

TITLE: BACTERIAL ISOLATES OF THE *Aedes aegypti* MIDGUT CAN USE HEME AS IRON SOURCE

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ABSTRACT: *Aedes aegypti* is vector of arboviruses such as dengue, urban yellow fever, chikungunya and Zika fever, representing a major challenge to public health around the world. Insect females are hematophagous. During blood digestion large amounts of heme is released in the midgut, which is potentially toxic to the host. Part of the free heme binds to the peritrophic matrix, reducing the oxidative stress. Studies carried on by our research group suggest that bacteria associated to the midgut epithelium participate in the blood degradation process and could contribute to the neutralization of the toxic effects of free heme. This work aimed to evaluate the ability of bacterial species isolated from the midgut of *Ae. aegypti* to use heme as iron source for growth by using fermentation assays. Apart of this, biochemical tests were made in order to investigate the catalase and peroxidase activity of the cells. Bacterial isolates (*Pantoea sp.*, *Chryseobacterium gleum* and *Pseudomonas putida*) were grown in shake flasks at 30 °C and 110 min⁻¹ in MC culture medium supplemented with increasing concentrations of heme. Dry cell weight was evaluated by turbidimetry using a calibration curve. Bacterial proteins were extracted and quantified using the 2D Quant-Kit (GE Healthcare®) method. Protein profiles were evaluated by SDS-PAGE and peroxidase activity by native polyacrylamide gel electrophoresis. The catalase activity was evaluated by mixing aliquots of 40 µL of cell suspension and H₂O₂ 3%. Positive teste is evidenced by rapid bubbles formation. The results shown that all three isolates can use heme to growth. The supplementation of the culture medium with 100 µM hemin had positive effect on the kinetic growth profiles of *Pantoea sp.* and *P. putida*, with higher growth rates and biomass production. Such a heme concentration is usually toxic to bacteria. Although none of the species presented peroxidase activity, all of them shown catalase activity. The data indicated that the intestinal microbiota of *Ae. aegypti* can play an important role on heme degradation during blood digestion, contributing to overcome the oxidative stress.

Keywords: *Aedes aegypti*, Heme, Oxidative stress, Microbiota

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