dermatitis, frente a 9 levaduras "killer", que agrupadas en tripletes generan un código de tres números (biotipos). La inhibición del crecimiento de las 30 cepas de *M. pachydermatis* por las levaduras "killer" empleadas, propició la obtención de 8 biotipos: 888 (33,3%), 212 (26,7%), 111 (16,7%), 312 (6,7%), 512 (6,7%), 242 (3,3%), 311 (3,3%) y 411 (3,3%). Los biotipos 888, 212 y 111 se presentaron con mayor frecuencia tanto en las muestras de oído como en las de piel.

Palabras clave

Malassezia pachydermatis, Perros, Fenómeno "killer", Otomicosis, Dermatotoxicosis

Gustafson [1] was the first researcher to associate *Malassezia pachydermatis* to canine otitis externa. After having classified this yeast as pathogenic for this animal species, several researchers have described dermatitis and otitis externa cases [2-5], as representing major diseases in a Small Animal Clinic. Studies in the basic area focusing on the agent itself are however scarce, thus leading to lack of information about the behaviour of *M. pachydermatis* in the major parasitized sites, namely ear and skin.

Some yeasts, known as killer yeasts, can produce toxins, substances which are lethal to strains of a same species or other species. Polonelli *et al.* [6] observed that the susceptibility of *Candida albicans* strains to killer yeasts could differentiate them into biotypes. As *M. pachydermatis* regards, this phenomenon was studied by Polonelli & Morace [7] though testing two strains only.

These data are to be broadened both for the biotypes to be able to be used as epidemiological markers and for the killer yeasts to be therapeutically employed.

This work therefore aimed at testing the susceptibi-

lity of *M. pachydermatis* isolates from canine otic secretion and skin against killer yeasts, checking whether there were any differences in behaviour in both parasitized sites.

MATERIAL AND METHODS

Thirty strains of *M. pachydermatis* originating in dogs, 15 isolated from otic secretion and 15 obtained from skin scraped from animals bearing symptoms and/or lesions suggesting malasseziasis have been studied.

Some otic secretion was collected by introducing a sterile swab into the ear canal, after cleaning external regions with some alcohol-ether solution. Skin scale and hairs were scraped from damaged areas with a scalpel. Following collection, the material was immediately sent to the laboratory for processing.

Yeasts capable of producing toxins lethal to yeast strains of the same or other species (killer yeasts) were used, as follows: K1-*Hansenula* sp. (Stumm 1034); K2-*Pichia* sp. (Stumm 1035); K3-*Hansenula anomala* (UM); K4-*H. anomala* (CBS 759); K5-*H. anomala* (Ahearn UN 866); K6-*Hansenula californica* (Ahearn C 40); K7-*Hansenula canadensis* (Ahearn WC 41); K8-*Hansenula dimennae* (Ahearn WC 44); K9 -*Hansenula mrakii* (Ahearn WC 51).

The technique employed for the research of biotypes using the killer system was the one recommended by

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Polonelli *et al.* [6] for *C. albicans*. *M. pachydermatis* samples were suspended in a 2 ml saline solution adjusted to tube 3 of the Mc Farland Scale. Such suspension was poured onto a Petri plate along with the killer medium melted, cooled at 45-50°C and homogenized. After solidification, the 9 killer yeasts were uniformly spotted onto the medium surface. The plates were incubated at 37°C, with readings after 24, 48, 72 and 96 hours. A zone of growth inhibition and/or a region of bluish-colored cells around the killer yeasts were taken as a positive result, whereas the absence of such observations was considered negative.

Polonelli *et al.* [6] also suggested some code used here for the reading of this test. It uses the combined effect of the 9 killer yeasts arranged in triplets.

As a means of controlling the cultivation medium and the action of the killer yeasts, standard *C. albicans* sample - from the collection of the cultures at the Biomedical Science Institute, Universidade de São Paulo (*C. albicans* -ICB-12A)- was utilized.

RESULTS

Figure 1 shows that the effect of the killer toxins in *M. pachydermatis* samples was easily detected by observing the clear zone of growth inhibition around the killer yeasts.

Table 1 shows the biotypes and the percentages of occurrence found in the otic secretion and skin samples.

As the susceptibility to the killer yeasts regards, eight biotypes were observed for *M. pachydermatis* (111,



Figure 1. *Malassezia pachydermatis* biotype 111, showing susceptibility to all killer yeasts (K₁ to K₉).

212, 242, 311, 312, 411, 512 and 888), three of which occurred more frequently: 888 (33.3%), 212 (26.7%) and 111 (16.6%), both in the samples originating in the skin and in the otic secretion (Table 1).

DISCUSSION

The killer toxin acts on the membrane cell determining the loss of its integrity due to the formation of permeable canals which allow for potassium, amino acids and glucose to leave through pores to the medium, thus inhibiting the metabolic processes of macromolecules and leading to a cellular death [8,9]. These molecular phenomena will be macroscopically translated on the reading plates by a growth inhibition zone of the sensitive yeast around the killer yeast, ranging from slightly yellowish to deep blue, represented by the dead cells, evidenced by methylene blue.

Table 1. Biotypes presented by *Malassezia pachydermatis* strains originating in otic secretion and skin, based on the presence of the killer phenomenon, after 96 hours.

Biotype	Otic Secretion Strains		Skin Strains		Total	
	Absolute Number	%	Absolute Number	%	Absolute Number	%
888	4	26.7	6	40	10	33.3
212	4	26.7	4	26.7	8	26.7
111	3	20	2	13.3	5	16.7
312	0	0	2	13.3	2	6.7
512	2	13.3	0	0	2	6.7
242	1	6.7	0	0	1	3.3
311	1	6.7	0	0	1	3.3
411	0	0	1	6.7	1	3.3
Total	15	100	15	100	30	100

The plates to be read in order to verify the killer phenomenon were incubated at 37°C for 96 hours once the readings were quite clear within this interval. For the killer toxin maximum effect to occur, the present work proved the 37°C temperature to be adequate for observing the behaviour of *M. pachydermatis* strains in the presence of killer yeasts, even though Bendová [9] recommends around 20-25°C for the incubation to be carried out. Polonelli *et al.* [10] had already verified that both 25°C and 37°C temperature values served this test regarding *Malassezia* spp.

The gathering of 8 biotypes in this research, out of which 3 (888, 212 and 111) represent 76.7 % of the 30 strains studied, proves that *M. pachydermatis* behaviour in the presence of killer yeasts can adapt to epidemiological studies. Differences among the samples originating in the otic secretion and skin were not detected as yet (Table 1).

It is hard to compare the results achieved to those by other researchers for literature is scarce. In a referred study of two *M. pachydermatis* strains, the researchers obtained the 114 and 118 biotypes [7], none of which was verified here. This should not come as a surprise once the code system proposed for *C. albicans* and here adopted can lead to hundreds of different biotypes [6].

As demonstrated for *C. albicans* [6], these biotypes can become a practical method easy to conduct in epidemiological investigations, particularly in hospital infections. In systemic infection outbreak by *M. pachydermatis* reported in neonatal intensive care units, Michelsen *et al.* [11] believe *M. pachydermatis* to be endemic in the nursing staff working in the hospital studied. They however reported difficulties in stating whether or not the infection source represented dissemination from one only strain, exactly because of the lack of either phenotypic or genotypic epidemiological markers for *M. pachydermatis*. The typifying of this yeast using the killer system would be of great assistance for this and other cases.

Still, as experimentally demonstrated when Beagles inoculated with *M. pachydermatis* in their ears presented remission when treated with a *H. anomala* killer strain, killer yeasts could be therapeutically used [10].

Having said that, it is believed killer yeasts might be used in the biotyping of *M. pachydermatis* and that further studies should be carried out, in order to obtain data which can be applied both in Human and Veterinary Medicine Clinics.

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