Original Article

A preliminary study on diversity of midgut microbiota in *Aedes aegypti* (Linnaeus) and *Culex quinquefasciatus* (Say) collected from Bangkok, Thailand

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Abstract

Aedes aegypti and *Culex quinquefasciatus* are important mosquito vectors for many infectious diseases. A number of factors affect the vector competence of these mosquitoes for a specific pathogen. The bacteria harbored in the midgut are known to influence mosquito physiology and can alter the response to various pathogens. Bacteria from the midgut of *Ae. aegypti* and *Cx. quinquefasciatus* were cultured and identified using bacteriological and molecular techniques in this study in which two groups of mosquitoes were examined. The first group was laboratory-reared, and the second group were field-collected mosquitoes from Bangkok. Twelve bacterial genera (i.e., *Acinetobacter, Agrobacterium, Bacillus, Cellulomonas, Chryseomicrobium, Dietzia, Enterobacter, Klebsiella, Microbacterium, Pantoea, Pseudomonas,* and *Staphylococcus*) were identified from laboratory-reared *Ae. aegypti* and eight bacterial genera (i.e., *Bacillus, Cellulomonas, Microbacterium, Micrococcus, Moraxella, Neisseria, Staphylococcus,* and *Streptococcus*) were determined from field-collected *Ae. aegypti*. Five bacterial genera (i.e., *Microbacterium, Micrococcus*, *Paenibacillus*, *Pseudomonas,* and *Staphylococcus*) were identified from laboratory-reared *Cx. quinquefasciatus* and 13 bacterial genera (i.e., *Acinetobacter*, *Actinomyces*, *Bacillus, Chryseobacterium, Kocuria*, *Microbacterium, Micrococcus, Novosphingobium*, *Pantoea*, *Providencia*, *Pseudomonas*, *Rhodococcus*, and *Staphylococcus*) were examined from field-collected *Cx. quinquefasciatus*. The variation of these midgut microbiota may influence mosquito vector competence for a specific pathogen. However, further studies need to be performed to indicate this relationship.

Keywords: Bacteria, Midgut, *Aedes aegypti*, *Culex quinquefasciatus*, Thailand

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Introduction

Aedes aegypti (Linnaeus) and *Culex quinquefasciatus* (Say) are important mosquitoes that can be found worldwide and throughout Thailand. They are nuisance insects and important vectors for many infectious diseases. They feed on various animal hosts and humans, and sometimes bite more than one host or one person within each gonotrophic cycle (Harrington et al., 2014). *Ae. aegypti* exclusively feed on humans in a single host species and most of multiple-host bloodmeals include at least one human host. Humans, dogs and swine are preferred hosts but bovines and chickens are avoided as hosts for *Ae. aegypti* in Thailand (Ponlawat and Harrington, 2005). *Cx. quinquefasciatus* is most frequently found in and around human habitations and is most prevalent during the rainy season. It prefers to feed on human blood than that of other animals (Azmi et al., 2015).

Ae. aegypti play an important role as vectors for many filarial nematodes and viruses particularly the Zika and dengue virus (Watson and Kay, 1999; Tiawsirisup and Nithiuthai, 2006; Diallo et al., 2008; Ariani et al., 2015; da Moura et al., 2015; Monaghan et al., 2016; Richard et al., 2016). *Cx. quinquefasciatus* are also important vectors for filarial nematodes, protozoa, and many viruses such as canine heartworm caused by *Dirofilaria immitis*, avain malaria caused by *Plasmodium gallinaceum*, West Nile virus and Rift Valley fever virus (Vargas and Beltran, 1941; Ahid et al., 2000; Tiawsirisup and Nithiuthai, 2006; Turell et al., 2007; Sudeep et al., 2015; Yurayart et al., 2017).

Vertebrate hosts, pathogens and mosquito vector factors affect the vector competence of mosquitoes for a specific pathogen. Pathogen infection in mosquitoes can occur only in the mosquito midgut since the structure and function of the midgut are different from the foregut and hindgut (Houk et al., 1981; Mercado-Curiel et al., 2008). The mosquito gut is the first point of contact between ingested pathogens and the mosquitos epithelial surface. The midgut is an important location for host-pathogen interaction and pathogen survival or elimination is thought to be an outcome of this relation. The midgut of the mosquito vector contains not only pathogens but also a diverse microbiota (Dennison et al., 2014). Midgut microbiota are bacteria that have co-evolved and developed symbiotic relationships with mosquitoes or the bacteria that are acquired from the mosquito's breeding water or nectar sources and they have adapted to persist within the mosquitoes. These bacteria influence the mosquitoes' physiology, the susceptibility of the mosquitoes to specific pathogens, the response to various pathogens, and the ability to transmit the pathogen. In the same vein, internal factors of the mosquito might modulate the composition of its midgut bacterial population. Midgut structure, pH, digestive enzymes and ingested food are factors shown to significantly influence the diversity of the microbial community of the mosquito (Oliveira et al., 2011; Ludvigsen et al., 2015). Midgut microbiota are also diverse depending on species, sex, developmental stage, ecological factors, and geographic location. Mosquitoes are exposed to a variety of microbes during their lifecycle, some of which are needed for their successful development into the adult stage. However, a reduction in bacteria diversity can be found during the metamorphosis (Kim et al., 2015).

The involvement of midgut microbiota in various important functions in relation to host and pathogen interaction has been reported. Studies on midgut microbiota diversity and the ability to modulate host-pathogen interaction have become a focus of research (Boissiere et al., 2012; Ramirez et al., 2012; Apte-Deshpande et al., 2014). Some studies have suggested a potential role of microbiota in the biology and vector competence of mosquitoes (Dennison et al., 2014). The midgut microbiota can modulate the mosquito's immune response and affect vector competence and can also manipulate mosquito competence by impairing pathogen infection through resource competition or antipathogen molecule secretion (Dennison et al., 2015). Ramirez et al. (2012) posited that field isolated bacteria from the mosquito midgut exert a harmful effect on dengue virus infection. The effect is demonstrated through the action of the mosquito immune system, which is activated by microbiota. On the other hand, dengue virus infection induces immune responses in the mosquito midgut tissue that act against the natural mosquito midgut microbiota. These observations have encouraged the recent development of new mosquito control methods based on the use of symbiotically-modified mosquitoes to interfere with pathogen transmission or reduce host reproduction and life span (Minard et al., 2013).

Studying the diversity of the midgut microbiota will address the basic knowledge for the advance study of the relationship between midgut microbiota and specific pathogen infection and transmission in the mosquito vectors. Understanding the role of microbiota in modulating infections with pathogens is important. However, information on midgut microbiota of mosquitoes from Thailand is limited. This study was conducted to access the diversity of midgut microbiota of *Ae. aegypti* and *Cx. quinquefasciatus* from Thailand. This is basic and important information for the advanced research on mosquito vector competence and mosquito control in Thailand.

Materials and Methods

***Mosquitoes*:** Laboratory-reared and field-collected *Ae. aegypti* and *Cx. quinquefasciatus* were examined in this study. The laboratory mosquitoes were reared and maintained with 10% sucrose at the Parasitology Unit, Department of Veterinary Pathology, Faculty of Veterinary Science, Chulalongkorn University. They were originally collected from Bangkok, Thailand and maintained for more than ten generations. Field mosquitoes were collected from Suanluang and Laksi districts in Bangkok, Thailand in 2013 using a BG-Sentinel mosquito trap (Biogents®, Germany). The species, source and number of tested mosquitoes is demonstrated in Table 1. The mosquitoes were then kept at 4°C for 20 min and identified under a light microscope. Only female mosquitoes were examined in this study. The sampling mosquitoes were washed in 70% ethanol for 5 min and rinsed with phosphate buffer saline (PBS). The midgut was then dissected

from each mosquito under a light microscope, kept in 300 µl of 60% glycerol in PBS, ground using a sterile plastic pestle, and kept at -80°C until tested. This study was conducted in 2013, then mosquitoes were not included among the experimental animals that needed approval from the Chulalongkorn University Animal Care and Use Committee.

Table 1 The species, source, and number of tested mosquitoes.

Mosquito species	Source of mosquitoes	Number of tested mosquitoes	Number of mosquitoes with midgut microbiota
Aedes aegypti	Laboratory	16	13
	Suanluang district	5	4
	Laksi district	7	6
Culex quinquefasciatus	Laboratory	11	10
	Suanluang district	10	7
	Laksi district	11	11

***Bacterial isolation and identification*:** Bacteria from the midguts of *Ae. aegypti* and *Cx. quinquefasciatus* were cultured and identified using bacteriological and molecular techniques.

***Bacteriological* technique:** The ground midgut from each mosquito was separated into three parts. For the first part, 50 µl of the ground midgut was examined for the total colonies using pour plate technique. The sample was mixed with plate count agar (PCA) and poured into a plastic petri dish. The petri dish was kept at 37°C for 24-48 hr and assessed for bacterial colonies. If the total colonies of bacteria were higher than 250 colonies, the ground midgut was diluted and the total colonies were examined again using pour plate. For the second and third part, 50 µl of the ground midgut was examined for bacterial colonies by spread plate technique using trypticase soy agar (TSA) with 5% sheep blood and MacConkey agar (MAC) with 5% sheep blood being used in this technique, respectively. The midgut was streaked over an agar surface by the four-way cross streak method and the streaked plate was kept at 37°C for 24-48 hr and assessed for bacterial colonies. The bacterial colonies were indicated as colony forming units (CFU) per mosquito.

Each bacterial colony from the spread plate technique was subcultured over TSA with 5% sheep blood by four-way cross streak method and the streaked plate was kept at 37°C for 24-48 hr. Pure bacterial colonies were examined using gram staining and molecular techniques. Before examination by molecular technique, the pure bacterial colony was cultured in Luria-Bertani (LB) broth and stock of the pure colony was kept in stock media at 37°C for 24 hr and transferred to room temperature. Duplicate samples of each mosquito midgut were analyzed.

***Molecular technique*:** Due to the various sizes and shapes of the colony of each bacterial genus, the molecular technique using DNA sequencing was mainly used to indicate the bacterial genus. Bacterial DNA was extracted using the boiling method. The pure bacterial colony was cultured in two ml of LB broth at 37°C for 24-48 hr. It was then centrifuged at 14,000 rpm for 15 min and the bacterial pellet was washed one time in distilled water. A total of 40 µl of distilled water was added into the bacterial pellet and it was kept at 100°C for 10 min, cooled down in the ice basket, and centrifuged at 14,000 rpm for 10 min. The supernatant which was extracted DNA was kept at -80°C until being tested using polymerase chain reaction and sequencing.

***Polymerase chain reaction technique*:** The extracted bacterial DNA was examined using polymerase chain reaction (PCR) technique. Two universal primers for 16S ribosomal RNA (16S rRNA) gene of bacteria were used in this study. The first pair of primers were 16S Forward 5'-AGT TTG ATC CTG GCT CAG-3' and 16S Reverse 5'-GCT ACC TTG TTA CGA CTT C-3' (Dinparast Djadid et al., 2011) and the second pair of primer were 63F 5'-CAG GCC TAA CAC ATG CAA GTC-3' and 1387R 5'-GGG CGG WGT GTA CAA GGC-3' (Marchesi et al., 1998). PCRs were performed in 25 µl-reaction. The PCR consisted of 1.5 µl of DNA template, 0.2 µl of *Taq* DNA polymerase (Platinum *Taq* DNA polymerase high fidelity, Invitrogen, USA), 2.5 µl of 10X PCR buffer, 0.5 µl of 10mM dNTPs, 1 µl of 50 mM $MgSO_4$, 10 µM of forward primer, 10 µM of reverse primer and 17.3 µl of distilled water. DNA was amplified using thermocycler (Perkin Elmer Cetus 9600, Perkin Elmer, Waltham, MA).

Reaction for the first primer consisted of the initial denaturation at 94°C for 2 min, the amplification was carried out for 35 cycles with the following temperature cycling parameters: 94°C for 30 s of denaturation, 55°C for 30 s of annealing, and 68°C for 1 min 30 s of extension. The final amplification cycle included an addition of 10 min extension at 72°C. Reaction for the second primer consisted of the initial denaturation at 94°C for 2 min, the amplification was carried out for 30 cycles with the following temperature cycling parameters: 95°C for 1 min of denaturation, 55°C for 1 min of annealing, and 72°C for 1 min 30 s of extension. The final amplification cycle included an addition of 5 min extension at 72°C. The PCR product was mixed with loading buffer (BlueJuice™ Gel Loading Buffer, Invitrogen, USA) and analyzed in 1.5% agarose gel (UltraPure™, Invitrogen, Carlsbad, CA) with an expected 1.5 and 1.3 kilobase pair band, respectively.

***Bacterial DNA sequencing*:** After DNA amplification, the PCR product in agarose gel was purified using Gel/PCR DNA Fragments Extraction Kit (Geneaid, Taiwan) according to the manufacturer's recommendation. The purified DNA was sequenced (First BASE Laboratories, Singapore), analysed using Molecular Evolution Genetics Analysis (MEGA) 5.1, and blasted with the data in GenBank.

Results

This study was conducted to examine the genera and diversity of bacteria in the midguts of *Ae. aegypti* and *Cx. quinquefasciatus* mosquitoes. Laboratory-reared and field-collected mosquitoes were examined in this study. The field mosquitoes were collected from Bangkok, Thailand using BG-Sentinel mosquito traps. Bacteria in the mosquito midguts were cultured and identified by using bacteriological and molecular techniques. A total of 22 genera were identified, belonging to 4 phyla: Actionobacteria, Bacteroidetes, Firmicutes, and Proteobacteria. Proteobacteria was the dominant bacterial phylum followed by Actinobacteria, Firmicutes, and Bacteroidetes, respectively. Found in this study by using the bacteriological technique, some bacterial colonies could not be identified using molecular technique. This was due to the limitation of the boiling method that was used for bacterial DNA extraction.

Bacterial isolates from the midgut of Ae. Aegypti: Sixteen laboratory-reared *Ae. aegypti* were examined and 12 bacterial genera (i.e., *Acinetobacter, Agrobacterium, Bacillus, Cellulomonas, Chryseomicrobium, Dietzia, Enterobacter, Klebsiella, Microbacterium, Pantoea, Pseudomonas,* and *Staphylococcus*) were identified from this mosquito group (13/16). The number of bacterial colonies ranged from 1-142 CFU/mosquito. Three mosquitoes were free from the culturable bacteria in the midguts (3/16). The most common bacteria found in this mosquito group were *Microbacterium* (4/16). Proteobacteria was the dominant bacterial phylum followed by Actinobacteria and Firmicutes, respectively.

Twelve field-collected *Ae. aegypti* from Bangkok were examined and eight bacterial genera (i.e., *Bacillus, Cellulomonas, Microbacterium, Micrococcus, Moraxella, Neisseria, Staphylococcus,* and *Streptococcus*) were identified from this mosquito group (10/12). The number of bacterial colonies ranged from 1-2,900 CFU/mosquito. Two mosquitoes were free from the culturable bacteria in the midgut (2/12). The most common bacteria found in this mosquito group were *Staphylococcus* (6/12). Actinobacteria and Firmicutes were the dominant bacterial phyla followed by Proteobacteria (Table 2 and 4).

Table 2 Number of bacterial colonies found in laboratory-reared and field-collected *Aedes aegypti* from Bangkok, Thailand.

ID	Source	No. of bacterial colonies per mosquito (CFU)			Closest related bacterial genera
		Pour plate	MAC plate	TSA plate	
1	Laboratory	7	0	8	*Microbacterium*
2	Laboratory	19	0	1	*Enterobacter*
3	Laboratory	8	0	2	*Pantoea*
			0	3	*Klebsiella*
			0	2	*Acinetobacter*
			0	2	*Agrobacterium*
4	Laboratory	4	0	1	-
5	Laboratory	2	0	4	*Bacillus*
6	Laboratory	3	0	4	*Microbacterium*
7	Laboratory	1	0	1	*Dietzia cinnamea*
8	Laboratory	20	0	14	*Microbacterium*
			0	5	*Pseudomonas*
			0	1	*Chryseomicrobium*
9	Laboratory	1	0	5	-
10	Laboratory	0	0	3	*Bacillus*
11	Laboratory	11	0	12	*Cellulomonas*
12	Laboratory	3	0	30	-
13	Laboratory	0	0	19	*Staphylococcus*
			0	1	*Pseudomonas*
14	Laboratory	1	0	142	*Microbacterium*
15	Laboratory	5	0	18	*Staphylococcus*
16	Laboratory	4	0	13	*Staphylococcus*
17	Field (Suanluang)	0	0	1	-
18	Field (Suanluang)	1	0	1	*Staphylococcus*
19	Field (Suanluang)	5	0	1	*Staphylococcus*
			0	2	*Micrococcus*
20	Field (Suanluang)	4	0	33	*Bacillus*
21	Field (Suanluang)	3	0	1	*Micrococcus*
22	Field (Laksi)	0	0	5	*Micrococcus*
			0	11	*Moraxella*
23	Field (Laksi)	1	0	1	*Microbacterium*
			0	2	*Staphylococcus*
24	Field (Laksi)	1	0	1	*Cellulomonas*
25	Field (Laksi)	4	0	2	*Staphylococcus*
26	Field (Laksi)	470	0	2,500	*Staphylococcus*
			0	700	*Neisseria*
			0	2,900	*Streptococcus*
27	Field (Laksi)	1	0	1	-
28	Field (Laksi)	1	0	41	*Staphylococcus*

Bacterial isolates from the midgut of Cx. quinquefasciatus: Eleven laboratory-reared *Cx. quinquefasciatus* were examined and five bacterial genera (i.e., *Microbacterium, Micrococcus*, *Paenibacillus*, *Pseudomonas,* and *Staphylococcus*) were identified from this mosquito group (10/11). The number of bacterial colonies ranged from 1-140 CFU/mosquito. One mosquito was free from the culturable bacteria in the midgut (1/11). The most common bacteria found in this mosquito group were *Staphylococcus* (6/11). Actinobacteria was the dominant bacterial phylum followed by Bacteroidetes, Firmicutes, and Proteobacteria.

Twenty-one field-collected *Cx. quinquefasciatus* from Bangkok were examined and 13 bacterial genera (i.e., *Acinetobacter*, *Actinomyces*, *Bacillus, Chryseobacterium, Kocuria*, *Microbacterium, Micrococcus, Novosphingobium*, *Pantoea*, *Providencia*, *Pseudomonas*, *Rhodococcus*, and *Staphylococcus*) were identified from this mosquito group (18/21). The number of bacterial colonies ranged from 1-18,800 CFU/mosquito. Three mosquitoes were free from the culturable bacteria in the midgut (3/21). The most common bacteria found in this mosquito group were *Micrococcus* (5/21). Proteobacteria was the dominant bacterial phylum followed by Actinobacteria and Firmicutes, respectively (Table 3 and 4).

Table 3 Number of bacterial colonies found in laboratory-reared and field-collected *Culex quinquefasciatus* from Bangkok, Thailand.

ID	Source	No. of bacterial colonies per mosquito (CFU)			Closest related bacterial genera
		Pour plate	MAC plate	TSA plate	
1	Laboratory	1	0	70	-
2	Laboratory	4	0	35	*Micrococcus*
3	Laboratory	4	0	11	*Staphylococcus*
			0	1	*Micrococcus*
			0	40	*Microbacterium*
4	Laboratory	20	0	9	*Microbacterium*
			0	1	*Staphylococcus*
5	Laboratory	1	0	1	*Staphylococcus*
6	Laboratory	96	0	2	*Paenibacillus*
			0	140	*Microbacterium*
7	Laboratory	0	0	1	*Microbacterium*
8	Laboratory	14	0	3	*Paenibacillus*
			0	62	*Microbacterium*
9	Laboratory	3	0	5	*Staphylococcus*
			1	0	*Pseudomonas*
10	Laboratory	26	0	2	*Staphylococcus*
11	Laboratory	3	0	3	*Staphylococcus*
12	Field (Suanluang)	608	1,400	18,800	*Providencia*
			240	0	*Pantoea*
13	Field (Suanluang)	28	0	7	*Micrococcus*
14	Field (Suanluang)	52	0	56	-
			18	0	-
15	Field (Suanluang)	0	0	1	*Chryseobacterium*
16	Field (Suanluang)	288	86	1,420	-
17	Field (Suanluang)	224,000	394	0	*Pantoea*
18	Field (Suanluang)	20	0	1	*Micrococcus*
19	Field (Suanluang)	4	0	1	*Staphylococcus*
			0	1	*Microbacterium*
20	Field (Suanluang)	5	0	1	*Micrococcus*
		5	0	1	*Microbacterium*
21	Field (Suanluang)	0	0	1	-
22	Field (Laksi)	0	0	1	*Pseudomonas*
23	Field (Laksi)	3	0	2	*Acinetobacter*
			0	9	*Staphylococcus*
			0	1	*Pseudomonas*
24	Field (Laksi)	1	0	1	*Actinomyces*
25	Field (Laksi)	12	0	17	*Staphylococcus*
26	Field (Laksi)	5	0	2	*Novosphingobium*
27	Field (Laksi)	91	0	82	*Pantoea*
28	Field (Laksi)	4	0	1	*Staphylococcus*
			0	1	*Kocuria*
			0	4	*Micrococcus*
29	Field (Laksi)	3	0	1	*Bacillus*
30	Field (Laksi)	1	0	9	*Kocuria*
			0	8	*Pantoea*
31	Field (Laksi)	6	0	1	*Microbacterium*
			0	1	*Bacillus*
32	Field (Laksi)	5	0	1	*Rhodococcus*

Table 4 Comparison of the occurrence of different bacterial genera in laboratory-reared and field-collected *Aedes aegypti* and *Culex quinquefasciatus* from Bangkok, Thailand.

Phylum	Closest related bacterial genera[a]	Percentage of occurrence			
		Laboratory-reared *Ae. aegypti*	Field-collected *Ae. aegypti*	Laboratory-reared *Cx. quinquefasciatus*	Field-collected *Cx. quinquefasciatus*
Actinobacteria	*Actinomyces*	0	0	0	4.8 (1/21)
	Cellulomonas	6.3 (1/16)	8.3 (1/12)	0	0
	Dietzia	6.3 (1/16)	0	0	0
	Kocuria	0	0	0	9.5 (2/21)
	Microbacterium	25.0 (4/16)	8.3 (1/12)	45.5 (5/11)	14.3 (3/21)
	Micrococcus	0	25.0 (3/12)	18.2 (2/11)	23.8 (5/21)
	Rhodococcus	0	0	0	4.8 (1/21)
Bacteroidetes	*Paenibacillus*	0	0	18.2 (2/11)	0
Firmicutes	*Bacillus*	12.5 (2/16)	8.3 (1/12)	0	9.5 (2/21)
	Staphylococcus	18.8 (3/16)	50.0 (6/12)	54.5 (6/11)	19.0 (4/21)
	Streptococcus	0	8.3 (1/12)	0	0
Proteobacteria	*Acinetobacter*	6.3 (1/16)[b]	0	0	4.8 (1/21)
	Agrobacterium	6.3 (1/16)	0	0	0
	Chryseobacterium	6.3 (1/16)	0	0	4.8 (1/21)
	Enterobacter	6.3 (1/16)	0	0	0
	Klebsiella	6.3 (1/16)	0	0	0
	Moraxella	0	8.3 (1/12)	0	0
	Neisseria	0	8.3 (1/12)	0	0
	Novosphingobium	0	0	0	4.8 (1/21)
	Pantoea	6.3 (1/16)	0	0	19.0 (4/21)
	Providencia	0	0	0	4.8 (1/21)
	Pseudomonas	12.5 (2/16)	0	9.1 (1/11)	9.5 (2/21)

[a] All bacterial genera were identified on the basis of a percent identity higher than 99%

[b] Percentage of occurrence (no. occurred/tested)

Discussion

The mosquito midgut is a site of complex interactions among mosquitoes, pathogens, and resident microbiota. The variation of these bacteria may influence mosquito biology and vector competence for a specific pathogen (Chandel et al., 2013; Minard et al., 2013). It is one of the factors responsible for the difference in disease transmission rate or vector competence within the mosquito population. Previous studies indicate the role and relationship between mosquito midgut microbiota and vector competence for specific pathogens (Dennison et al., 2014). However, the information about midgut microbiota and the relationship between these bacteria and pathogens from Thailand is limited. This study was performed to initiate basic information about midgut microbiota from Thailand's mosquitoes. Laboratory mosquitoes were originally collected from Bangkok, Thailand and maintained for more than ten generations and field mosquitoes were also collected from Bangkok to indicate the effect of environmental conditions on the variation of midgut microbiota. Molecular analysis of the 16S ribosomal RNA gene of bacteria was used for bacterial identification in this study. Individual mosquitoes harbor extremely diverse gut bacteria in their gut. All of the laboratory-reared mosquitoes in this study were raised and fed in the same manner. However, the midgut microbiota identified from these mosquitoes was different. The microbiota in each generation of mosquitoes was likely to become more diverse during the course of the experiment in laboratory condition.

Twelve bacterial genera were identified from laboratory-reared female *Ae. aegypti* and eight bacterial genera were determined from field-collected female *Ae. aegypti*. Five bacterial genera were identified from laboratory-reared female *Cx. quinquefasciatus* and 13 bacterial genera were examined from field-collected female *Cx. quinquefasciatus*. The most common bacteria found in laboratory-reared and field-collected *Ae. aegypti* were *Microbacterium* and *Staphylococcus*, respectively and the most common bacteria found in laboratory-reared and field-collected *Cx. quinquefasciatus* were *Staphylococcus* and *Micrococcus*, respectively. The difference in bacterial genera identified from each field-collected mosquito might be attributable to environmental conditions.

Chandel et al. (2013) studied the midgut microbiota of female *Cx. quinquefasciatus* mosquitoes collected from India. The 16S ribosomal DNA from culturable microflora were examined and revealed the presence of 83 bacterial species belonging to 31 genera. Proteobacteria was the most dominant phylum, followed by Firmicutes and Actinobacteria. *Staphylococcus* was the largest genus represented by 11 species whereas *Enterobacter* was the most prevalent genus and recovered from most field stations. However, only 13 bacterial genera were identified from field-collected *Cx. quinquefasciatus* from Thailand in this study. Chandel et al. (2015) also isolated *Vagococcus fluvial*is from *Cx. quinquefasciatus* mosquito midgut collected from India where these bacteria were known from domestic animal and human sources only. This finding might confirm the hypothesis that microbiota is acquired from food sources of the mosquito (Ludvigsen et al., 2015). However, no *Vagococcus* was isolated from our present study from Thailand.

The study by Valiente Moro et al. (2013) showed the bacterial isolates from female *Ae. albopictus* mosquitoes were mostly Proteobacteria followed by Firmicutes and Actinobacteria phylum. On the other hand, Actinobacteria was the most abundant phylum

in male *Ae. albopictus* followed by Proteobacteria and Firmicutes. *Pantoea* was the most common genus in both females and males from all sampling sites. In our present study, *Pantoea* was isolated from laboratory-reared female *Ae. aegypti* and field-collected female *Cx. quinquefasciatus* from Thailand. The study of midgut microbiota of *Ae. albopictus* and *Ae. aegypti* collected from India by Yadav et al. (2015) found 24 bacterial species from 13 genera of four major phyla using 16S rRNA gene sequence analysis. Phylum Proteobacteria was dominant followed by Firmicutes, Bacteroidetes, and Actinobacteria. The bacteria belonging to the phylum Proteobacteria and Firmicutes were identified from both *Ae. albopictus* and *Ae. aegypti*, while, bacteria belonging to phylum Bacteroidetes and Actinobacteria were isolated only from *Ae. albopictus* and *Ae. aegypti*, respectively. *Enterobacter* was the dominant bacterial genus in both *Ae. albopictus* and *Ae. aegypti*. The same result was found in this present study where bacteria in phylum Bacteroidetes was only found in laboratory-reared female *Cx. quinquefasciatus*.

For the midgut microbiota of *Anopheles* mosquitoes, the majority of the identified bacteria from *An. stephensi* and *An. maculipennis* from Iran belonged to proteobacteria phylum, including *Pseudomonas* and *Aeromonas*. The other bacteria found in these mosquitoes were *Pantoea, Acinetobacter, Brevundimonas, Bacillus, Sphingomonas, Lysinibacillus*, and *Rahnella* (Dinparast Djadid et al., 2011). *Pantoea agglomerans* has anti-*Plasmodium* effector proteins that reduce mosquito refractoriness to malaria infection and engineered *P. agglomerans* strains are able to inhibit *Plasmodium falciparum* development (Wang et al., 2012). Other studies showed mosquitoes with an important microbiota seem more resistant to infections and certain bacteria, such as *Enterobacter* partially or totally and inhibit ookinete, oocyst, and sporozoite formation (Cirimotich et al., 2011). Co-infection between *Serratia marcescens* and *Plasmodium vivax* in *An. albimanus* resulted in only 1% of mosquitoes being infected with oocysts (Gonzalez-Ceron et al., 2003). *Acinetobacter, Bacillus, Enterobacter*, and *Pseudomonas* are also found in the mosquitoes from Thailand which was indicated by the present observation.

Another important bacterial endosymbiont in mosquitoes is *Wolbachia* (Tiawsirisup et al., 2008). These bacteria block the transmission of arbovirus by *Aedes* mosquitoes and this is currently being evaluated for the control of the disease outbreak (Mousson et al., 2010; van den Hurk et al., 2012; Hussain et al., 2013; Raquin et al., 2015). *Wolbachia* induces cytoplasmic incompatibility that results in the developmental failure of offspring (Segoli et al., 2014). However, only agar-culturable bacteria were examined in this study. *Wolbachia* infection in *Ae. aegypti* and *Cx. quinquefasciatus* cannot be indicated by the methodology used in this study. In addition, the presence of antibiotics in the blood of infected people presents a new risk of disease transmission increasing. Antibiotics in malaria-infected people enhances the susceptibility of *An. gambiae* to malaria infection by disturbing their midgut microbiota. Antibiotics exposure also increases mosquito survival and fecundity, which are known to increase vector competency (Gendrin et al., 2015). Diverse bacterial genera identified from different mosquito species and locations were caused by both external factors (e.g., environmental conditions) and internal factors. This microbiota diversity could indicate the differences in vector competence among mosquito species and strains. Future studies of the role of culturable bacteria in the biological role in the invasiveness of *Ae. aegypti* and *Cx. quinquefasciatus* need to be performed. Isolated bacteria should be characterized to better understand its genetic contents and any possible to influence on *Ae. aegypti* and *Cx. quinquefasciatus* vector competence.

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บทคัดย่อ

การศึกษาเบื้องต้นเกี่ยวกับความหลากหลายของแบคทีเรียในทางเดินอาหารส่วนกลาง ของยุงลายบ้านและยุงรำคาญจากเขตกรุงเทพมหานคร ประเทศไทย

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ยุงลายบ้านและยุงรำคาญเป็นแมลงพาหะที่นำเชื้อที่สำคัญหลายชนิด ปัจจัยที่มีผลต่อศักยภาพของยุงในการนำเชื้อนั้นมีหลายอย่าง แบคทีเรียในทางเดินอาหารส่วนกลางของยุงนั้นมีผลต่อลักษณะทางสรีรวิทยาของยุง และมีบทบาทในการเปลี่ยนแปลงการตอบสนองต่อเชื้อโรคของยุง การศึกษานี้เป็นการตรวจหาชนิดของเชื้อแบคทีเรียจากทางเดินอาหารส่วนกลางของยุงลายบ้านและยุงรำคาญโดยวิธีทางแบคทีเรียวิทยาและอณูชีววิทยา ตัวอย่างยุงที่นำมาศึกษาแบ่งออกเป็น 2 กลุ่ม คือ กลุ่มที่หนึ่งเป็นตัวอย่างยุงที่ได้จากการเพาะเลี้ยงในห้องปฏิบัติการ และกลุ่มที่สองเป็นตัวอย่างยุงที่เก็บมาจากพื้นที่ในกรุงเทพมหานคร การศึกษานี้ตรวจพบเชื้อแบคทีเรียจำนวน 12 สกุล ได้แก่ *Acinetobacter, Agrobacterium, Bacillus, Cellulomonas, Chryseomicrobium, Dietzia, Enterobacter, Klebsiella, Microbacterium, Pantoea, Pseudomonas* และ *Staphylococcus* จากยุงลายบ้านที่เพาะเลี้ยงในห้องปฏิบัติการ และตรวจพบเชื้อแบคทีเรียจำนวน 8 สกุล ได้แก่ *Bacillus, Cellulomonas, Microbacterium, Micrococcus, Moraxella, Neisseria, Staphylococcus* และ *Streptococcus* จากยุงลายบ้านที่เก็บตัวอย่างจากพื้นที่ในกรุงเทพมหานคร สำหรับยุงรำคาญที่เพาะเลี้ยงในห้องปฏิบัติการนั้นตรวจพบเชื้อแบคทีเรียจำนวน 5 สกุล ได้แก่ *Microbacterium, Micrococcus*, *Paenibacillus*, *Pseudomonas* และ *Staphylococcus* และยุงรำคาญที่เก็บตัวอย่างจากพื้นที่ในกรุงเทพมหานครนั้นตรวจพบเชื้อแบคทีเรียจำนวน 13 สกุล ได้แก่ *Acinetobacter*, *Actinomyces*, *Bacillus, Chryseobacterium, Kocuria*, *Microbacterium, Micrococcus, Novosphingobium*, *Pantoea*, *Providencia*, *Pseudomonas*, *Rhodococcus* และ *Staphylococcus* ความหลากหลายของเชื้อแบคทีเรียที่ตรวจพบในทางเดินอาหารส่วนกลางของยุงอาจมีผลต่อศักยภาพของยุงในการนำเชื้อชนิดต่างๆ อย่างไรก็ตามจำเป็นต้องมีการศึกษาเพิ่มเติมเพื่อบ่งชี้ถึงความสัมพันธ์นี้

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