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Isolation and evaluation of bacteria associated with entomopathogenic nematode *Heterorhabditis* spp. against the spider mite, *Tetranychus truncatus* Ehara (Acari: Tetranychidae)

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Abstract

Background: Entomopathogenic nematodes (EPNs) belonging to the *Heterorhabditis* spp. harbour symbiotically associated bacteria which are toxic to a wide range of insect pests. Isolation, purification, characterization and mass multiplication of such bacteria will be a promising strategy in the management of the pests. This study was carried out to isolate the EPN from different locations, isolate and purify the bacterial colonies, characterize the bacteria through morphological and molecular strategies and to test the efficacy of different bacteria in the control of polyphagous *Tetranychus truncatus* Ehara mites.

Results: EPNs were isolated from soil samples at 11 localities of Kerala State, India, and used to infect the *Galleria mellonella* L. larvae. Bacteria associated with the haemolymph of the infected larvae were isolated, which on NBTA medium have produced circular to irregular, entire, opaque and smooth colonies. Sequence characterization of the *16S rRNA* revealed nine isolates namely: one symbiotic bacterium *Photorhabdus luminescens*, two *Pseudomonas aeruginosa*, five *Ochrobactrum* sp. and one *Stenotrophomonas maltophilia.* Phylogenetic analysis using the sequences has further confirmed the bacterial identity. Evaluation of the cell suspension (CS) and cell-free supernatant (CFS) of *P. luminescens*, *P. aeruginosa* and *Ochrobactrum* sp. for their adulticidal and ovicidal efficiencies on *T. truncatus* had identified significant adulticidal effects by *P. luminescens,* followed by *P. aeruginosa.* After 96 h of treatment, *P. luminescens* at 10^8 cells/ml resulted in a significantly higher mortality rate of adult mites (64.00 and 60.67%, respectively, for CFS and CS), compared to that resulted by *P. aeruginosa* (38.67 and 33.33%).

Conclusions: Results of this study showed that *P. luminescens* associated with the EPN *Heterorhabditis* spp. is a promising biocontrol agent for *T. truncatus*.

Keywords: *16S rRNA*, Biocontrol, Entomopathogenic bacteria, *Photorhabdus luminescens*, *Pseudomonas aeruginosa*

Background

Entomopathogenic nematodes (EPNs) belonging to the families Steinernematidae and Heterorhabditidae are obligate insect parasites which can be used as potential biocontrol agents against important soil dwelling pests (Lacey and Georgis 2012). The bacteria symbiotically associated with EPNs are toxic to a wide range of insect

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pests (Guo et al. 1999). Nematodes protect the bacteria from the host's immune system and help in their transportation by forming a special vesicle in the infective L3 juvenile stage, as in case of Steinernematidae, or retain throughout the intestine as in case of Heterorhabditidae. The bacteria help in maintaining ideal conditions for the reproduction of the nematodes by releasing some of the nutrients. They also release antimicrobial substances, which prevent the growth of other bacteria (Boemare et al. 1996).

Photorhabdus luminescens is the main bacterial species symbiotically associated with the EPN *Heterorhabditis* (Boemare et al. 1996). Pathogenicity of this bacterium against insect pests has been well documented by earlier scientists (Bowen et al. 1998). Additionally, few studies report the pathogenicity of this bacterium to mite species (Kulkarni et al. 2017). However, many other bacteria are also associated with EPNs (Babic et al. 2000). Entomopathogenicity of some of these bacteria associated with *Heterorhabditis* spp. has also been reported (Salgado-Morales et al. 2019).

Spider mites (Tetranychidae) are serious sucking pests of many agricultural and horticultural crops. Among the spider mites, *Tetranychus truncatus* Ehara (Acari: Tetranychidae) is a major species infesting economically important crops of Kerala State, India (Bennur et al. 2015). In this study, bacteria associated with the EPNs *Heterorhabditis* spp. were evaluated under laboratory conditions for their bio-efficacy against *T. truncatus*.

Methods

Collection of soil samples

Soil samples were collected randomly from 11 localities of Kerala State, India. From each locality, 500 g soil was collected from 15 cm depth, sealed and labelled in polybags and brought to the laboratory.

Isolation of *Heterorhabditis* from the soil

Following the baiting technique (Bedding and Akhurst 1975), EPNs were isolated from the soil samples. Larvae of greater wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae) obtained from the culture maintained by All India Network Project on Agricultural Acarology (AINPAA), Kerala Agricultural University, India, were used for the isolation and maintenance of EPNs. Four to five healthy last-instar larvae of *G. mellonella* were released separately into plastic containers of 250 ml size, containing the soil samples collected from different locations. The soil samples were moistened before transferring to the plastic container to maintain adequate moisture content. Mouth of the container was covered with muslin cloth, placed upside down and stored at room temperature. Infected larvae which turned to brick red colour and died within 24–48 h, indicating infection by the EPNs were picked and washed thoroughly with distilled water and transferred to White's trap (White 1927) and kept in dark at 28 °C. For the multiplication and maintenance of EPNs, infective juveniles (IJs) released from the cadavers along the sides of the Petri plate were collected, stored in vials and used as stock for infecting the *Galleria* larvae. To get infected, four to five larvae were released into Petri plate lined with filter paper, moistened with IJ suspension (1 ml), incubated at 28 °C in a BOD incubator and observed for mortality. The dead cadavers were used for the isolation of EPNs-associated bacteria.

Isolation of bacteria from the EPNs

Bacteria were isolated from the EPNs infected cadavers and inoculated on NBTA plates. Within 24–48 h of death, the cadavers were collected and surface sterilized with 70% ethanol under aseptic conditions. The cadavers were dissected with a sterile blade, and a drop of haemolymph was streaked on Petri plates containing 10–20 ml of NBTA media (peptone 5 g, beef extract 3 g, NaCl 5 g, bromothymol blue dye 0.025 g, 2,3,5 triphenyl-tetrazolium chloride (TTC) 0.04 g, agar 15 g and distilled water 1000 ml) using a sterile inoculation loop (Woodring and Kaya 1988). Same medium was used for isolation, purification and maintenance of the bacteria. The Petri plates were then sealed tightly by using parafilm and kept in BOD incubator for 24–48 h at the optimum temperature of 28 °C and dark conditions and observed for colony proliferation. Individual pure colonies were selected, streaked on NBTA plates and subcultured continuously to get pure colonies of uniform size and morphology.

Identification of the bacteria

Bacteria associated with EPNs were studied for their cultural characters and *16S rRNA* sequences. The colony characters such as shape, colour, edge, margin, elevation and surface as well as change in the colour of media, were recorded. The isolates were also subjected to Gram's reaction.

For molecular characterization, *16S rRNA* gene was amplified and sequenced through colony PCR on fresh cultures of 24–48 h. Reaction mixture (50 µl) was prepared by adding EmeraldAmp GT PCR Master mix (25 µl), water (23 µl), forward and reverse primers (1 µl each) and bacterial culture was added by picking a single colony with tooth pick. Universal primer sequences (27f/1492r) 5′ AGAGTTTGATCCTGGCTC AG 3′ (forward) and 5′ ACGGCTACCTTGTTACGA CTT 3′ (reverse) were used (Mulla et al. 2017). Thermal program followed had initial denaturation at 94 °C for 4 min, followed by 35 cycles consisting of denaturation at 94 °C for 30 s, annealing at 55.7 °C for 45 s

and extension at 72 °C for 45 s and the final extension at 72 °C for 8 min (Veriti, Thermo Fisher Scientific). Products were electrophoresed (1.4% agarose gel) and sequenced. Contigs of *16S rRNA* gene were analysed for homology and phylogenetic relations along with 25 accessions of *Ochrobactrum* spp., seven accessions of *Photorhabdus luminescens*, 10 sequences of *P. aeruginosa* and five accessions of *Stenotrophomonas* spp. retrieved from GenBank. Phylogenetic tree was constructed by neighbour joining method with 500 bootstrap replications.

Bioassay of EPN-associated bacteria against *Tetranychus truncatus*

Bacterial cells as well as cell-free supernatant (CFS) of five bacterial isolates (one isolate of *P. luminescens*, two isolates of *P. aeruginosa* and two isolates of *Ochrobactrum*) were evaluated independently against the eggs and adults of *T. truncatus* at 10^4, 10^5, 10^6, 10^7 and 10^8 cells/ml concentrations. The *Pseudomonas* and *Photorhabdus* isolates were selected because of their high pathogenicity against some of the insect pests (Salgado-Morales et al. 2019). Since *Ochrobactrum* spp. is reported to be pathogenic (Fu and Liu 2019) and non-pathogenic (Babic et al. 2000) to insects, its isolates were also included. The emerging multidrug-resistant opportunistic pathogen *Stenotrophomonas* (Brooke 2012) was excluded from the study. Experiments were laid out in completely randomized design with 26 treatments including control and three replications per treatment.

For the ovicidal assay, ten gravid females of *T. truncatus* were transferred on the leaf bit of mulberry (5×5 cm^2 each), placed in a Petri plate lined with wet cotton and allowed for laying eggs. After 24 h, the mites were removed and 25 eggs were retained per leaf bit. For adulticidal assay, three leaf bits with 25 gravid female mites were used for each treatment. Sterile liquid broth of NBT (150 ml) was taken in a conical flask and a loop full of fresh bacterial culture was added aseptically, sealed and maintained in an orbital shaker at 200 rpm for 24–48 h and used as mother culture. Serial dilution, plating and colony counting method were followed to assess the initial concentration of the mother culture. Based on the initial concentration, 10^4, 10^5, 10^6, 10^7 and 10^8 cell/ml were prepared by serial dilution. Ten millilitre each of these dilutions was centrifuged at 4000 rpm for 20 min for the preparation of cell suspension and cell-free supernatant and sprayed separately on leaf bits with egg and adult mites, using a hand atomizer (2 ml/leaf bit). Per cent mortality was recorded by observing under a stereo binocular microscope (LEICA EZ4 HD) at 24, 48, 72 and 96 h of spraying.

Results

Eleven isolates of EPNs *Heterorhabditis* spp. were isolated from the soil samples by baiting method. The infective juvenile suspension prepared from the EPNs isolates (Fig. 1A) was used for infecting healthy *Galleria* larvae (Fig. 1B–D). Bacteria were isolated from the infected cadavers (Fig. 1E) and cultured on NBTA media. When haemolymph from the infected cadavers of *G. mellonella* was streaked on NBTA plates, bacterial colonies developed within 24–48 h. Eleven bacteria were isolated from the EPN-infected *Galleria* cadavers and coded based on the locality from where soil was collected for EPN isolation (Table 1).

Cultural characterization of the bacteria

The colony characteristics of the isolates KL1 and KT1 were similar on NBTA medium (Table 2), with circular shape, irregular edges, opaque, flat and smooth surface. Similarly, the isolates MP1, MT1, FR1, EKM1, HI1, HQ1 and HS1 were similar, but differed from the isolates, KL1 and KT1 in colour of the colony. The colour varied from red (FR1, MT1, HI1) to pinkish red (HS1, MP1, EKM1, HQ1). Elevation of the colonies varied from flat (KL1. KT1, FR1, EKM1), low convex (MP1, HS1) to raised (MT1, CF1, HI1, HQ1, WH1). Isolates WH1 and CF1 were similar, producing dark red colonies with white margin and the media turned from yellowish to blue (Fig. 1F). Circular to irregular, opaque, raised, smooth colonies were produced. Under 100X oil immersion microscope, the cells appeared red in colour (Fig. 1G) and were identified as Gram's negative. However, the shape of the cell varied among the isolates from short to long rods.

Molecular characterization

Using the universal primers, *16S rRNA* gene from eleven isolates was amplified (Fig. 2) and sequenced. Out of the eleven isolates, only nine isolates were identified, as the sequencing information for the remaining two isolates (WH1 and MT1) was incomplete even after repeated sequencing. Of the nine isolates identified, isolates KL1 and KT1 were identified as *Pseudomonas aeruginosa,* while the isolate HQ1 was identified as *Stenotrophomonas maltophilia* (Table 3). The isolate CF1 showed maximum sequence homology with *Photorhabdus luminescens*, while the remaining isolates MP1, FR1, EKM1, HI1 and HS1 belonged to *Ochrobactrum* (FR1—*O. pseudogrignonensis*, HI1—*O. anthropi,* EKM1, MP1 and HS1—undescribed species).

The phylogenetic analysis of *16S rRNA* gene from the nine isolates along with 47 related accessions from the GenBank has resulted in a phylogenetic tree with two clusters (Fig. 3). Cluster A had subcluster A1

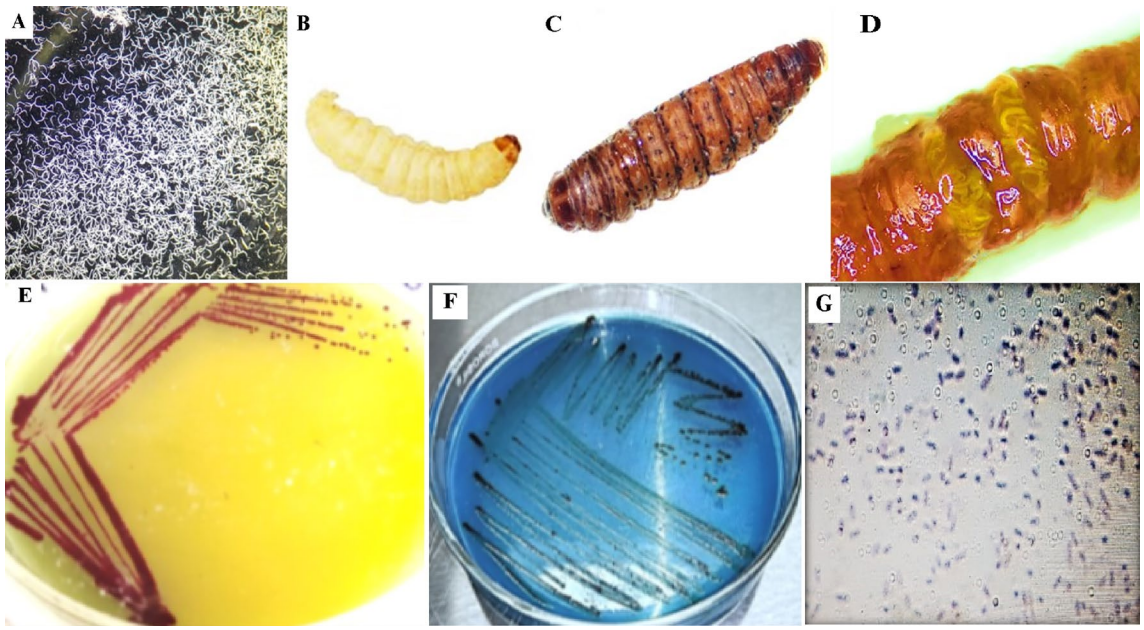

Fig. 1 **A** Infective juvenile suspension of nematodes collected in the White's trap. **B** Healthy *Galleria* larva. **C** Entomopathogenic nematode infected cadaver. **D** Nematodes inside the host body. **E** Colonies produced by bacteria on NBTA media. **F** Change in media colour due to pigmentation by CF1 isolate. **G** Gram-stained bacterial cells (100X magnification)

Table 1 Details of the isolates of *Heterorhabditis* spp. isolated from soil

Accession number	Location	District	GPS	
			Longitude	Latitude
KL1	Kaladi	Ernakulam	76°42′65″E	10°15′31″N
KT1	Kottapadam	Palakkad	76°39′14″E	10°99′79″N
MT1	Mattathur	Thrissur	76°19′15″E	10°22′45″N
FR1	Fruit Research Station	Thrissur	76°28′57″E	10.54′65″N
EKM1	Angamaly	Ernakulam	76°24′0″E	10°11′60″N
CF1	Coconut Farm	Thrissur	76°28′57″E	10°54′65″N
HI1	KAU main campus 1	Thrissur	76°28′57″E	10°54′65″N
MP1	Nilambur	Malappuram	76°13′33″E	11°16′37″N
HS1	KAU main campus 2	Thrissur	76°28′57″E	10.54′65″N
HQ1	KAU main campus 3	Thrissur	76°28′57″E	10.54′65″N
WH1	KAU Waterhouse	Thrissur	76°28′57″E	10.54′65″N

accommodating all sequences of *Ochrobactrum* and A2 accommodating the accessions of *Stenotrophomonas*. Cluster B had subcluster B1 accommodating *Photorhabdus luminescens* accessions and B2 accommodating *P. aeruginosa* accessions.

Bioassay of the EPN-associated bacteria against *Tetranychus truncatus*

Five bacterial isolates (CF1—*P. luminescens,* KL1—*P. aeruginosa* strain 1, KT1—*P. aeruginosa* strain 2, HI1—*Ochrobactrum* sp. strain 1 and FR1—*Ochrobactrum* sp. strain 2) were evaluated for their biocontrol efficacy. Though the isolates had non-significant ovicidal action (Fig. 4), substantial adulticidal effects were recorded (Fig. 5, Additional file 1: Tables S1 and S2). The highest egg mortality of 30.67% was observed at 96 h, in the treatment with 10^8 cells/ml of *P. luminescens*. In adulticidal treatments, no mortality was observed up to 24 h but after 48 h, mortality rate had increased. The *P. luminescens* isolate was significantly superior over other isolates in both CS and CFS forms. At 96 h, treatment with 10^8 cells/ml of *P. luminescens* recorded significantly higher mortality of 60.67%. The same treatment recorded 64.00% mortality in its CFS form. This was followed by the other treatments of *P. luminescens* at 10^7 cells/ml (52.00% in CS and 56.00% in CFS), 10^5 cells/ml (50.67% in CS and 53.33% in CFS), 10^6 cells/ml (49.33% in CS and 52.00% in CFS) and 10^4 cells/ml (49.33% in CS and 48.00% in CFS).

Among treatments of *P. aeruginosa*, the highest mortality of 38.67% in CFS and 33.33% in CS was recorded in the treatment with 10^8 cells/ml of strain 2 (KT1 isolate). No significant mortality rate was observed in any of the treatments of *Ochrobactrum* spp.

Table 2 Cultural characters of bacteria associated with entomopathogenic nematodes, *Heterorhabditis* spp.

Sl. No.	Isolates	Shape	Edge	Opacity	Elevation	Surface	Colour of colonies on NBTA	Colour of NBTA media (after 3–4 days)	Gram reaction	Cell shape
1	KL1	Circular	Irregular	Opaque	Flat	Smooth	Red	Yellowish turned to blue	Gram negative	Rod shape
2	KT1	Circular	Irregular	Opaque	Flat	Smooth	Red	Yellowish turned to blue	Gram negative	Rod shape
3	MT1	Irregular	Entire	Opaque	Raised	Smooth	Red	Yellowish green	Gram negative	Long rods
4	FR1	Circular	Undulate	Opaque	Flat	Smooth	Red	Yellowish green	Gram negative	Rod shape
5	EKM1	Circular	Entire	Opaque	Flat	Smooth	Pinkish red	Yellowish green	Gram negative	Rod shape
6	CF1	Circular	Entire	Opaque	Raised	Smooth	Dark red colony with white margin	Yellowish turned to blue	Gram negative	Short rods
7	HI1	Circular	Entire	Opaque	Raised	Smooth	Red	Yellowish Green	Gram negative	Short to long rods
8	MP1	Circular	Entire	Opaque	Low convex	Smooth	Pinkish red	Yellowish green	Gram negative	Short to long rods
9	HS1	Circular	Entire	Opaque	Low convex	Smooth	Pinkish red	Yellowish green	Gram negative	Rod shape
10	HQ1	Circular	Entire	Opaque	Raised	Smooth	Pinkish red	Yellowish turned to greenish blue	Gram negative	Rod shape
11	WH1	Irregular	Entire	Opaque	Raised	Smooth	Dark red colony with white margin	Yellowish turned to Greenish blue	Gram negative	Short to long rods

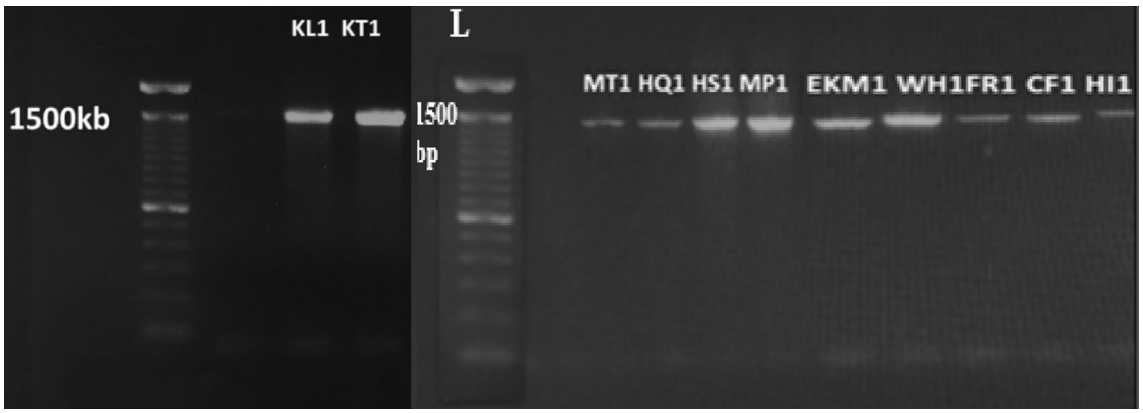

Fig. 2 *16S rRNA* gene amplified from different isolates (1.5 Kb)

Table 3 Homology of *16S rRNA* gene sequences of bacteria in NCBI database

Bacterial Isolate	NCBI Identity	Per cent identity	Query coverage (%)	Accession no. in GenBank	*E* value
KL1	*Pseudomonas aeruginosa* strain QK-3	99.76	100	MH7461061	0.0
KT1	*Pseudomonas aeruginosa* strain PA0504	100	100	MK607451.1	0.0
MP1	*Ochrobactrum* sp. strain QY-1	99.85	100	MN263248.1	0.0
FR1	*Ochrobactrum pseudogrignonensis* strain OsEnb HZB H6	100	100	MN889402.1	0.0
EKM1	*Ochrobactrum* sp. strain QY-1	99.92	100	MK351298.1	0.0
HI1	*Ochrobactrum anthropi* strain DP5	100	100	MG550982.1	0.0
HQ1	*Stenotrophomonas maltophilia*	99.86	100	MK600536.1	0.0
HS1	*Ochrobactrum* sp. strain QY-1	99.77	100	MN263248.1	0.0
CF1	*Photorhabdus luminescens* strain SG-HR4	99.56	100	JX221723.1	0.0

Discussion

In this study, nine bacterial isolates were isolated from the haemolymph of EPNs (*Heterorhabditis* spp.) infected *G. mellonella* cadaver. This included one species of symbiotic bacteria, *P. luminescens* and eight non-symbiotic/associated bacteria namely two isolates of *P. aeruginosa*, five isolates of *Ochrobactrum* and one isolate of *Stenotrophomonas maltophilia*.

Photorhabdus is the predominant genus of entomopathogenic bacteria, which is a natural symbiont of the EPN, *Heterorhabditis* spp. (Boemare et al. 1993). It is Gram negative and bioluminescent. The bacterium supports growth of the nematode in the association, but is a deadly pathogen to most of the insect pests (Clarke 2008). They reside in the gut of the nematodes and get released into the insect haemocoel soon after the EPN infects the insect and kill them within 24–48 h. Other than the symbionts, few associated bacteria also reside within the system of *Heterorhabditis.* Bacterial species such as *Alcaligenes aquatilis, Alcaligenes faecalis, Enterococcus mundtii, Pseudomonas protegens, Serratia nematodiphila, Serratia marcescens* and *Stenotrophomonas maltophilia* were previously isolated from the *Heterorhabditis* infected *Galleria* cadaver (Ruiu et al. 2017).

Ochrobactrum is a Gram-negative opportunistic bacterium (Brucellaceae) which mostly occurs singly. This bacterium is related to *Brucella, Phyllobacterium, Rhizobium* and *Agrobacterium.* Occurrence of natural dixenic association between the bacterial symbiont *P. luminescens* and the bacterium *Ochrobactrum* sp. in *Heterorhabditis* species was reported by Babic et al. (2000). In this study, five isolates of *Ochrobactrum* were found in association with the EPN-infected *Galleria*. Akhurst (1982) reported the antibiotic properties of the *Xenorhabdus* sp. (now *Photorhabdus*) which inhibits the growth of other microorganisms. However, Aujoulat et al. (2019) observed the ability of *Ochrobactrum* sp. to grow well even in the presence of *P. luminescens* due of its resistance to the antibiotics produced by the *Photorhabdus*.

Pseudomonas includes disease-causing opportunistic bacteria. *P. aeruginosa* is a free living, Gram-negative

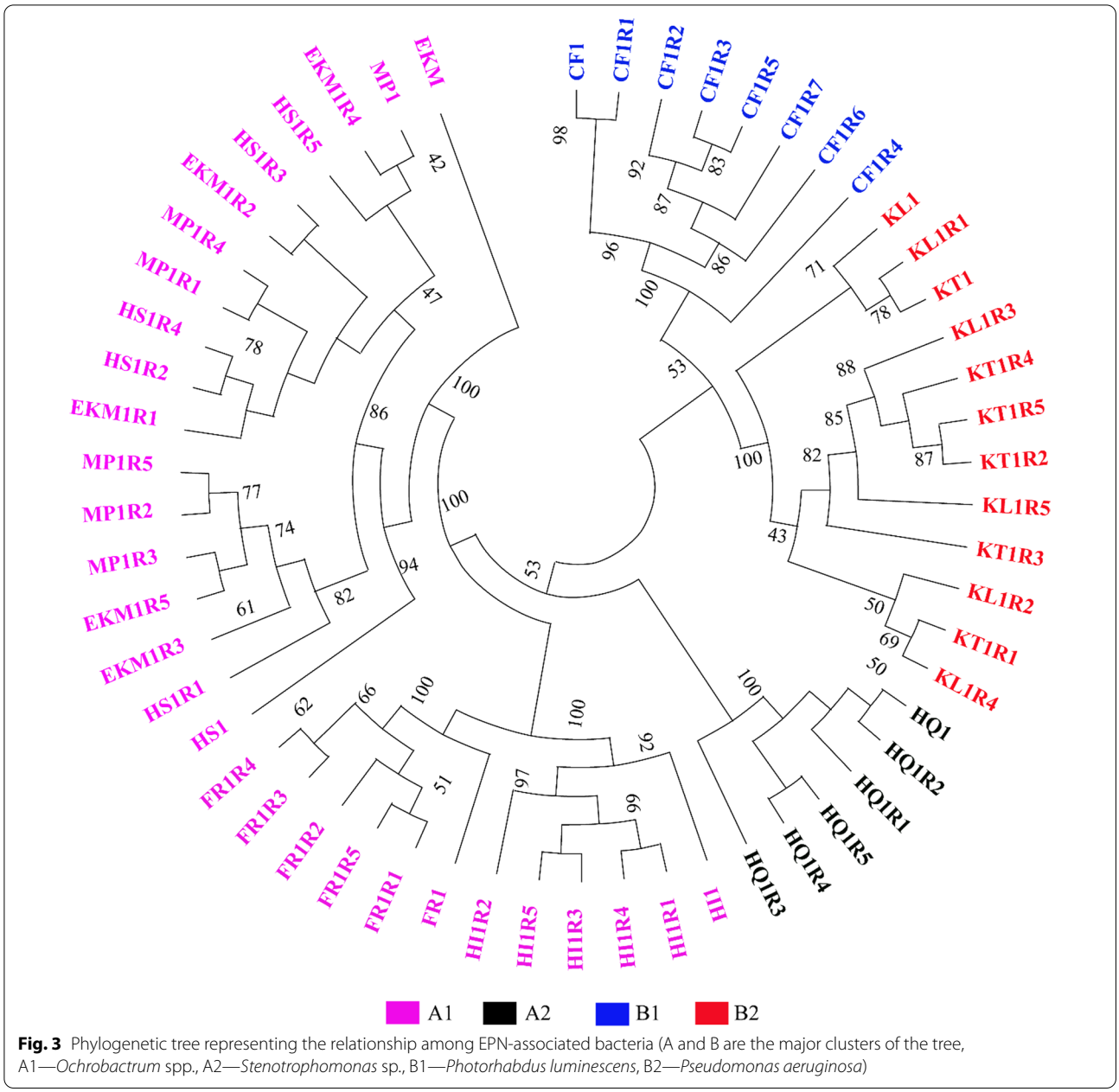

Fig. 3 Phylogenetic tree representing the relationship among EPN-associated bacteria (A and B are the major clusters of the tree, A1—*Ochrobactrum* spp., A2—*Stenotrophomonas* sp., B1—*Photorhabdus luminescens*, B2—*Pseudomonas aeruginosa*)

bacterium having extensive metabolic diversity. The *Stenotrophomonas* spp., formerly isolated as *P. maltophilia*, is an emerging multidrug-resistant opportunistic pathogen. Ruiu et al. (2017) isolated *S. maltophilia* from the *Heterorhabditis* infected *Galleria* cadaver.

In the phylogenetic tree, the sequences of *Ochrobactrum* spp. and *Stenotrophomonas* spp. formed two subclades indicating the divergence of these genera from a common ancestry. In clade 2, *Photorhabdus* and *Pseudomonas* subclustered, indicating the evolutionary relationship of the two species. Velasco et al. (1998) reported the similarity of *O. anthropi* and *O. intermedium* with *Photorhabdus luminescens* subsp. *akhurstii*. All the subclades have further branched into subclusters within the species, indicating significant inter-specific diversity.

Efficacy of the symbiotic bacterium *P. luminescens* against different insect pests was reported by several workers (Kumar et al. 2014). However, studies on the efficacy of this bacterium on mites are limited. Due to the virulent properties and the ability to infect a wide range of insect hosts, it is a promising candidate for agricultural use as a mass-produced biological control agent (Gerdes

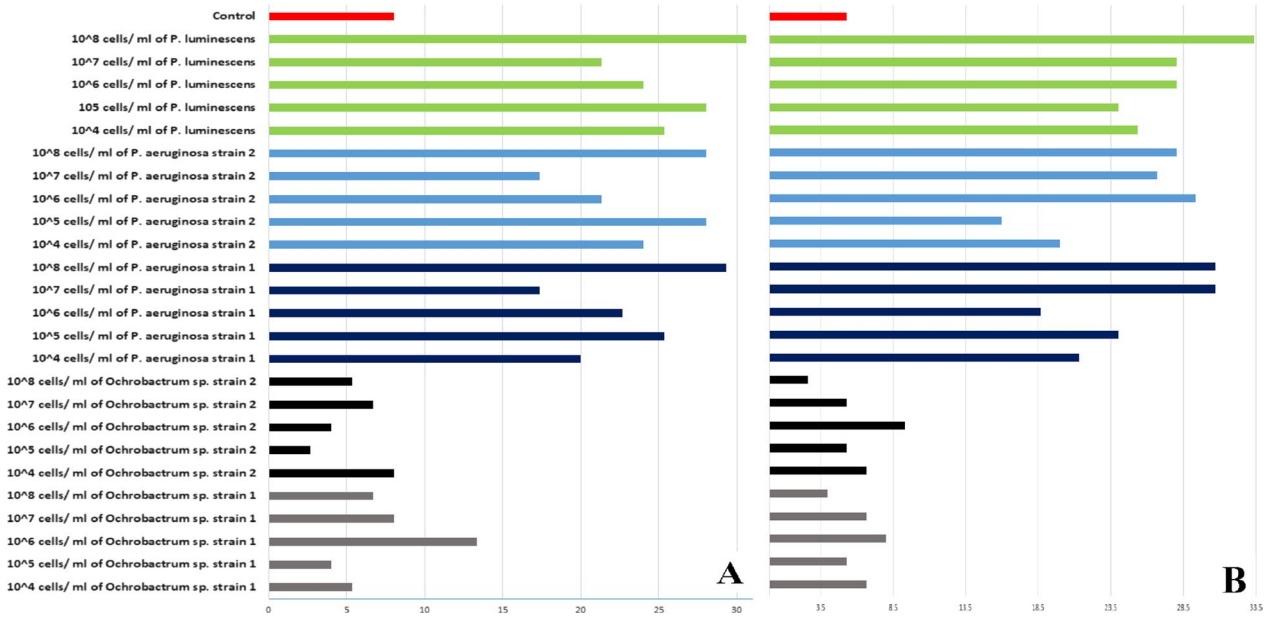

Fig. 4 Ovicidal effect (mortality %) of cell suspension (**A**) and cell-free supernatant (**B**) of different bacterial isolates against *Tetranychus truncatus* at 96 h

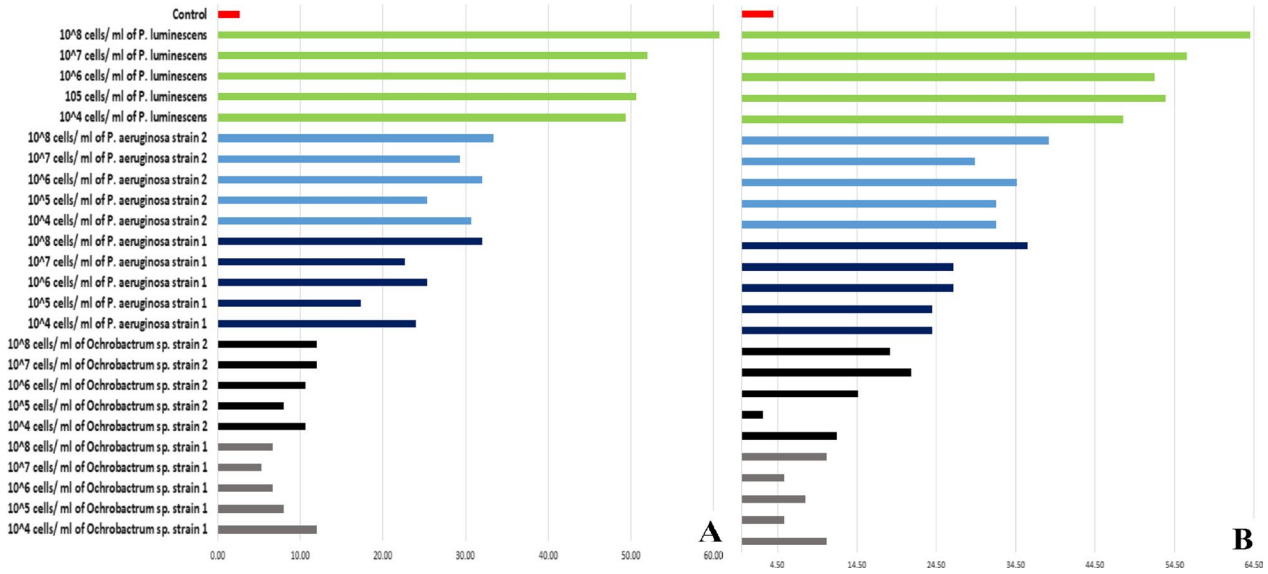

Fig. 5 Adulticidal effect (mortality %) of cell suspension (**A**) and cell-free supernatant (**B**) of different bacterial isolates against *Tetranychus truncatus* at 96 h

et al. 2015). Kumar et al. (2014) studied the bio-efficacy of broth cultures of different isolates of *P. luminescens* against some sucking pests (*Aphis gossypii* and *Tetranychus macfarlanei*) and defoliators (*Plutella xylostella* and *Spodoptera litura*). The isolate Z-8–1 was found to be effective against *A. gossypii* after 24 h with 100% nymphal mortality and the isolate Z-3–1 was effective against *T. macfarlanei* with 100% mortality after 36 h. None of the isolates were pathogenic to defoliators.

In this study, adult mortality rate was higher by *P. luminescens* treatment, compared to *P. aeruginosa* and *Ochrobactrum*. At the highest concentration of 10^8 cells/ml, *P.*

luminescens has resulted in 60.67% mortality. Mortality was negligible with the other isolates. This clearly showed that *P. luminescens* is more effective against *T. truncatus* than the other bacteria. The EPN-associated bacteria: *Ochrobactrum* as well as *P. aeruginosa* were also reported to possess insecticidal activity against few pests.

Salgado-Morales et al. (2019) assessed the pathogenicity of *P. luminescens* HIM3 and *P. aeruginosa* NA04 isolated from *Heterorhabditis indica* against *G. mellonella* and few other insect pests. Both the isolates were found to be highly virulent to *G. mellonella* with nearly complete mortality after 24 h. *P. luminescens* was virulent to both of the insect hosts but the pathogenicity of *P. aeruginosa* varied with the hosts. They also noticed that a high concentration of *P. aeruginosa* was required for infecting *Tenebrio molitor*. *P. aeruginosa* recorded only (10%) mortality of *Diatraea magnifactella* Dyar after 36 h of treatment, but mortality was complete with *P. luminescens.* In the present study, mortality of *T. truncatus* was less when treated with *P. aeruginosa* compared to *P. luminescens*. The bacterium *Ochrobactrum tritici* isolated from the EPN *Oscheius chongmingensis* was reported to cause 93.33% mortality of *G. mellonella* (Fu and Liu 2019). But *Ochrobactrum* isolates did not exhibit appreciable activity against *T. truncatus* in this study.

Conclusions

This study identified a potential isolate of *P. luminescens* with significant adulticidal action against *T. truncatus.* Techniques for mass-multiplying *P. luminescens* are to be standardized for utilization of this bacterial isolate in mite pest management.

Abbreviations

BOD: Biological oxygen demand; CFS: Cell-free supernatant; CS: Cell suspension; EPN: Entomopathogenic nematodes.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s41938-022-00586-8.

Additional file 1: Table S1. Adulticidal effect of cell suspension (CS) of bacterial isolates against *Tetranychus truncatus*. **Table S2.** Adulticidal effect of cell-free supernatant (CFS) of bacterial isolates against *Tetranychus truncatus*

Acknowledgements

Not applicable.

Author contributions

HB conceived the research hypothesis, supervised the research progress, performed the morphological characterization of EPN and mites, reared and maintained the mites and revised the manuscript. AMN performed the experiments and wrote the draft manuscript. DM designed the experiments for the molecular characterization of the bacterial isolates, performed the bioinformatics analyses and revised the manuscript. GD designed the experiments for the isolation, purification and morphological characterization of bacteria. SMR designed sequence characterization experiments and managed the funding. All authors read and approved the final manuscript.

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Availability of data and materials

Additional data associated with this study can be accessed from the Master's thesis submitted by AMN. The bacterial cultures and the pure lines of mites are maintained at Kerala Agricultural University, India.

Declarations

Ethics approval and consent to participate

Not applicable, because our manuscript reports neither studies involving human participants nor human data or human tissue.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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