Genetic variability and phospholipase production of Malassezia pachydermatis isolated from dogs with diverse grades of skin lesions

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Little detailed information is available on the association of Malassezia pachydermatis genotypes and the extent of skin damage that they cause. In the present study, isolates of M. pachydermatis, recovered from the skin of healthy dogs and dogs with dermatitis in Brazil, were characterized on the basis of partial sequencing of the large subunit (LSU), first internal transcribed spacer (ITS-1) and chitin synthase 2 gene (chs-2). The determination of phospholipase production was also included in the investigations. The severity of lesions and hyperpigmentation of dogs with skin disease were evaluated. For each locus, two main sequence types were designated as genotypes A and C. Two other minor sequence types (A2, C2) were also recorded and defined for the ITS-1. Genotype A isolates were the most prevalent, being recovered from healthy and diseased animals. No significant difference was detected among genotypes or ITS-1 sequence types and grades of skin damage or hyperpigmentation in the dogs with skin lesions. The number of M. pachydermatis isolates that produced phospholipase was statistically higher for diseased dogs than for strains found in healthy animals. The present study reveals that multiple genetic variants of M. pachydermatis occur in dogs and that the distribution patterns of particular genotypes on the skin of dogs in Brazil might be related to environmental and ecological factors which maintain distinctive genotype assemblages in specific geographical areas.

Keywords dog, Malassezia pachydermatis, molecular characterization, phospholipase, skin damage

Introduction

Malassezia pachydermatis can be recovered from the skin of dogs with various forms of dermatitis, as well as from healthy dogs. This yeast is often described as highly pro-inflammatory [1] due to its capacity to produce enzymes such as phospholipases [2,3] and/or metabolites that cause inflammation reactions or hypersensitivity [4–6]. It has also been demonstrated that multiple genetic variants of M. pachydermatis occur on dog skin [7–12] and that the sequencing of Malassezia spp. nuclear ribosomal DNA regions (e.g., large subunit, LSU, and first internal transcribed spacer, ITS-1) and chitin synthase 2 gene (chs-2) are useful in genetic and epidemiological investigations of the occurrence of the yeast in dog populations [10,11]. To our knowledge, the association between M. pachydermatis genotypes and the skin damage they cause has never been investigated. Thus, the present investigation aimed at: (i) identifying M. pachydermatis genotypes isolated from the skin of dogs with and without dermatitis in Brazil, (ii) studying the association between Malassezia...
genotypes and grade of skin damage and, (iii) evaluating the phospholipase activity with respect to the genotype of isolates recovered from diseased and clinically healthy dogs.

Materials and methods

One hundred and sixty-eight *Malassezia* isolates (three from each dog) were recovered from skin samples of 56 Brazilian dogs with or without skin lesions and were maintained on modified Dixon agar [3]. These test strains were divided into the following two groups; Group 1 = 84 isolates collected from skin sites on 28 healthy dogs and Group 2 = 84 isolates collected from skin lesions of 28 dogs with dermatitis. The extent of the lesions of dogs in Group 2 was evaluated using the Canine Atopic Dermatitis Extent and Severity Index (CADESI-03) and reported as severe (>16) and middle (≤16) with 20 as maximum score value for each dog [13]. Hyperpigmentation was also evaluated and reported as ‘none, mild or severe’. *M. pachydermatis* isolates were identified microscopically, based on their morphology and ability to grow on media without lipid supplementation (Sabouraud dextrose agar, Liofilchem Diagnostici®, Teramo, Italy) and molecularly classified based on LSU, ITS-1 and chs-2 partial sequencing as previously reported [10,12]. Sequences were compared with the *M. pachydermatis* sequences available in the GenBank™ database for each locus investigated (i.e., *M. pachydermatis* accession nos. DQ915500, DQ915501, DQ915502, DQ915503, DQ915504, DQ915505, DQ915506, DQ915507, DQ915508, DQ915509, EU158827, EU158828 and EU158829). Sixty-one isolates (29 from Group 1 and 32 from Group 2) were employed in studies of phospholipase production using the semiquantitative egg-yolk plate method [4]. The Chi-square test was used to compare the occurrence of *M. pachydermatis* sequence-types and phospholipase-positive strains within each group, between groups and between animals with different grades of skin damage and hyperpigmentation. A value of $P \leq 0.05$ was considered to be statistically significant.

Discussion

The results of this study indicate that multiple genotypes/sequence types of *M. pachydermatis* occur on the skin of dogs with and without dermatitis in Brazil, and that they may, as previously reported, be retrieved from the same animals [7,8,10].

In particular, genotype A was the most prevalent in both groups, while genotype C was the least frequently encountered. These results are not consistent with those of previous reports in which the occurrence of genotype A was associated with otitis [9] or skin lesions [10], while genotype C was linked with healthy skin sites of dogs with localized lesions [10]. This discrepancy may be due to the fact that the sampling procedure employed in the present study differs from previously reported methods, in which different areas of the same animal were sampled in order to differentiate *Malassezia* genotypes at different skin sites [10].

Multiple genotypes and/or sequence types were isolated from the skin of five dogs (one from Group 1 and four from Group 2) (Table 1).

Isolates of genotypes A and C were obtained in culture from skin samples of dogs from both Group 1 and Group 2, with genotype A as the most prevalent in both groups ($P < 0.05$). No statistically significant difference in the prevalence of genotypes was detected among groups.

ITS-1 sequence type A2 and C1 were detected only in dogs of Group 2. No significant differences were detected between *M. pachydermatis* genotypes or ITS-1 sequence types and grades of skin damage or hyperpigmentation (Table 1). Nonetheless, *M. pachydermatis* ITS-1 sequence types A2 were most frequently isolated from dogs with severe skin damage (≥16), but no relationship with hyperpigmentation was observed. *Malassezia pachydermatis* ITS-1 sequence types C1 was retrieved from only one dog with skin damage < 16 and mild hyperpigmentation (Table 1).

All four *M. pachydermatis* sequence types, exclusively defined based on ITS-1, produced phospholipase. Overall, the number of *M. pachydermatis* isolates producing phospholipase was statistically lower for Group 1 than for Group 2 ($P < 0.05$). No relationship was observed between phospholipase production and grades of skin damage in Group 2 dogs.
all ITS-1 sequence types are equally distributed among dogs with skin lesions. Dogs with skin lesions were also divided according to the grade of severity of skin damage (i.e., <16 mild or moderate, ≥16 severe) and hyperpigmentation. Identical superscript letters indicate statistically significant differences (P < 0.05).

Table 1  Number and percentage (in brackets) of Malassezia pachydermatis sequence types for each gene (i.e., LSU, ITS-1, and chs-2 designated by subscripts L, I, and C, respectively) occurring on healthy dogs and dogs with skin lesions. Dogs with skin lesions were also divided according to the grade of severity of skin damage (i.e., <16 mild or moderate, ≥16 severe) and hyperpigmentation. Identical superscript letters indicate statistically significant differences (P < 0.05).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Grade of severity</th>
<th>Number of dogs</th>
<th>Number of isolates</th>
<th>LSU</th>
<th>ITS-1</th>
<th>chs-2</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>A1</td>
<td>C1</td>
<td>A1</td>
</tr>
<tr>
<td>Group 1</td>
<td>Healthy</td>
<td>28</td>
<td>84</td>
<td>67&lt;sup&gt;a&lt;/sup&gt; (79.8)</td>
<td>17&lt;sup&gt;a&lt;/sup&gt; (20.2)</td>
<td>67&lt;sup&gt;b&lt;/sup&gt; (79.8)</td>
</tr>
<tr>
<td></td>
<td>CADESI 3 Index</td>
<td>10</td>
<td>30</td>
<td>24&lt;sup&gt;a&lt;/sup&gt; (80.0)</td>
<td>4&lt;sup&gt;b&lt;/sup&gt; (20.0)</td>
<td>21&lt;sup&gt;a&lt;/sup&gt; (70.0)</td>
</tr>
<tr>
<td></td>
<td>&lt;16</td>
<td>18</td>
<td>54</td>
<td>40&lt;sup&gt;a&lt;/sup&gt; (74.1)</td>
<td>14&lt;sup&gt;a&lt;/sup&gt; (25.9)</td>
<td>39&lt;sup&gt;a&lt;/sup&gt; (72.2)</td>
</tr>
<tr>
<td>Group 2</td>
<td>Hyper-pigmentation</td>
<td>8</td>
<td>24</td>
<td>18&lt;sup&gt;c&lt;/sup&gt; (75.0)</td>
<td>6&lt;sup&gt;c&lt;/sup&gt; (25.0)</td>
<td>17&lt;sup&gt;c&lt;/sup&gt; (70.8)</td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>10</td>
<td>30</td>
<td>22&lt;sup&gt;c&lt;/sup&gt; (73.3)</td>
<td>8&lt;sup&gt;c&lt;/sup&gt; (26.7)</td>
<td>20&lt;sup&gt;c&lt;/sup&gt; (66.7)</td>
</tr>
<tr>
<td></td>
<td>Mild</td>
<td>10</td>
<td>30</td>
<td>24&lt;sup&gt;c&lt;/sup&gt; (80.0)</td>
<td>6&lt;sup&gt;c&lt;/sup&gt; (20.0)</td>
<td>23&lt;sup&gt;c&lt;/sup&gt; (76.7)</td>
</tr>
<tr>
<td></td>
<td>Severe</td>
<td>10</td>
<td>30</td>
<td>131&lt;sup&gt;c&lt;/sup&gt; (78.0)</td>
<td>37&lt;sup&gt;c&lt;/sup&gt; (22.0)</td>
<td>127&lt;sup&gt;c&lt;/sup&gt; (75.6)</td>
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<tr>
<td>Total</td>
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<td>56</td>
<td>168</td>
<td>131&lt;sup&gt;c&lt;/sup&gt; (78.0)</td>
<td>37&lt;sup&gt;c&lt;/sup&gt; (22.0)</td>
<td>127&lt;sup&gt;c&lt;/sup&gt; (75.6)</td>
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</tbody>
</table>
(including bacterial flora, pH, salts, immune response, biochemistry and physiology) play a significant role in the adherence, establishment and growth of this yeast [18]. In addition, it has been speculated that *Malassezia* has the paradoxical ability to both stimulate and suppress the immune response directed against it, which is manifested as a balance between commensalism and pathogenicity [6]. Again, it has been demonstrated that the biochemical composition of canine skin may differ, depending on skin site and its health and integrity [5], and that it might influence the pathogenetic role of *M. pachydermatis* [17].

Finally, the absence of genotype B in dogs from Brazil might suggest that the occurrence of distinctive genotype assemblages is related to environmental and ecological factors according to the geographical area.

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**References**


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