

Molecular Characterization of Midgut Bacteria in Larval and Adult Stages of *Aedes albopictus* in Gampaha District, Sri Lanka

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Bacterial species that are acquired from the aquatic larval and adult stages are established in the midgut of mosquitoes, exhibiting different functional tasks retaining in the gut as symbiotic species. The present study aimed on screening of midgut bacteria of larval and adult *Ae. albopictus*, as a fundamental pre-requirement to support the Sterile Insect Technique (SIT) and Incompatible Insect Technique (IIT) approaches which are in progress, in Sri Lanka. In novel techniques such as SIT, IIT or the use of genetically modified mosquitoes need artificial rearing of the life cycle stages of disease vectors followed by open releases into the environment and thereby reduce vector densities through population replacement or suppression. Sampling sites included Brandiyamulla, Gampaha, and Miriswaththa in Gampaha Medical Office of Health (MOH) area of Sri Lanka. Unfed adults and 3rd instar larvae, 250 in number were sacrificed using a cold shock and 70% Ethanol respectively. 70% ethanol followed by phosphate buffer saline (PBS) were used for surface sterilization. A homogenized lysate was prepared in sterile PBS (250µL), by pooling dissected midguts of ten individuals of larvae/adult mosquitoes. A dilution series (100- 10⁻⁷) was made from lysate and 100 µL from each dilution was plated on Plate Count Agar and pure cultures for each microbe were obtained. Isolated bacteria were subjected to 16S rRNA amplification. A total number of 6 bacterial strains (*Microbacterium trichothecenolyticum*, *Kocuria kristinae*, *Elizabethkingia miricola*, *Staphylococcus sciuri*, *Pantoea dispersa*, *Neisseria flavescens*) were identified from 5 bacterial families; Flavobacteriaceae (22.05%), Neisseriaceae (11.76%), Micrococcaceae (10.29%), Staphylococcaceae (14.70%), and Erwiniaceae 35.29%) from field-collected adults, while 6 strains (*Agromyces* sp., *Microbacterium paraoxydans*, *Microbacterium* sp., *Bacillus megaterium*, *Bacillus nanhaiensis*, *Bacillus* sp.) were identified from the field-captured larvae. Species composition of gut microbes isolated from larvae was dominated by family Bacillaceae (76.76%). *Pantoea dispersa* and *Bacillus megaterium* were the most prominent bacterial species isolated from midgut of adults and larvae respectively. *Microbacterium* genera was found as common for both adults and larvae, although no common bacteria were found up to species level. Midgut bacteria belonged to Bacteroidetes (*Elizabethkingia miricola*) and Proteobacteria (*Pantoea dispersa*, *Neisseria flavescens*) were only recorded from the midgut of adults. Larvae and adults in *Ae. albopictus* denoted different midgut bacterial species.

Keywords: insect, lysate, microbe, mosquito, strains