Malaysian Journal of Microbiology, Vol 20(4) 2024, pp. 459-471
DOI: http://dx.doi.org/10.21161/mjm.230455

Malaysian Journal of Microbiology
Published by Malaysian Society for Microbiology
(In SCOPUS since 2011)

Insecticidal potential of wax-degrading bacteria derived from fermented *Ficus* extract against *Phenacoccus solenopsis* Tinsley

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Received 5 October 2023; Received in revised form 26 March 2024; Accepted 19 April 2024

ABSTRACT

Aims: *Phenacoccus solenopsis* is a pest of many agricultural and horticultural crops, causing considerable losses in Asia and other parts of the world. Managing *P. solenopsis* is challenging due to a waxy covering body and high reproductive potentiality. This study was aimed to explore the potential use of wax-degrading bacteria isolated from fermented *Ficus* extract against *P. solenopsis*.
Methodology and results: A total of ten isolates of wax-degrading bacteria were isolated from fermented *Ficus* extract (*Ficus* FPE) using a selective medium, Davis minimal agar (DMA), which was enriched with mealybug wax. The wax-degrading ability of the bacteria was evaluated through a turbidimetry test using 2, 6-dichlorophenol indophenol (DCPIP). *Bacillus* sp. 42PB3 demonstrated the highest wax-degrading potential, with 64.78% mealybug wax degraded and 73.85% paraffin wax degraded. The bioassay was conducted with three bacterial concentrations (10^8, 10^7 and 10^6) tested against third-instar nymphs of *P. solenopsis* on *Hibiscus rosa-sinensis* by top application technique in completely randomized design (CRD) design with ten replications. The cell suspension and supernatant of *Klebsiella pneumonia*, *Paenibacillus* sp. 21F1E3, *Bacillus* sp. 35F1C1 and *Bacillus* sp. 42F1B3 isolates caused 70-83% and 60-66% mortality (supernatant) after 168 h post-treatment. *Klebsiella pneumonia* badly affected the offspring production of the treated mealybug with the lowest number of crawlers (80.2), followed by *Paenibacillus* sp. 21F1E3 isolate (111).
Conclusion, significance and impact of study: In the present study, all tested bacteria isolated from fermented *Ficus* extract showed varying levels of wax-degrading ability and insecticidal activity against *P. solenopsis*. These bacterial isolates show promise as an environmentally friendly biocidal agent for managing *P. solenopsis*, potentially curbing crop losses and ensuring food quality through sustainable pest management practices in the future.

Keywords: Biopesticides, fermented plant extract, mealybug, *Phenacoccus solenopsis*, wax-degrading bacteria

INTRODUCTION

Phenacoccus solenopsis, commonly known as cotton mealybugs, is a sap-feeding pest feeding on 154 host plant species in different world regions (Alam *et al.*, 2011). It secretes toxic saliva into the plant tissues and sometimes becomes a vector to transmit viral diseases to infested plants (Hoffman *et al.*, 2011). Plants infested with *P. solenopsis* become stunted, cease the photosynthesis process and reduce vigour (Ibrahim *et al.*, 2015). Chemical insecticides are the primary mode for mealybug control. However, the management of mealybugs is always challenging due to their cryptic lifestyle, high reproductive potential and presence of wax coatings on the body that hinder the penetration of insecticides (Nagrare *et al.*, 2011; Salunkhe *et al.*, 2013). Attention is needed to explore alternative and effective biocontrol measures to reduce the yield loss caused by mealybugs.

Wax-degrading bacteria are a vital group of microorganisms that are capable of using wax as a sole carbon source for their growth and development. This group of bacteria utilizes and emulsifies waxy substances (hydrocarbons) mainly by several mechanisms, such as the synthesis of microbial surfactants, pseudo-solubilization, adhesion and enzyme release.

The quick selection of hydrocarbon-degrading microorganisms using redox indicator 2, 6-dichlorophenol indophenol (DCPIP), is a widely accepted technique once it detects an oxidation of NADH to NAD^+. In the presence of hydrocarbon, when the change in colour of DCPIP from

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ISSN (print): 1823-8262, ISSN (online): 2231-7538

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DOI: http://dx.doi.org/10.21161/mjm.230455

its original blue (oxidised state) turns to colourless, the test is deemed positive (Bidoia *et al*., 2010; Lima *et al*., 2019). Previously, numerous studies have documented some microorganisms isolated from soil (Elisa *et al.*, 2006), agroindustrial wastes (Nnolim *et al.*, 2020), insect pests (Arunkumar *et al.*, 2017; Ateyyat *et al.*, 2010; Salunkhe *et al.*, 2013), oil wells (Xu *et al.*, 2013) using hydrocarbons as a sole source of carbon and energy for their growth.

Wax-degrading bacteria such as *Alcaligenes*, *Acinetobacter*, *Actinomycetes*, *Arthrobacter*, *Bacillus*, *Lactobacillus*, *Corynebacteria*, *Flavobacterium*, *Micrococcus*, *Neisseria*, *Noicardia*, *Plesiomonas*, *Proteus*, *Pseudomonas*, *Rhodococcus*, *Xanthomonas*, *Erwinia* and *Zoogloea* are capable of wax degradation that offer a novel approach to the management of insect pests with waxy coating (Lynch and Hobbie, 1988; Ridgway *et al*., 1990; Roper, 2004; Mobaiyen *et al.*, 2013; Xu *et al*., 2013; Arunkumar *et al.*, 2017). According to Salunkhe *et al.* (2013), wax-degrading bacteria such as *Serratia marcescens* and *Pseudomonas aeruginosa* isolated from the cadavers of pink hibiscus mealybug, *Maconellicoccus hirsutus*, had reduced the wax content of the mealybugs significantly. Arunkumar *et al.* (2017) also isolated wax-degrading bacteria such as *Bacillus*, *Enterobacter*, *Pseudoxanthomonas*, *Pseudomonas* and *Serratian* from the cadavers of *Phenacoccus solenopsis* Tinsley and *Ferrisia virgata* Cockerell and had proven their ability in surfactant production to degrade the wax layer of mealybug. Thus, the use of bacteria as bio-pesticides against waxy-covering hemopteran insects, especially mealybugs, has drawn special attention due to their specificity, safety, sustainability and simplicity of manufacturing. This is a new report on these wax-degrading bacteria isolated from fermented *Ficus* extract (*Ficus* FPE) and their efficacy against *P. solenopsis*. Therefore, the study was aimed to isolate wax-degrading bacteria from fermented *Ficus* and evaluate their efficacy in causing mortality to *P. solenopsis* as well as their ability to degrade the mealy wax that will accelerate the activity of insecticides. The application of wax-degrading bacteria in crop protection programs will reduce the usage of chemical insecticides for agricultural insect pests, ensuring an eco-friendly environment and human health issues.

MATERIALS AND METHODS

Insect

The females of *P. solenopsis* were collected from *Hibiscus rosa-sinensis* in Serdang, Selangor and released onto the green okra B501 with a camel hairbrush to generate F1 population. *Phenacoccus solenopsis* was maintained under room temperature at 27 ± 2 °C and relative humidity (RH) of 65 ± 5% (El-Zahi and Farag, 2017). The plastic tray containing mealybugs was covered with fine mesh fastened with a rubber band to prevent contamination and escape of insects.

Preparation of fermented plant extracts

The leaves of *Ficus* (*Ficus hispida)* were collected from the vicinity of Universiti Putra Malaysia (UPM), Selangor, based on its insecticidal potency (Ahmadi *et al.*, 2012) and brought back to the Laboratory of Insect Pathology, Faculty of Agriculture, Universiti Putra Malaysia in 2021. The leaves were rinsed with tap water and then in distilled H_2O twice to remove debris. The leaves were chopped with a sterile sharp knife on a chopping board and then placed into airtight plastic containers, mixed with molasses and distilled H_2O at a ratio of 3:1:10 (w:v:v). The tightly closed containers were stored in a dry, cool and shady place for fermentation.

Wax extraction from mealybug

Mealybug wax extraction was performed following the method of Shalunkhe *et al.* (2013) with some modifications. The cuticular wax of the mealybugs was obtained by submerging the insects in chloroform for 1 min. A total of 1,500 mealybugs were soaked in 100% chloroform. The dissolved wax was filtered through an Acrodisc® syringe filter with a 28 mm diameter and 0.2 µm pore size. The filtrate was air-dried to evaporate the chloroform at room temperature. After drying, the white wax substance was collected and used to isolate wax-degrading bacteria.

Isolation of wax-degrading bacteria from fermented plant extract

Modified Davis minimal agar (DMA) was used to isolate the wax-degrading bacteria from the *Ficus* FPE (Salunkhe *et al*., 2013). The modified DMA contained 2.0 g/L of mealybug wax, 1.0 g/L of ammonium sulphate, 0.7 g/L of dipotassium phosphate, 0.1 g/L of magnesium sulphate and 15 g of bacteriological agar. One mL of *Ficus* FPE was diluted to 10 mL with distilled water up to 10^{-6}. A total of 100 µL diluted *Ficus* FPE was spread on the DMA plates and the plates were incubated at 37 °C for 48 h in an inverted position following the method of Sirisha *et al.* (2017). Single pure colonies were sub-cultured on DMA with three replications. Morphologically distinct colonies were processed for microscopic examination and molecular identification. Isolation of wax-degrading bacteria was carried out at 7day intervals from day 0 until day 63 with three replicates. During fermentation, the pH of the *Ficus* FPE was recorded. Gram staining was also conducted as described by Sirisha *et al.* (2017), to observe the colour (blue or red) of the bacterial cells.

Microscopic examination of wax-degrading bacteria

The size, shape, colour, edge pattern and opacity of the colonies, as well as the cell arrangement of bacteria, were examined under Wild Heerbrugg M3Z Stereo microscope (Leica, Switzerland) and Olympus CX31RBSF compound microscope (Olympus Optical Co. Ltd, Philippines)

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 20(4) 2024, pp. 459-471
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connected to Dino-Eye eyepiece camera (ANMO Electronics Corporation, Taiwan).

Molecular identification of wax-degrading bacteria

DNA of the bacterial isolates was extracted using the Nucleo Spin® Tissue Total DNA Extraction Mini Kit (Macherey-Nagel, Germany). The 16S rRNA gene of bacteria isolates was successfully amplified using forward primer 27F (5'-AGA GTT TGA TCM TGG CTC AG-3') and reverse primer 1525R (5'-TAC GGY TAC CTT GTT ACG ACT T-3') (Salunkhe *et al.*, 2013). The cycling program of PCR was carried out using a thermal cycler under the following conditions: 94 °C for 2 min, 30 cycles of 94 °C for 30 sec, 55 °C for 30 sec, 72 °C for 1 min, 72 °C for 5 min to yield 1,400 bp PCR product. The amplified product was then purified by NEXprep™ PCR/GEL Purification Mini Kit (NEX Diagnostics, Korea) prior to nucleotide sequencing.

Bioinformatics protocol

The sequencing results were analyzed and edited using Sequence Scanner 2, BioEdit program version 7.0.5 (Hall, 2010). The newly derived nucleotide sequences were subjected to a homology search using BLASTn (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and aligned with the ClustalW MEGAX program version 10.1.8 (Kumar *et al.*, 2018). Phylogenetic analyses were constructed using MEGAX (Kumar *et al.*, 2018). The Neighbor-joining (NJ) method was used for the clustering of bacteria isolates among themselves with 1,000 bootstrap replications to evaluate the branching confidence (Felsenstein, 1985). Bootstrap values above 60 were regarded as significant, and the same cluster would most likely form in a phylogenetic tree (Hillis and Bull, 1993; Park *et al.*, 2010).

Wax degradation test

The bacterial isolates were evaluated for their ability to degrade mealybug wax in Bushnell Haas Broth (HiMEDIA, India) which composed of KH_2PO_4 (1 g), K_2HPO_4 (1 g), NH_4NO_3 (1 g), $MgSO_4 \cdot 7H_2O$ (0.2 g), $FeCl_3$ (0.05 g), $CaCl_2 \cdot 2H_2O$ (0.02 g) yeast extract (5 g) and DCPIP (1 g/L) as indicator (Okoye *et al.*, 2019). The wax degradation ability of the bacteria was assessed by turbidimetry test using 2, 6-dichlorophenol indophenol (DCPIP) (redox potential +0.217 V) at 600 nm according to the protocol described by Okoye *et al.* (2019) with a few alterations. Bacterial inoculum was prepared by inoculating a loopful of bacterial culture in Luria Bertani (LB) broth and incubated for 18 h at 37 °C with agitation at 120 rpm. A total of 100 µL of bacterial culture was then inoculated into 9 mL of Bushnell Haas Broth (HiMEDIA, India) containing 100 mg of mealybug wax as the sole carbon source and 0.5 mL of 0.1% (v/v) DCPIP. Bushnell Haas Broth containing 100 mg of mealybug wax and 0.5 mL of 0.1% (v/v) DCPIP but without bacterial inoculums was used as a negative control. Bushnell Haas broth containing bacterial inoculum, 0.5 mL of paraffin oil and 0.5 mL of 0.1% (v/v) DCPIP served as the positive control. The absorbance of the medium was measured at 600 nm at 0, 3, 6, 9 and 12 days of inoculation. Wax degradation is confirmed positive when the DCPIP dye changes from blue to colourless (Bidoia *et al.*, 2010). The percentage of dye reduction was calculated using the formula given below (Patel and Lakshmi, 2016):

$$\% \text{ Dye reduction} = (C - T) \times 100/C$$

Where C is the control and T is the test culture.

Bioassay

Bacterial suspension for bioassay was prepared according to the protocol of Krishnamoorthy *et al.* (2020) with a few changes. One loopful of bacterial isolate was inoculated into 50 mL LB broth in a 250 mL flask and incubated at 37 °C overnight in a shaker bath at 125 rpm. The overnight cultures were centrifuged at 4,225× *g* for 10 min and the cell pellet was resuspended in 10 mL sterile saline solution. The optical density (OD) of the suspension was measured trice by Multiskan GO Spectrophotometer (ThermoFisher Scientific, Finland) at 600 nm and viable cell count was determined by plate count method (Harley and Prescott, 2002). Cell suspension of 10^8, 10^7 and 10^6 colony forming units (CFU)/mL in sterile saline solution was prepared (Blackburn *et al.*, 1998; Waterfield *et al.*, 2001). The supernatant resulting from the centrifugation at 4,225× *g* was harvested and filtered through a 0.2 µM Millipore syringe filter. Both the filtrate and the cell pellet were ready for bioassay.

Insecticidal bioassay

The insecticidal potentiality of bacteria isolates was tested against the third-instar nymph of *P. solenopsis* following the method of Krishnamoorthy *et al.* (2020) with minor amendments. Disease and chemical-free, non-infested, fresh hibiscus leaves were collected from plants and surface-sterilized by washing with 0.1% (v/v) NaOCl for 10 min. The leaves were rinsed twice with sterile distilled water and then left for air drying at room temperature under a laminar airflow. The petiole of each leaf was wrapped with wet cotton wool to keep the leaf turgid for a more extended period. The cell pellet suspension was added with 0.05% Arabic gum and 0.02% Tween 80 (10 µL per mL of cell suspension). Sterile distilled water containing only 0.05% Arabic gum and 0.02% Tween 80 served as a control for the experiment. LB medium served as a control for the treatment with filtrate (supernatant) generated from centrifugation of bacterial culture. The bioassay was laid out according to a completely randomized design (CRD) design with 10 replications per treatment. The leaves and mealybugs were put in 9 cm diameter Petri dishes and sprayed with test solutions (equal spray volume (0.5 mL/leaf) of bacterial solution and supernatant) by using a hand atomizer. The mealybugs were maintained at a temperature of 27 ± 2 °C

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 20(4) 2024, pp. 459-471
DOI: http://dx.doi.org/10.21161/mjm.230455

and relative humidity of 65 ± 5%. The mortality of individual mealybugs was recorded at 24, 48, 72, 96, 120, 144 and 168 h post-treatment (Kumar *et al.*, 2012). *Phenacoccus solenopsis* which failed to show movements after a gentle touch with a camel hairbrush, was considered a dead insect under Dino-Eye eyepiece camera. The percent mortality of mealybug was calculated using Henderson-Tilton's formula (Henderson and Tilton, 1955).

$$\% \text{ Corrected mortality} = [1 - (T_a \times C_b)/(T_b \times C_a)] \times 100$$

Where T_a = No. of alive insects after treatment in the treated area; T_b = No. of live insects before treatment in the treated area; C_a= No. of the living insects after treatment in the control area; C_b= No. of alive insects before treatment in the control area.

Effect of bacterial cell suspension on population growth of mealybugs

The female *P. solenopsis* was used to evaluate the efficacy of the bacterial isolates on the population expansion of *P. solenopsis* by assessing the number of crawlers and freshly emerging adults. In this test, fresh, tender, untreated, disease-free and non-infested hibiscus leaves were used in a 9 cm Petri dish to feed the mealybugs. Second-instar nymphs of *P. solenopsis* were treated with 10^5 CFU/mL of bacterial cell suspension by topical spray. One treated mealybug was transferred to one Petri dish with the help of a camel hairbrush on a hibiscus leaf in the Petri dish. The hibiscus leaf was replaced with fresh leaf in 3-day intervals until the end of the experiment. The mealybug exposed to sterile dH_2O was used as the control. Mealybug culture was conducted at a temperature of 25 ± 2 °C and relative humidity of 65 ± 10% following CRD design with 10 replications. Data on a number of newly emerged crawlers and adults from each treated mealybug were recorded.

Statistical analysis

The recorded data on mortality were subjected to analysis of variance (ANOVA) and the mean comparison among the multiple treatment levels with the control was computed by Tukey at $p<0.001$ using the statistical program SAS® software (SAS Institute, North Carolina State University, USA, Version 9.4, 2012).

RESULTS

The temperature varied from 27 to 32 °C daily during fermentation. pH of the fermented *Ficus* extract gradually dropped over time and became acidic in nature as the fermentation time increased. At 0 days, the initial pH was 6.17. However, the pH decreased gradually as the fermentation time increased and dropped to 3.71 at 63 days of fermentation. A total of 42 bacterial isolates were isolated and examined for their morphological characteristics, such as colony colour, shape, margin, elevation, Gram nature, cell shapes and cell arrangement (Table 1). Among them, 28, 14, 40, 2 and 1 isolates were Gram-positive, Gram-negative, rod-shaped, cocci and coccobacilli bacteria, respectively. Among Gram-positive bacteria, 24 and only 1 isolate had round and irregular colony, respectively. The colonies of the Gram-positive bacteria were either white, milky white, opaque or pale yellow in colour, except the isolates 35F3B3 and 35F2B5 were pinkish white in colour. Their cell arrangement was either single, paired or in chain. All the Gram-negative bacteria were rod-shaped bacteria. Their colonies were round in shape and the colour was either opaque, white or transparent. The Gram-negative bacterial cells were either single or paired in majority. The number of Gram-negative bacteria decreased after 28 days of fermentation. Based on phenotypic features, 13 different types of bacteria isolates were suspected initially; those were subjected to molecular identification.

Molecular characterization

The 16S rRNA PCR amplification of all wax-degrading bacterial isolated from the fermented *Ficus* extract produced a DNA band with a size of 1400 bp. The sequence analysis of bacteria isolates using the BLASTn program of NCBI is presented in Table 2. The 16S rRNA sequence analysis has confirmed the identity of the bacterial isolates as 1 isolate of *Klebsiella pneumoniae* (0F1B1, 99.17% sequencing homology), 1 isolate of *Staphylococcus gallinarum* (0F1S1, 100%), 1 isolate of *Bacillus velezensis* (0F1S2, 99.86%), 1 isolate of *Priestia megaterium* (7F2A4, 98.47%), 3 isolates of *Bacillus cereus* (14F1C3, 35F3B1 and 35F2B5; 99.38%, 99.53% and 99.08%, respectively), 1 isolate of *Paenibacillus* sp. (21F1E3, 99.17%) and 5 isolates of *Bacillus* sp. (35F1C1, 42F2B2, 49F1B1, 42F1B3 and 49F1C1; 99.64%, 93.85%, 97.64%, 97.06% and 100%, respectively).

The phylogenetic tree was constructed with sequences of the 16S rRNA regions (Figure 1). Phylogenetic analysis showed 10 different monophylogenetic clades formed between newly generated sequence and GenBank sequences of bacterial isolates, namely *Bacillus velezensis* (Clade 1), *Paenibacillus* sp. (Clade 2), *Klebsiella pneumoniae* (Clade 3), *Bacillus* sp. 35F3B1 (Clade 4), *Bacillus* sp. 49F1B1 (Clade 5), *Priestia megaterium* (Clade 6), *Bacillus cereus* (Clade 7), *Bacillus* sp. 42F2B2 and *Bacillus* sp. 49F1C1 closely related to *B. paranthracis* (Clade 8), *Bacillus* sp. 42F1B3 (Clade 9) and *Staphylococcus gallinarum* (Clade 10).

***In vitro* wax degradation test**

The bacterial isolates were further screened for their ability to degrade the long-chain hydrocarbons of paraffin wax and mealybug wax by using DCPIP dye detection (Figure 2 and Figure 3). The *Bacillus* sp. 42F1B3 demonstrated the significantly highest wax-degradability by showing DCPIP decoloration after 12 days, followed by *Bacillus* sp. 35F1C1, *Paenibacillus* sp. 21F1E3 and *K.*

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 20(4) 2024, pp. 459-471
DOI: http://dx.doi.org/10.21161/mjm.230455

Table 1: Morphological characteristics of bacteria isolated from fermented *Ficus* extract.

Bacterial isolate	Gram nature	Bacterial colony				Bacterial cell	
		Colour	Shape	Margin	Elevation	Shape	Arrangement
0F1B1, 0F3A2, 0F2B1	-ve	Opaque	Round	Entire	Convex	Rod	Paired or single or cluster
0F1S1, 0F3S1	+ve	Dull white	Irregular	Undulate	Flat	Cocci	single, paired, cluster
0F1S2	+ve	White	Irregular	Undulate	Flat	Rod	Single or chain
7F2A4	+ve	Milky white	Round	Entire	Raised	Rod	Chain
7F1B1, 7F2A3, 7F3B1	-ve	Opaque	Round	Entire	Convex	Rod	Paired or single or cluster
14F1C3, 14F3A3	+ve	White	Round	Undulate	Flat	Rod	Single or chain
14F1A4, 14F2B3	+ve	Milky white	Round	Entire	Raised	Rod	Chain
21F1E3, 21FB3, 21F2E1	-ve	Opaque	Round, punctiform	Entire	Raised	Rod	Single or pair
21F2D2, 21F1A3	+ve	White	Round	Undulate	Flat	Rod	Single or chain
28F3B1	-ve	Opaque	Round, punctiform	Entire	Raised	Rod	Single or pair
28F2A2, 28F3A3, 28F3B3	+ve	White	Round	Undulate	Flat	Rod	Single or chain
35F1C1	-ve	White, transparent	Round, punctiform	Entire	Raised	Rod	Single or pair
35F3B3, 35F2B5	+ve	Pinkish white	Round	Undulate	Raised	Rod	Single or chain
35F3B1	+ve	Opaque	Round	Entire	Raised	Rod	Single or pair
42F2B2	+ve	Pale yellow	Round	Undulate	Flat	Rod	Chain
42F1A3, 42F1C2	-ve	White, transparent	Round, punctiform	Entire	Raised	Rod	Single or pair
42F1B3	+ve	White	Round	Undulate	Raised	Cocco-bacilli	Single or chain or pair
49F3A2, 49F1C1, 49F2C2	+ve	Pale yellow	Round	Undulate	Flat	Rod	Chain
49F1B1	-ve	White, transparent	Round, punctiform	Entire	Raised	Rod	Single or pair
56F3A1, 56F2A2, 56F1B4, 56F2B5	+ve	Pale yellow	Round	Undulate	Flat	Rod	Chain
63F1B2, 63F2A1, 63F3B4	+ve	Pale yellow	Round	Undulate	Flat	Rod	Chain

Table 2: Molecular identity of wax-degrading bacteria isolated from fermented *Ficus* extract using 16S rRNA gene.

Isolate	NCBI accession number	% Query coverage	Closest match in GenBank	Sequence homology (%)	Bacterial ID
0F1B1	ON024774	100	OU548745	99.70	*Klebsiella pneumoniae*
0F1S1	ON024785	100	MG836019	100.00	*Staphylococcus gallinarum*
0F1S2	ON024784	100	ON791803	99.86	*Bacillus velezensis*
7F2A4	ON024775	100	ON773822	98.47	*Priestia megaterium*
14F1C3	ON024776	99	KY312802	99.38	*Bacillus cereus*
21F1E3	ON024777	100	KF479598	99.67	*Paenibacillus* sp.
35F3B1	ON024778	100	ON892078	99.53	*Bacillus cereus*
35F3B2	ON024779	99	ON786602	99.53	*Bacillus cereus*
35F1C1	ON024780	100	KT308203	99.64	*Bacillus* sp. 35F1C1
42F1B3	ON025801	96	OM333628	100.00	*Bacillus* sp. 42F1B3
42F2B2	ON024781	99	ON024783	94.09	*Bacillus* sp. 42F2B2
49F1B1	ON024781	100	KF441707	97.64	*Bacillus* sp. 49F1B1
49F1C1	ON024783	100	KR029294	97.06	*Bacillus* sp. 49F1C1

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

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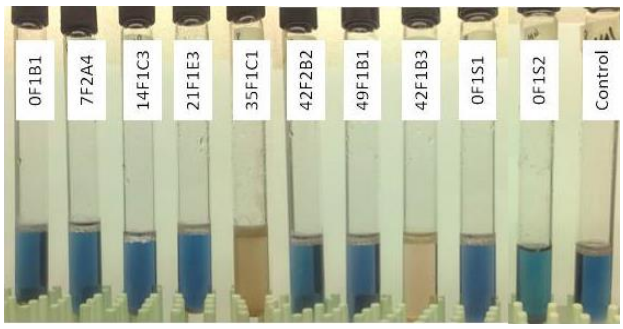

Figure 2: Mealybug wax degradation test using DCPIP as an indicator after 12 days of incubation.

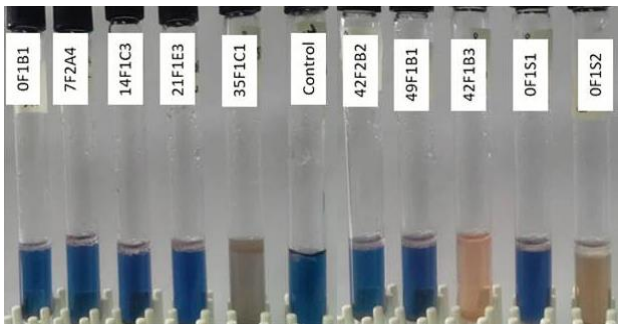

Figure 3: Paraffin wax degradation test using DCPIP as an indicator after 12 days of incubation.

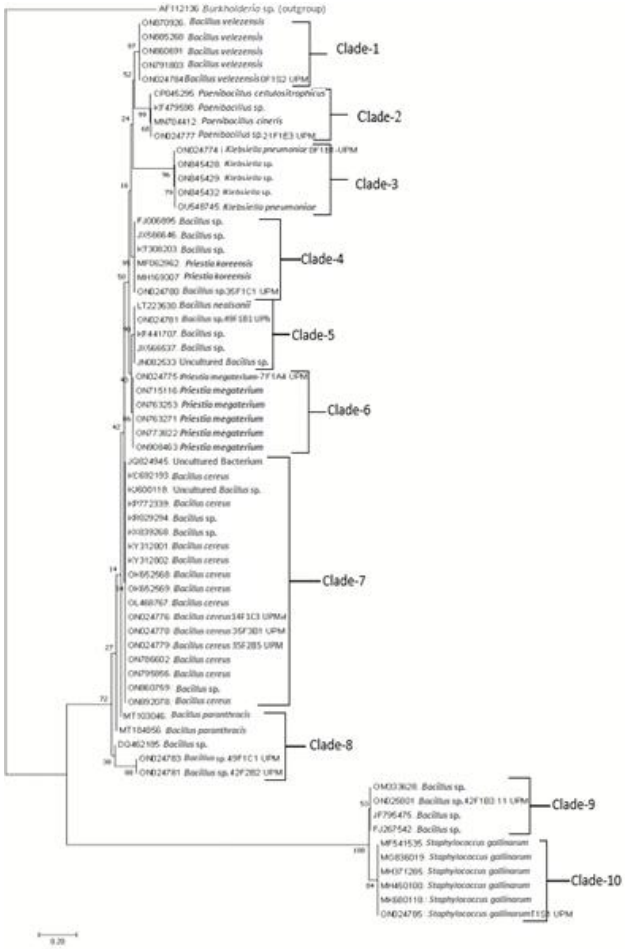

Figure 1: Neighbor-joining tree based on 16S rRNA gene sequences showing relationships of the bacteria in genera *Klebsiella*, *Bacillus*, *Staphylococcus*, *Paenibacillus*, *Priestia* and related type strains. The numbers on the branches denote the percentage bootstrap values of 1000 replicates. Scale bar 0.20 indicates substitutions per nucleotide position.

pneumoniae compared to the control in both mealybug wax and paraffin wax treated tubes. The *Bacillus* sp. 49F1B1 showed the lowest wax-degradability of mealybug wax. The wax degradation score (calculated in % reduction dye) of different bacterial isolates is presented in Figure 4. All bacterial isolates were statistically different from the control in paraffin wax degradation, while bacteria isolate 49F1B1 showed the lowest mealybug wax-degrading potential that was not statistically different from the control.

The percentage of dye reduction is positively correlated with the wax degradation rate. The bacterial isolates showed higher degradation in the paraffin wax than in the mealybug wax (Figure 4). *Bacillus* sp. 42F1B3 demonstrated the highest performance in the percent reduction of dye (73.85% paraffin wax degraded, 64.78% mealybug wax degraded), while *Bacillus* sp. 49F1B1 and *K. pneumoniae* showed the lowest performance.

Toxicity of bacterial isolates

The results on the toxicity of bacteria isolates against *P. solenopsis* recorded at 24 h intervals until 168 h are presented in Table 3. In the study, the mortality of *P. solenopsis* was significantly ($F=74.45$; $df=10$; $p<0.001$) higher in the bacterial treatments compared to the control. The nymphal mortality increased with the increase in treatment concentration and time of exposure. There was no significant difference in all treatments with a bacteria concentration of 10^6 CFU at 24 h post-treatment. All the treatments with bacteria at 10^6 CFU exhibited less than 60% nymphal mortality. When the bacterial concentration was increased to 10^7 CFU, only two bacterial isolates (*K. pneumoniae* and *Paenibacillus* sp. 21F1E3) induced more than 60% nymphal mortality at 168 h post-treatment. Bacterial suspension at 10^8 CFU showed better results at shorter treatment times. *Klebsiella pneumoniae* showed more than 60% nymphal mortality (64 ± 3.71%) as early as 72 h post-treatment, followed by *Paenibacillus* sp. 21F1E3 (69 ± 1.79%) after 120 h post-treatment, *Bacillus* sp. 35F1C1 (70 ± 2.58%) and *Bacillus* sp. 42F1B3 (75 ± 2.68%) at 168 h post-treatment. Amongst these bacteria, *K. pneumoniae* 0F1B1 was the top performer (83 ± 2.1%) at 10^8 CFU at 168 h post-treatment, whereas *B. paranthracis* was the least performer (35 ± 2.23%) among the treatments of bacteria isolates.

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

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DOI: http://dx.doi.org/10.21161/mjm.230455

Table 3: Toxicity of bacteria isolated from fermented *Ficus* extract against third-instar nymphs of *P. solenopsis*.

Isolates	% Mean mortality of *P. solenopsis* (Mean ± S.E)											
	24 h			72 h			120 h			168 h		
	10^8	10^7	10^6	10^8	10^7	10^6	10^8	10^7	10^6	10^8	10^7	10^6
*K. pneumoniae**	44 ± 3.71^{a}	19 ± 2.33^{ab}	8 ± 2.90^{ab}	64 ± 3.71^{a}	36 ± 5.20^{ab}	14 ± 3.71^{b}	75 ± 2.68^{a}	45 ± 5.82^{ab}	25 ± 3.07^{b}	83 ± 2.13^{a}	66 ± 3.05^{a}	45 ± 2.23^{ab}
P. megaterium	14 ± 1.63^{d}	6 ± 1.63^{cd}	3 ± 1.52^{b}	24 ± 1.63c^{d}	15 ± 1.66^{cd}	10 ± 1.49^{bc}	34 ± 1.63^{c}	18 ± 2.00^{c}	11 ± 1.79^{de}	45 ± 2.23^{cd}	27 ± 2.60^{de}	19 ± 2.33^{d}
B. cereus	12 ± 2.49^{de}	8 ± 2.49^{cd}	4 ± 1.63^{b}	18 ± 1.33^{d}	14 ± 1.63^{cd}	8 ± 1.33^{bcd}	34 ± 1.63^{c}	22 ± 1.33^{c}	12 ± 1.33^{cde}	46 ± 1.63^{cd}	32 ± 2.49^{cde}	26 ± 2.66^{cd}
Paenibacillus sp. *21F1E3**	36 ± 3.71^{ab}	25 ± 3.41^{a}	14 ± 2.66^{a}	58 ± 2.21^{a}	40 ± 3.48^{a}	27 ± 2.60^{a}	69 ± 1.79^{a}	51 ± 4.33^{ab}	36 ± 3.71^{a}	79 ± 2.33^{ab}	66 ± 3.05^{a}	48 ± 4.89^{a}
Bacillus sp. 35F1C1*	30 ± 3.94^{bc}	13 ± 3.34^{bc}	5 ± 2.68^{b}	44 ± 2.21^{b}	21 ± 3.48^{cd}	7 ± 2.60^{bcd}	51 ± 3.154^{b}	29 ± 4.06^{bc}	15 ± 2.23^{bcde}	70 ± 2.58^{b}	53 ± 3.00^{ab}	34 ± 3.05^{bc}
Bacillus paranthracis	12 ± 1.33^{de}	8 ± 1.33^{cd}	6 ± 1.63^{ab}	18 ± 1.33^{d}	13 ± 1.52^{cde}	9 ± 1.79^{bcd}	20 ± 1.49^{d}	16 ± 2.21^{cd}	14 ± 1.63^{cde}	35 ± 2.23^{d}	25 ± 2.23^{e}	20 ± 2.10^{d}
Bacillus sp. 49F1B1	22 ± 2.49^{cd}	12 ± 1.33^{bc}	4 ± 1.63^{b}	29 ± 2.33c^{d}	18 ± 2.49^{cd}	8 ± 2.49^{bcd}	39 ± 3.48^{c}	28 ± 2.90^{bc}	22 ± 1.33^{cde}	54 ± 2.66^{c}	46 ± 3.71^{b}	38 ± 2.90^{abc}
Bacillus sp. 42F1B3*	30 ± 4.21^{bc}	7 ± 2.60^{cd}	4 ± 1.63^{b}	45 ± 4.01^{b}	26 ± 4.26^{bc}	5 ± 1.66^{bcd}	53 ± 3.66^{b}	30 ± 4.94^{bc}	10 ± 2.10^{def}	75 ± 2.68^{ab}	45 ± 3.41^{bc}	36 ± 2.21^{abc}
S. gallinarum	10 ± 2.10^{de}	6 ± 1.63^{cd}	3 ± 1.52^{b}	32 ± 1.33^{c}	14 ± 1.63^{cd}	9 ± 1.00^{bcd}	36 ± 1.63^{c}	22 ± 3.88^{c}	18 ± 2.49^{bcd}	49 ± 2.33^{c}	40 ± 2.10^{bcd}	27 ± 3.95^{cd}
B. velezensis	11 ± 3.14^{de}	0 ± 0^{d}	0 ± 0^{b}	32 ± 3.26^{c}	10 ± 2.98^{de}	2 ± 1.33^{cd}	42 ± 3.88^{bc}	18 ± 5.33^{c}	6 ± 2.66^{ef}	50 ± 4.21^{c}	28 ± 3.26^{de}	16 ± 1.63^{d}
Control (water)	0 ± 0^{e}	0 ± 0^{d}	0 ± 0^{b}	0 ± 0^{e}	0 ± 0^{e}	0 ± 0^{d}	0 ± 0^{d}	0 ± 0^{d}	0 ± 0^{f}	0 ± 0^{e}	0 ± 0^{f}	0 ± 0^{e}

Values (mean ± SE) in the same column bearing the same letters are not significantly different at $p<0.001$ (Tukey test). *Potential isolates.

Toxicity of bacterial supernatant

The insecticidal activity of the bacterial supernatant was also tested against third-instar nymphs of *P. solenopsis* (Table 4). The supernatant significantly reduced the number of *P. solenopsis* ($F=31.99$; df=10; $p=0.001$) over the control. At 24 h and 72 h post-treatment, *Bacillus* sp. 42F1B3 supernatant exhibited the highest nymphal mortality (27 ± 3.00% and 3 5± 4.01%, respectively) among the tested isolates, exhibiting no significant difference with the supernatants of *K. pneumoniae*, *Bacillus* sp. 35F1C1 and *Bacillus* sp. 49F1B1. At 120 h post-treatment, *K. pneumoniae* supernatant exhibited the highest nymphal mortality (52 ± 4.16%) of *P. solenopsis* that was not statistically different from *Bacillus* sp.42F1B3 (47 ± 4.22%), *Paenibacillus* sp. 21F1E3 (39 ± 3.78%), *Bacillus* sp.35F1C1 (46 ± 3.40%), *Bacillus* sp.49F1B1 (38 ± 4.89%) and *S. gallinarum* (34 ± 3.40%). At 168 h post-treatment, *Bacillus* sp.42F1B3 had exhibited the highest nymphal mortality (66 ± 3.40%) without significant variation with those of *K. pneumoniae* (64 ± 2.23%), *Paenibacillus* sp. 21F1E3 (64 ± 3.71%), *Bacillus* sp.35F1C1 (63 ± 4.22%) and *S. gallinarum* (52 ± 1.33%), whereas the rest of all demonstrated below 50% nymphal mortality.

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 20(4) 2024, pp. 459-471
DOI: http://dx.doi.org/10.21161/mjm.230455

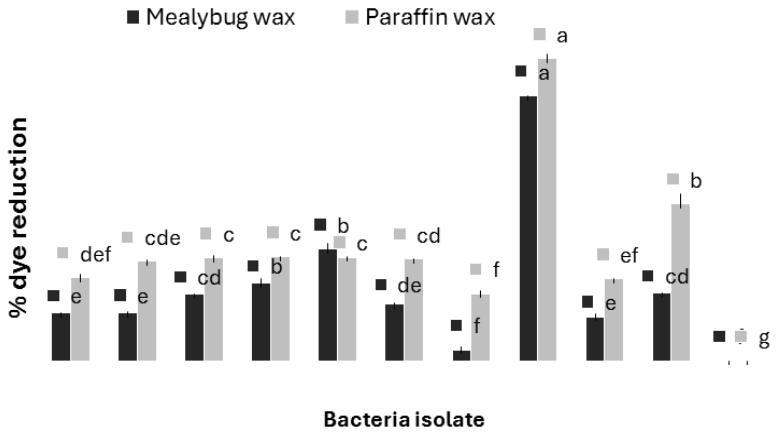

Figure 4: Biodegradation profile of bacteria isolated from fermented *Ficus* extract (0F1B1 = *K. pneumoniae*, 7F2A4 = *P. megaterium*, 14F1C3 = *B. cereus*, 21F1E3 = *Paenibacillus* sp., 35F1C1 = *Bacillus* sp.1, 42F2B2 = *B. paranthracis*, 42F1B3 = *Bacillus* sp. 2, 49F1B1 = *Bacillus* sp. 3, 0F1S1 = *S. gallinarum*, 0F1S2 = *B. velezensis* and Control = water).

Table 4: Toxicity of bacterial supernatant against third-instar nymphs of *P. solenopsis*.

Bacterial supernatant	% Mean nymphal mortality (Mean ± S.E)			
	24 h	72 h	120 h	168 h
*K. pneumoniae**	21 ± 3.48^{abc}	35 ± 3.07^{a}	52 ± 4.16^{a}	64 ± 2.23^{ab}
P. megaterium	8 ± 2.49^{def}	24 ± 2.66^{ab}	27 ± 4.48^{d}	49 ± 2.33^{bc}
B. cereus	10 ± 2.10^{cdef}	20 ± 2.58^{ab}	32 ± 1.33^{bcd}	50 ± 5.16^{bc}
Paenibacillus sp. 21F1E3*	15 ± 2.68^{bcde}	27 ± 3.00^{ab}	39 ± 3.78^{abcd}	64 ± 3.71^{ab}
Bacillus sp. 35F1C1*	17 ± 1.52^{abcd}	31 ± 3.14^{ab}	46 ± 3.40^{abc}	63 ± 4.22^{ab}
B. paranthracis	10 ± 2.10^{cdef}	20 ± 2.58^{ab}	28 ± 3.88^{cd}	43 ± 2.13^{c}
Bacillus sp. 49F1B1	22 ± 3.26^{ab}	30 ± 2.98^{ab}	38 ± 4.89^{abcd}	49 ± 1.00^{bc}
Bacillus sp. 42F1B3*	27 ± 3.00^{a}	35 ± 4.01^{a}	47 ± 4.22^{ab}	66 ± 3.40^{a}
S. gallinarum	6 ± 2.66^{def}	24 ± 3.39^{ab}	34 ± 3.40^{abcd}	52 ± 1.33^{abc}
B. velezensis	4 ± 1.63^{ef}	18 ± 6.46^{b}	22 ± 6.79^{d}	38 ± 5.33^{c}
Control (water)	0 ± 0^{f}	0 ± 0^{c}	0 ± 0^{e}	0 ± 0^{d}

Values (mean ± SE) in the same column bearing the same letters are not significantly different at p<0.001 (Tukey test). *Potential isolates.

Table 5: Effect of bacterial suspension on the offspring production of *P. solenopsis* at 10^5 CFU/mL.

Bacteria	No of emerged crawler	No of emerged adult
K. pneumoniae	80.7 ± 4.32^{d}	37.0 ± 2.44^{c}
Paenibacillus sp. 21F1E3	111.3 ± 7.11^{c}	40.8 ± 1.93^{c}
Bacillus sp. 35F1C1	161.2 ± 3.95^{b}	60.3 ± 3.78^{b}
Bacillus sp. 42F1B3	159.4 ± 5.37^{b}	52.9 ± 4.18^{b}
Control (water)	248.8 ± 5.53^{a}	190.7 ± 3.81^{a}

All measurements were expressed as the mean ± SE (n=10). Values in the same column bearing the same letters are not significantly different at p<0.001 (Tukey test).

Effect of bacteria suspension on offspring production of mealybugs

Four bacterial isolates namely *K. pneumonia* 0F1B1, *Paenibacillus* sp. 21F1E3, *Bacillus* sp. 35F1C1 and *Bacillus* sp. 42F1B3 demonstrated above 60% nymphal mortality at 168 h post-treatment. Therefore, these four bacterial isolates were subjected to evaluate the impact of bacteria isolates on offspring production of mealybugs. The impacts of bacterial treatment on the offspring production of *P. solenopsis* are presented in Table 5. The result revealed that the impact of bacterial isolates on offspring production was significantly different from the control treatment. Among the test isolates, *K. pneumoniae* was recorded as the statistically efficient microbial agent for lowering the number of crawlers and adults generated from treated females. The mean number of crawlers (80.2) generated from *K. pneumoniae*-treated females were the lowest, followed by *Paenibacillus* sp. 21F1E3 (111), *Bacillus* sp. 35F1C1 (161) and *Bacillus* sp. 42F1B3 (159). The control (water) showed the highest score (248.8) in the mean number of crawlers. The

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 20(4) 2024, pp. 459-471
DOI: http://dx.doi.org/10.21161/mjm.230455

development of adults from crawlers was the highest in the control (190.9), followed by *Bacillus* sp. 35F1C1 (60.3), *Bacillus* sp. 42F1B3 (52.9), *Paenibacillus* sp.21F1E3 (40.8) and *K. pneumoniae* (37.0).

DISCUSSION

In the present investigation, the pH of fermented *Ficus* extract decreased during the fermentation process. The acidic condition in the fermented *Ficus* extract was developed due to the accumulation of byproducts during the microbial fermentation process. This result is concomitant with the finding of Judoamidjojo *et al.* (1989), who reported that the decrease in pH was caused by the accumulation of acidic compounds by some microorganisms present in the palm juice. A total of 42 bacterial isolates were isolated from the fermented *Ficus* extract. A total of 13 bacterial isolates were confirmed based on the colony's morphological characteristics. Out of thirteen bacterial isolates, nine were Gram-positive bacteria and four were Gram-negative bacteria. Based on both morphological and phenotypic characteristics, 10 different types of bacterial isolates were confirmed belong to the genera *Bacillus* (Beaumont, 2002), *Staphylococcus* (Azokpota *et al.*, 2006), *Paenibacillus* (Nguyen *et al.*, 2017) and *Klebsiella* sp. (Ghanem *et al.*, 2007). Among them, 6 different types of bacterial isolates (*S. gallinarum* 0F1S1; *B. velezensis* 0F1S2; *K. pneumoniae* 0F1B1; *P. megaterium* 7F1A4; *B. cereus* 14F1C3; *B. cereus* 35F3B1; *B. cereus* 35F2B5; *B. paranthracis* 42F2B2 and *B. paranthracis* 49F1C1) were confirmed at the species level, while 4 bacterial isolates were confirmed at the genus level (*Bacillus* sp. 21F1E3, *Bacillus* sp. 35F1C1, *Bacillus* sp. 42FB3 and *Bacillus* sp. 49F1B1). The 16S rRNA gene is an important identification tool for bacterial classification (De Clerck *et al.*, 2004). The 16S rRNA gene is present in all bacteria, and it is large enough (~1400 bp) to provide a stable and reliable region for genus and species identification (Janda and Abbott, 2007). According to phylogenetic analysis, the isolate 0F1B1 is closely related to *K. pneumoniae* (GenBank accession no: OU548745), 0F1S1 is closely related to *S. gallinarum* (GenBank accession no: MG836019), 0F1S2 is closely related to *B. velezensis* (GenBank accession no: ON791803), 7F2A4 is closely related to *P. megaterium* (GenBank accession no: ON773822), 14F1C3, 35F3B1and 35F2B5 are closely related to *B. cereus* (GenBank accession no: KY312802), 21F1E3 is closely related to *Paenibacillus* sp. (GenBank accession no: KF479598), 42F2B2 and 49F1C1 are closely related to *B. paranthracis* (GenBank accession no: ON024783), and 35F1C1, 42F1B3, and 42F1B1 are closely related to different *Bacillus* sp. All 10 bacterial isolates were subjected to a wax-degrading potential test and bioassay to evaluate their efficacy in controlling *P. solenopsis*.

The capability of bacterial isolates to utilize paraffin wax and mealybug wax as carbon sources was determined at varying levels. All isolates had induced a colour change from blue to colourless, indicating their ability to degrade the paraffin wax and mealybug wax. The isolated *Bacillus* sp. 42F1B3 exhibited the highest percentage of wax degradation potential, followed by *Bacillus* sp.35F1C1, *Paenibacillus* sp. 21F1E3 and *K. pneumoniae*. In the current investigation, the *Bacillus* genus included the majority of bacteria isolates that consumed wax (hydrocarbons). *Bacillus* species were effective long-chain hydrocarbon degraders, making them perfect for wax removal agents to improve oil industry productivity (Sood and Lal, 2008; Deng *et al.*, 2014). Previous reports also stated that *Bacillus* sp. was the most common isolate of all the crude oil-utilizing bacteria isolated from highly polluted soil samples with 30-40% crude oil contamination (Sorkhoh *et al.*, 1993; Annweiler *et al.*, 2000; Ijah and Antai, 2003). Besides *Bacillus*, *Proteus*, *Psuedomonas*, *Micrococcus*, *Enterobacter* spp. and *Corynebacterium* (Balogun *et al.*, 2015; Obiakalaije *et al.*, 2015) and *Paenibacillus* sp. (Shibulal *et al.*, 2017) were capable of degrading hydrocarbons. Chikere *et al.* (2016) reported some of the bacterial genera (*Bacillus*, *Pseudomonas*, *Klebsiella*, *Corynebacterium*, *Proteus* and *Micrococcus*) as hydrocarbon utilizing bacteria isolated from crude oil-contaminated soils. Obiakalaije *et al.* (2015) also isolated ten hydrocarbon-degrading bacteria, including *Bacillus*, *Pseudomonas* and *Corynebacterium*, in a study of bioremediation of crude oil-polluted soil using animal waste. Mealybugs possess a waxy layer on their body that protects them from the penetration of insecticides (Meyerdirk *et al.*, 1998; Kairo *et al.*, 2000). The wax degradation by bacteria from the insect bodies will facilitate easy penetration of biopesticides to accelerate the insecticidal function of the biopesticides on the insects. Regnault-Roger *et al.* (2012) documented that elimination of the waxy layer from the insect cuticle could cause dehydration of the membrane cells, which may result in death and thus microbial agents could be promising an option to control them.

The bioassay study revealed that all the bacteria exhibited insecticidal properties against the nymphs of *P. solenopsis*. The mortality of mealybugs was dose-dependent, with the highest nymphal mortality occurring at 10^8 CFU/mL at 168 h post-treatment. *Klebsiella pneumoniae*, *Paenibacillus* sp. 21F1E3, *Bacillus* sp. 42F1B3 and *Bacillus* sp. 35F1C1 showed more than 70% nymphal mortality. The bacterial supernatant, which was separated from the bacterial cells, also showed 28-66% nymphal mortality at 168 h post-treatment. The supernatant of *Bacillus* sp. 42F1B3 had induced more than 60% nymphal mortality at 168 h post-treatment. Thus, these bacterial isolates could be effective as bio-insecticides either in the form of bacterial cells or supernatants.

Klebsiella pneumoniae was the most virulent entomopathogenic bacteria (induced 83 ± 2.13% mortality) in controlling *P. solenopsis* in this study. The results agreed with those reported by Insua *et al.* (2013), who reported that an inoculum of 10^6 CFU/mL of *K. pneumoniae* 48136 could kill 100% of *Galleria mellonella* larvae within 24 h. The efficacy of *K. pneumoniae* on *G. mellonella* larvae was evaluated by cell death, phagocyte avoidance and reduced defence responses.

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

He *et al.* (2019) documented 50% mortality of *Trichoplusia ni* larvae after 3 days post ingestion with *K. pneumonia*. Wand *et al.* (2013) also reported that 68% of 50 *K. pneumoniae* strains produced in the fermentation broth induced 50% mortality of *G. mellonella* larvae at 1 × 10^5 CFU/mL after 5 days post-infection. *Paenibacillus* sp. 21F1E3, *Bacillus* sp. 42F1B3 and *Bacillus* sp. 35F1C1 were the second higher group of bacteria, which exhibited 70-79% nymphal mortality of *P. solenopsis*. Mohamedova *et al.* (2017) documented the efficacy of *B. amyloliquefaciens* and *Paenibacillus polymyxa* against citrus mealybugs with 84.29% and 90.37% mortality after being treated with 10^8 CFU/mL, respectively. Ruiu (2015) reported that the spore-formers, *P. popilliae* and *P. lentimorbus*, were the agents of milky disease in the coleopteran species. Besides the insect pests, *Paenibacillus* sp. has been reported to cause disease in beneficial insects such as honeybees. Davidson (1973) reported that the genus *P. larvae* subsp. *larvae* had induced the honeybee disease American Foulbrood (AFB) in the honeybee colonies.

In the present study, *Bacillus* species induced 46-75% nymphal mortality of *P. solenopsis*. Among the *Bacillus* species, *Bacillus* sp. 42F1B3 induced significantly higher mortality compared to *Bacillus* sp. 49F1B1, *B. velezensis* 0F1S2, *B. cereus* 14F1C3 and the lowest nymphal mortality was recorded by *B. velezensis* 0F1S2. Ali *et al.* (2017) reported that *Bacillus* sp. CAF3 and *Enterobacter* sp. L3 induced 95% and 81% mortality of *Phenacoccus parvus* mealybug, respectively. Similarly, Krishnamoorthy *et al.* (2020) reported 78.7% and 74.0% mealybug mortality induced by *B. cereus* PYP3 and *Bacillus* sp. PLB1, respectively, where both the isolates showed chitinase and protease activity along with wax-degrading ability. Ibargutxi *et al.* (2008) also reported that members of the genus *Bacillus* are widely used as biological control agents for many insect pests due to their inherent ability to create endospores and tolerance to harsh environments. Besides the bacterial cells, the supernatant of all 10 bacteria was tested against *P. solenopsis*. The nymphal mortality increased with treatment time. *Bacillus* sp. 42F1B3 induced more than 60% nymphal mortality at 168h post-treatment. Dahmana *et al.* (2020) reported a high insecticidal activity of *B. clausii* supernatant with 75% of mosquito larval mortality. Shawer *et al.* (2018) tested the efficacy of the supernatant of *Photorhabdus luminescens* against *Drosophila suzukii* third-instar larvae and pupal stages and the results showed that it induced 24% mortality of larvae at 4 days post-treatment, 44% pupal mortality at 10 days post-treatment and 74% of all individual cumulative mortality at 10 days post-treatment, while 10-fold diluted supernatant did not show significant insecticidal performance.

The impact of the bacteria on *P. solenopsis* was also investigated. *Klebsiella pneumoniae* (0F1B1) had badly affected the offspring production of *P. solenopsis*, followed by *Paenibacillus* sp. 21F1E3, *Bacillus* sp. 42F1B3 and *Bacillus* sp. 35F1C1 isolates. Devi *et al.* (2022) reported that larvae of *A. hemolyticus* fed on a diet treated with *K. pneumoniae* and *P. paralactis* gave increased bacteria compared to *E. mundtii* and E. *casseliflavus*, resulting in dominance in larval infection and growth inhibition. This finding revealed that these bacteria would be a promising biocontrol tool for the containment of *P. solenopsis* population. Nevertheless, further research is needed to evaluate the effectiveness and formulation stability of products based on bacterial suspensions in various crops and environmental situations when applying this technology in open-field conditions.

CONCLUSION

In this study, 10 wax-degrading bacteria were isolated from fermented *Ficus* extract including *K. pneumoniae* (0F1B1), *S. gallinarum* (0F1S1), *B. velezensis* (0F1S2), *P. megaterium* (7F2A4), *B. cereus* (14F1C3), *Paenibacillus* sp. (21F1E3), *B. paranthracis* (42F2B2), *Bacillus* sp.(35F1C1), *Bacillus* sp. (42F1B3) and *Bacillus* sp. (49F1B1). Among them, the isolates *K. pneumoniae*, *Paenibacillus* sp. 21F1E3, *Bacillus* sp. 35F1C1 and *Bacillus* sp. 42F1B3 exhibited promising results in the *P. solenopsis* control as a wax degrader with insecticidal activity. These species can be potential candidates for developing eco-friendly biopesticides against *P. solenopsis*. Further investigation on field trials with bacterial mass production should be carried out to assess their efficacy and economic feasibility.

ACKNOWLEDGEMENTS

The authors are thankful to the National Agricultural Technology Program-Phase II Project (NATP-2), BARC Component, Bangladesh Agricultural Research Council, for funding this project under grant number 6282508-10201 and UPM for providing research facilities during the study.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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