

TITLE: BIOFILM PRODUCTION ON ABIOTIC SURFACES BY *Corynebacterium pseudodiphtheriticum* AND COLONIZATION IN AN ANIMAL HOST.

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ABSTRACT:

Corynebacterium pseudodiphtheriticum is a gram positive bacillus, characterized as an opportunistic pathogen, found in the microbiota of the respiratory tract and skin. Currently reports of cases caused by this microorganism have been frequent, and may affect both immunocompetent and immunocompetent patients. The virulence factors of this pathogen have not yet been fully elucidated and, therefore, the present study aimed to elucidate biofilm formation on hydrophobic and hydrophilic surfaces. We have also investigated the effect of bacterial colonization on mortality of meal beetle larvae. Strains were isolated from the nasopharynx (n=5) and oropharynx (n=2) of nosocomial patients in Maranhão, Brazil. Biofilm formation on negatively charged polystyrene surfaces was determined quantitatively in 96-well flat-bottomed microtitre plates. Bacterial suspensions were added to the microplate wells. After incubation, the contents of each well were aspirated and the remaining attached bacteria were fixed with methanol and stained with crystal violet. The bound dye was then solubilised with glacial acetic acid and the OD of the solution was measured using an enzyme immunosorbent assay reader. Glass-adherent bacteria created a confluent coat of cells on the sides of the tube. To evaluate biofilm formation on hydrophilic surfaces, bacterial strains were inoculated into glass tubes containing broth culture medium and incubated without shaking over a period of 48 hours. Supernatant with non-adherent bacterial cells was discarded, and the procedure was repeated twice. The results showed that *C. pseudodiphtheriticum* was able to adhere to hydrophilic (glass) and hydrophobic (polystyrene) abiotic surfaces at different levels of intensity and independent of the isolation site. In addition, *C. pseudodiphtheriticum* strains induced melanin synthesis and caused the death of *Tenebrio molitor* larvae, but at varying intensities and times. Thus, *C. pseudodiphtheriticum* is a species that can not be neglected because biofilm production may contribute to the establishment of healthcare-associated infections. Since the innate immune system is evolutionarily conserved in insects, the use of

these animals in research is gaining ground. This study demonstrated the potential of *T. molitor* larvae as a model host that can be used to study corynebacterial virulence.

Keywords: hydrophilic surface, hydrophobic surface, *Tenebrio molitor*, Maranhão.

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