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Research article

Diversity of midgut microbiota in laboratory-colonized and field-collected *Aedes albopictus* (Diptera: Culicidae): A preliminary study

Ranida Tuanudom [a,c], Nichapat Yurayart [a], Channarong Rodkhum [b], Sonthaya Tiawsirisup [a,*]

[a] *Animal Vector-Borne Disease Research Unit, Veterinary Parasitology Unit, Department of Veterinary Pathology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330, Thailand*
[b] *Department of Veterinary Microbiology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330, Thailand*
[c] *Interdisciplinary Program of Biomedical Science, Faculty of Graduate School, Chulalongkorn University, Bangkok 10330, Thailand*

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ABSTRACT

Aedes (Ae.) albopictus is an important vector for many pathogens. Previous studies have revealed a role for midgut bacteria during pathogen infection in mosquitoes; however, studies of *Ae. albopictus* midgut bacteria are limited. We examined the diversity of midgut bacteria in female laboratory-colonized and field-collected *Ae. albopictus*. A total of 31 bacterial genera were identified representing 10 and 28 genera of laboratory-colonized and field-collected *Ae. albopictus*, respectively. The predominant bacterial genera in the laboratory-colonized *Ae. albopictus* were *Staphylococcus* and *Micrococcus*, whereas the bacterial diversity in the field-collected *Ae. albopictus* exhibited a higher proportion of *Rhizobium* and *Agrobacterium* as the dominant genera. However, only *Staphylococcus* showed a significant difference between laboratory-colonized and field-collected *Ae. albopictus*. The midgut bacterial species were identified from 30 laboratory-colonized *Ae. albopictus* mosquitoes. A total of 16 bacterial species were identified and the predominant bacterial species was *Micrococcus luteus*, followed by *Staphylococcus epidermidis* and *Agrobacterium tumefaciens*. Field mosquitoes were collected from the Sing Buri, Chumphon, and Yala Provinces of Thailand. The midgut bacterial species identified from the 10 *Ae. albopictus* collected from the Sing Buri Province included *Bacillus subtilis, Staphylococcus haemolyticus, Staphylococcus hominis,* and *Serratia marcescens. Serratia marcescens* was the only bacteria identified from this area. Midgut bacterial species were identified from 40 filed-collected *Ae. albopictus* from Chumphon Province. A total of 25 bacterial species were identified and the predominant species were *Enterobacter cloacae, Micrococcus luteus,* and *Providencia rettgeri.* Only 15 bacterial species were identified from the mosquitoes collected from Chumphon Province. A total of 18 bacterial species were identified from 30 *Ae. albopictus* collected from Yala Province and the predominant species were *Rhizobium pusense* and *Agrobacterium tumefaciens.* Only 12 bacterial species were found in mosquitoes collected from Yala Province. These findings indicate changes in the midgut bacteria population in *Ae. albopictus* from various locales, which may result from variability in the blood-meal source, diet, or habitat. A comprehensive survey of the midgut bacteria community prevalence in wild populations is critical for not only gaining a better understanding of the role of this bacterium in shaping the microbial community in *Ae. albopictus,* but also for informing current and future mosquito and disease control programs.

1. Introduction

Aedes (Ae.) albopictus (Skuse) or the Asian tiger mosquito is a mosquito that acts as a potential disease vector for the transmission of many filarial, protozoan, and viral pathogens including canine heartworm, avian malaria, chikungunya, dengue, West Nile, and yellow fever virus (Tiawsirisup et al., 2004, 2005; Tiawsirisup and Kaewthamasorn, 2007; Thavara et al., 2009; Chompoosri et al., 2016; Tuanudom et al., 2017; Yurayart et al., 2017). This mosquito is native to the tropical and subtropical areas of Southeast Asia and has spread to many countries by modern transportation (Craven et al., 1988). It does not exhibit any specific ecological specialization and has succeeded in colonizing temperate zones, such as in the United States and Europe (McHugh and Hanny, 1990; Dalla Pozza et al., 1994). *Ae. albopictus* exhibits both distinct cold tolerant and tropical strains, and it overwinters in the egg

* Corresponding author.
E-mail addresses: sonthaya.t@chula.ac.th, sonthayatiaw@hotmail.com (S. Tiawsirisup).

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stage in temperate climates, but is active throughout the year in tropical and subtropical habitats (Hanson and Craig, 1995).

The digestive tract of the mosquito consists of three parts which include the foregut, midgut, and hindgut. The midgut serves as the first contact area between pathogens and the epithelial surface. Midgut microbiota are bacteria that are harbored or colonize the midgut of mosquitoes. Recently, studies have focused on the role of bacterial communities on the fitness and competence of various mosquito vectors and pathogen transmission (Pumpuni et al., 1996; Diallo et al., 1999; Dillon and Dillon, 2004; Azambuja et al., 2005; Gusmao et al., 2007). Some midgut bacteria play an important role in disease transmission, host-parasite interaction, and vector competence (Apte-Deshpande et al., 2014; Mohlmann et al., 2020). They may increase or decrease vector competence through various mechanisms including enhancement of the immune response or by obstructing the development of pathogens (Kalappa et al., 2018; Monteiro et al., 2019). Midgut bacteria may attenuate the expression of molecules directed against infecting pathogens, which could be used as a novel strategy for disease control (Shane et al., 2018). They are also known to increase the immune response of mosquitoes (Pumpuni et al., 1996; Meister et al., 2005; Dong et al., 2009), in which immunocompetent mosquitoes are less likely to transmit pathogens, which could also be useful for disease control (Abdul-Ghani et al., 2012).

The diversity of the midgut microbiota has been examined in different species of mosquitoes (Tiawsirisup et al., 2008, 2018; Guegan et al., 2020; Zoure et al., 2020). Previous studies have evaluated the influence of colonized microbiota on the susceptibility of mosquitoes to virus and parasitic infection (Pumpuni et al., 1996; Dillon and Dillon, 2004; Azambuja et al., 2005; Gusmao et al., 2007). However, studies on *Ae. albopictus* regarding the midgut microbiota identification and their interaction with pathogens are limited. In the present study, we examined the diversity of midgut bacteria in laboratory-colonized and field-collected *Ae. albopictus* from different locations in Thailand. The results provide important information for other basic and advanced studies on mosquito biology, midgut microbiota, and pathogen infection.

2. Materials and methods

2.1. Mosquitoes

Female laboratory-colonized and field-collected *Ae. albopictus* were examined. The laboratory-colonized mosquitoes were originally collected from Nonthaburi Province and colonized at the Department of Medical Sciences, Ministry of Public Health, Thailand. They were subsequently maintained in the Parasitology Unit, Department of Veterinary Pathology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand for more than 10 generations. Field-collected mosquitoes were collected from the Sing Buri, Chumphon, and Yala Provinces of Thailand. This study was conducted in 2014, at which time mosquitoes were not included as experimental animals that required approval from the Chulalongkorn University Animal Care and Use Committee.

2.2. Mosquito dissection and bacterial isolation

Bacteria were isolated from the midguts of laboratory-colonized and field-collected *Ae. albopictus*. The mosquitoes were dissected and bacterial isolation was performed within 24 h. The mosquitoes were euthanized at −20 °C and dissected under sterile conditions. Each mosquito was washed with 70% ethanol for 5 min and then twice with phosphate-buffered saline before midgut dissection and bacterial cultivation. The midgut was homogenized in 300 μL of 60% glycerol and a 100 μL aliquot of the suspension was spread on tryptone soya agar supplemented with 5% sheep blood and incubated at 37 °C for 24 h. Pure bacterial isolates from the midgut of each mosquito were subcultured in 2 mL of tryptone soya broth and incubated at 37 °C for 24 h. The bacteria were collected by centrifugation at 20,000 rcf for 15 min and the bacterial pellet was washed with distilled water.

2.3. Bacterial DNA extraction

A total of 40 μL of distilled water was added to the bacterial pellet, resuspended by vortexing, incubated at 100 °C for 10 min, cooled on ice, and centrifuged at 20,000 rcf for 10 min. The supernatant (extracted DNA) was stored at −80 °C until further use.

2.4. Midgut bacterial identification

The small ribosomal RNA (16S) gene was amplified by PCR from the extracted DNA of the isolates using two pairs of eubacteria-specific primers. The first primer pair was 16SF (5′–AGTTTGATCCTGGCTCAG–3′) and 16SR (5′–GCTACCTTGTTACGACTT C-3′) (Dinparast Djadid et al., 2011), whereas the second pair was 63F (5′–CAGGCCTAACACATGCAAGTC–3′) and 1387R (5′–GGGCGGWGTGTACAAGGC–3′) (Marchesi et al., 1998), which yielded amplicons of expected sizes of 1.5 and 1.3 kb, respectively. The amplified fragments were purified using the Gel/PCR DNA Fragments Extraction Kit (Geneaid, Taiwan) and submitted to First BASE Laboratories (Singapore) for sequencing.

All partial 16S rRNA gene sequences were assembled and analyzed using the Lasergene package version 5.03 (DNASTAR, Inc., Madison, Wisconsin, USA). The sequences obtained were compared against GenBank using the BLAST algorithm. Homologous sequences were retrieved from GenBank (BLASTn search) and aligned using the ClustalW program. Phylogenetic relationships were determined by tree reconstruction using the Neighbor-Joining (NJ) method with the Kimura-2 parameter for distance calculation, which is incorporated into the MEGA 7.0.26 package. The robustness of the phylogenetic tree was examined through 1,000 bootstrap replicates and the consensus tree was used for analysis. All sequences were submitted to the National Centre for Biotechnology and Information GenBank sequence database with accession numbers, SUB3724128: MG996794-MG996888, SUB3733025: MG997080-MG997092, SUB3782911: MH050409-MH050425, and SUB3782990: MH050699-MH050738.

2.5. Data analysis

Differences in the midgut bacterial genera infection rates between female laboratory-colonized and field-collected *Ae. albopictus* were determined by a Chi-square test. Differences in the proportion of the community of Actinobacteria, Firmicutes, and Proteobacteria phyla isolated from the midgut between female laboratory-colonized and field-collected *Ae. albopictus* were determined by a Chi-square test. Differences in the proportion of the community of Actinobacteria, Firmicutes, and Proteobacteria phyla isolated from the midgut among female laboratory-colonized and field-collected *Ae. albopictus* from Sing Buri, Chumphon, and Yala Provinces were determined by ANOVA. *P* values less than 0.05 were considered statistically significant.

3. Results

3.1. Diversity of cultured bacterial genera from laboratory-colonized and field-collected Ae. albopictus

The midgut bacterial genera identified from female laboratory-colonized and field-collected *Ae. albopictus* are summarized in Table 1. A total of 31 bacterial genera were identified which included 10 and 28 genera in laboratory-colonized and field-collected *Ae. albopictus*, respectively. The majority of the isolated bacteria were Gram-negative bacteria. The predominant bacterial genera in the laboratory-colonized

Table 1. Comparison of midgut bacterial infection rates between female laboratory-colonized and field-collected *Aedes albopictus*.

Bacterial phylum	Bacterial genus (gram)	Infection rate (infected/tested mosquitoes)	
		Laboratory-colonized	Field-collected
Actinobacteria	*Actinomyces* (+)	0 (0/30)	1.3 (1/80)
	Brachybacterium (+)	0 (0/30)	1.3 (1/80)
	Leucobacter (+)	3.3 (1/30)	0 (0/80)
	Microbacterium (+)	13.3 (4/30)	3.8 (3/80)
	Micrococcus (+)	16.7 (5/30)	6.3 (5/80)
	Nocardioides (+)	0 (0/30)	1.3 (1/80)
Firmicutes	*Bacillus* (+)	0 (0/30)	6.3 (5/80)
	Staphylococcus (+)*	26.7 (8/30)	3.8 (3/80)
Proteobacteria	*Acinetobacter* (-)	3.3 (1/30)	5.0 (4/80)
	Agrobacterium (-)	13.3 (4/30)	7.5 (6/80)
	Beijerinckia (-)	0 (0/30)	1.3 (1/80)
	Brevundimonas (-)	0 (0/30)	1.3 (1/80)
	Burkholderia (-)	0 (0/30)	2.5 (2/80)
	Candidatus Rhizobium (-)	0 (0/30)	2.5 (2/80)
	Chryseobacterium (-)	0 (0/30)	2.5 (2/80)
	Enhydrobacter (-)	3.3 (1/30)	0 (0/80)
	Enterobacter (-)	0 (0/30)	5.0 (4/80)
	Erwinia (-)	0 (0/30)	1.3 (1/80)
	Klebsiella (-)	3.3 (1/30)	3.8 (3/80)
	Massilia (-)	0 (0/30)	1.3 (1/80)
	Moraxella (-)	0 (0/30)	1.3 (1/80)
	Novosphingobium (-)	0 (0/30)	1.3 (1/80)
	Pandoraea (-)	3.3 (1/30)	0 (0/80)
	Pantoea (-)	0 (0/30)	2.5 (2/80)
	Pectobacterium (-)	0 (0/30)	1.3 (1/80)
	Providencia (-)	0 (0/30)	3.8 (3/80)
	Pseudomonas (-)	6.7 (2/30)	3.8 (3/80)
	Rahnella (-)	0 (0/30)	1.3 (1/80)
	Rhizobium (-)	0 (0/30)	8.8 (7/80)
	Serratia (-)	0 (0/30)	1.3 (1/80)
	Sphingomonas (-)	0 (0/30)	2.5 (2/80)

*Significant difference in the midgut bacterial infection rates between female laboratory-colonized and field-collected *Aedes albopictus* was determined by a Chi-square test ($P < 0.05$).

Ae. albopictus were *Staphylococcus* (26.7%) and *Micrococcus* (16.7%), whereas the bacterial genera diversity in field-collected *Ae. albopictus* was much greater with *Rhizobium* (8.8%) and *Agrobacterium* (7.5%) representing the slightly dominant genera. Only *Staphylococcus* exhibited a statistically significant difference between laboratory-colonized and field-collected *Ae. albopictus* ($P < 0.05$) (Table 1). Besides, most of the identified bacterial genera belong to the Proteobacteria phylum, and there were only two bacterial genera, *Bacillus* and *Staphylococcus,* in the Firmicutes phylum.

3.2. Diversity of cultured bacterial species from laboratory-colonized Ae. albopictus

The midgut bacterial species identified from the 30 laboratory-colonized *Ae. albopictus* are summarized in Table 2. Most of the identified bacterial species from laboratory-colonized *Ae. albopictus* belong to the Actinobacteria phylum (Figure 1). A total of 16 bacterial species were identified and the dominant bacterial species were *Micrococcus luteus* (16.7%), followed by *Staphylococcus epidermidis* (13.3%) and *Agrobacterium tumefaciens* (13.3%). The highest average number of total bacterial colonies per mosquito was found in *Microbacterium dextranolyticum* at 212 (4–621).

Table 2. The midgut bacterial infection rates in female laboratory-colonized *Aedes albopictus*.

Bacterial phylum	Closest related bacterial species*	Infection rate (infected/tested mosquitoes)	Average number of total colonies per mosquito (range)
Actinobacteria	*Leucobacter chironomi*	3.3 (1/30)	12
	Microbacterium dextranolyticum	10.0 (3/30)	212 (4–621)
	Microbacterium laevaniformans	3.0 (1/30)	2
	Micrococcus luteus	16.7 (5/30)	44 (16–86)
	Micrococcus yunnanensis	6.7 (2/30)	22 (12–32)
Firmicutes	*Staphylococcus arlettae*	6.7 (2/30)	11 (3–18)
	Staphylococcus epidermidis	13.3 (4/30)	45 (6–130)
	Staphylococcus pasteuri	3.3 (1/30)	12
	Staphylococcus warneri	6.7 (2/30)	92 (3–180)
Proteobacteria	*Acinetobacter variabilis*	3.3 (1/30)	14
	Agrobacterium tumefaciens	13.3 (4/30)	78 (6–258)
	Enhydrobacter aerosaccus	3.0 (1/30)	10
	Klebsiella pneumoniae	3.3 (1/30)	14
	Pandoraea sputorum	3.3 (1/30)	292
	Pseudomonas aeruginosa	3.3 (1/30)	16
	Pseudomonas luteola	3.3 (1/30)	92

* All bacterial species were identified based on a 16S DNA sequence similarity of greater than 99%.

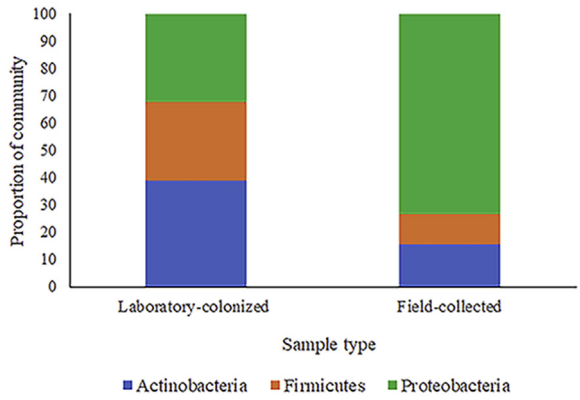

Figure 1. Proportion of the community of identified bacterial species belonging to their respective phylum isolated from the midgut of female laboratory-colonized and field-collected *Aedes albopictus* from Thailand. There were differences in the proportion of the community of Actinobacteria, Firmicutes, and Proteobacteria phyla isolated from the midgut between laboratory-colonized and field-collected *Ae. albopictus* from Thailand, as determined by a Chi-square test ($P < 0.05$).

3.3. Diversity of cultured bacteria species from field-collected Ae. albopictus

The field mosquitoes were collected from Sing Buri, Chumphon, and Yala Provinces, which are representative of the central, upper southern, and lower southern areas of Thailand, respectively. Midgut bacterial species were identified from 10 *Ae. albopictus* collected from the Sing Buri

Province. The predominant bacterial species belonged to the Firmicutes phylum (Figure 2) and included *Bacillus subtilis* (10%), *Staphylococcus haemolyticus* (10%), *Staphylococcus hominis* (10%), and *Serratia marcescens* (10%). *Serratia marcescens* was the only species identified from this area. The highest average number of total bacterial colonies per mosquito found in *Staphylococcus hominis* was 66 (Table 3).

The midgut bacterial species were identified from 40 filed-collected *Ae. albopictus* from Chumphon Province, and most identified bacterial species belonged to the Proteobacteria phylum (Figure 2). A total of 25 bacterial species was identified, with the dominant bacterial species being *Enterobacter cloacae* (7.5%), *Micrococcus luteus* (7.5%), and *Providencia rettgeri* (7.5%). Fifteen bacterial species were identified from mosquitoes collected from Chumphon Province. The highest average number of total bacterial colonies per mosquito found in *Enterobacter cloaca* was 98 (3–273) (Table 4).

A total of 18 bacterial species were identified from 30 *Ae. albopictus* collected from the Yala Province and most of the identified bacterial species belonged to the Proteobacteria phylum (Figure 2). The predominant bacterial species were *Rhizobium pusense* (16.7%) and *Agrobacterium tumefaciens* (13.3%). A total of 12 bacterial species were found only in the mosquitoes collected from Yala Province. The highest average number of total bacterial colonies per mosquito found in *Pectobacterium carotovorum* was 546 (Table 5).

There were differences in the proportion of the community of Actinobacteria, Firmicutes, and Proteobacteria phyla isolated from the midgut between laboratory-colonized and field-collected *Ae. albopictus* from Thailand, as determined by a Chi-square test ($P < 0.05$) (Figure 1). In addition, there were differences in the proportion of the community of Firmicutes and Proteobacteria phyla isolated from the midgut among laboratory-colonized and field-collected *Ae. albopictus* from Sing Buri, Chumphon, and Yala Provinces of Thailand, as determined by ANOVA ($P < 0.05$) (Figure 2). A total of 53 phylotypes were observed in the NJ phylogenetic tree using a 99% DNA sequence similarity as the cut-off, and the 16S rRNA gene sequences from a variety of phylogenetic groups are shown in Figure 3.

4. Discussion

The diversity of midgut microbiota has been studied and identified from various species of mosquitoes, primarily *Anopheles* mosquitoes (Tainchum et al., 2020; Zoure et al., 2020). In contrast, studies of *Aedes* and *Culex* mosquitoes are limited (Tiawsirisup et al., 2018; Muturi et al., 2020; Seabourn et al., 2020). In the present study, we examined the diversity of midgut bacteria in female laboratory-colonized and field-collected *Ae. albopictus*, the latter of which was sampled from three different locations in Thailand. There were concerns that the field-collected mosquitoes were only tested for CHIKV, but none of them

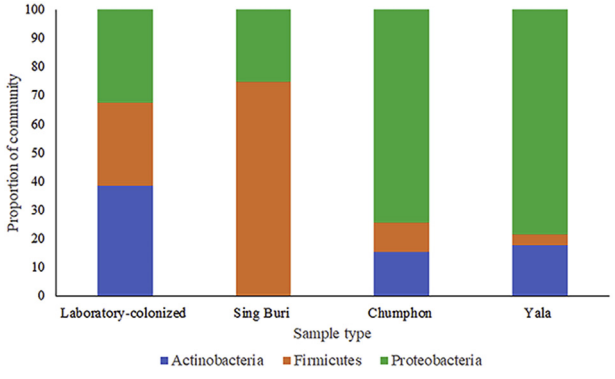

Figure 2. Proportion of the community of identified bacterial species belonging to their respective phylum isolated from the midgut of female laboratory-colonized and field-collected *Aedes albopictus* from Sing Buri, Chumphon, and Yala Provinces, Thailand. There were differences in the proportion of the community of Firmicutes and Proteobacteria phyla isolated from the midgut among laboratory-colonized and field-collected *Ae. albopictus* from Sing Buri, Chumphon, and Yala Provinces, Thailand, as determined by ANOVA ($P < 0.05$).

Table 3. The midgut bacterial infection rates in female field-collected *Aedes albopictus* from Sing Buri Province.

Bacterial phylum	Closest related bacterial species*	Infection rate (infected/tested mosquitoes)	Average number of total colonies per mosquito (range)
Firmicutes	*Bacillus subtilis*	10.0 (1/10)	3
	Staphylococcus haemolyticus	10.0 (1/10)	3
	Staphylococcus hominis	10.0 (1/10)	66
Proteobacteria	*Serratia marcescens*#	10.0 (1/10)	3

Represents the bacterial species that were only found in this group.
* All bacterial species were identified based on a 16S DNA sequence similarity of greater than 99%.

Table 4. The midgut bacterial infection rates in female field-collected *Aedes albopictus* from Chumphon Province.

Bacterial phylum	Closest related bacterial species*	Infection rate (infected/tested mosquitoes)	Average number of total colonies per mosquito (range)
Actinobacteria	*Actinomyces oris*#	2.5 (1/40)	24
	Microbacterium dextranolyticum	2.5 (1/40)	3
	Microbacterium yannicii#	2.5 (1/40)	15
	Micrococcus luteus	7.5 (3/40)	8 (3–15)
Firmicutes	*Bacillus kochii*#	5.0 (2/40)	(3–6)
	Bacillus pocheonensis#	2.5 (1/40)	9
	Staphylococcus epidermidis	2.5 (1/40)	3
Proteobacteria	*Acinetobacter lwoffii*#	2.5 (1/40)	3
	Acinetobacter variabilis	5.0 (2/40)	3
	Agrobacterium tumefaciens	5.0 (2/40)	3
	Chryseobacterium taklimakanense#	5.0 (2/40)	8 (3–12)
	Enterobacter cancerogenus#	2.5 (1/40)	279
	Enterobacter cloacae#	7.5 (3/40)	98 (3–273)
	Enterobacter hormaechei#	2.5 (1/40)	3
	Enterobacter mori#	2.5 (1/40)	3
	Erwinia tasmaniensis#	2.5 (1/40)	3
	Klebsiella pneumoniae	5.0 (2/40)	5 (3–6)
	Klebsiella quasipneumoniae#	2.5 (1/40)	30
	Klebsiella variicola#	2.5 (1/40)	18
	Moraxella osloensis	2.5 (1/40)	9
	Novosphingobium panipatense	2.5 (1/40)	3
	Pantoea dispersa#	5.0 (2/40)	8 (3–12)
	Providencia rettgeri	7.5 (3/40)	9 (3–15)
	Pseudomonas psychrotolerans#	5.0 (2/40)	8 (6–9)
	Rhizobium pusense	5.0 (2/40)	47 (12–81)

Represents the bacterial species that were only found in this group.
* All bacterial species were identified based on a 16S DNA sequence similarity of greater than 99%.

Table 5. The midgut bacterial infection rates in female field-collected *Aedes albopictus* from Yala Province.

Bacterial phylum	Closest related bacterial species*	Infection rate (infected/tested mosquitoes)	Average number of total colonies per mosquito (range)
Actinobactira	*Brachybacterium nesterenkovii*	3.3 (1/30)	9
	Microbacterium aoyamense#	3.3 (1/30)	9
	Micrococcus luteus	3.3 (1/30)	3
	Micrococcus yunnanensis	3.3 (1/30)	3
	Nocardioides zeae#	3.3 (1/30)	9
Firmicutes	*Bacillus altitudinis*#	3.3 (1/30)	3
Proteobacteria	*Acinetobacter radioresistens*	3.3 (1/30)	3
	Agrobacterium tumefaciens	13.3 (4/30)	4.5 (3–6)
	Beijerinckia fluminensis#	3.3 (1/30)	3
	Brevundimonas aurantiaca#	3.3 (1/30)	3
	Burkholderia seminalis#	6.7 (2/30)	3
	Candidatus Rhizobium massiliae#	6.7 (2/30)	9
	Massilia timonae#	3.3 (1/30)	18
	Pectobacterium carotovorum#	3.3 (1/30)	546
	Pseudomonas oleovorans#	3.3 (1/30)	6
	Rahnella aquatilis#	3.3 (1/30)	15
	Rhizobium pusense	16.7 (5/30)	3
	Sphingomonas sanguinis#	6.7 (2/30)	3

Represents the bacterial species that were only found in this group.
* All bacterial species were identified based on a 16S DNA sequence similarity of greater than 99%.

were infected with CHIKV. However, they were not tested for dengue virus, Zika virus, and other pathogens, which may affect the microbiota of field-collected mosquitoes. Because of the limitation of the bacterial culture method, this study focused only on the characterization of culture-dependent aerobic bacteria from the mosquito midguts. Unfortunately, the effect of bacteria on inducing or inhibiting pathogen infection was not determined. *Wolbachia* is one of the most important symbiont bacteria found in different species of mosquitoes [e.g., *Ae. albopictus* and *Culex (Cx.) gelidus*] (Kitrayapong et al., 2002; Tiawsirisup et al., 2008); however, it was not examined in this study because of the limitation of the culture method and these bacteria are primarily found in the reproductive system of mosquitoes.

Proteobacteria was the predominant phylum found in field-collected *Ae. albopictus*, whereas Actinobacteria was the predominant phylum found in the laboratory-colonized *Ae. albopictus*. The predominant bacterial genera in the laboratory-colonized *Ae. albopictus* were *Staphylococcus* and *Micrococcus*, whereas the bacterial genera diversity in field-collected *Ae. albopictus* was much greater and included *Rhizobium* and *Agrobacterium*. However, only *Staphylococcus* showed a statistically significant difference between laboratory-colonized and field-collected *Ae. albopictus*.

A high diversity in the microbiota of the midgut was observed in the field-collected *Ae. albopictus* compared with those colonized in the laboratory. These discrepancies may result from differences in the source of the blood meal, diet, or the environment in which they live (Chen et al., 2020; Lee et al., 2020; Scolari et al., 2021). *Acinetobacter, Bacillus, Enterobacter, Pseudomonas,* and *Raoultella* bacterial isolates were found in larval feeding and sugar solution and *Enterobacter* was found to successfully immigrate to both larval and adult stages of *Ae. albopictus* (Chen et al., 2020). *Bacillus* is one genus of the midgut bacteria that was isolated from mosquitoes collected from all three locations, but none from the laboratory-colonized mosquitoes. *Bacillus* also dominated the isolated bacterial taxa from wild *Ae. albopictus* and *Ae. aegypti* collected in Madagascar, with *Acinetobacter, Agrobacterium, and Enterobacter* following closely behind (Zouache et al., 2011).

The midgut microbiota community may experience interference and undergo change because of the blood meal and food received at each stage of the mosquitoes, which subsequently affects pathogen infection, dissemination, and transmission in the mosquitoes (Muturi et al., 2019). The microbiota composition is affected by the developmental stage in mosquitoes (Scolari et al., 2021). However, trans-stadial passage of some bacteria from the larva to the adult stage has been shown in *Ae. albopictus* and *Anopheles (An.) albimanus* (Yadav et al., 2016; Chen et al., 2020; Galeano-Castaneda et al., 2020).

Previous research using bacterial culture and denaturing gel electrophoresis revealed that Proteobacteria and Firmicutes were the dominant bacterial communities associated with *Ae. albopictus* in the Indian Ocean, and that bacterial diversity and composition were influenced by the mosquitoes' habitat (Zouache et al., 2009, 2011). A taxonomic microarray targeting a broader range of bacterial taxa revealed that the bacterial community in *Ae. albopictus*, which originated on La Réunion, was more diverse than previously described, and that the various endosymbionts could interact with one another and with the chikungunya virus within the mosquitoes (Zouache et al., 2009).

Staphylococcus (i.e., *S. arlettae, S. epidermidis, S. pasteuri,* and *S. warneri*) was the predominant genus found in the laboratory-colonized *Ae. albopictus*, which was significantly different from the field-collected *Ae. albopictus*. Mixed infection of *S. arlettae* and *S. epidermidis* was also found in one laboratory-colonized mosquito. *S. haemolyticus* and *S. hominis* were found in mosquitoes collected from Sing Buri Province and *S. epidermidis* was found in mosquitoes collected from Chumphon Province. In contrast, there was no *Staphylococcus* isolated from mosquitoes collected from Yala Province. *Staphylococcus* has also been identified from other species of mosquitoes (e.g., *Ae. aegypti, An. albimanus*, and *Cx. quinquefasciatus*) (Tiawsirisup et al., 2018; Galeano-Castaneda et al., 2020).

Our study found *Enterobacter* at 5% of the midguts of field-collected *Ae. Albopictus,* whereas *E. cloacae* was found in the *Ae. albopictus* collected from Chumphon Province. The previously reported data indicate the involvement of *E. cloacae* in the inhibition of *Plasmodium berghei* development in *Anopheles stephensi* (Eappen et al., 2013). Both *Micrococcus luteus* and *M. yunnanensis* were identified from laboratory-colonized and field-collected *Ae. albopictus.* Previous studies showed that *Micrococcus* produces proteins for antibiotic tolerance, re-emergence from latent infections and even quorum sensing and biofilm formation (Mali et al., 2017). Another important bacterial genus identified from *Ae. albopictus* was *Acinetobacter*, which is known to take part in blood digestion by mosquitoes (Gaio Ade et al., 2011). These bacteria have been isolated from various environments, including soil samples, potato plants, and dried seaweed, as well as from the air (Groth et al., 1996; Zhang et al., 2010). The mosquitoes may receive these bacteria with food that the mosquito larvae feed upon or in the sheep blood provided to the adult mosquitoes.

The mosquito midgut microbiota may decrease or facilitate the development of a pathogen in mosquitoes (Mohlmann et al., 2020). In the present study, *Serratia marcescens* were only isolated from *Ae. albopictus* collected from Sing Buri Province; however, our previous study indicated there was no *S. marcescens* isolated from laboratory-reared and field-collected *Ae. aegypti* and *Cx. quinquefasciatus* from Bangkok, Thailand (Tiawsirisup et al., 2018). These bacteria have also been isolated from lab-reared and field-caught adult females and larvae of *Anopheles stephensi* (Rani et al., 2009). *Serratia marcescens* are important

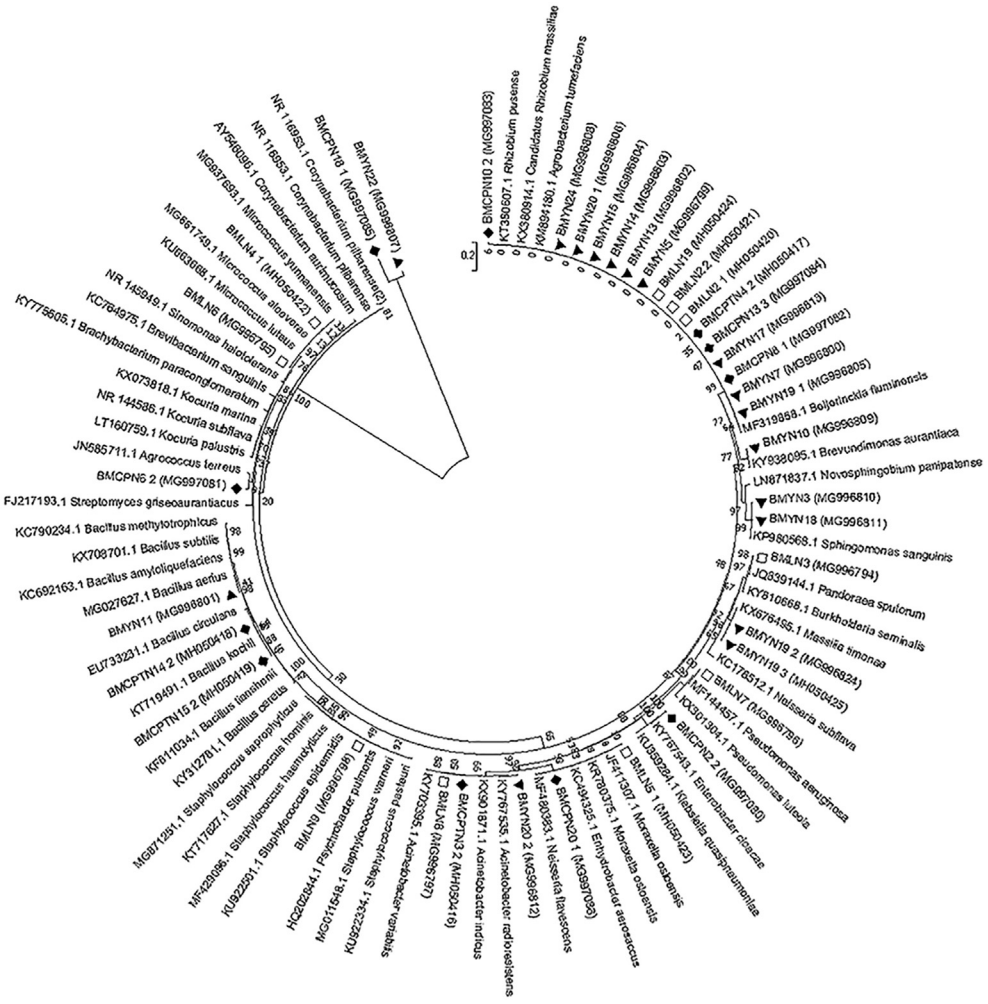

Figure 3. Phylogenetic tree (NJ) constructed from the partial 16S rRNA gene fragment sequences (1,500 bp) of isolates cultured from laboratory-colonized and field-collected *Ae. albopictus* with BS values provided at the nodes. Entries with a black square represent reference names and accession numbers (in parentheses). Entries from this study are represented as strain number and accession number (in parentheses) (■ reference names, □ laboratory-colonized *Ae. albopictus*, △ field-collected from Chumphon, ▲ field-collected from Yala).

because they facilitate arboviral infection and enhance viral dissemination through a secreted protein that digests membrane-bound mucins on the mosquito gut epithelium (Wu et al., 2019). The susceptibility of *Ae. aegypti* to CHIKV and dengue viruses increases in the presence of *Serratia odorifera* because of the suppression of the immune response of *Ae. aegypti* (Apte-Deshpande et al., 2012, 2014). *Ae. aegypti* are more susceptible to DENV-2 when fed with *Aeromonas* and *Escherichia coli* (Apte-Deshpande et al., 2014). However, these bacteria reduce the *Plasmodium* parasite load in *Anopheles* mosquitoes (Bando et al., 2013).

A thorough study of the role of midgut bacteria may result in a better understanding of the microbiota's direct or indirect involvement in mosquito immune response and reproduction. This may contribute to the enhancement of current vector and disease control efforts. Certain bacteria that live in the midgut are critical for disease transmission, host-parasite interactions, and vector competence. They can reduce or improve vector competence in a variety of ways, including by enhancing the immune response or by inhibiting parasite development. The midgut is the first point of contact between parasites and the epithelial surface, where parasite populations are significantly reduced (Azambuja et al., 2005). The midgut microbiota may be able to genetically alter the expression of anti-parasite compounds, which might be employed as a novel vector control method. They are also known to enhance the immune response of mosquitoes (Pumpuni et al., 1996; Meister et al., 2005; Dong et al., 2009), in which the immunocompetent mosquitoes are less likely to transmit parasites and could be useful for disease control (Abdul-Ghani et al., 2012). Given the well-established link between specific bacterial taxa and vector susceptibility to a variety of mosquito-borne pathogens, changes in gut microbial communities in response to host blood meal source may have a profound effect on pathogen transmission and may be a key determinant of variation in vector competence (Muturi et al., 2019). Further studies are needed to assess the role of each midgut bacteria and specific pathogen infection, dissemination, and transmission in *Ae. albopictus* mosquitoes, which may improve vector and disease control strategies.

5. Conclusions

This study examined the diversity of midgut bacteria in female laboratory-colonized and field-collected *Ae. albopictus*. A total of 31 bacterial genera were identified which included 10 and 28 genera in laboratory-colonized and field-collected mosquitoes, respectively. A total of 16 bacterial species were identified from the laboratory-colonized mosquitoes and a total of 4, 25, and 18 midgut bacterial species were identified from the mosquitoes collected from Sing Buri, Chumphon, and Yala Province, respectively. These discrepancies may result from differences in the source of the blood meal, diet, or environment, all of which may affect pathogen infection, dissemination, and transmission in mosquitoes. An extensive survey of the midgut bacteria community prevalence in wild populations is necessary to not only improve our understanding of the role of bacteria in shaping the microbial community in mosquitoes, but also for providing essential information for current and future mosquito and disease control programs.

Declarations

Author contribution statement

Ranida Tuanudom: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Nichapat Yurayart: Performed the experiments.

Channarong Rodkhum: Analyzed and interpreted the data.

Sonthaya Tiawsirisup: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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