NOTE Internal Medicine

Homogeneous Cell Suspension of *Malassezia pachydermatis* Obtained with an Ultrasonic Homogenizer

Tae MURAI1), Yuka NAKAMURA1,2)*, Rui KANO1), Shinichi WATANABE1) and Atsuhiko HASEGAWA3)

1)Department of Dermatology, Teikyo University School of Medicine, 11–1 Kaga-2, Itabashi, Tokyo 173–8605, 2)Departments of Veterinary Internal Medicine and 3)Pathobiology, Nihon University School of Veterinary Medicine, 1866 Kameino, Fujisawa, Kanagawa 252–8510, Japan

(Received 5 October 2001/Accepted 27 December 2001)

ABSTRACT. It is difficult to produce homogeneous cell suspensions of *Malassezia pachydermatis*, since yeast cells paste up and form many clumps. However, homogeneous fungal suspensions are required for susceptibility examinations and biochemical analyses. Although several types of trials have been carried out using glass homogenizers and many types of agents to obtain homogeneous fungal suspension. They have not yielded good results. We therefore attempted to use an ultrasonic homogenizer to separate clumps of yeast cells into separate individual cells. We succeeded in this fashion in producing homogeneous cell suspensions of *M. pachydermatis*. These results indicate that an ultrasonic homogenizer can be used to prepare homogeneous fungal suspensions of *M. pachydermatis*.

KEY WORDS: homogenous suspension, *Malassezia*, ultrasonic homogenizer.


*Malassezia* species are microbiological flora of human and other mammalian skin [2, 9, 10]. These yeasts are etiological agents of systemic infection as well as skin disorders such as pityriasis versicolor and *Malassezia* folliculitis in humans [3, 5, 12]. *Malassezia* yeasts, especially *M. pachydermatis*, have been reported to be related to skin diseases such as seborrhea and atopic dermatitis as well as otitis externa in dogs and cats [4]. Several susceptibility testing methods for antifungal drugs against yeasts have been developed for determination of minimum-inhibitory concentrations (MIC) [1, 6, 7]. However, it is difficult with these methods to obtain results with sufficient reproducibility, because they use fungal suspensions with chunky yeast cells for assay. We therefore developed a practical method to prepare homogeneous suspensions of *M. pachydermatis* using an ultrasonic homogenizer.

Five clinical isolates (three isolates from canine otitis externa and the other two isolates from feline otitis externa) and one authenticated isolate from Centraalbureau voor Schimmecultures of *M. pachydermatis* were used in this study. Isolates from animal patients were identified by a conventional method [8] and molecular analysis [11]. These isolates were maintained by culture on modified Dixon agar at 30°C for one week. The colonies (180 ± 46 CFU) of various sizes and shapes are observed with the fungal suspension made by the glass homogenizer (Fig. 2a). The colonies (110 ± 18 CFU) were smaller in size and increased in number after treatment of the suspension with the ultrasonic homogenizer (Fig. 2b). Colony growth was not observed in the destroyed yeast cells obtained with an output level of 12 W (Fig. 2c).

Since the yeast cells were surrounded by substances resulting in pasting together of individual yeast cells, cells did not exist separately [13]. Attempts to obtain homogeneous yeast cell suspensions using ethyl alcohol, protease and several types of surfactant failed. Therefore, a physical method rather than a chemical method was used to prepare homogeneous *Malassezia* cell suspensions. As a result, it was proven possible to produce homogeneous *Malassezia* suspension without destroying cells by treatment with an ultrasonic homogenizer.
Fig. 1. Many yeast cells were in a large clump in Fig. 1a. On the other hand, almost yeast cells were existed individually in Fig. 1b. (Magnification × 400)

Fig. 2. The colonies from fungal suspension made by the glass homogenizer varied size and shape in Fig. 2a. On the other hand, increased number of small colonies from the suspension made by the ultrasonic homogenizer within output level at 8W, for more than 15 seconds were observed in Fig. 2b. No colony growth from the suspension made by the ultrasonic homogenizer within output level at 12 W was detected in Fig. 2c.
HOMOGENOUS SUSPENSION OF MALASSEZIA

Table 1. Yeast cells of *Malassezia pachydermatis* treated with an ultrasonic homogenizer

<table>
<thead>
<tr>
<th>Duration of operation (sec)</th>
<th>15</th>
<th>30</th>
<th>60</th>
<th>90</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 Clumped</td>
<td>Clumped</td>
<td>Clumped</td>
<td>Clumped</td>
<td>Clumped</td>
<td>Clumped</td>
</tr>
<tr>
<td>8 Partly separated</td>
<td>Separated</td>
<td>Separated</td>
<td>Separated</td>
<td>Separated</td>
<td>Separated</td>
</tr>
<tr>
<td>12 Destroyed</td>
<td>Destroyed</td>
<td>Destroyed</td>
<td>Destroyed</td>
<td>Destroyed</td>
<td>Destroyed</td>
</tr>
</tbody>
</table>

Clumped: Most yeast cells existed in a clump. Partly separated: Most yeast cells existed individually, although several clumped cells also existed. Separated: Almost all yeast cells existed individually and no destroyed cells were detected. Destroyed: Many destroyed yeast cells were observed, although a small number of cells existed separately.

REFERENCES