

TITLE: Biofilm formation by *Malassezia pachydermatis* isolates

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ABSTRACT:

The capacity to form biofilm in the ear canal is one of the key factors to ensure *Malassezia pachydermatis* permanence in this microenvironment, being associated with more resistance to challenges faced in the auricular region. This factor is also correlated with less response to antifungal treatments, just like already know for bacteria. In this research, twelve *M. pachydermatis* isolates, obtained from auricular clinical samples, were tested to verify biofilm formation. A crystal violet staining technique was adapted for the evaluation. The samples were cultured in Modified Dixon Agar at 32 °C for 3 days and transferred later to Yeast-Peptone Extract Broth, incubated at the same temperature and period. After the standardization of the culture in 1×10^6 UFC/mL, 150 µL of each vial was transferred to 96-well sterile microplates, being incubated for 24 hours at 32 °C. Subsequently, the suspended cells were aspirated, and each well was washed twice with 150 µl of PBS (Phosphate Buffered Saline, pH 7.2), followed by the addition of 200 µl of Yeast-Peptone Extract Broth incubated during 4 days at 32 °C. The culture medium was changed on a daily basis. Finally, the microplates were washed twice with 200 µl of PBS and allowed to air dry. Then, the wells were filled with 150 µL of aqueous crystal violet solution (0.5%) and washed with 200 µL of sterile distilled water after 45 minutes. Afterward, 200 µl ethanol (95%) was used for 45 minutes to promote the discoloration, followed by the transfer of 100 µL from each well to a new microplate. The results were obtained by spectrophotometer reading at 595 nm wavelength. The test was performed in duplicate and the negative control results were subtracted from the tested samples in a way to minimize interference from the bottom of the microplates. All samples showed the ability to form a biofilm *in vitro*. The biofilm production has already been reported by authors, who highlighted the ability of this yeast to promote the biofilm formation in catheters, representing the possibility to cause systemic infections, especially in neonates. Considering cases of chronic ear infections, there is a possibility of progression to otitis media. When biofilm formation occurs in this anatomical region, the treatment becomes much more difficult, both due to the access of the antifungal drug and the protection offered by the fungal structure itself. Often surgical intervention is required for washing and biofilm removal.

Keywords: Virulence factors, Biofilm formation, *Malassezia pachydermatis*

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