TITLE: PHOSPHOLIPASE, PROTEINASE, LIPASE AND COLLAGENASE PRODUCTION BY *Malassezia pachydermatis* ISOLATES

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ABSTRACT:

Malassezia pachydermatis, a non-lipophilic, non-mycelial and unipolar yeast, is one of the most frequent etiological agents isolated from skin and ear disorders in dogs. This commensal yeast may become pathogenic under the influence of predisposing factors, such as changes in the superficial cutaneous microclimate or host defenses, causing otitis and different clinical forms of dermatitis and otitis in domestic animals. The processes involved in the colonization and infection include adherence to the *stratum corneum*, secretion of enzymes, as well as the influence of the innate and adaptive immune responses of the host. There is a production of enzymes, such as esterase, lipase, acid phosphatase, lipoxygenase, protease and phospholipase, which are recognized as virulence factors. In the present study, the activity of the enzymes phospholipase, lipase, collagenase and protease were verified in 12 *M. pachydermatis* strains obtained from dogs with skin lesions (6), from dogs with otitis (3) and from an asymptomatic free-living *Didelphis* spp. (3). Phospholipase activity was detected using egg yolk agar (peptone-1%, dextrose-2%, NaCl-5.73%, $CaCl_2$-0.05%, bacteriological agar-2% and egg yolk-4%). To verify the presence of the enzyme collagenase, a test medium containing agar (2%) and gelatin (1%) was used. To verify the presence of the lipase enzyme, a medium containing bacteriological agar (2%), peptone (1%), NaCl (0.5%), $CaCl_2$ (0.01%) and Tween 20 (1%) was used. Protease activity was verified with a medium containing bacteriological agar (2%) and skim milk (10%). All tests were incubated for 10 days at 32 °C and performed in triplicate. All isolates were able to express the tested enzymes. The isolates showed a very high extracellular activity of the protease enzyme, expressed by precipitation zones (PZ) between 0.39 and 0.44. All the isolates had a high levels of lipase (PZ < 1) and phospholipase activity (PZ between 0.40 and 0.53), with an intense precipitation halo. Even the isolates from the free-range *Didelphi*s spp. without symptomatology showed high extracellular activity of these enzymes. No differences were observed in the enzymatic activity between the groups of pathogenic and commensal isolates in this and in previous studies, suggesting that the production of enzymes could not be a determining factor in the process of infection. In conclusion, there was no difference between the enzymatic activities of *M. pachydermatis* isolates from canine otitis, canine dermatitis and from a *Didelphis* spp. healthy ear.

Keywords: *Didelphis* spp., canine dermatitis, canine otitis, virulence factors.