

## CHAPTER V

### Conclusion and Suggestion

Morphological changes of the *An. dissidens* midgut showed that the tissue was still immature in newly emerged mosquitoes. The midgut was surrounded by a YB that covered the MV. At the mature stage (likely 3-5 days after emergence), the apical side of the midgut was covered by MN during sugar and post blood feeding. A deteriorative cell was not seen in this study. Initial PM formation was revealed within 3 h after blood feeding and became highly compacted between 6 and 8 h. The mosquitoes took around 72 h to digest the blood meal, in which time the midgut returned to its previous structure.

Sixty-six midgut protein spots were identified by nano LC-MS, including proteins involving carbohydrate metabolism, blood feeding, ovarian development, cytoskeleton apparatus, innate immunity, and detoxification.

Changes in the salivary gland morphology of *An. dissidens* during adult development were observed. The salivary glands developed rapidly and matured within 3 days. Degenerative changes, including loss of stored secretion and increased cytoplasmic vacuolation, as well as concentric lamellar structures, were observed from day 16 post emergence, which correlated with mosquito development. The secretions in all secretory cavities were released immediately after blood feeding through a canal that had smaller secretory cavities and puckered basal lamina.

For salivary gland proteins, seventeen major spots appeared from newly emerged mosquitoes to 21 days after emergence. Protein expressions were high following the aging process. Diverse expressions were found surprisingly on day 12. Moreover, fifteen protein spots showed significant depletion after blood feeding, with the percent amount of depletion ranging from 8.5% to 68.11%. This work provides candidate proteins that may be involved in sporozoite maturation and transmission, including the putative mucin-like proteins, anti-platelet protein, long form D7 salivary proteins, putative gVAG

protein precursor, D7-related 3.2 protein, gSG7 proteins, and gSG6 protein.

In total, sixteen bacterial species from laboratory reared *An. dissidens* mosquitoes were identified. The bacteria belonged to Gammaproteobacteria (such as *Enterobacter*, *Thorsellia*), Alphaproteobacteria (such as *Asaia*) and Firmicutes (such as *Enterococcus*). The identification of bacterial species were performed using the culture dependent and culture independent methods, it could be summarized that both methods complemented each other. Further study should investigate the horizontal, vertical and transstadial transfer of bacteria in the midgut of *An. dissidens*.

Overall, this study reported the initial influence on vector competence from changes in physiological characteristics and persistence of microorganisms in tissues. These observations are substantial evidence that supports the holistic knowledge of underlying relationships between events (during the aging process and gonotrophic cycle) and several factors affecting *Plasmodium* transmission. However, cause and effect cannot be established by using only data such as these. To understand further the properties and functions of tissue barrier or supporting factors applied to *Plasmodium*, meta-analyses need to be pursued. These would hopefully reveal factors that play an important role in malaria transmission and produce candidates for targeting its interruption.

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