CHAPTER V
DISCUSSIONS AND CONCLUSIONS

1. Bed bug (*C. hemipterus*) *Wolbachia*

We were capable to amplify the *wsp* fragment of Wolbachia infected of bed bug *C. hemipterus* collected in Bangkok, Thailand. The F-supergroup *Wolbachia* 16S rDNA gene was amplified using primers INTF2 and INTR2 (19, 47). From this study, we found that the amplified gene was 136 bp which has the same sizes as pervious report (19, 47). Sequencing analysis of the amplified PCR product was demonstrated that, more than 98% identity to *Wolbachia* of *C. hemipterus* reported in the GenBank. We were not able to obtain the sequences of the 16S rDNA from reported in the previous studies, so we could not compare the sequences of our study and the sequences of the previous reports.

2. *Wolbachia* transinfected

Embryonic microinjection is commonly used to establish *Wolbachia* infections in desirable insects because it allows *Wolbachia* placement within developing pole cells and yields high rates of establishment of successful transinfected lines (15, 21). Direct adult microinjection was used to establish *Wolbachia* infection in *Ae. aegypti*. It is much simpler and has been several benefits, including no need for the expertise required for embryonic microinjection (15). In this study we used direct microinjections to introduce F-supergroup *Wolbachia* from bed bug *C. hemipterus* into virgin adult females of naturally uninfected *Ae. aegypti*. We were able to establish isofemale lines from the injection. The results indicated that, *Wolbachia* bacteria were able to transfer into the new hosts even across the insect Order.

Recently, Ruang-areerate T and Kittayapong P reported the transmission efficiency of the *Wolbachia* from *Ae. albopictus* superinfectd in *Ae. aegypti*. They capable to establish of *Wolbachia*-superinfected and transmission efficiency has maximum at G_6 (85%, n = 27) and was stably maintained until G_{26} (81.8%, n =11) (15). In this study, we capable to produced the transmission efficiency of *Wolbachia* reached

maximum at G_1 (55.55%, n = 45) and G_4 (54.71%, n = 159). At the time of this report, transinfected lines of total 10 generations post-injection are stably infected.

Relative *Wolbachia* density was determined in different tissues, including ovaries, testes, Malpighian tubule, midgut, thoracic muscles, head and spermatheca to demonstrate tissues tropism of transinfected, infection was found in the reproductive tissues; ovaries and testes only (15). Imply that *Wolbachia* prefers reproductive tissues over nonreproductive tissues. In this study, *Wolbachia* density was determined in the whole of host only.

To determine, relative of the level of maternal transmission and the *Wolbachia* copy number in the host cell. *Wolbachia* density was measured by using quantitative real-time PCR based upon SYBR Green I. We found that, *Wolbachia* density has highest in G_3 (100.5×10^{-1}) which associate with high efficiency to transinfected in G_4 (54.71%, n = 159). Additionally, *Wolbachia* density has lowest in G_8 (3.69×10^{-1}) which corresponding with low efficiency to tranisinfected in G_9 (4.2%, n = 189). It could be conclude that, the level of transinfection correlated to the level of *Wolbachia* density in the host *Ae. aegypti* population.

3. DNA sequencing

From sequencing experiment to confirm the F-supergroup *Wolbachia* transinfection, the nucleotide bases were determined from each generation. We found that, the nucleotide base alteration occurred at the same sites and the same based under the different generation but slightly different in nucleotide base change 1 region at the 55 fragment position from bed bug *C. hemipterus Wolbachia* microinjection is "A" to "G". Moreover, the amino acid alteration displayed at the same sites and the same amino acid under the different generation but distinct in amino acid 1 region at the 81 fragment position from bed bug *C. hemipterus Wolbachia* microinjection is "M (Methionine)" to "G (Guanine)" that are both in non polar side chain. This finding, we assume that *Wolbachia* may be have adapted to live in the new hosts.

In order to study the changing in nucleotide sequences of the *Wolbachia* in the new hosts, longer sequence of this gene or other genes should be study.

4. CI expression

Lower CI expression in transinfected lines has been observed in many studies and suggests that CI expression depend on host factors, *Wolbachia* adaptation or a threshold level for *Wolbachia* densities (15, 21). In recent years, there has been observed incomplete CI expression of *Wolbachia* superinfection of *Ae. aegypti* and suggest that, level of CI expression corresponds with *Wolbachia* copy number within the host (15). In the experiment, comprising single-pair copulations with one male and one female, they found that crosses between transinfected male and naturally uninfected female produced 50.85% egg hatch, which was significantly lower than that for naturally uninfected crosses produced 87.53% egg hatch [$P = 0.002$] (15). In this study, the express CI was performed in G_2 and G_4. Transinfected males with naturally uninfected females compare with naturally uninfected crosses, transinfected crosses and transinfected female crosses with uninfected male. This pattern is similar to the unidirectional CI. Result showed significantly lower hatch rate than other test crosses [P value < 0.05] but was not able to induce complete CI expression.

The result suggests that *Wolbachia*-induced incomplete CI could be used as a means for vector control of naturally uninfected *Ae. aegypti* population. In addition, we assume that the level of CI expression may be corresponded with the *Wolbachia* copy number in their hosts (un-experiment). Therefore, to develop optimum of *Wolbachia* as a vector for gene driving system, the transinfected lines of *Wolbachia* should be repeatedly mating for all generation to increase the level of maternal transmission and the *Wolbachia* density, which may be lead to high levels of CI expression or complete CI.

5. Future prospect

The fitness cost of transinfection mosquitoes could be determined by percentage of egg hatch, percentage of survival of larvae, pupae and adults and number of eggs laid compared with naturally uninfected. From this study, there were not significant difference for percentage of egg hatch, percentage of pupal survival and percentage of adult survival, whereas a significant difference was found for the number of eggs laid. The fitness costs are important to be sure of beneficial effects of

Wolbachia on transinfected (15). So, to increase efficiency of *Wolbachia* transmission to offspring, we suggest that, other parameters of fitness cost should be determined.

This study demonstrated that *Wolbachia* bacteria are a potential for using as a gene driving strategy to manipulate vector population by suppression or population replacement. Although we were successfully demonstrated cross transinfection of the *Wolbachia* across the insect Order, only incomplete CI expression was showed. In order to use *Wolbachia* as an effective gene driving system for the vector population replacement, *Wolbachia* from other hosts (insects) rather than bed bug must be explored.