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ISOLATION AND IDENTIFICATION OF THE BACTERIAL COMMUNITY ASSOCIATED WITH THE ROVE BEETLE, *Paederus fuscipes* CURTIS (COLEOPTERA: STAPHYLINIDAE) IN PENANG

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ABSTRACT

The rove beetle, *Paederus fuscipes* Curtis has caused tremendous dermatitis once unintentionally crushed against human skin due to their potent toxic released called paederin produce by the endosymbiont bacteria. The genus *Pseudomonas* was hypothesized to be the endosymbiont bacteria involved in the production of paederin in *Paederus*. However, no extensive studies have been performed to investigate the existence of other bacteria, and the relationship between the endosymbiont counts amongst male and female *P. fuscipes* beetles. We found that *P. fuscipes* harbours surprising numbers of bacteria diversity with a total of 20 different bacterial species identified. *Bacillus*, *Serratia*, *Staphylococcus*, and *Pseudomonas* bacteria were the dominated genus found in all collected beetles from all light trap locations. However, only *Pseudomonas aeruginosa* Migula is the medically important species and is commonly known to cause severe dermatitis lesions to humans. Our results revealed the *P. aeruginosa* counts cultured on *Pseudomonas* medium was found higher total mean bacteria counts in *P. fuscipes* females ($2.58 \times 10^5 \pm 2.73 \times 10^4$ cell) compared to male beetles ($5.68 \times 10^4 \pm 3.70 \times 10^3$ cells). We demonstrated that a large diversity of bacterial community has been isolated from both male and female *P. fuscipes*, however *P. aeruginosa* colonies were frequently detected in females compared to male beetles. These results can be used in future studies to investigate the possible impact of these bacterial counts on the concentration of paederin produced in *P. fuscipes*.

Key words: Endosymbionts, *Paederus*, *Paederus fuscipes*. *Pseudomonas*, 16srRNA gene

INTRODUCTION

The family Staphylinidae includes some of the world's most significant insect species that can cause important clinical manifestations via vesicant toxin to their victims (Haddad *et al*., 2012). Although mosquitoes, tsetse flies, and sand flies are mainly recognized as the vectors of tropical diseases associated with mortality, the rove beetle of the genus *Paederus* is also a threat to mankind, due to the toxic hemolymph paederin (Ghoneim, 2013). Paederin is an extremely potent vesicant polyketide cytotoxin that causes paederus dermatitis (also known as dermatitis linearis) skin lesion once unintentionally crushed against human skin (Piel, 2002; Gibbs, 2015). Severe dermatologic, ophthalmologic, and systemic symptoms are shown by a paederus dermatitis victim (Cressey *et al.,* 2013). The first reported case of paederus dermatitis was in the summer back to the late nineteenth century at Anjet-Kidoel lighthouse in Java, Indonesia (Vorderman, 1901). Since then, it has become a nuisance pest due to its significant public health problems to humans (Bong *et al.,* 2015).

Nonetheless, the benefits provided by paederin for *Paederus* beetles are contradicted with the problems caused to human beings. In tropical regions of Asia, massive outbreak cases were frequently reported in human-dominated areas because these insects live exclusively on moist environmental conditions such as near river margins, lakes, and rice field areas which are built close to human residential premises (Bong *et al*., 2015). Bright illumination is one of the factors in causing *Paederus* beetle infestation into human settings

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(Maryam *et al*., 2017b). Interestingly, most incidences of infestation were mostly observed in the northern region of Peninsular Malaysia (Bong *et al*., 2013).

Remarkably, it was indicated that paederin is not produced by the beetle themselves but relies on some species of an endosymbiont bacteria within the *Paederus* spp. which is known to be phylogenetically related to *Pseudomonas aeruginosa* (Kellner, 2002). The capability to synthesize paederin is limited to only certain female adults, whereas the males and larvae can acquire the toxin only by ingestion or transovarially (Kellner & Dettner, 1995). According to Dillon and Dillon (2004), bacterial association with insects affects their hosts in various ways from making a nutritional contribution, aids food digestion, reproduction and protection against pathogenic colonization. For instance, paederin plays an important role in protecting *Paederus* larvae through the chemical defense to survive the attacks against predation by wolf spiders (Kellner & Dettner, 1996). In this study, we isolated, cultured, and identified the symbiont bacteria from *P. fuscipes* to obtain a more precise estimation of their occurrence in both male and female beetles.

MATERIALS AND METHODS

Sampling site and *Paederus fuscipes* samples

Paederus fuscipes specimens were collected from Telok Air Tawar rice fields (N 5° 29' 9.3171" E 100° 23' 1.3012") in mainland Penang, Malaysia. CDC Fay-Prince suction light trap baited with a 4W blacklight UV bulb hereafter called the "UV light trap" was used as a visual cue to capture *P. fuscipes* beetles in the field. Each UV light trap (named as LT 1, LT 2, LT 3, and LT 4) was deployed on every four sides of the square experimental rice field plots measuring 100 m in height by 130 m in width. LT 1 and LT 3 are located closer to human housing premises, whereas LT 2 and LT 4 are more to the center of the rice field areas. All of the UV light traps were operated at the same time from dusk, and insect trapping was conducted over a 2 h period from 2015 to 2215 h. A specific time was selected due to the peak time of *P. fuscipes* dispersal flight (Maryam *et al.,* 2017a). Captured individuals placed in labeled collection jar, brought back to the Medical Entomology Laboratory, Universiti Sains Malaysia, and beetles were sexed apart. Two adult beetles (one of each sex) from each different UV light trap were tested for the isolation of bacteria. Sampling collection was replicated three times (n=3) on different occasions.

Isolation and enumeration of bacteria

A day prior to the experiment, *P. fuscipes* beetles were captured and were then killed using 70% chloroform. Beetles were individually placed in a sterile 1.5 mL microcentrifuge tube, washed in a series of three baths containing sterile distilled water, 70% ethanol, and vortexed (approx. 1 to 2 min), and then rewashed again using sterile distilled water. Using standard aseptic techniques, sterilized beetles were then transferred into a new 1.5 mL microcentrifuge tube containing 200 µL of sterile saline (0.85% NaCI), individually mashed using a sterile forcep, and was briefly vortexed. The microcentrifuge tube was then centrifuged, to separate the debris from the mashed beetles. All necessary material for handling *P. fuscipes* beetle was autoclaved at 121°C for 20 min prior to the experiment.

In order to assess the distribution and population levels of bacterial communities in a *Paederus* beetle, an aliquot of 100 µL of each extract samples was then transferred to several bijou bottles with a concentration of 10^{-1}, 10^{-2} and 10^{-3} respectively. The bijou bottles were then vortexed for 1 min to ensure a homogeneous suspension of bacterial cells. Finally, 100 µL of each homogenized serial dilutions were directly plated on two selected microbiological media, (1) Nutrient Agar (NA), and (2) *Pseudomonas* Agar Base with the addition of CFC supplement for the selective isolation of *Pseudomonas* species. Firstly we ran the bacterial identification on NA in order to understand the range and amount of different species of bacterial communities found in *P. fuscipes* beetle. On the second batch of the experiment, *Pseudomonas* agar base with added CFC supplement was used to identify and confirm all possible *Pseudomonas* bacteria found in both male and female *P. fuscipes* beetles. Different *P. fuscipes* individuals were used for each experimental design. All of the media were prepared according to the manufacturers' instructions (HIMEDIA). The spread plate method was run on triplicates for each concentration. Plates were incubated at 37°C for 24 h. Microbial enumeration was done in triplicates by counting the colony-forming units (CFU) per beetle.

Colony characteristics of isolates

Microorganisms were first screened based on (a) colony characteristics (pigmentation, size, shape, opacity, margin, elevation, viscosity, surface texture) and (b) Gram's staining of the isolates for Gram-positive and Gram-negative identification was conducted by following a standardized Gram staining procedure by Claus (1992). Morphologically distinct and well-isolated

colonies were aseptically picked by using a sterile inoculation loop, restreaked onto new media plates and subcultured twice to ensure the purity or until pure colonies were obtained. A representative of each colony type was then selected from each sample for sequencing. Isolate names were labeled as follows; (1) R1, R2, and R3 represent the number of sampling replicates, (2) M and F represent male and female beetles, and (3) 1, 2, 3, and 4 represents the location of the UV light traps.

Genomic DNA extraction

A single pure colony of 24 h culture of the different isolated bacterium on each media was inoculated in 50 mL of sterile nutrient-rich (NR) broth in separate 250 mL flask and incubated overnight at 30°C with the agitation of 200 rpm in a shaking incubator. After 24 h, a total amount of 1 mL cultured bacteria cells was then transferred to a 1.5 mL sterile microcentrifuge tube. The cultures were centrifuged at 15,000× g for 1 min, and the supernatant was discarded. DNA extraction was performed using a HiYield Genomic DNA Mini Extraction Kit (RBC, Taiwan) according to the manufacturer's instructions with some modification on the wash step and DNA elution. Briefly, following the cell lysis, DNA binding and wash step, 100 µL of pre-heated Elution buffer was added to the dried GD Column which was previously centrifuged for additional 5 min during the wash step to avoid any residual ethanol carryover and was finally let to stand for at least 5 min for complete absorption of the elution buffer. To elute the purified DNA, centrifugation at 15,000× g for 30 sec was done, and the genomic DNA was stored at -20°C until further analysis.

Polymerase chain reaction (PCR)

The 16S rRNA gene was amplified from bacterial colonies by PCR, using universal primer pairs 60 F (TNANACATGCAAGTCGAAKCG) and 1392 R (ACGGGCGGTGTGTRC) (My TACG Bioscience Enterprise, Selangor, Malaysia). The PCR reaction mixture (25 µL) was performed by adding 1 µL of template DNA, 0.25 µL of each forward and reverse primers, and 11.0 µL of nuclease-free water to 12.5 µL of EconoTaq PLUS GREEN 2X Master Mix (Lucigen, USA). The 25 µL PCR reaction mixture was transferred individually into a clean 0.2 mL PCR tubes.

Before the PCR was performed in a PCR thermocycler (MyCycler, Bio-Rad, USA), each reaction mixture was mixed gently by vortexing and a brief spin. Cycling parameters for PCR reactions were carried out according to the following protocol: an initial denaturation step at 94°C for 2 min, followed by 30 cycles of a denaturation step at 94°C for 30 sec, a primer annealing step at 54°C for 30 sec, an extension step at 72°C for 1 min, and a final extension step of 10 min at 72°C. All PCR products were then subjected to gel electrophoresis.

Agarose gel electrophoresis

Agarose gel electrophoresis was performed as described by Sambrook and Russell (2001) to separate and identify DNA fragments after DNA extraction and PCR procedure. Aliquots of 6 µL (1:5 ratios of 6X Bromophenol Blue and Xylene Cyanol loading dye to DNA samples) of each product were resolved electrophoretically on 0.8% (w/v) agarose gel using 0.5x TBE buffer stained with 3 µL of Health View nucleic acid. The gel electrophoresis was run for 70 V for 40 min and was then visualized under UV transilluminator. The DNA molecular weight marker, a 1 kb DNA ladder (Lucigen, USA) was used to determine the size of the DNA fragments. Once a single band was observed on an agarose gel, the non-purified PCR product was sent to Genomics Bioscience and Technology Laboratory, Taiwan for DNA sequencing. The obtained DNA sequences were assembled through manual alignment using BioEdit software (Hall, 1999). The aligned sequences were then analyzed using the standard nucleotide Basic Local Alignment Search Tool (BLAST) server at the National Center for Biotechnology Information (NCBI) https://blast.ncbi.nlm.nih.gov/Blast.cgi.

Data analysis

An independent t-test was performed to determine any significant difference in the CFU counts of bacteria isolated from both male and female *P. fuscipes*. Prior to statistical analyses, all data were checked for normality of distribution at the 0.05 significance level by using the Shapiro-Wilk Normality test. Data were log-transformed prior to analysis. All analyses were conducted using SPSS version 20.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Characterisation of bacteria isolated from *P. fuscipes*, cultured on nutrient agar (NA)

Bacterial count on nutrient agar

In general, the total bacterial counts on the NA for female *P. fuscipes* were found lower as compared to the male *P. fuscipes* beetles after a 24 h incubation period with mean values of $5.15 \times 10^5 \pm 8.23 \times 10^4$ and $2.19 \times 10^6 \pm 2.70 \times 10^5$ respectively. T-test analysis showed no significant difference ($t = 1.52$; $df = 19$; $P = 0.145$) of the mean number of viable bacteria colonies among male and female *P. fuscipes* beetles on NA. The highest mean number

of viable bacterial count of female beetles with 1.18 x 10^6 ± 7.75 x 10^4 was collected from LT 3, whereas LT 4 possessed the lowest mean number of viable bacterial counts with 1.64 x 10^5 ± 7.75 x 10^4 (Table 1). However, at LT 1, the number of viable bacterial cells was TFTC in all female beetles throughout the three sampling occasions. In contrast, male beetles collected from LT 4 showed the lowest mean number of viable bacterial counts with 2.26 x 10^5 ± 2.96 x 10^4 of bacterial cells and the highest mean number of bacterial count of male beetles was with 6.31 x 10^6 ± 7.27 x 10^5 bacterial cells collected from LT 2 (Table 1).

Characteristics of the isolates on nutrient agar

On the nutrient agar, a total of 15 different isolates were selected and cultured from different individuals of *P. fuscipes* beetle (Table 2). Gram-negative rods were the major group of bacteria found in *P. fuscipes* individuals, represented by 7 isolates (out of 15). Many of the Gram-negative rods bacteria were found in LT 1 and LT 4 throughout the sampling period in both male and female beetles. Four isolates of each Gram-positive rods and cocci-shaped bacteria were also identified in all four light traps. However, the number of cocci-shaped bacteria found in male beetles (R2M3, R3M3, and R3M4) was higher compared to female beetles (R3F1). Based on the morphological characteristics, an estimation of 15 different species of bacteria is found in *P. fuscipes.*

Molecular identification of the bacterial isolates on nutrient agar

Fifteen species of bacteria were identified from nine different genera isolated from *P. fuscipes* at different light trap locations on NA. Isolates were

Table 1. The mean number of viable bacterial cells of *P. fuscipes* individual on nutrient agar (NA)

	Paederus beetles	CFU per beetle
Light trap 1	Female	TFTC
	Male	1.34 x 10^6 ± 1.74 x 10^5
Light trap 2	Female	7.17 x 10^5 ± 1.63 x 10^5
	Male	6.31 x 10^6 ± 7.27 x 10^5
Light trap 3	Female	1.18 x 10^6 ± 7.75 x 10^4
	Male	8.72 x 10^5 ± 1.48 x 10^5
Light trap 4	Female	1.64 x 10^5 ± 7.75 x 10^4
	Male	2.26 x 10^5 ± 2.96 x 10^4

TFTC indicates too few too count.

Table 2. Characteristics of isolated bacteria from *P. fuscipes* beetle, cultured on nutrient agar (NA) at different collection points

Isolate	Colony Morphology								Gram's Stain
	Pigmentation	Size	Shape	Opacity	Margin	Elevation	Viscosity	Surface texture	
R1F2	White	Medium	Circular	Opaque	Entire	Convex	Yes	Glistening	Rod, -ve
R1F4	Dark orange	Small	Circular	Opaque	Entire	Flat	Yes	Glistening	Rod, -ve
R1M1	Yellow	Small	Circular	Opaque	Entire	Flat	Yes	Smooth	Rod, +ve
R1M4	Fair white	Medium	Circular	Opaque	Entire	Convex	Yes	Smooth	Rod, -ve
R2F1	Fair white	Medium	Circular	Translucent	Entire	Flat	No	Rough	Rod, +ve
R2F4	White	Large	Circular	Opaque	Entire	Raised	Yes	Dull	Rod, +ve
R2F4	Yellowish	Medium	Circular	Opaque	Entire	Raised	Yes	Smooth	Rod, -ve
R2M1	Slightly yellowish	Medium	Circular	Opaque	Entire	Flat	Yes	Smooth	Rod, -ve
R2M3	Clear white	Small	Circular	Transparent	Entire	Convex	Yes	Glistening	Coccus, +ve
R2M4	Fair white-yellowish	Medium	Circular	Translucent	Entire	Flat	Yes	Smooth	Rod, -ve
R3F1	Greenish	Large	Circular	Translucent	Entire	Grow in medium	Yes	Glistening	Rod, -ve
R3F1	Orange-yellow	Medium	Irregular	Opaque	Undulate	Flat	No	Rough	Coccus, +ve
R3F4	Creamy-white	Large	Filamentous/ irregular	Opaque	Filamentous	Flat	Yes	Rough	Rod, +ve
R3M3	Creamy-orange	Medium	Circular	Opaque	Entire	Flat	Yes	Glistening	Coccus, +ve
R3M4	Fluorescent-yellow	Small	Circular	Opaque	Entire	Convex	Yes	Glistening	Coccus, +ve

subjected to 16S rRNA gene sequence analysis and the following bacterial genera were identified as *Serratia, Chryseobacterium, Microbacterium, Pseudomonas, Paenibacillus, Bacillus, Stapylococcus, Acinetobacter*, and *Micrococcus* (Table 3). *Bacillus*, *Serratia*, *Staphylococcus*, and *Pseudomonas* bacteria were identified most commonly in all light trap locations in both male and female beetles based on the number of bacterial colonies grown on the NA media. Nonetheless, bacterial species of the genus *Staphylococcus* were commonly found in male beetles (R2M3, R3M3) compared to females (R3F1).

However, *P. aeruginosa* colony was only identified on a female beetle from LT 1 location. This bacterium exhibits a slimy texture of green pigments on NA. Nevertheless, other *Pseudomonas* species*; Pseudomonas nitritireducens* Lizula and Komagata, *Pseudomonas mosselii* Dabboussi, and *P. taiwanensis* Wang were also identified in both female and male beetles and were obtained from 10 out of 24 individuals sampled from LT 1, LT 2, LT 3, and LT 4 location.

Characterisation of bacteria isolated from *P. fuscipes*, cultured on *Pseudomonas* agar

Bacterial count on Pseudomonas agar

To confirm the existence of different *Pseudomonas* species found, the selective *Pseudomonas* medium was used to inoculate and culture bacteria. The total mean number of viable bacterial counts on males was found generally lower as compared to females on the selective *Pseudomonas* agar with $8.59 \times 10^5 \pm 1.29 \times 10^5$ and $1.32 \times 10^6 \pm 3.92 \times 10^5$ bacterial cells respectively. At LT 2, the lowest mean number of the bacterial count with $2.70 \times 10^5 \pm 5.08 \times 10^4$ bacterial cells were observed on male beetle, while the highest CFU counts in males were found at LT 3 with $2.70 \times 10^6 \pm 4.48 \times 10^5$ bacterial cells. Whereas, females accounted for the highest mean number of viable bacterial count of $4.00 \times 10^6 \pm 1.45 \times 10^6$ bacterial cells at LT 3 location, and the lowest in females was with $4.85 \times 10^5 \pm 5.06 \times 10^4$ bacterial cells at LT 4.

Conversely, the specific green coloured pigment produced from *P. aeruginosa* species was found collected from *P. fuscipes* female at LT 1 with the highest mean number of CFU counts of $7.75 \times 10^5 \pm 7.08 \times 10^4$. While, the least mean number of *P. aeruginosa* counts on male beetle was from LT 4 with $2.27 \times 10^5 \pm 1.48 \times 10^4$ bacterial cells. Overall, numerous *P. aeruginosa* colonies were found from three *P. fuscipes* females compared to only two male beetles, however, this difference was not significant ($t = 1.69$; $df = 14$; $P = 0.113$; Table 4).

Characteristics of the isolates on Pseudomonas agar

A total of six different bacterial isolates were cultured from *P. fuscipes* on the selective *Pseudomonas* agar from all light trap locations. All isolates were Gram-negative rod-shaped bacteria which indicates the possibility of *P. aeruginosa* species. The colony and cell morphologies based on Gram's staining of the bacteria on *Pseudomonas* agar were summarized in Table 5 with an estimation of a possibility of six identified *Pseudomonas* species.

Molecular identification of the bacterial isolates on Pseudomonas agar

Three out of six identified species were found to be among the *Pseudomonas* species. Besides *P. aeruginosa*, *Pseudomonas oryzihabitans* Kodoma, and *Pseudomonas entomophila* were the identified

Table 3. The 16S rRNA gene sequence of isolated bacteria from *P. fuscipes* on nutrient agar (NA)

Isolate	Bacteria	Maximum Identity (%)	Query Coverage (%)	E value	Accession Number
R1F2	*Serratia nematodiphila*	99	100	0.0	MK 163508
R1F4	*Chryseobacterium vietnamense*	98	100	0.0	MK 163509
R1M1	*Microbacterium paraoxydans*	99	100	0.0	MK 163510
R1M4	*Pseudomonas nitritireducens*	97	100	0.0	MK 163511
R2F1	*Paenibacillus glycanilyticus*	94	99	0.0	MK 163512
R2F4	*Bacillus pacificus*	98	100	0.0	MK 163513
R2F4	*Pseudomonas mosselii*	99	100	0.0	MK 163514
R2M1	*Pseudomonas taiwanensis*	99	100	0.0	MK 163515
R2M3	*Staphylococcus cohnii* subsp. *urealyticus*	99	98	0.0	MK 163516
R2M4	*Acinetobacter soli*	98	100	0.0	MK 163517
R3F1	*Pseudomonas aeruginosa*	98	100	0.0	MK 163518
R3F1	*Staphylococcus gallinarum*	98	100	0.0	MK 163519
R3F4	*Bacillus aerius*	98	100	0.0	MK 163520
R3M3	*Staphylococcus sciuri*	98	99	0.0	MK 163521
R3M4	*Micrococcus yunnanensis*	98	100	0.0	MK 163522

Table 4. The number of viable bacterial cells of an individual *P. fuscipes* female and male on selective *Pseudomonas* agar

	Paederus beetles	CFU per beetle	CFU of *Pseudomonas aeruginosa* per beetle
Light trap 1	Female	$7.75 \times 10^5 \pm 7.08 \times 10^4$	$7.75 \times 10^5 \pm 7.08 \times 10^4$
	Male	TFTC	N/A
Light trap 2	Female	TFTC	N/A
	Male	$2.70 \times 10^5 \pm 5.08 \times 10^4$	TFTC
Light trap 3	Female	$4.00 \times 10^6 \pm 1.45 \times 10^6$	TFTC
	Male	$2.70 \times 10^6 \pm 4.48 \times 10^5$	N/A
Light trap 4	Female	$4.85 \times 10^5 \pm 5.06 \times 10^4$	$2.58 \times 10^5 \pm 3.85 \times 10^4$
	Male	$4.65 \times 10^5 \pm 2.02 \pm 10^4$	$2.27 \times 10^5 \pm 1.48 \times 10^4$

TFTC indicates too few too count. N/A indicates not available.

Table 5. Characteristics of isolated bacteria from *P. fuscipes* beetle on selective *Pseudomonas* agar

Isolate	Colony Morphology								Gram's Stain
	Pigmentation	Size	Shape	Opacity	Margin	Elevation	Viscosity	Surface texture	
F3	Greenish	Medium	Circular	Translucent	Entire	Grow in medium	Yes	Glistening	Rod, -ve
F3	Yellowish	Small	Circular	Opaque	Entire	Flat	No	Rough	Rod, -ve
M2	Clear white	Small	Circular	Opaque	Entire	Convex	No	Glistening	Rod, -ve
F4	Clear white	Small	Circular	Opaque	Entire	Flat	No	Glistening	Rod, -ve
M4	Clear white	Small	Circular	Opaque	Entire	Convex	Yes	Glistening	Rod, -ve
M4	Fair white-yellowish	Small	Circular	Opaque	Entire	Convex	Yes	Glistening	Rod, -ve

Table 6. The 16S rRNA gene sequence of isolated bacteria from *P. fuscipes* on selective *Pseudomonas* agar

Isolate	Description	Maximum Identity (%)	Query Coverage (%)	E value	Accession Number
F3	*Pseudomonas aeruginosa*	98	95	0.0	MK 163523
F3	*Pseudomonas oryzihabitans*	99	99	0.0	MK 163524
M2	*Enterobacter hormaechei*	95	97	0.0	MK 163525
F4	*Stenotrophomonas maltophilia*	99	100	0.0	MK 163526
M4	*Stenotrophomonas pavanii*	98	100	0.0	MK 163527
M4	*Pseudomonas entomophila*	98	96	0.0	MK 163528

Pseudomonas species. However, our results showed that *P. aeruginosa* was the dominant species identified on the selective *Pseudomonas* agar which existed in five out of eight individuals sampled from all four light trap location, except for *P. fuscipes* female beetle in LT 3 where the *P. oryzihabitans* colonies were found to be slightly higher in number with $4.00 \times 10^6 \pm 1.45 \times 10^6$ CFU counts per beetle (Table 4). On the other hand, *P. entomophila* was identified in both female and male beetles from LT 4 but the number of colonies was too few to be counted (TFTC) as compared to *P. aeruginosa*. Interestingly, in addition to the genus *Pseudomonas*, *Stenotrophomonas*, and *Enterobacter* are other genera which were also cultured on *Pseudomonas* agar, and identified from female beetle at LT 2 and both female and male beetles at LT 4 location (Table 6).

DISCUSSION

A large diverse community of bacteria has been isolated from *P. fuscipes* beetle captured from all light trap locations with an overall of 11 genera and 20 species. Based on the colony appearance on the isolation medium, most captured beetles were shown to carry a variety of bacteria, and some were of

medical importance such as *Staphylococcus cohnii urealyticus* corrig. Kloos and Wolfshohl, and *P. aeruginosa* species (Soldera *et al*., 2013; Wick *et al.,* 1990).

The current study showed that the number of colony-forming units (CFU) count was found the highest in male beetles, in contrast to female beetles on the NA. Thus, it suggested that *P. fuscipes* male houses a diversity of bacterial symbionts compared to female beetles. None of the *P. aeruginosa* strains were detected in *P. fuscipes* male beetle cultured in nutrient agar except, from a female in LT 1 location but with a low number of *P. aeruginosa* colony. However, other *Pseudomonas* species such as *P. nitritireducens, P. mosselii,* and *P. taiwanensis* was commonly detected from *P. fuscipes* population in all light trap locations with higher number of bacterial colonies.

In contrast, once the selective *Pseudomonas* medium was used for the isolation of bacteria from *P. fuscipes*, *P. aeruginosa* colony was not only identified in *P. fuscipes* females (LT 1, LT 3, and LT 4), but also male beetles (LT 2, LT 4). This showed that while it was possible to isolate *Pseudomonas* using a standard media such as NA, the culture medium does not support the growth of *P. aeruginosa* strains from *P. fuscipes*. The use of suitable selective *Pseudomonas* medium is recommended in increasing the efficiency of *P. aeruginosa* detection in *P. fuscipes* beetle. The results of our study showed that the CFU counts in each female beetle on the *Pseudomonas* medium were generally higher compared to male beetles. This could be due to the higher frequencies of *P. aeruginosa* colonies detected in *P. fuscipes* females. A study conducted by Maleki Ravasan *et al*. (2018) also reported that the paederin producing bacteria was also found to be four times higher in females compared to *P. fuscipes* males. This is rational because *Paederus* females transmit the paederin producing bacteria to their offspring from predation by natural enemies (Kellner & Dettner, 1995; 1996). However, our findings on *P. aeruginosa* species on each light trap location was not consistent because of the low sample size of studies.

Based on the morphological characteristics of *P. aeruginosa*, the bacterial strain was in good agreement with the general description of *P. aeruginosa* colonies on the laboratory cultures. The colonies were described as Gram-negative, rod-shaped, and produce water soluble pigments which diffuse through the culture medium (Gahlout *et al.,* 2017). It contains extracellular pigments known as pyocyanin (blue-green) and pyoverdine (yellow-green). As stated by Gahlout *et al*. (2017), the colour blue in pyocyanin turns stationary phase cultures of *P. aeruginosa* into the colour green. This indicates the green coloured pigment isolated from *P. fuscipes* beetle in our study is of *P. aeruginosa* species.

Although *P. nitritireducens, P. mosselii, P. taiwanensis, P. oryzihabitans,* and *P. entomophila* are other *Pseudomonas* spp. identified among the *P. fuscipes* beetles, based on the previous study conducted by Kellner (2002) at Central European on *P. sabeus*, the endosymbiont responsible for the production of toxin paederin was found to have the closest similarity with the *P. aeruginosa* species only in the "paederin-producing" (+)-females. Interestingly, the results of the present study also revealed that *P. aeruginosa* was the dominant symbiont species detected among female beetles on the selective *Pseudomonas* medium. It is therefore probable that the primary symbiont bacteria found in *P. fuscipes* female is *P. aeruginosa,* since they harbor significant numbers of these microorganisms in the current study.

It is notoriously known that among the genus *Pseudomonas* (sensu stricto), *P. aeruginosa* is the only fluorescent pseudomonad pathogenic to mammals including mankind (Stanier *et al.,* 1966). According to Brock *et al.* (1994), *P. aeruginosa* is typically opportunistic, and is an important pathogen in human infection of various organs but is not an obligate parasite. Lyczak *et al*. (2000) stated that the ubiquitous occurrence of *P. aeruginosa* in the environment is due to its ability to utilize a wide range of simple organic compounds as energy sources. Given the widespread occurrence of *P. aeruginosa* in soil and water, it is possible for its symbiosis with *Paederus* beetles as they are found normally in moist environmental conditions such as the rice field areas (Bong *et al.,* 2015). Hence, *P. aeruginosa* might indeed be the postulated producer of paederin biosynthesis which was responsible for the numerous dermatitis outbreak cases documented worldwide (Gibbs, 2015; Uzunoğlu *et al.,* 2016; Cáceres *et al.,* 2017). Moreover, it is also known that *P. aeruginosa* bacteria is the most common causal agent that contributes to the pathogenesis of burn wound infection (Lyczak *et al.,* 2000). This might explain the pain or burning sensation felt by some severe form of paederus dermatitis victims throughout the world (Ghoneim, 2013). Consequently, we can deny the possibility of other *Pseudomonas* species that is responsible for causing paederus dermatitis infestation to human.

According to the 16S rRNA sequence, the pathogenic or potentially pathogenic bacteria isolated from both male and female beetles were discovered to be from the genus *Staphylococcus* and *Pseudomonas* species. *Staphylococcus cohnii* subsp. *urealyticus,* and *Pseudomonas aeruginosa* are the clinically significant strains that may cause severe infections and often life-threatening diseases to

humans (Stanier *et al.,* 1966; Bottone *et al.,* 2010; Soldera *et al.,* 2013). However, bacterial isolates from the genus *Serratia, Chryseobacterium, Microbacterium, Paenibacillus, Bacillus, Acinetobacter*, *Micrococcus, Stenotrophomonas*, and *Enterobacter* were also found present in *P. fuscipes* beetles. The existence of these bacteria in *P. fuscipes* might cause severe secondary bacterial infections to paederus dermatitis victims.

CONCLUSION

This investigation indicated variations among the bacterial isolates found in male and female *P. fuscipes* beetles. *Pseudomonas aeruginosa,* the paederin producing bacteria is not only found in females, but also in male *P. fuscipes* beetles. Since the low sample size was analyzed in this study, metagenomic study on bacterial community associated with *P. fuscipes* can be conducted to understand the mechanism of endosymbiont involved in the production of paederin in *Paederus* females, and also to investigate the existence of other bacterial species that can cause secondary infection to paederus dermatitis victims.

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