

Bacterial Diversity in the Midgut of Field Caught *Culex tritaeniorhynchus*

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Ingestion of blood meal by female mosquitoes triggers a series of physiological processes in midgut where symbiotic microbes also exist. These symbiotic microbes can be engineered to produce molecules that inhibit pathogens; through paratransgenic approach. Little is known about the midgut microbes of *Culex* mosquitoes and no attempt has been made so far in Sri Lanka. *Cx. tritaeniorhynchus* mosquitoes were separated from the entomological surveys conducted at Kelaniya Medical officer of Health (MOH) area from June – August 2019. Unfed adult female mosquitoes were sacrificed using a cold shock and were surface sterilized using 70% ethanol followed by rinsing with phosphate buffer saline (PBS). Midgut of mosquitoes were dissected and midgut of ten mosquitoes were pooled in sterile PBS (250 µL) to make a homogenized lysate. A dilution series (10⁰- 10⁻⁷) was made from lysate. 100 µL from each dilution was plated on Plate Count Agar (PCA) and were incubated for 48 hours at 37 °C. Pure cultures for each microbe were obtained from the primary plates using streak plate method, sub culturing in Nutrient Agar. The experiment set up was repeated 25 times with ten mosquito pools at each effort. Colony separation was done based on phenotypical differences and basic biochemical tests. Stab cultures of isolates were sequenced for 16S ribosomal RNA partial gene. To identify the closest related sequence, obtained sequences were analyzed by Bioedit software package and completely aligned sequences were compared with the BLAST database. The evolutionary history was inferred using the neighbor-joining method and the evolutionary analyses were conducted in MEGA X. A total of eight bacterial strains namely; *Staphylococcus pasteurii*, *Bacillus megaterium*, *Staphylococcus cohnii*, *Pantoea dispersa*, *Staphylococcus chromogenes*, *Bacillus aquimaris*, *Staphylococcus arlettae*, *Staphylococcus scuirii* was isolated from *Cx. tritaeniorhynchus* (n=250). All of these species were belonged to two phyla; Firmicutes and Proteobacteria. Phylum Firmicutes was the dominant phyla which include seven species. The evolutionary distances which were computed using Tajima-Nei method were used to infer the phylogenetic tree. It represented a close relationship between the species of two genera; *Staphylococcus* and *Bacillus* while the relationship was distant for genus *Pantoea*. The present data strongly encourage further investigations to explore the potential usage of these microbes through the paratransgenic approach which is a novel eco-friendly vector control strategy.

Keywords: evolutionary; microbe; mosquito; paratransgenic; symbiotic

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