CHAPER IV

Discussion

4.1 Changes of the midgut from female *An. dissidens* during adult development and blood feeding

4.1.1 Midgut morphology

The midgut of female mosquitoes not only plays an important role in blood digestion for egg development, but also is the first place for *Plasmodium* development and multiplication. When the midgut lumen is in a degradative environment filled with protease and other digestive enzymes, parasites must leave it as fast as possible. In the complex life cycle of *Plasmodium*, its incursion of the midgut creates the first invasive bottleneck. To understand the environmental factors better, changes in the apical epithelial surface of the female midgut, underlying two different events (during the adult development and after blood feeding), were observed.

It is generally considered that morphology of the midgut epithelium of *An. dissidens* is similar to that described in other Culicidae (Hecker et al. 1977; Clements 1992; Siden-Kiamos and Louis 2004; Abraham and Jacobs-Lorena 2004; Okuda et al. 2002, 2005; Ferreira et al. 2008). Furthermore, the morphological comparison between the hematophagous and non-hematophagous mosquito (*Toxorhynchites theobaldi*) has shown that the anterior midgut of the latter is more slender and longer than that of the former, while the posterior midgut of the latter is smaller than that of the former. Although a difference of the midgut morphology has been found in both mosquito groups, its functions are similar in food digestion and absorption (Godoy et al. 2015).

The epithelial cells of *An. dissidens* were not distinguished completely soon after emergence (0-2 days old). This corresponded to *Ae. aegypti* and *An. gambiae* (Hecker et al. 1974 and 1977), in that the apical surface of their midgut was found to be

covered by YB and highly irregular in shape, with few MV. This reflected constraint in the function of food digestion and absorption. The YB is possibly a meconial peritrophic matrix (MPM), containing sloughed larval midgut epithelium replaced by regenerative cells. There are two forms of MPM, including MPM1 that is found from late in the 4 th larval stage until early in the pupal stage, and MPM2 which forms around the blood meal in adult female mosquitoes (Moncaryo et al. 2005; Inthakhan et al. 2015). The role of MPM may involve protection of developing adult midgut epithelium from microbes in the midgut lumen. Furthermore, Moll et al. (2001) revealed the occurrence and number of bacteria within MPM in newly emerged mosquitoes. The results supported the property of MPM1, which is important in sterilization of the mosquito midgut between the 4th larval and early pupal stage, and it might be a source of nutrients for newly emerged mosquitoes.

After dislodging YB from the apical surface, the single cell layer epithelium was observed on the luminal surface, containing two epithelium cell types. Firstly, the epithelium cell displays brush borders that have numerous MV on the apical cell. Secondly, the epithelium cell displays none or a few MV on the apical cell, which is called a bare cell. This cell is found often singularly or in small clusters on the posterior of the midgut. Although studies on the function of bare cells have been performed, the cell function is unclear (Zieler and Dvorak 2000; Vlachou et al. 2004; Baton and Ranford-Cartwright 2005; Whitten et al. 2006). Shahabuddin and Pimenta (1998) revealed that the specific cell type in the *Ae. aegypti* midgut, named Ross cells, is the target for *P. gallinaceum* ookinate. Characterization of the Ross cell has shown low numbers of MV, a highly expressed level of vesicular ATPase, and lack secretory granules. Meanwhile, the invasion of *P. berghei* ookinetes and *P. gallinaceum* ookinates on *An. stephensi*, and *An. stephensi* and *Ae. aegypti* midgut cells, respectively, have taken different routes (Shahabuddin and Pimenta 1998; Han et al. 2000; Gupta et al. 2005). These findings showed the species-specific mechanism in the invasions of parasites on mosquito vectors.

On day 3, MN strands were woven into a sheet covering the MV area on the entire surface of the luminal side in the *An. dissidens* midgut, which corresponded with those of *Ae. aegypti* and Hemiptera (Zieler et al. 2000; Silva et al. 2007). However, there are various types of production. For instance, the MN of *Ae. aegypti* and *Brontocoris*

tabidus has been found in the midgut at all times, while that of *Rhodnius prolixus* has materialized a few hours after feeding (Zieler et al. 2000; Fialho et al. 2009). A main function of MN is probably protecting the midgut epithelium directly from phagocytes and toxic products of blood digestion.

Previous studies on the MN of *R. prolixus* have demonstrated that it is involved in supporting the journey of trypanosomatids to the gut, suggesting that its components induce significant colonization of *Trypanosoma cruzi* in the midgut vector (Gonzalez et al. 2006; Alves et al. 2007). Furthermore, Vega-Rodriguez et al. (2014) have revealed inhibition of *Plasmodium* ookinete invasion of the *An. gambiae* midgut, by binding both ookinete surface enolase and a specific midgut receptor of *An. gambiae*, termed SM1, which is only detectable on the luminal side of the female mosquito epithelium. Therefore, an alternative function of the MN might be involved in trapping pathogens in the midgut lumen in order to invade the midgut epithelium. However, further studies should identify and characterize the proteins of MN in other mosquito vectors, to determine whether they are involved in supporting parasite traversal of the midgut epithelium.

The time of blood digestion in mosquitoes (*Anopheles*, *Aedes* and *Culex*) during the gonotrophic cycle is approximately 60-70 h depending on the ambient temperature, blood source, meal size, and several other factors (Lehane 2005). When *An. dissidens* digested blood meals completely, their midgut returned to the characteristics observed before blood feeding within 72 h.

In *Cx. quinquefasciatus*, secretion of substances, such as PM precursor granules and digestive enzymes, have been synthesized in the epithelium of midgut cells before blood feeding (Okuda et al. 2002). The substances have been released immediately as PM formation began to happen in the midgut lumen (Okuda et al. 2005). A factor of released substances probably involves stretching the epithelium.

The PM in *An. dissidens* lined the apical side of the epithelial and compacted in 3 h and in 8-10 h after blood feeding, respectively. However, the PM formation in *An. darlingi* and *Cx. quinquefasciatus* has been formed completely within 12 h (Okuda et al. 2002; Cázares-Raga et al. 2014). The PM component (Ag-Aper1 protein) in *An. gambiae*

has been first observed at 60 h (Devenprot et al. 2004), while that in *Ae. aegypti* became evident at around 4-8 h after blood feeding (Perrone and Spielman 1988; Intakhan et al. 2014) and *Ochlerotatus togoi* formed around 45 min after blood feeding (Jariyapan et al. 2013). It should be noted that the PM in each species takes different times to form.

There has also been evidences that PM formation has involved in the infection process. Infectivity of *P. gallinaceum* in *Ae. aegypti* has been reduced when thickness of the PM increases (Billingsley and Rudin 1992). The mechanisms by which the PM has been formed and its stage of complexity or consolidation at the time of ookinete maturation differ between anopheline and aedine species (Billingsley and Rudin 1992). PM formation is significantly slow when *P. berghei* invades *An. gambiae* (Huber et al. 1991). However, the non-hematophagous mosquito (*T. theobaldi*) has presented PM in the midgut lumen, as it is not related to blood feeding (Godoy et al. 2015). An important role of the PM is to protect the midgut epithelium from abrasive food particles, digestive enzymes, toxic products, and pathogenic infections per se.

4.1.2 Midgut proteins

Many mosquito tissues have well-developed a few days after emergence by control of juvenile hormones for mosquito development and reproduction (Hagedorn 1994; Raikhel et al. 2005). Our study also investigated the midgut protein expression of female *An. dissidens* (3-5 days old). The most of abundant proteins in the *An. dissidens* midgut involved carbohydrate metabolism such as malate dehydrogenase (SN6), dihydrolipoamide succunytransferase component of 2-oxoglutarate dehydrogenase (SN12), pyruvate kinase (SN13), hexokinase (SN16), phosphoglycerate kinase (SN17), enolase (SN18), fructose-bisphosphate aldolase class I (SN24-26), glycerol-3- phosphate dehydrogenase (NAD+) (SN32), isocitrate dehydrogenase (NAD+) (SN33), 2,3-bisphosphoglycerate-dependent phosphoglycerate mutase (SN43), triosephosphate isomerase (SN45, 46), galactokinase (SN48), and oxidative phosphorylationolism such as NADH dehydrogenase (ubiquinone) Fe-S protein1 (SN3), dihydrolipoamide dehydrogenase (SN8, 10), aldehyde dehydrogenase (SN11), F0F1-type ATP synthase beta subunit (SN14), ATP synthase beta subunit (SN15), aldehyde reductase (SN28), proton donor (SN29), V-type H^+ transporting ATPase subunit E (SN41), NADH dehydrogenase (ubiquinone) flavoprotein 2 (SN42), cytochrome b-c1 complex subunit

Rieske mitochondrial (SN47), cytochrome c oxidase subunit Va precursor (SN66). The results corresponded to the proteins expressed in *Ae. aegypti* and *Ae. albopictus* (Popova-Butler et al. 2009; Saboia-Vahia et al. 2012).

Proteins involved in amino acid metabolism such as wd-repeat protein (SN5), glutamine synthase (SN21), serpin (SN22), arginine kinase (SN23), coproporphyrinog en III oxidase (SN34), cytosol aminopeptidase (SN37), guanine nucleotide- binding protein subunit beta-2- like 1 protein (SN38), superoxide dismutase1 (SN52), eukaryotic translation initiation factor 5A (SN55), 40S ribosomal protein S12 (SN60), and proteins involved in lipid metabolism as mitochondrial electron transfer flavoprotein subunit alpha (SN36) also were found in the *An. dissidens* midgut. The proteins expressed could be correlated with several parts of the mosquito life span, including protein associated with basal metabolism, and proteins that play important roles in blood feeding and ovarian development (Sawabe and Moribayashi 2000).

Proteins involved in detoxification such as glutamine synthase (SN21) and superoxide dismutase (SN52) were observed. The midgut has an overwhelming number of toxic compounds (such as free radicals, or ammonia) during sugar or blood feeding; however, the midgut could detoxify the compounds (Scaraffia et al. 2010; Sim and Denlinger et al. 2011). The level of glutamine synthase activity, due to environmental toxins, has been associated with resistance to all major classes of insecticides by catalyzing the conjugation of glutathione (Ranson and Hemingway 2005). Furthermore, *Chironomus riparius* has expressed thioredoxin reductase during this period (Nair and Choi 2012).

Proteins related to cytoskeleton apparatus such as actin (SN19, 20), tropomyosin (SN27), muscular protein 20 (SN56, 58), profilin (SN62), found in all eukaryotic cells, were observed in *An. dissidens.* The proteins play important roles in cytokinesis, cell shape, phagocytosis, movement of the cell and chromosome, and intracellular signaling (Pollard and Cooper 2009).

4.2 Changes in the female salivary glands of *An. dissidens* during adult development and after blood feeding

4.2.1 Salivary gland morphology

This study revealed the clearly investigation of morphological salivary gland changes, both during adult aging and blood feeding. In general, the female salivary gland showed similarities with the salivary glands of *Aedes*, *Culex* and *Anopheles* species in terms of cell shape and organ location (Orr et al. 1961; Wright 1969; Janzen and Wright 1971; Barrow et al. 1975; Moreira-Ferro et al. 1999).

Following emergence, development of the *An. dissidens* salivary glands of the newly emerged mosquito was progressive. The glands accumulated secretory material rapidly and developed completely within day 3, corresponding to previous studies on salivary gland proteins in *Ae. aegypti*, *An. cracens*, and *Cx. quinquefasciatus* (Beckett 1990; Nascimento et al. 2000; Jariyapan et al. 2007).

In most of mosquito species, on the first day post emergence, adult *Anopheles* spp. females need to feed on sugar to meet the energy demands of basal metabolism and flight. After the third day of adult life, they must feed on human or animal blood as they require nutrients in the blood to stimulate growth of ovaries and encourage creation of eggs (Clements 1992). The rate of protein accumulation in the salivary glands is highest on day 2 of adult development, reaching a peak in week two and then beginning to decline at week three (Racioppi and Spielman, 1987).

Degenerative changes including loss of stored secretion, increase of cytoplasmic vacuolation, and concentric lamellar structures were observed from 16 days post emergence that correlate with total amount of the salivary gland proteins determined during adult development. This result suggests that the cytological changes in the salivary glands are a natural phenomenon due to aging as reported in *Ae. aegypti* and *Ae. togoi* (Beckett 1990). However, study on details of morphological and cytopathological changes of long-term malarial infected salivary glands of *An. dissidens* using TEM would improve our understanding on the mosquito cellular respond to malarial infection as they

relate to pathogen transmission as a study in *Cx. quinquefasciatus* infected with West Nile virus (Girard et al. 2005).

4.2.2 Salivary gland proteins

Variation in the physiological states of mosquitoes according to their age, blood feeding status and pathogenic infection have all been shown to be important factors in determining salivary protein amounts and composition (Nascimento et al. 2000; Siriyasatien et al. 2005; Choumet et al. 2007; Phumee et al. 2011; Jariyapan et al. 2012; Wasinpiyamongkol et al. 2012; Cotama et al. 2013; Zocevic et al. 2013; Sor-suwan et al. 2014). In the present study it was demonstrated that ageing affected the expression of proteins in the salivary glands of female *An. dissidens*. The expression of salivary gland proteins in the mosquitoes varied from 0 to 21 days post emergence. The 17 major salivary gland proteins identified were detectable from day 1 to day 21 post emergence, suggesting that the mosquitoes may be capable of blood feeding within 24 h of emergence. However, other factors, such as the maturation of the mosquito midgut, and juvenile mosquito hormone levels, are involved in digestive regulation and facilitation (Billingsley 1990; Moll et al. 2001; Moncayo et al. 2005; Noriega et al. 1997; Zhou et al. 2011). Our results are consistent with previous studies in *An. barbirostris* species A2 and *An. gambiae*. It was shown that the expression of *An. barbirostris* species A2 salivary gland proteins is affected by ageing, although the proteome profiles were analyzed only 0 to 60 h post emergence (Jariyapan et al. 2012). The salivary proteome of female *An. gambiae* mosquitoes aged 21 days after emergence were shown to be more diverse than the mosquitoes aged 8 days old (Choumet et al. 2007).

From several studies in mosquitoes infected by malaria parasites, *Plasmodium* sporozoites have been discovered in the salivary glands of the mosquito vectors from day 9 to 21 post infection, depending on the species of both parasites and mosquitoes and the ambient temperature (Beier 1998; Myung et al. 2004; Akaki and Dvorak 2005; Sato et al. 2014). In *An. dissidens* mosquitoes, they have been found in the salivary glands of the infected mosquitoes at 14 days after infection (Thongsahuan et al. 2011). For this reason, one purpose of this study was to investigate the salivary proteins that may influence sporozoite maturation using the evidence from the expression patterns of the major salivary gland proteins during adult development. Our results indicate that

the proteins in Group 1, which started to show maximum expression in the mosquitoes aged 12 days old or 16 days old onwards, and Group 3, which showed maximum expression on day 12 of adult life, are potentially the most interesting with respect to sporozoite maturation. These proteins include a putative mucin-like protein (SN4), a long form D7 salivary protein (SN10), a putative gVAG protein precursor (SN11), a short form D7r1 salivary protein (SN13), gSG7 salivary proteins (SN15 and 16), an anti-platelet protein (SN8), a short form D7r1 salivary protein (SN12), a D7-related 3.2 protein (SN14), and a gSG6 protein (SN17). These proteins might provide a suitable environment for early stage sporozoites to develop into mature sporozoites. However, most of these proteins including the long form D7, gVAG, short form D7r1, gSG7, anti-platelet protein, D7 related 3, and gSG6, have been characterized as being involved in anti-platelet aggregation, anti-vasoconstriction and anti-inflammatory responses (Ribeiro and Francischetti 2003; Calvo et al. 2004; Boisson et al. 2006; Isawa et al. 2007; Yoshida et al. 2008; Lombard et al. 2009; Das et al. 2010; Alvarenga et al. 2010; Assumpcao et al. 2013).

For Group 2, their expression pattern together with the function of apyrase as an anti-platelet aggregation protein (Sun et al. 2006) and a long form D7 salivary protein (D7L2) that has been shown to reduce blood feeding capacity (Das et al. 2010), suggests that apyrase homologues (SN1, 2, and 3), a putative mucin-like protein (SN5), a putative mucin-like protein (SN7), and a long form D7 salivary protein homologue (SN9) may be involved in blood feeding. Regarding a putative mucin-like protein (SN6; Group 4), it was expressed at its highest level on day 3, then steadily falling thereafter suggesting that this protein might not be involved in the maturation of the parasites. Further studies on the roles of these salivary gland proteins involving the regulation of infectivity of sporozoites should be performed.

Choumet et al. (2012) have recorded activity during the blood-feeding phase of *An. gambiae* and shown that young mosquitoes aged 8 days old fed more rapidly than older mosquitoes aged 23 days old. In the *An. dissidens* mosquitoes aged 12 to 21 days old, proteins that are homologous to salivary apyrase (SN3), anti-platelet protein (SN8), a long form D7 protein (SN9), D7r1 (SN12), D7 related 3 (SN14), and gSG6 (SN17) were found with decreased in expression on day 12 or 16 of adult life. One possibility is that

the decrease in amount of these proteins associated with blood feeding could help in promoting transmission, by providing a longer probing time so giving more opportunity for the sporozoites to enter the skin of a new host, but this requires experimental investigation.

Another purpose of this study was to investigate for proteins that may influence sporozoite transmission, by looking for those that show a high level of depletion immediately after blood feeding. The major proteins which were depleted significantly (more than 30%) after blood feeding in this mosquito species were the homologue proteins to apyrase (SN3), putative mucin-like proteins (SN4, 5 and 7), anti-platelet protein (SN8), long form D7 proteins (SN9 and 10), gVAG protein precursor (SN11), D7 related 3 (SN14), gSG7 (SN15 and 16), and gSG6 (SN17). The results correspond with the findings of the studies into *An. gambiae* and *An. camprestris*-like (Choumet et al. 2007; Sor-suwan et al. 2014). It should also be noted that salivary proteins are not always evenly distributed within the glands and may be present in different amounts in different lobes (Phattanawiboon et al. 2014; Suwan et al. 2002; Sor-Suwan et al. 2013) and this may explain the differential depletion of some proteins compared to others after blood feeding.

Researches into sporozoite biology have uncovered the skin stage of the *Plasmodium* life cycle (Sidjanski and Vanderberg 1997; Matsuoka et al. 2002; Vanderberg and Frevert 2004; Amino et al. 2006; Yamauchi et al. 2007). The sporozoites do not directly enter the host bloodstream during a blood meal of mosquitoes. Later they leave the site of injection and find their way to the bloodstream independently (Sidjanski and Vanderberg 1997; Matsuoka et al. 2002). Studies by Amino et al. (2006) revealed that about half of the sporozoites remain in the skin for up to seven h, and Yamauchi et al. (2007) showed that 15-20% of the sporozoites entered the lymphatic system. Matsuoka et al. (2015) have confirmed that sporozoites can stay in the skin site for more than 42 h when deposited there by infective mosquitoes. Many sporozoites remain motile for at least 30 min at the bite site (Vanderberg and Frevert 2004). Since sporozoites can reside in the skin at the bite sites for h after injection, the co-inoculation of salivary proteins together with the sporozoites might help in maintaining or supporting optimal conditions

for the parasites, before migrating to a blood vessel for passage to the liver or to the draining lymph node.

As discussed above, the results of this study has identified proteins that may have a role in sporozoite maturation and transmission, including the putative mucin-like protein (SN4), the anti-platelet protein (SN8), the long form D7 salivary protein (SN10), the putative gVAG protein precursor (SN11), the D7-related 3.2 protein (SN14), gSG7 salivary proteins (SN15 and 16), and the gSG6 protein (SN17). Studies on infected mosquitoes, including other mosquito species not yet examined, might provide better understanding of the interaction between the salivary proteins identified by depletion following blood feeding, and skin stage sporozoites. Further investigation on the functions of the salivary gland proteins on skin invasion of *Plasmodium* sporozoites could be performed using transient RNA interference (RNAi) gene-silencing assays on the salivary transcribed genes in the mosquito vectors together with the fluorescently labeled *P. berghei* parasites (Choumet et al. 2012; Frischknecht et al. 2004, 2006). Real-time imaging would allow visualization of gliding motility and invasion of the released salivary gland sporozoites at the bite sites and determine probing time and feeding quality and quantity of the mosquitoes.

4.3 Bacterial diversity of female *An. dissidens* midguts

Microflora (MF) in mosquito midgut have come out as an important factor contributing vector competence for human pathogens. In particular, the colonized MF have shown to both directly and indirectly kills *Plasmodium* parasites in *An. gambiae* (Dong et al. 2009). This study was an attemp to investigate the bacterial community of female *An. dissidens* (lab-reared strain). Comparative characterization of cultivable and uncultivable MF of female *An. dissidens* midguts yields an interesting result that the MF compositions were different. There are three possible explanations for the differences; 1) DNA competition from divergent bacteria favoring the ones of higher abundance in the PCR; 2) no primer pairs for the 16S rRNA gene exist that can amplify all bacteria present in the databases (Baker et al. 2003); 3) Ability of bacteria to grow on the LB media, because some bacteria require special nutrients to support their growths. This is similar to previous studies (Pidiyar et al. 2004; Lindh et al. 2005; Rani et al. 2009; Djadid et al. 2011).

The predominant bacteria in lab-reared female *An. dissidens* midgut displayed 16S rRNA gene similarity to class of gammaproteobacteria, i.e., *Thorsellia anophelis* (uncultivable bacteria) and *Enterobacter* sp. (cultivable bacteria). The results were similar to several previous studies on *An. albimanus*, *An. fuestus*, *An. gambiae*, *An. darlingi*, *An. stephensi*, *An. barbumbrosus*, *An. crawfordi*, *An. gigas*, *An. maculatus* (Pumpuni et al. 1996; Straif et al. 1998; Gonzalez-Ceron et al. 2003; Terenius et al. 2008; Rani et al. 2009; Ngo et al. 2015). The gammaproteobacteria are water and soil dwelling bacteria, suggesting that *An. dissidens* picked up MF from the natural water used and/or ingested food. In *An. gambiae* mosquitoes captured from central Kenya (nearby rice paddy environment), the dominant bacterium in the midgut such as *T. anophelis* has been also found in high abundance in rice paddies and surface of rice paddies water where *An. gambiae* s.l. larvae feed on (Briones et al. 2008). It is propable that *T. anophelis* in the midgut of *An. dissidens* might also be from transstadial transfering from the natural water that used for the rearing larvae.

In the *An. dissidens* midgut, *Asaia* sp. was identified. The bacteria isolate commonly exist in nectar (Moore et al. 2002; Tucker and Fukami 2014). Interestingly, the bacteria isolate can horizontally and vertically transmit between *Anopheles* mosquitoes (Crotti et al. 2009). Moreover, the bacteria isolate can be found in *An. stephensi* ovaries and eggs, thus allowing transmission pass on the bacteria from mother to offspring (Favia et al. 2007).

A bacterium, *Thermoactinomyces daqus*, is a new species of *Thermoactinomyces* that has been a fermentation starter for the production of Chinese liquors. *T. daqus* is thermophilic bacteria in a group of Actinobacteria. This bacterial group includes some of the most commonly found in soil and freshwater (Yao et al. 2015). Interestingly, *T. daqus* was also detected in the *An. dissidens* midguts. Actinobacteria have been extensively studied for secondary metabolite production with high pharmacological and commercial interest (Anandan et al. 2016). Moreover, it has been known that secondary metabolites are used potentiable control of mosquito-parasitic nematodes and mosquito larvae, and are completely harmless to other nontarget organisms and environment (Pampiglione et al. 1985; Zizka et al. 1988; Rao et al. 1990; Ikeda and Omura 1995).

Recently, several evidences have shown that the bacterial communities influence vector competence for human pathogen (Dong et al. 2009; Rodrigues et al. 2010; and Ramirez et al. 2014). Dong et al. (2009) has revealed that sterile mosquito (mosquito which the midgut bacteria were depleted by antibiotic) is more susceptible to *Plasmodium* infection than septic mosquitoes, suggesting that bacteria in the midgut affect to *Plasmodium* infection. Moreover, Ramirez et al. (2014) has found that secondary metabolites from *Chromobacterium* sp. isolated from the midgut of field-collected *Ae. aegypti* mosquitoes, contain anti-*Plasmodium* and anti-dengue activity. On the other hand, *Serratia odarifera* has been found the potential influence of dengue virus susceptibility of *Ae. aegypti* female (Apte-Deshpande et al. 2012).

Moreover, *Elizabethkingia* sp. has been detected as the predominant species in *An. gambiae* and *An. stephensi*, and the prevalence of *Elizabethkingia* sp. is correlated with the reduction of bacterial diversity during larvae to adult metamorphosis (Boissière et al. 2012; Akhouayri et al. 2013; Ngwa et al. 2013). This reduction in bacterial diversity eventually result in higher susceptible to *Plasmodium* infection.

The understanding of the interactions between MF and mosquito midgut could be potentially used for the development of novel transmission intervention strategy for arthropod-borne diseases. Further study should address the anti-human pathogen properties of bacterial isolates from *An. dissidens* mosquito. Additionally, these bacterial isolates should be determined for capacity to colonize and/or persist in the midgut, the impact of bacteria isolates on mosquito longevity, as these factors can determine the success of transmission intervention.