# Molecular mechanism of virulence in the bacterium, *Photorhabdus luminescens* (Thomas and Poinar) against *Tetranychus truncatus* Ehara (Prostigmata: Tetranychidae)

By

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**(2018-11-006)**

**DEPARTMENT OF PLANT BIOTECHNOLOGY**

**COLLEGE OF AGRICULTURE**

**VELLANIKKARA, THRISSUR- 680 656**

**KERALA, INDIA**

**2021**

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*by*

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**(2018-11-006)**

**THESIS**

**Submitted in partial fulfillment of the requirements for the degree of**

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**DEPARTMENT OF PLANT BIOTECHNOLOGY**

**COLLEGE OF AGRICULTURE**

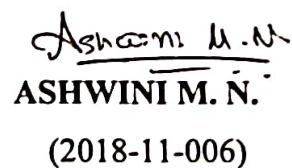
**VELLANIKKARA, THRISSUR-680 656**

**KERALA, INDIA**

**2021**

# DECLARATION

I, hereby declare that this thesis entitled **"Molecular mechanism of virulence in the bacterium, *Photorhabdus luminescens* (Thomas and Poinar) against *Tetranychus truncatus* Ehara (Prostigmata: Tetranychidae)"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.



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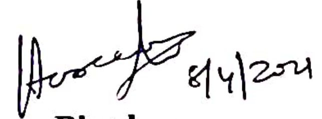
# CERTIFICATE

Certified that this thesis entitled **"Molecular mechanism of virulence in the bacterium, *Photorhabdus luminescens* (Thomas and Poinar) against *Tetranychus truncatus* Ehara (Prostigmata: Tetranychidae)"** is a record of research work done independently by **Ms. Ashwini M. N.** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.



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We, the undersigned members of the advisory committee of **Ms. Ashwini M. N.** for the degree of **Master of Science in Agriculture** with major field in **Plant Biotechnology**, agree that the thesis entitled **"Molecular mechanism of virulence in the bacterium, *Photorhabdus luminescens* (Thomas and Poinar) against *Tetranychus truncatus* Ehara (Prostigmata: Tetranychidae)"** may be submitted by **Ms. Ashwini M. N.** in partial fulfillment of the requirement for the degree.

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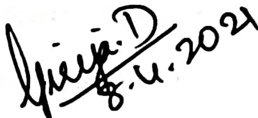


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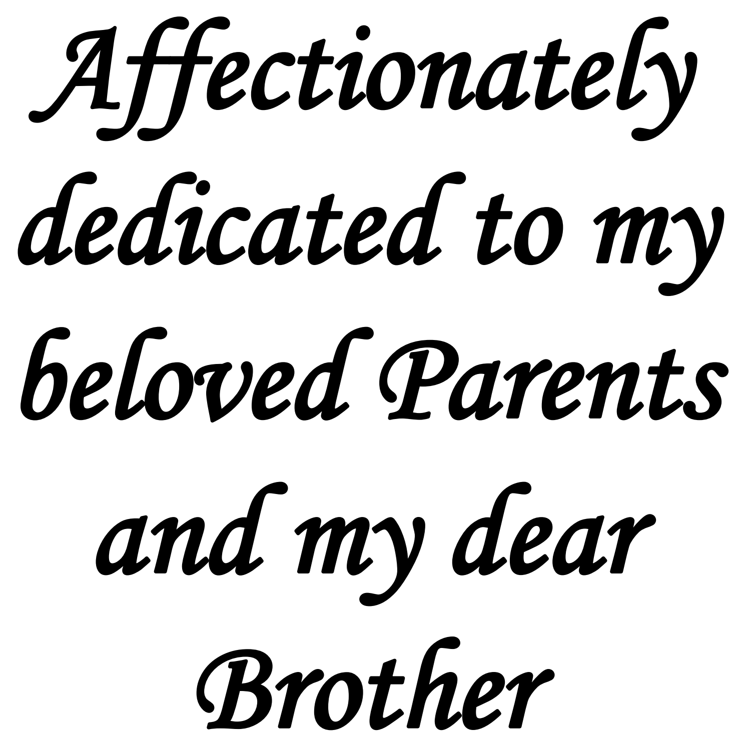
***(Ashwini M. N.)***


Affectionately dedicated to my beloved Parents and my dear Brother

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**ABBREVIATIONS**

| | |
|---|---|
| % | Percentage |
| µg | Microgram |
| µl | Microliter |
| BLASTn | Nucleotide basic local alignment search tool |
| bp | Base pair |
| Cm | Centimeter |
| CPBMB | Centre for Plant Biotechnology and Molecular Biology |
| DNA | Deoxyribonucleic acid |
| DNase | Deoxyribonuclease |
| dNTPs | Deoxyribo Nucleoside Triphosphate |
| EDTA | Ethylene Diamine Tetra Acetic acid |
| g | Gram |
| KAU | Kerala Agricultural University |
| Kb | Kilo base pairs |
| L | Litre |
| M | Molar |
| MEGA | Molecular evolutionary genetic analysis |
| mg | Milligram |
| ml | Millilitre |
| mM | Milli Molar |
| NCBI | National Center for Biotechnology Information |
| ng | Nano gram |
| $^{o}C$ | Degree Celsius |
| PCR | Polymerase Chain Reaction |
| pH | Hydrogen ion concentration |
| rRNA | Ribosomal RNA |
| rpm | Revolutions per minute |
| SNP | Single Nucleotide Polymorphism |

| | |
|---|---|
| TAE | Tris Acetate EDTA |
| TE | Tris EDTA |
| UV | Ultra Violet |
| V | Volts |

Introduction

# 1. INTRODUCTION

Spider mites (Tetranychidae) are considered as one of the most serious sucking pests of crops worldwide. Many agricultural and horticultural crops including greenhouse, field and fruit crops are damaged by the spider mites. Among the different species of spider mites, *Tetranychus truncatus* Ehara has emerged as the predominant mite species infesting economically important crops of Kerala (Bennur *et al*., 2015; Arunima, 2017).

Mite management in crops largely depends on the use of chemical pesticides. Chemical pesticide encourages flare up of spider mites by killing the beneficial insects that prey on them. The intensive use of acaricides has also led to the development of resistance in many mite species around the globe, making mite management difficult. Mite populations have also developed resistance to newly introduced compounds after few years of use (Vassiliou and Kitsis, 2013). In Kerala, novel acaricides are being extensively used for managing mite infestation in vegetables, ornamental plants, rice and banana. Recent studies conducted jointly by the Department of Plant Biotechnology and All India Network Project on Agricultural Acarology (AINPAA), Department of Agricultural Entomology, College of Agriculture found that *T. truncatus* has developed significant level of resistance to three commonly used acaricides in Kerala (Bachhar *et al*., 2019). The study suggests that there is a need for alternative strategies in mite pest management to avoid the adverse effects of chemical acaricides.

The bacterium, *Photorhabdus luminescens* (Thomas and Poinar) is an endosymbiont of entomopathogenic nematodes (EPNs) *Heterorhabditis* spp. which is Gram negative, bioluminescent, motile, non-spore forming, facultative anaerobic bacteria, belonging to the family Enterobacteriaceae. It produces an array of toxin complexes and enzymes which possess a wide range of insecticidal actions against both sucking and chewing arthropod pests of agricultural crops including mites. Application of the bacterial solution as topical pesticide was found to be advantageous

in terms of safety, potency and specificity compared to chemical spray, besides being bio-degradable.

The toxins produced by *Photorhabdus luminescens* include the Toxin Complexes (TCs), "Makes caterpillars floppy" (McF) toxins, the *Photorhabdus* Virulence Cassettes (PVC), *Photorhabdus* insect-related toxins (Pir) *etc*. (Kushwah and Somvanshi, 2015). Among the different toxins, the Toxin Complexes (TCs) have been reported to possess appreciable insecticidal activity against many insect pests. The high molecular weight TCs are encoded by several loci which produce complexes *viz*., *tca, tcb, tcc*, and *tcd*. Most of the orally active toxins which are responsible for the insecticidal activity are encoded by *tca* and *tcd* (Blackburn *et al*., 1998), while the *tcc* genes are responsible for the putative virulence functions (Yang *et al.*, 2006).

In this context, the present study "Molecular mechanism of virulence in the bacterium, *Photorhabdus luminescens* (Thomas and Poinar) against *Tetranychus truncatus* Ehara (Prostigmata: Tetranychidae)" was undertaken to characterize the toxin complex gene involved in the virulence of *P. luminescens* against the spider mite.

The objectives of the study are

1. To identify the virulent strain of *P. luminescens* against *T. truncatus*
2. To elucidate the molecular mechanism of virulence against *T. truncatus*

Review of
literature

# 2. REVIEW OF LITERATURE

Entomopathogenic nematodes (EPN) in association with their symbiotic bacteria are one of the most promising biocontrol agents, which can effectively control wide range of insect pests with lower risk to the environment than the conventional chemical pesticides. Number of studies are in progress over the world to understand the mechanism behind their toxicity against insect pests and also to find out the ways to use them as potential bio pesticides. In this study, we assessed the molecular mechanism of virulence of one such symbiotic entomopathogenic bacteria, *Photorhabdus luminescens*, isolated from the EPN *Heterorhabditis.* Some of the studies regarding diversity, isolation and characterization of different symbiotic bacteria associated with EPN, their bio efficacy against various pests and the virulence mechanism are discussed below.

## 2.1 Diversity of symbiotic bacteria associated with the EPN

The infective juveniles of EPN invade the body of the host and release the symbiotic bacteria in to the body cavity in which they multiply and within two to three days of infection, the host gets killed. Steiner in 1923 described the first entomopathogenic nematode called *Aplectana kraussei* (now *Steinernema kraussei*). Steinernematidae and Heterorhabditidae are the two important entomopathogenic nematode families which play a major role in balancing the population of some insect pests in the environment (Ferreira and Malan, 2014). *Xenorhabdus* and *Photorhabdus* are the two major genera of bacteria which are associated with them respectively (Akhurst and Boemare, 1988; Thomas and Poinar, 1979; Akhurst *et al*., 1996; Boemare *et al*., 1993; Fischer-LeSaux *et al*., 1999). Both bacteria and the nematode which are in symbiotic association will share an evolutionary diversity and systematics (Adams *et al.,* 2006).

Khan and Brooks (1977) reported the first isolate of *Photorhabdus* (initially *Xenorhabdus*) from an entomopathogenic nematode *Chromonema heliothidis* (earlier name of *Heterorhabditis bacteriophora*). The bacteria was described as gram negative, rod shaped, bioluminescent bacteria which produced cream-colored colonies on nutrient agar and gradually turns to pink-red. They reported that the bacteria was

similar to *Achromobacter nematophilus* in most of the characteristics except for the bioluminescent properties.

Transformation from the EPN symbiotic state to entomopathogenic state is an interesting phenomenon and the bacteria *Photorhabdus* and *Xenorhabdus* serves as excellent models for the study of signal transduction, transcriptional and post-transcriptional regulation of gene expression involved in the process of transformation (Forst *et al*., 1997).

*Photorhabdus* is the main bacterial genera which is symbiotically associated with the EPN *Heterorhabditis* spp. However, some reports shown that, there are some other bacterial groups other than the unique symbiont in the gut of EPN which are mostly opportunistic in nature (Babic *et al*., 2000).

Jackson and co-workers in 1995 have isolated both *Providencia rettgeri* and *Photorhabdus* spp. from either infective juvenile stage nematodes (*Heterorhabditis* sp.) or from the nematode infected cadavers of *Galleria mellonella* and studied them by comparing each other. The growth of *P. rettgeri* was very fast (twice as that of *Photorhabdus*) and it was the only species recovered from the cadaver when both the bacterial species were co-injected to the same *G. mellonella* larvae.

Bacteria which are associated naturally with *P. luminescens* were isolated from the EPN, *H. Indica* and studied by Babic and co-workers in 2000. They characterised the bacteria by phenotypic analysis, RFLP assay and also by using *16S rDNA* analysis and the results indicated the relatedness with the *Ochrobactrum* sp. They also tested the pathogenicity of *Ochrobactrum* against *Galleria mellonella* and *Spodoptera littoralis* which was found to be negative or non-entomopathogenic.

Enright *et al*. (2003) isolated and characterised a novel endospore-forming bacteria *Paenibacillus nematophilus* from the EPN *Heterorhabditis* sp. All the phenotypic and chemotaxonomic properties as well as the *16S rRNA* gene sequence analysis were studied to identify the bacteria. Genomic heterogeneity among the isolates was revealed by DNA–DNA similarity studies.

Quiroz-Castaneda *et al*. (2015) have isolated and identified a bacterium, *Alcaligenes faecalis* strain MOR02 from the EPN infected cadaver of *G. mellonella*. They studied the association of *A. faecalis* MOR02 and EPN (*Steinernema* and *Heterorhabditis)* in the view of dispersion and colonization. The bacteria colonized the oesophagus and intestine region of the nematodes and used different strategies to multiply and kill the insect host to their own benefit.

Ruiu *et al*. (2017) isolated new bacterial species from the haemolymph of EPN infected *Galleria mellonella* larvae to assess their oral toxicity against two important insect orders namely Diptera (*Musca domestica* and *Ceratitis capitata*) and Lepidoptera (*Lymantria dispar* and *Malacosoma neustria*). The bacteria were identified as *Alcaligenes aquatilis, Alcaligenes faecalis, Enterococcus mundtii, Pseudomonas protegens, Serratia nematodiphila, Serratia marcescens* and *Stenotrophomonas maltophilia*. *Ochrobactrum* sp. of bacteria can grow well even in the presence of *Photorhabdus luminescens* because of its resistance power to the antibiotics produced by the *Photorhabdus* (Aujoulat *et al*., 2019).

Skowronek *et al.* (2020) isolated the midgut colonizing bacteria (*Pseudomonas chlororaphis*, *Citrobacter murliniae*, *Acinetobacter calcoaceticus*, *Chryseobacterium lathyri*, *Chryseobacterium* sp., *Serratia liquefaciens*, and *Serratia* sp.) from the insect cockchafer *Melolontha melolontha* larva which were treated with the EPN (*Steinernema* and *Heterorhabditis* sp. separately) and studied their antimicrobial properties. They reported that the gut bacteria will also play a role in the insect immune response against the EPN associated bacteria *Xenorhabdus* and *Photorhabdus*.

## 2.2 Symbiotic bacterium, *Photorhabdus luminescens*

In 1979, Thomas and Poinar reported a new bacterial genus, *Xenorhabdus* which is gram-negative, rod-shaped, facultative anaerobic, entomopathogenic and belongs to the family Enterobacteriaceae. They also described about a novel bioluminescent species *Xenorhabdus luminescens* which was isolated from the intestinal lumen of an entomogenous nematode, *Heterorhabditis bacteriophora* which

differs from other species of *Xenorhabdus* in terms of bioluminescence and catalase activity.

Akhurst (1980) reported about the two forms of *Xenorhabdus* sp., symbionts of *Neoaplectana* and *Heterorhabditis* nematodes by observing their characteristics colony morphology on NA as well as on NBTA media. The two forms had shown equal pathogenicity when injected to *G. mellonella.* However, in comparison with the secondary form, the primary form provides better condition for the growth and reproduction of the nematodes. The primary forms has produced white, opaque, convex and circular colonies with an irregular margin on NA media and colonies with red core overlaid by dark blue, surrounded after 3-5 days by a clear zone on NBTA media. Whereas, the colonies of secondary forms were translucent, flatter and of greater diameter than the primary form on NA and red coloured with no clear zone on NBTA.

Akhurst (1982) reported the antibiotic properties of the *Xenorhabdus* sp. which inhibit the growth of other microorganisms in its primary form. It will be stable even after autoclaving but loose its activity after dialysis. The bacteria did not exhibit the antibiotic property if they are incubated anaerobically.

Gerritsen *et al*. (1992) observed four different variants of *X. luminescens* XE-87.3 which was isolated from EPN *Heterorhabditis* strain NLH-E87.3. The four variants namely XE-red, XE-pink, XE-yellow and XE-white. XD-red was considered as the primary form and was luminescent which had the ability to release red pigments and some antibiotics into the media. XE-pink, was similar to XE-red only with the colour exception. XE-yellow produced yellow pigmentation with only few antibiotics, less luminescent and highly unstable can turn to primary form very soon. XE-white is almost similar to XE-yellow except the production of any pigment or antibiotics. All the four variants were genetically similar but differ in the protein pattern.

Wang and Dowds (1993) studied the phase variation mechanism in the bacteria *Xenorhabdus luminescens* by considering the differential activity of the lipase enzyme.

Boemare *et al*. (1993) studied the mutualistic association between the bacteria *X. luminescens* and its symbiotic host EPN (*Heterorhabditis* sp.). They also estimated the DNA relatedness among different strains of *Xenorhabdus* and proposed to transfer the bacterial group *X. luminescens* to a new genus called *Photorhabdus,* as it was distinct from other strains of *Xenorhabdus* by both molecular and phenotypic characteristics.

Chaston *et al*. (2011) analysed the divergent evolution of *Xenorhabdus* and *Photorhabdus* from a common ancestor that arrived at convergent lifestyles. Both of these are mutualists with nematodes and pathogenic to the insects but the genetic and physiological studies revealed that they used different mechanisms to complete their lifecycle. They are capable to survive the antimicrobial peptide (AMP), a production of the insect immune system. *Photorhabdus* uses lipopolysaccharide (LPS) to inhibit the action of AMP. Whereas *X. nematophilia* prevents production of insect AMP. Genomic comparison studies revealed 94 per cent similarity in the *16S rDNA* region in them, which indicates their close relatedness unlike their nematode hosts (*Steinernema* and *Heterorhabditis*), which are very distantly related.

Smigielski and Akhurst (1994) postulated the efficiency of phase 1 cells of *Photorhabdus* and *Xenorhabdus* for better adaptation in the insect and the nematode and phase 2 will perform better as free living organisms in the soil.

Krasomil-Osterfeld in 1995, studied the process of phase shifting in *P. luminescens* bacteria and its influencing factors like temperature, supply of oxygen, pH, osmotic pressure differences and light in the growing environment and proved only the low-osmolarity condition in the sub culturing medium can significantly trigger the shifting of phases from phase 1 to phase 2.

Han and Ehlers in 2001 studied the effects of phase variation of the entomopathogenic bacteria *P. luminescens* on the growth and multiplication of its nematode hosts *H. bacteriophora* and *S. carpocapsae* both in *in vivo* and *in vitro*. They suggested that intensity of the bacterial colony colour, colour of the media due to pigmentation, pathogenicity, presence of crystalline proteins and antibiotic

substances and also the bioluminescence are the major factors to identify the phases. Retention of the phase 1 cells by the dauder juvenile provides the best condition for the reproduction of the nematodes than phase 2. Phase II cells were characterized by loss of pathogenicity which is a distinguishable property from Phase I cells, and in between an intermediate state where the bacteria starts to lose its pathogenicity.

The nematode cannot kill the insect without the bacteria, hence the Phase II cells affects the pathogenicity (Turlin *et al*., 2006).

Silva *et al*. (2002) artificially infected the larval *Manduca sexta* with *P. luminescens* subsp. *akhurstii* strain W14 which was labelled with Green Fluorescent Protein (GFP). They established an infection model for the bacteria which is very specific for set of stages. They studied the growth rate of the bacteria inside different tissues of the insect host by tracking the bacterial movements through GFP, during the course of infection. Initially the bacteria colonize at the anterior portion of the midgut then gradually spread to the posterior part. Once after the destruction of the midgut they move to the other tissues such as the fat body and destroy.

Ciche and Ensign (2003) studied the transmission pattern of the bacteria *P. luminescens* through its nematode vector by labelling the cells of the bacteria with Green fluorescent Protein (GFP) in order to trace out the process through which the EPN regurgitates the cells of the bacterial symbionts. They published the first report on this aspect. The bacteria colonized at the region behind the basal bulb in the anterior portion and extending throughout the DJ intestine. Bacteria takes a pulsatile movement through the pharynx, towards the mouth and exited out.

*Photorhabdus* initially in symbiotic phase with its nematode and once after releasing into the host insect's body becomes pathogenic. After the release, the bacteria and the nematode will cause the death of the insect cooperatively. Many large genomic islands present in the bacterial genome are responsible for the symbiosis and virulence (Ffrench-Constant *et al*., 2003)

Munch *et al.* (2008) used the Differential Fluorescence Induction (DFI) based promoter-trap library screening method along with some bioinformatics tools for the

identification of the induced operons or corresponding genes in *P. luminescens* upon infection to the host *G. mellonella*. They stated that the bacteria is capable to sense and adapt according to the changing host environment.

Molecular changes adopted by the bacteria, *Photorhabdus* will help them to adopt for the complex life cycle (Waterfield *et al*., 2009).

Orozco-Hidalgo and co-workers in 2019 evaluated the growth kinetics of *P. luminescens* subsp. *akhurstii* SL0708 in Colombia by considering the pathogenicity and the changes in the metabolic phases (phase I, intermediate phase, phase II) of the bacteria. The initial phase after the inoculation until 24h was logarithmic phase which was having the phase I cells with maximum bioluminescence compared to the other two phase II.

## 2.3 Isolation and morphological characterisation of the bacterial isolates

Bleakley and Nealson (1988) studied the properties of *X. luminescens* strain Hm in both of its primary and secondary form. After prolonged incubation the primary forms changed in to secondary form which was less luminous than primary. Extracellular antibiotics, protease and lipase activities were also found to be less in the later secondary form. On LB agar, initially yellowish opaque colonies were produced by the primary form which gradually turned to brownish red with pigmentation. While the secondary forms were creamy in appearance and were less opaque. No pigmentation was observed either in LB or in NA.

Akhurst *et al*. (1996), isolated different strains of *Photorhabdus* from wide range of nematode host of different locations and also from human clinical specimens. They used DNA relatedness and phenotypic characterization methods to analyse the taxonomic structure of the bacteria. All the strains studied were found to be congeneric. They also found that the genus *Photorhabdus* is more homogenous than *Xenorhabdus.*

Bondi *et al.* (1999) studied the biological or the morphological characteristics of two isolates of *P. luminescens* (MU1 and MU2) and also the activity of their products,

AADA (antimicrobial agar disulphide agents). MU1 showed two variant forms phase 1 and phase 2, while MU2 showed three form variants like phase 1 phase 2 and an intermediate phase I like MU2. They reported the characteristic changes in the form with time and also the changes in biological and biochemical properties of the bacteria according to the form. They observed large amount of β-lactamase, urease, bioluminescens and AADA in phase I of both the isolates and lesser amount in intermediate phase I like MU2 and absent in phase II of both the isolates.

Hazir *et al*. (2004) studied the taxonomy of two different novel subspecies of *P. luminecens* (*P. luminescens* subsp. *kayaii* and *P. luminescens* subsp. *thracensis*) which were isolated from EPN *H. bacteriophora* collected from Turkish soil and characterized genotypically by riboprint analysis and also based on the metabolic properties.

Singh *et al*. (2012) characterised the *P. luminescens* bacteria and used it as a food source or the developmental signals for the mass production of the EPN *H. bacteriophora* in a bioreactor. Usage of Phase II cells will affect the recovery of the nematodes so they specifically identified the viable phase I cells which are very much suitable for the mass production of nematodes. Initially they isolated the bacteria and screened for bioluminescence. Phase I were highly luminescent for up to 7 h than phase II. NBTA and MAC agar media were used for confirmation of the phase. Phase I produced brick/dark red colonies on NBTA and red colonies on MAC agar,  Microbial growth was measured in terms of fresh pellet weight (biomass), bioluminescence, pigmentation and pH of medium once after the bacterial growth.

Pervez and co-workers (2015) in Kozhikode (Kerala), recovered an entomopathogenic bacteria from *Heterorhabditis* sp. (IISR-EPN 01). Morphologically the isolate was Gram negative, rod shaped, facultative and motile. The produced colonies were of red colour with off white margins, raised, circular and opaque. The isolate was found to be positive for the biochemical tests like citrate utilization, methyl red, urease and carbohydrate fermentation tests and was negative for indole production, oxidase and VogesProskauer tests. They also amplified and sequenced the ITS region of the *16S rDNA*. By considering all these morphological, biochemical and

molecular results, the isolate was confirmed as *P. luminescens* subsp. *akhrustii*. This was the first report on this bacteria, isolated from the ginger rhizosphere in India.

### 2.4 Molecular characterisation of the bacterial isolates

Woodman (2008) briefly explained the procedure for colony PCR which is an easy and quick process for DNA sequence identification.

Brunel *et al*. (1997) proved that the *16 sRNA* analysis by PCR amplification along with the restriction digestion of the products will be an efficient tool for the identification of the bacterial symbionts of EPN. They identified 13 different strains of *Xenorhabdus* and 14 different strains of *Photorhabdus* by using the same.

Szallas *et al*. (1997) compared the *16S rRNA* gene sequences of different bacterial symbionts isolated from different EPN (40 isolates isolated from *Heterorhabditis* sp., seven isolates from *Steinernema* sp. and a strain of *Xenorhabdus japonicas*). They used phylogenetic analysis and DNA-DNA hybridization studies of selected strains from the isolates for the separation of *Photorhabdus* genus from *Xenorhabdus* and further classified the genus.

Fischer-LeSaux *et al.* (1999) examined the taxonomic position of the genus *Photorhabdus* based on the results of DNA relatedness, maximum growth temperature, comparison of *16S rRNA* gene sequences and also by phenotypic characterization. They also proposed for the subdivision of *P. luminescens* and *P. temperate* into their subspecies.

Drancourt *et al.* (2000) evaluated the *16S rDNA* sequence analysis as an excellent tool for molecular identification of unidentifiable isolates through morphological characters. Sequencing of *16S rDNA* provides unambiguous data even for rare isolates, which can be reproducible in and between laboratories unlike phenotypic identification.

Toth and Lakatos (2008) isolated and characterized the symbiotic bacterial strain $3107^{T}$ from Hungarian entomopathogenic nematode by using *16S rRNA* gene analysis but the results were uncertain because of the low boot strap values.

Phylogenetic analysis by using *gyrB* sequences positioned the isolates in to species, *Photorhabdus temperate*. Further by analysing physiological properties and carbon-source utilization profiles, they proposed the strain as a new subspecies *Photorhabdus temperata* subsp. *conferee.*

Emelianoff *et al.* (2008) have isolated EPN along with their natural symbiont bacteria from the soils of Herault and Gard (Southern France). Three *Steinernema* species (*S. feltiae*, *S. affine* and an undescribed species) with their bacterial symbionts, *X. bovienii, X. Kozodoii* and one *Heterorhabditis* species (*H. bacteriophora*) with the symbiont *P. luminescens* were isolated and described by *16S rRNA* gene sequences analysis.

Aly and Mona (2009) have isolated four different local symbiotic bacterial isolates (three *Photorhabdus luminescens* and one *Xenorhabdus nematophila)* from the EPN collected from the Egyptian soil samples. Cultural properties, cellular morphology and *16S rRNA* gene analysis were used as the tools for characterisation. They also found the 32 nucleotide positional differences between the *Photorhabdus* isolate (FJ755891) and other isolates of *P. luminescens* and *X. nematophila* based on the *16S rRNA* gene similarity.

Hsieh *et al*. (2009) isolated and characterized the EPN, *H. brevicaudis* and its bacterial symbiont *P. luminescens* subsp. *akhurstii* in Taiwan for the first time. Bacteria was plated on MacConkey's agar and NBTA media and they absorbed dye in both media. Biochemical tests and *16S rDNA* analysis confirmed the bacterial identity.

Peat *et al*. (2010) examined the phylogenetic relationships among different species of *Photorhabdus* by analysing three genetic loci *16S rRNA* gene, *gyrB*, and *glnA*, which produced most robust phylogenetic hypothesis in the genus. They noticed the gradual decrease in the intensity of bioluminescence along with the evolution, which may be a trait acquired under aquatic condition

An and Grewal (2010) isolated a new subspecies (strain GPS11) of *P. temperate* from *H. bacteriophora* and characterized by using *16S rRNA* gene sequence analysis

and physiological trait analysis. But there were four subgroups in the species (*P. temperate*) as per the phylogenetic tree. The new strain differed in *16S rRNA* and *gyrB* gene, physiological traits, nematode hostspecies, and geographic origin with other groups. So they proposed the strain GPS11 as new subspecies: *Photorhabdus temperata* subsp. *stackebrandtii*.

Tailliez *et al*. (2010) used the multigene approach (sequences from the genes; *recA, gyrB, dnaN* and *gltX*) in order to compare and study phylogeny of the bacteria *Photorhabdus* and *Xenorhabdus* and proposed four new subspecies namely *P. luminescens* subsp. *caribbeane*nsis, *P. luminescens* subsp. *Hainanensis*, *P. temperate* subsp. *khanii*, and *P. temperate* subsp. *tasmaniensi*s.

Ferreira *et al*. (2013) isolated a bacterial isolate AM7, a symbiont of nematode *Heterorhabditis*. The symbiont was bioluminescent, gram negative and the cells were catalase-positive, oxidase-negative and produced pigmented colonies. Multigene approaches like *16S rRNA, recA, gyrB, dnaN, gltX* and *infB* suggested their relatedness with the strains *P. luminescens* subsp. *caribbeanensis*, *P. luminescens* subsp. *akhursti*i and *P. luminescens* subsp. As the proteomic studies revealed their pattern of deviation, they proposed as a novel subspecies *P. luminescens* subsp. *noenieputensi*s

Ferreira *et al*. (2014) isolated two new bacterial symbionts of the EPN *H. zealandica* from the soils of South Africa. By reconsidering the results of multigene approach (including the *gyrB* sequence) and the DNA–DNA hybridization experiments they proposed the strains as *Photorhabdus heterorhabditis*.

Srinivasan *et al*. (2015) studied the comparison of *16S rRNA* gene based identification with the non-*16S* based clinical identification. In overall, between *16S* gene based and clinical identities they got 96 per cent of genus-level concordance rate and an 87.5 per cent of species-level concordance rate and also concluded that a model-based approach is superior to an alignment based method.

Mandadi *et al.* (2015) reported the draft whole genome sequences of the three novel isolates (NBAII PLHb105, NBAII HiPL101 and NBAII H75HRPL105) of

*Photorhabdus luminescens* which are isolated from the entomopathogenic nematode, *Heterorhabditis* species in southern India and the nucleotide sequence information was deposited in NCBI Nucleotide database with accession numbers AZAB00000000, JTHJ00000000 and JXUR00000000 respectively.

Geldenhuys *et al.* (2016) isolated the first isolates of *Photorhabdus lumin*escens subsp. *laumondii* from EPN (*Heterorhabditis africana* SF281 and *H. bacteriophora* SF351) of soils of South Africa and used *16S rDNA*, *recA, gyrB* and *gltX* sequence analysis for characterization.

Palma *et al.* (2016) reported the draft genome sequence of *Photorhabdus luminescens* strain DSPV002N. The genome contained 5,518143 nucleotides, 4,701 protein coding genes (CDSs), of these 27 CDSs produces insecticidal toxin proteins which are similar to *P. luminescens* subsp. *laumondii* TT01.

*Photorhabdus* sp. can readily exchange their nematode host at intra- and interspecies level which clearly indicates the different symbiotic associations between *Heterorhabditis - Photorhabdus (*Kazimierczak *et al.,* 2017). In this study they have used five different genes like *16S rRNA, gyrB, recA, gltX* and *dnaN* for phylogenetic analysis. They isolated six bacterial symbionts from two species of EPN, *H. bacteriophora* and *H. megidis.* The bacteria isolated from *H. megidis* were identified as *P. temperate* sub sp. *temperate* and *P. temperate* sub sp. *cinerea.* The isolates which were isolated from the *H. bacteriophora* were identified as *P. luminescens* sub sp. *kayaii* and *P. temperate* sub sp. *cinerea*.

Salgado-Morales *et al*. (2017) isolated *Photorhabdus luminescens* strain HIM3 (from *Heterorhabditis indica* MOR03 EPN) collected the soil from the sugarcane field and reported the draft genome sequence of 5.47 Mb in size.

Machado *et al*. (2019) have reported two new strains of *Photorhabdus*, MEX20-17T and MEX47-22T which were isolated from *H. atacamensis* and *H. mexicana* nematodes respectively. They were Gram-negative, rod-shaped, non-spore-forming bacteria. They conducted *16S rRNA* gene sequence analysis, housekeeping-gene

based analysis and whole-genome based phylogenetic reconstruction studies for deeper analyses. By considering all the results they proposed the new strains, MEX20-17T as *P. khanii* subsp. *guanajuatensis* and MEX47-22T strain as *P. luminescens* subsp. mexicana subsp.

**2.5 Bio efficacy of the bacterial isolates against insect pests and mites**

Due to the virulent properties and the ability to infect a wide range of insect hosts, *P. luminescens* is a promising candidate for agricultural use as a mass produced biological control agent (Gerdes *et al.,* 2015).

The isolates of *P. luminescens* showed higher pathogenicity against the pupae of diamondback moth (*Plutella xylostella*) than *X. Nematophilus* isolates. They showed 60 and 40 per cent mortality, respectively (Abdel-Razek, 2003).

Mohan *et al*. (2003) used *Photorhabdus luminescens* suspension ($10^8$ CFU per ml) as foliar spray, in order to assess the pathogenicity of the bacteria against cabbage butterfly *Pieris brassicae.* They observed 100 per cent mortality within 24 h of the spray.

Au *et al*. (2004) studied the response of the *Photorhabdus* against the immune system of *Manduca sexta* both in vivo and in vitro with *E. coli* as a control. They proved that the bacteria can easily escape the immune response of the phagocytes which are present in the insect haemocoel by killing them and by suppressing the phagocytosis.

Gerritsen *et al*. (2004) tested the oral toxicity of excretion products of several *Photorhabdus* and *Xenorhabdus* strains on two thrips species. The thrips were killed after sucking sap from leaves covered with the toxins. They also suggested the possibilities of creating transgenic plants expressing *Photorhabdus* toxins so that when thrips suck from these plants, they take up the toxin and gets killed.

Gerritsen *et al*. (2005) used forty-six different *Photorhabdus* isolates and six different isolates of *Xenorhabdus* bacteria in order to test their oral toxicity in controlling two species of thrips, *Frankliniella occidentalis* and *Thrips tabaci*. After

seven days of treatment, only six species of *P. temperata* showed 90 per cent of mortality and thus proved the efficacy.

Pinheiro and Ellar (2007) tested the virulence/ toxicity of *Yersinia pseudotuberculosis* IP32953 and *Photorhabdus luminescens* against *M. sexta* larvae and found the latter bacterium as more potential one.

Heermann and Fuchs (2008) performed a comparative genome analysis between two bacterial species in which one is representative of entomopathogenic group which was not human pathogenic (*P. luminescens*) and the other one represents the group of bacteria which was highly pathogenic to human and also showed some virulence to insects (*Y. enterocolitica*), in order to study evolution of bacterial pathogenicity. The results proved that both bacteria shared a set of common factors involved in invertebrate to vertebrate infection. They also showed partial difference and partial similarity during course of evolution.

Ansari *et al*. (2008) tested the virulence of nine entomopathogenic nematodes against white grub. The isolates of *Steinernema glaseri* Belgian strain (SgBE) and *S. Glaseri* NC strain (SgNC), *Heterorhabditis bacteriophora* CLO51 strain (HbCLO51) and *H. megidis* VBM30 strain (HmVBM30) showed high level of virulence to third-instar larvae and pupae under laboratory experiments. They reported that the virulence varied with different developmental stages and the species of nematode.

Uma *et al.* (2010a) determined the toxicity of the primary and secondary forms of *P. luminescens* isolated from entomopathogenic nematode, *H. indica* against *Aphis gossypii.* They reported cell free supernatant to be more virulent than the bacterial cells and inferred that mortality through contact action was more applicable for *A. gossypii* which is a sucking pest. In both the primary and secondary forms, the secretion of cells played an important role in causing the pest mortality indicating the role of toxin compound produced by cells of *P. luminescens*.

The cells free supernatant (CFS) of both primary and secondary forms of *P. luminescens* were more virulent than cells against *Thrips palmi* Karny, a serious

pest on cotton causing high mortality within 24 to 48 h. The cells of primary and secondary form recorded 67.50 and 72.50 per cent mortality, whereas corresponding CFS recorded 85.00 and 80.00 per cent mortality, respectively at the concentration of $10^{10}$ Cells/ ml. The LC50 of both primary ($2.38\times 10^7$ Cells/ml) and secondary form cells ($2.35 \times 10^7$ Cells/ml) and CFS of *P. luminescens* decreased with increase in exposure time from 24 to 48 h (Uma *et al.,* 2010b).

Shahina *et al.* (2011) used the *Photorhabdus luminescens* bacteria (isolated from *Heterorhabditis bacteriophora* isolate 1743) against *Galleria mellonella* larvae and subterranean termite (*Macrotermis* sp.). Different observations were recorded in which, cell penetration in to the larva was observed in *G. Mellonella* and *Macrotermis* spp. within 9 and 12 min of spraying. The mortality rate increased as the concentration of bacterial cells increased. They observed 95 and 98 percent of mortality of *G. mellonella* and *Macrotermis* sp., respectively.

Somvanshi *et al.* (2012) inversion in the promoter sequence may cause the switching of pathogenic P form into mutualistic M form in the life cycle of *P. luminescens* bacteria. M form is one-seventh the volume of P form in size, which produced only few metabolites and less bioluminescence and were slow growing when compared to P form. In order to prove this, they generated several *mad* mutants by mutating subunits of the *mad* gene *madR, madA, madH madJ* and *madO* but only *madJ* and *madO* mutants failed to produce the M form.

Kumar *et al*. (2014) isolated eight different isolates of *P. luminescens* and used for the bioassay studies. They got two efficient strains, Z-8-1 which was effective against *Aphis gossypii* with 100 per cent nymphal mortality and Z-3-1 which was effective against *Tetranychus macfarlanei* with 100 per cent mortality.

Safeness of the bacteria *P. luminescens* to the community and to the environment when used as bio control agents was discussed by Gerdes and co-workers (2015). They mentioned the virulence factors associated and they suggested that the bacteria is safe to use, effective against wide range of insect hosts, cost effective and also its easiness of application.

Blackburn *et al*. (2016a) investigated the virulence modification (vmo) with the individual colonies. The results confirmed that pathogenicity will vary with the colony chosen for the bioassays. They reported that, media used for the bacterial growth and the selection of the colony will play a major role in virulence studies. They have used four different media composition to grow eight different strains from three species. The growth was good in media containing agar supplemented with Pyruvate. For vmo assays, they injected individual colonies of three different species of bacteria to healthy *Gall*eria larvae and observed for the infection.

Salazar-Gutierrez *et al*. (2017) assessed the pathogenicity of *P. luminescens* strain SL0708 (from *H. indica* SL0708) against *Spodoptera frugiperda* and *G. mellonella*. After 48 h, 100 per cent mortality was observed in both insects. They also reported the role of extracellular factors (proteases, esterases, ureases, hemolysins and siderophores) in the pathogenicity of the bacteria.

Silva *et al.* (2017) used the supernatant solution of *P. luminescens* and *X. Nematophila* for the bioassay against *Aedes aegypti*. The synergistic action of toxic compounds from the bacteria present in the supernatant results in the mortality of larva.

Kushwah *et al*. (2017) identified the most virulent strain of *Photorhabdus* against *G. Mellonella* among various native isolates of *Photorhabdus* from IARI New Delhi on the basis of LT50 and LC50 values. *P. luminescens* ssp. *Akhurstii* from Meghalaya (IARI-SGMG3) showed the lowest LC50 value (0.262) with high virulence.

The nanoparticulated cell free supernatant of *P. luminescens* exhibited superior pesticidal property against serious sucking pests of cotton, viz. *T. macfarlanei* and *A. gossypii*. On mites, nano particles recorded lower median lethal concentration (LC50: 0.0001 ppm) compared to normal form ($8.36 \times 10^2$ ppm) within 12 h of exposure. Similarly, on aphids, lower LC50 (LC50: 0.0027 ppm) was recorded by NPs compared to normal form (LC50: $2.12 \times 10^3$ ppm) (Kulkarni *et al*., 2017).

Yooyangke *et al.* (2018) isolated different isolates of symbiotic bacteria (*Xenorhabdus* and *Photorhabdus*) from the EPN collected from soils of Nam Nao National Park, Thailand. The bacterial suspension was used for the bioassay against *Aedes* sp. The strain *P. luminescens* subsp. *akhurstii* (bNN121.4TH) produced 98 per cent of larval mortality in *Ae. albopictus* after 96 h of treatment.

Shawer *et al*. (2018) used different concentrations of cell and cell free supernatant in order to study the insecticidal activity of *Photorhabdus luminescens* against *Drosophila suzukii* and the results proved the bacteria as a promising candidate for the biological control of insect pests with significant oral and contact toxicity in which 70-100 per cent of mortality was observed within 10 days of treatment.

Acaricidal effects of cell free supernatant of entomopathogenic bacteria *Xenorhabdus* and *Photorhabdus* sp. against *Tetranychus urticae* were studied by Eroglu *et al.* (2019). The experiment was conducted under controlled conditions of temperature (25 ± 1°C), relative humidity (70 ± 5 %) and a light cycle of 16 h. The results proved that the supernatant is less or not effective as ovicidal (4 % mortality) and effective in controlling other stages like larvae (46-97 % mortality), protonymphs (30-96 % mortality), deutonymphs (41-92 % mortality), adult males (92-100 % mortality) and for adult females (46-93 % mortality). Among all the bacteria examined, *X. szentirmaii* and *X. nematophila* were found to be more efficient with more than 90 per cent of mortality.

**2.6 Molecular mechanism of Toxin gene**

Bowen *et al.* (1998) studied about the insecticidal toxins from entomopathogenic bacteria *P. luminescens*. Four loci namely *tca, tcb, tcc* and *tcd* are involved in construing the toxin complex, in which *tca* and *tcd* both can be used as better alternatives for *Bt* because of their oral toxicity against *Manduca sexta.*

Richardson *et al.* (1988) characterized the antibiotic produced by the *Xenorhabdus luminescens* as 3, 5-dihydroxy-4-isopropylstilbene. They also isolated a pH sensitive pigment (yellow in <9 pH media and red in > 9 pH media) from the same

bacteria and characterized it as 1, 6-dihydroxy-4-methoxy-9, 10-anthraquinone which is a derivative of anthraquinone.

Insecticidal protein complex having molecular weight of 1,000,000 was isolated and purified from *Photorhabdus luminescens* by Bowen and Ensign (1998) and reported the efficiency of the secondary form of bacteria in killing *M. sexta* larvae which is same as primary form. The insecticidal protein complex included protease, phospholipase, or hemolytic activity and only a trace of lipase activity was observed. SDS PAGE analysis revealed the composition of the denatured protein complex as it was made up of several subunits (size may vary 30 to 200 kDa).

Ffrench-Constant and Bowen (1999) described the cloning of *TC* genes from *P. luminescens* and *X. nematophilus* and which are homologous to the toxins of *B. thuringiensis.*The histopathological effects of toxin proteins produced by the *TC* genes were similar to the other oral toxic proteins.

Ffrench-constant *et al.* (2000) identified different classes of virulence factors (like Tc insecticidal toxin complexes, Rtx-like toxins, proteases and lipases, colicin and pyocins, and various antibiotics) and they functionally analysed the genes responsible for the factors by targeted knockout and through this process they also studied how the factors will act on the insects.

Bowen and Ensign (2001) isolated and characterized the two distinct crystalline inclusion proteins from *Photorhabdus luminescens* and reported that those were not orally toxic to the insect larva. Type 1 was large rectangular inclusion and Type 2 was smaller bipyramid shaped inclusion and were readily soluble at pH 11 and 4 in 1 per cent (SDS) and in 8 M urea.

Bowen *et al*. (2000) separated three protease fractions (two related protein species were of 40 kDa and one was of 55 kDa) from the broth of *P. luminescens* culture and they demonstrated that these fractions were not involved in the oral toxicity as it is solely contributed by the proteins produced from Tc complex genes. The purification helps in further investigations of potential roles of these protease in bacterial virulence.

Daborn *et al*. (2002) created cosmid library from *P. luminescens* subsp. *akhurstii* strain W14 genomic DNA in order to identify the toxins capable of killing insects. They produced recombinant *E. coli* and infected fifth instar *M. sexta* for screening of the library and finally isolated a single cosmid called as H3, for which they described as a single *Photorhabdus* gene called *mcf* (makes caterpillars floppy). The gene causes apoptosis in both insect hemocytes and the midgut epithelium and helps the bacteria to kill the insect and persist within, thus helps the symbiotic EPN to multiply. The toxin was of high molecular weight and similar to other known proteins except the presence of BH3 domain. Insertional mutagenesis studies were done to confirm the ORF.

Waterfield *et al.* (2002) reported the genomic islands (*Tc* genes, makes caterpillars floppy' or *Mcf* genes, cytotoxic necrotising factor (CNF)-like toxin genes, designated *Pnf*, macrophage-toxin-like encoding gene and type III secretion system (TTSS) present in the *Photorhabdus* genome which are responsible for the virulence and the symbiotic association with the EPN. They found that nearly 53 per cent of the *Photorhabdus* genome was distinct from *Escherichia coli*. In order to isolate these unique islands they end-sequenced and arrayed the cosmid library and the pathogenic phenotypes were identified by comparing with the public databases.

Duchaud *et al*. (2003) successfully determined the genome sequence of strain TT01 of *P. luminescens*, a symbiont of the nematode *Heterorhabditis* sp. The complete genome sequence of the strain contains 4,839 predicted protein-coding genes which encodes a large number of proteins, adhesins, toxins, hemolysins, proteases and lipases, and contains a wide array of antibiotic synthesizing genes. Some of the newly identified insecticidal proteins were very effective for the control of insect pests.

Marokhazi *et al*. (2003) by using a DNA microarray and comparative genomics, showed that oral toxicity is associated with toxin complex genes *tcaAB*C and that this locus can be mobilized or deleted within different strains.

Sergeant *et al*. (2003) studied the interaction between the gene products which are produced by two to three different genes, involved in insecticidal activity of *Xenorhabdus nematophilus* PMFI296 against various insects.

Bennett and Clarke (2005) described the identification and characterization of a mutation in the pbgE1 gene of *P. luminescens* strain, TT01 which was predicted to be the fifth gene in the pbgPE operon. They showed that the mutant BMM305 was strongly attenuated in virulence against larvae of *Galleria mellonella* and was defective in symbiosis, as this mutant was unable to colonize in the gut of the IJ stage of the nematode. The study concluded that the pbgPE operon in *P. luminescens* is required for pathogenicity and symbiosis.

Yang *et al*. (2006) studied the expression of the "*Photorhabdus* virulence cassettes" (PVCs) by inserting the cassettes in the cosmids of *Escherichia* coli and proved their injectable toxicity against *Galleria mellonella*. Electron microscopy studies showed the PVC products resemble some of the bacteriocin but are modified to target the eukaryotic host cells.

Toxins produced by *Photorhabdus* and *Xenorhabdus* bacteria having both oral and injectable insecticidal activity. The PirAB groups of TCs are orally toxic to mosquitoes and some lepidopteran pests, other groups like makes caterpillars floppy (*Mcf*) (which causes apoptosis in cells) and *Photorhabdus* virulence cassettes (*PVCs*) toxins both are having only injectable activity( Ffrench-Constant *et al*., 2007).

The toxin genes in *P. luminescens* were characterized and grouped as: the Toxin Complexes (*TCs*),  "Makes caterpillars floppy" (*McF*) toxins, and the *Photorhabdus* Virulence Cassettes (*PVC*), *Photorhabdus* insect-related toxins (*Pir*) etc. (Kushwah and Somvanshi, 2015; Rodou *et al*., 2010).

Blackburn *et al*. (2011) revealed the presence of Toxin complex gene (*Tca)* in *Bacillus thuringiensis* (Bt) isolate which was very similar to the toxin complex gene of *Photorhabdus luminescens* bacteria. They screened nearly about eighty-one diverse Bt isolates for the presence of *tccC* gene (gene helps for the complete expression of

toxin complexes) and the gene was found in 17 isolates. Then they sequenced the *Tca* operon (17 kb) of two isolates which showed >99 per cent sequence identity

Sheets *et al*. (2011) proposed the structure of toxin complex from the bacteria *Xenorhabdus nematophilus* having three components like *XptA2, XptB1, and XptC1* which is related to the Tc complex of *P. luminescens. XptA2* binds to the membrane of the host and helps in translocation of the toxin component. *XptB1* and *XptC1* will form a binary complex which upon binding with the tetramer of *XptA2* produce a toxin complex which is completely active in nature.

*Photorhabdus luminescens* contains a toxin complex of three subunits namely *TcdA(A), TcdB(B)* and *TcdC(C)* which are in 5:1:1 stoichiometric ratio. For the secretion of these three subunits, N termini of both B and C are required and they cannot act independently (Yang and Waterfield, 2013).

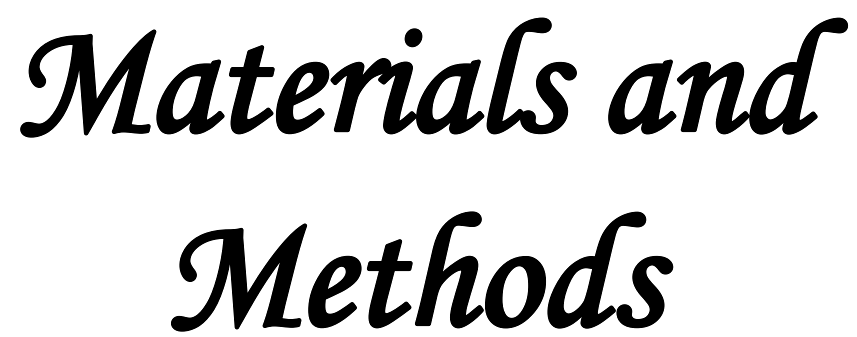
Once after the invasion of *P. luminescns* bacteria into the host, Toxin complexes of ABC-type (TCs) will be released along with other virulent factors. TCs will be functionally active only if it is complete by having all the three components like *TcA*, *TcB* and *TcC*. *TcC* is the actual component of the TCs which is responsible for its function. The other two, *TcA* (*TcdA*) and *TcB* will help in the translocation of *TcC*. Cryoelectron microscopy studies indicated the formation of transmembrane pore by *TcA* and also gave an idea about the protein translocation through syringe like mechanism (Gatsogiannis *et al*., 2013).

Meusch *et al.* (2014) studied the prepore and pore state structures of *TcA* subunit in high resolution which can form four receptor binding sites and a host specific neuraminidase-like region. Six-bladed b-propeller is the key component of the gate which will open up on binding of the *TcB/TcC* to *TcA* and thus helps in continuous translocation of the toxic proteins.

Blackburn *et al.* (2016b) studied the patterns of virulence evolution in *Photorhabdus* bacteria by using ancestral state reconstruction method. They used 18 different strains of the genus *Photorhabdus* with important taxonomic lineages for the study. They found that there was increase in virulence, with the evolution. By

considering the results of their research they suggested that, a thorough knowledge about the origin and maintenance of the virulence will help in understanding the mechanism of *Heterorhabditis-Photorhabdus* complexes which aid in selection of the effective strains for bio control.

Mulla *et al*. (2017) cloned insecticidal-protein-encoding gene, *tcdA* from a *P. luminescens* strain isolated from Western Ghats of Karnataka and expressed in *E. coli* by inserting the gene in pET32a (+) plasmid. They successfully purified the recombinant *tcdA* by Ni-NTA Affinity Chromatography.


Materials and
Methods

# 3. MATERIALS AND METHODS

The present work entitled "Molecular mechanism of virulence in the bacterium, *Photorhabdus luminescens* (Thomas and Poinar) against *Tetranychus truncatus* Ehara (Prostigmata: Tetranychidae)" was carried out at the Department of Plant Biotechnology and the Department of Agricultural Entomology, College of Agriculture, Vellanikkara, Kerala Agricultural University during 2018-2020. The objectives of the study were to identify the virulent strain of *P. luminescens* and to elucidate the molecular mechanism of virulence against *T. truncatus.*

The materials used and the methods followed for the study, and the details of the experiments carried out, based on the objective set forth are discussed below.

## 3.1 Isolation of bacterial isolates associated with the entomopthogenic nematodes

In this study, eleven different isolates of bacteria associated with the entomopthogenic nematode (EPN), *Heterorhabditi*s spp. were isolated. Seven isolates were obtained from the cultures being maintained in the Acarology laboratory, while four were isolated during the study (Table 12).

### 3.1.1 Collection of soil samples

To isolate entomopathogenic nematode (EPN) *Heterorhabditis*, soil samples were collected randomly from different localities of Kerala. From each locality, soil was collected at a depth of 15 cm and 500 g of soil was collected in a polythene bag. The soil samples were labelled with details of locality and date of collection, and brought to the laboratory for further isolating the EPN (Plate 1A).

### 3.1.2 Isolation of Entomopathogenic nematodes (EPNs)

Isolation of the EPN, *Heterorhabditis* from the collected soil samples was done following baiting method described by Bedding and Akhurst (1975). Larvae of greater wax moth, *Galleria mellonella* (Lepidoptera: Galleriidae) obtained from the culture maintained in the Acarology laboratory, All India Network project on Agricultural Acarology (AINPAA), Department of Agricultural Entomology were used for the

isolation and maintenance of the EPN. Four to five healthy last-instar larvae of *Galleria* were released separately into plastic containers of 250 ml size containing the soil samples. The soil sample was moistened before transferring to the plastic container to maintain adequate moisture content. The mouth of the container was covered with muslin cloth and the container was placed upside down and stored at room temperature (Plate 1B). At least three *G. mellonella* traps were kept for each soil sample. The containers were frequently inspected for infection of larvae with EPNs. The infected larvae which turned to brick red color and died within 24-48 h indicating infection by the EPN, *Heterorhabditis* were picked and washed thoroughly with distilled water and transferred to White's trap and kept in dark at 28 ºC (Plate 1C). The infective juveniles (IJs) of EPNs released were collected, stored in vials and used as stock culture for further infecting *Galleria* larva and maintenance of *Heterorhabditis* spp.

**3.1.3 Isolation bacterial isolates from the EPN**

**3.1.3.1 Preparation of culture media**

Nutrient Bromothymol Blue Agar (NBTA) media was used for isolation, culturing and characterization of different bacterial isolates. The composition of the NBTA media is furnished below (Table 1).

**Table 1. Composition of NBTA medium**

| Sl. No. | Composition | Quantity |
|---|---|---|
| 1. | Peptone | 5 g |
| 2. | Beef extract | 3 g |
| 3. | NaCl | 5 g |
| 4. | Bromothymol Blue dye | 0.025 g |
| 5. | 2,3,5 Tri phenyl – tetrazolium chloride (TTC) | 0.04 g |
| 6. | Agar | 20 g |
| 7. | Distilled water | 1000 ml |

### 3.1.3.2 Isolation of bacteria

The infective juvenile suspension prepared from the stock culture of EPN, was used for infecting *Galleria* larva. Four to five *Galleria* larvae were released into Petri plate lined with moist filter paper with infective juvenile suspension and incubated at 28 ºC in a BOD. The larvae were observed for mortality and dead larvae were further used for isolation of bacterial isolates.

Within 24 to 48 h of death, the cadaver was collected, surface sterilized with 70 per cent alcohol and passed over the flame three to four times by using sterile forceps under Laminar Air Flow chamber. The cadaver was dissected with sterile blade, and a drop of haemolymph was streaked onto the Petri plate containing NBTA media (10-20 ml) by using sterile inoculation loop. Care was taken not to damage the gut of the cadaver that otherwise would lead to contamination with the gut bacteria. The Petri plates were then sealed tightly by using Parafilm and kept in BOD incubator for 24-48 h to maintain the optimum temperature of 28 ºC and dark condition (Plate 2). The plates were observed for bacterial growth.

Single pure colonies of bacteria were selected and streaked on new NBTA plates and were continuously sub cultured to get pure colonies of uniform size and morphology.

#### 3.1.3.2.1 Maintenance of glycerol stocks for storage of the bacterial isolates

As the bacteria would lose viability very soon, each isolate was stored in glycerol stock for further studies. A 0.5 ml of the fresh bacterial culture was added to 0.5 ml of freshly prepared sterile glycerol aliquot (80 per cent) and the tubes were sealed and stored at -80 °C.

### 3.2 Identification of the isolates of bacteria

Eleven local isolates of bacteria associated with EPN were characterized based on cultural and molecular methods.

### 3.2.1 Cultural characterization

Each isolate was streaked onto Petri plates containing NBTA media and incubated for 2-4 days. The cultural characteristics *viz.*, colony shape, edge, margin, elevation, surface, color of the colonies as well as change in media color were recorded.

The bacterial isolates were studied for Gram reaction using 24 h old cultures. A thin smear of bacterial cells was prepared on clean glass slide separately for different isolates and were heat fixed. Few drops of crystal violet was added on the smear and washed with distilled water after 30 seconds. The smear was covered with Gram's iodine solution, left for about 60 seconds and washed with distilled water. The smear was then washed with 90 per cent alcohol drop by drop to remove all the excess color from the smear. After that, safranin was added and kept for about 30 seconds followed by washing with distilled water. The slides were then air dried after removing excess water by using blotting paper and observed under 100x oil immersion lens.

### 3.2.2 Molecular characterization of bacterial isolates

In order to identify the species of the bacterial isolates, the *16S rRNA* gene (the conserved sequence) was PCR amplified and sequenced. Colony PCR was done to amplify the *16S rRNA* gene.

Nuclease free water of 100 µl was added to all PCR tubes and a small amount of bacterial colony was added with the help of a small tip by just touching the colony. It was kept in PCR machine for initial denaturation at 94 ºC for 10 min. Then it was kept in ice for 5 min, followed by centrifugation at 11000 rpm for 4 min and then kept in refrigerator. The master mix (EmeraldAmp GT PCR Master mix- 2x Premix) along with bacterial colony and primers (Table 4) were mixed in 0.2 ml PCR tubes and subjected to thermal cycling (Table 3). The reaction mixture (50 µl) was prepared by adding the components as given in Table 2.

**Plate 1. Stages in isolation of EPN**
**(A) Soil samples (B) Isolation of EPN from soil (C) White trap**

**Plate 2. Incubation of bacteria in BOD at 28°C**

**Table 2. Composition of reaction mixture used for the PCR**

| Sl. No. | Reagents | Volume (µl) |
|---|---|---|
| 1. | Water | 19 |
| 2. | Master mix 2x | 25 |
| 3. | Reverse primer (10 picomol) | 1 |
| 4. | Forward primer (10 picomol) | 1 |
| 5. | Colony (in 100 µl water) | 4 |
| | Total | 50 |

**Table 3. Thermal program used for the PCR**

| Stage | Steps | Temperature | Duration | No. of cycles |
|---|---|---|---|---|
| 1 | Initial denaturation | 94 ºC | 4 min | 1 |
| 2 | Denaturation | 94 ºC | 30 sec | ×35 |
| 3 | Annealing | 55.7 ºC | 45 sec | |
| 4 | Extension | 72 ºC | 45 sec | |
| 5 | Final extension | 72 ºC | 8 min | 1 |
| 6 | Hold | 4 ºC | ∞ | |

**Table 4. Primer details for *16S rRNA* region**

| Sl no. | Locus | Sequence | Reference |
|---|---|---|---|
| 1. | Forward<br>Reverse | 5' AGAGTTTGATCCTGGCTCAG 3'<br>5' ACGGCTACCTTGTTACGACTT 3' | Mulla *et al.*, 2017 |

### 3.2.2.1 Assessment of PCR product

- The amplification of *16S rRNA* of bacterial isolates was assessed by agarose gel electrophoresis (1.4 % gel)
- Agarose gel was prepared by adding 0.84 g of agarose in 60 ml of 1X TAE buffer. After adding, the buffer mixture was slightly heated to melt the agarose and allowed to cool.
- After cooling (42°C to 45°C), 3 µl of ethidium bromide was added to visualize the DNA bands
- Gel casting tray was sealed properly after wiping with 70 per cent alcohol. The comb was placed in such a way that it should be 1 to 2 mm above the tray
- Then the gel was poured to the tray with care to avoid the bubble formation while pouring and kept for solidification
- Comb was removed from the solidified gel and the gel was transferred to the electrophoresis tank. 1x TAE buffer was poured into the tank till the buffer stands 1 cm above the gel and covered the wells completely.
- 5 µl each of the products and 100 bp ladder were loaded into the wells.
- Power cords were connected and a constant voltage of 80 V was maintained throughout the electrophoresis
- Once after the bands reached ¾th of the gel the power cords were removed
- BioRad gel documentation system (Quantity One software) was used for documentation of the bands where the gel was exposed to UV radiations and the image was saved in JPEG format.

### 3.2.2.2 Sequencing of PCR products

PCR products were sequenced at AgriGenome Labs, Pvt. Ltd., Cochin, Kerala.

### 3.2.2.3 Analysis of sequencing data by using *in- silico* tools

CAP3 sequence assembly programme was used to merge the forward and reverse sequences to form the contigs and were used for analyzing sequence

homology BLASTn (Basic Local Alignment Search Tool). Nucleotide sequences of individual accession were uploaded in BLASTn and the bacteria were identified by considering the maximum sequence identity percentage, query coverage and the lowest E value (expected value) of the accessions with NCBI accessions. MSA (Multiple Sequence Alignment)

#### 3.2.2.4 Multiple sequence alignment and construction of phylogenetic tree:

In order to estimate the relationship between all the nine samples used in the study, a phylogenetic tree was constructed. A total of forty seven similar sequences obtained from BLAST result (Table 5), including seven for CF1 isolate and five each for remaining eight isolates were retrieved from NCBI database. Forty seven sequences along with nine query sequences were used for construction of phylogenetic tree. They were named as R1, R2, R3, R4 and R5 in front of the query isolate name. These sequences were aligned by using MAFFT (Multiple Alignment using Fast Fourier Transform) tool. Based on this alignment, a neighbour joining tree was constructed (Saitou and Nei 1987) by using MEGA X software (version 10.2.2) Bootstrap value was kept at 500 replications. Branches showing more than 70 per cent of bootstrap values were considered as nodes.

**Table 5. Details of related sequences used for the construction of Phylogenetic tree**

| Sl. No. | NCBI accession ID | given Name | Description | Size of the *16S rRNA* sequence (base pairs) | Identity with the query sequence |
|---|---|---|---|---|---|
| 1. | MH746106.1 | KL1R1 | *Pseudomonas aeruginosa* strain QK-3 *16S ribosomal RNA* gene | 1447 | 99.76 % |
| 2. | KX778119.1 | KL1R2 | *Pseudomonas* sp. strain SAUF141 *16S ribosomal RNA* gene | 1389 | 99.76 % |
| 3. | MH010896.1 | KL1R3 | *P. aeruginosa* strain ABPL150 *16S ribosomal RNA* gene, partial sequence | 1424 | 99.68 % |
| 4. | KF984154.1 | KL1R4 | *P. aeruginosa* strain CJ2 *16S ribosomal RNA* gene, partial sequence | 1458 | 99.68 % |
| 5. | MT598026.1 | KL1R5 | *P. aeruginosa* strain AB18 *16S ribosomal RNA* gene, partial sequence | 1446 | 99.68 % |
| 6. | MT646431.1 | KT1R1 | *P. aeruginosa* strain MLTBM2 *16S ribosomal RNA* gene, partial sequence | 1391 | 100 % |
| 7. | MT633047.1 | KT1R2 | *P. aeruginosa* strain OIS 4.8.1 *16S ribosomal RNA* gene, partial sequence | 1480 | 100 % |
| 8. | MT626658.1 | KT1R3 | *P. aeruginosa* strain S-04 *16S ribosomal RNA* gene, partial sequence | 1439 | 100 % |

**Table 5. continued**

| | | | | | |
|---|---|---|---|---|---|
| 9. | MT598024.1 | KT1R4 | *P. aeruginosa* strain AP17 *16S ribosomal RNA* gene, partial sequence | 1448 | 100 % |
| 10. | MT598022.1 | KT1R5 | *P. aeruginosa* strain AP04 *16S ribosomal RNA* gene, partial sequence | 1420 | 100 % |
| 11. | MN889402.1 | FR1R1 | *Ochrobactrum. pseudogrignonense* strain OsEnb-HZB-H6 *16S ribosomal RNA gene,* partial sequence | 1359 | 100 % |
| 12. | MN889385.1 | FR1R2 | *O. pseudogrignonense* strain OsEnb-HZB-F10 *16S ribosomal RNA* gene, partial sequence | 1353 | 100 % |
| 13. | MN889382.1 | FR1R3 | *O. pseudogrignonense* strain OsEnb-HZB-F5 *16S ribosomal RNA* gene, partial sequence | 1367 | 100 % |
| 14. | MN889372.1 | FR1R4 | *O. pseudogrignonense* strain OsEnb-HZB-E1 *16S ribosomal RNA* gene, partial sequence | 1359 | 100 % |
| 15. | MK165125.1 | FR1R5 | *Ochrobactrum* sp. strain PI-23 *16S ribosomal RNA* gene, partial sequence | 1359 | 100 % |
| 16. | KR149612.1 | EKM1 R1 | *O. pseudintermedium* strain 1-4-b-7 *16S ribosomal RNA* gene, partial sequence | 1322 | 99.92 % |
| 17. | KJ018990.1 | EKM1 R2 | *O. pseudintermedium* strain SDT62 *16S ribosomal RNA* gene, partial sequence | 1341 | 99.92 % |
| 18. | KF026284.1 | EKM1 R3 | *O. pseudintermedium* strain RA7 *16S ribosomal RNA* gene, partial sequence | 1352 | 99.92 % |

**Table 5. continued**

| | | | | | |
|---|---|---|---|---|---|
| 19. | JX646632.1 | EKM1 R4 | *Ochrobactrum* sp. Y2 *16S ribosomal RNA* gene, partial sequence | 1393 | 99.92 % |
| 20. | MT742989.1 | EKM1 R5 | *Ochrobactrum* sp. strain DM1 *16S ribosomal RNA* gene, partial sequence | 1407 | 99.92 % |
| 21. | JX221723.1 | CF1R1 | *Photorhabdus luminescens* strain SG-HR4 *16S ribosomal RNA* gene, partial sequence | 1576 | 99.56 % |
| 22. | AY278644.1 | CF1R2 | *P. luminescens* subsp. *akhurstii* strainEG2 *16S ribosomal RNA* gene, partial sequence | 1500 | 99.56 % |
| 23. | KF780170.1 | CF1R3 | *P. luminescens* strain NBAII Hb105 *16S ribosomal RNA* gene, partial sequence | 1545 | 99.49 % |
| 24. | KT963833.1 | CF1R4 | *P. luminescens* subsp. *laumondii* strain SF 281 *16S ribosomal RNA* gene, partial sequence | 1528 | 98.55 % |
| 25. | Y17605.1 | CF1R5 | *P. luminescens 16S rRNA*gene | 1503 | 99.42 % |
| 26. | JN200818.1 | CF1R6 | *P. luminescens* strain LPP30 *16S ribosomal RNA* gene, prtial sequence | 1499 | 99.42 % |
| 27. | AB355866.1 | CF1R7 | *P. luminescens* subsp. *akhurstii* gene for *16S rRNA*, partial sequence, strain: LN2 | 1499 | 99.20 % |
| 28. | MT534544.1 | HI1R1 | *Ochrobactrum anthropi* strain DP5 *16S ribosomal RNA* gene, partial sequence | 1381 | 100 % |

**Table 5. continued**

| | | | | | |
|---|---|---|---|---|---|
| 29. | MT083950.1 | HI1R2 | *O. anthropi* strain TY171-20 *16S ribosomal RNA* gene, partial sequence | 1344 | 100 % |
| 30. | MT081283.1 | HI1R3 | *O. anthropii* strain XG-2B *16S ribosomal RNA* gene, partial sequence | 1345 | 100 % |
| 31. | MG550982.1 | HI1R4 | *Ochrobactrum.* sp. strain Ktm-7 *16S ribosomal RNA* gene, partial sequence | 1353 | 100 % |
| 32. | MN252068.1 | HI1R5 | *O. anthropi* strain A8 *16S ribosomal RNA* gene, partial sequence | 1388 | 100 % |
| 33. | KF826289.1 | MP1R1 | *Ochrobactrum* sp. W6 *16S ribosomal RNA* gene, partial sequence | 1402 | 99.77 % |
| 34. | AM490610.1 | MP1R2 | *O. pseudintermedium* partial *16S rRNA* gene, strain CCUG 43465 | 1389 | 99.77 % |
| 35. | DQ305290.1 | MP1R3 | *Ochrobactrum* sp. CGL-X *16S ribosomal RNA* gene, partial sequence | 1451 | 99.85 % |
| 36. | MG008507.1 | MP1R4 | *Ochrobactrum* sp. strain CSL1 *16S ribosomal RNA* gene, partial sequence | 1340 | 99.77 % |
| 37. | MF062571.1 | MP1R5 | *Ochrobactrum* sp. strain LJ-C *16S ribosomal RNA* gene, partial sequence | 1454 | 99.77 % |
| 38. | MN263248.1 | HS1R1 | *Ochrobactrum* sp. strain QY-1 *16S ribosomal RNA* gene, partial sequence | 1347 | 99.77 % |

**Table 5. continued**

| | | | | | |
|---|---|---|---|---|---|
| 39. | MK351298.1 | HS1R2 | *O. pseudintermedium* strain AIM7 *16S ribosomal RNA* gene, partial sequence | 1346 | 99.77 % |
| 40. | KT992334.1 | HS1R3 | *Ochrobactrum* sp. strain YE2-10 *16S ribosomal* RNA gene, partial sequence | 1378 | 99.77 % |
| 41. | KX832688.1 | HS1R4 | *O. pseudintermedium* strain C9 *16S ribosomal RNA* gene, partial sequence | 1388 | 99.77 % |
| 42. | LN851900.1 | HS1R5 | *Ochrobactrum* sp. Vr39 partial *16S rRNA* gene, strain Vr39 | 1327 | 99.77 % |
| 43. | MK600536.1 | HQ1R1 | *Stenotrophomonas* sp. strain LSB20 *16S ribosomal RNA* gene, | 1440 | 99.86 % |
| 44. | MT000012.1 | HQ1R2 | *S. maltophilia* strain XS 8-4 *16S ribosomal RNA* gene, partial sequence | 1441 | 99.86 % |
| 45. | MN714632.1 | HQ1R3 | *Stenotrophomonas*sp. strain M11 *16S ribosomal RNA* gene, partial sequence | 1446 | 99.86 % |
| 46. | KM893074.1 | HQ1R4 | *Stenotrophomonas maltophilia* strain LH15 *16S ribosomal RNA* | 1450 | 99.86 % |
| 47. | KF542911.1 | HQ1R5 | *S. maltophilia* strain *S4 16S ribosomal RNA* gene, partial sequence | 1423 | 99.86 % |

### 3.3 Bio efficacy of bacterial isolates against *Tetranychus truncatus*

Five isolates of bacteria associated with EPN, *Heterorhabditis* sp. isolated in the study were evaluated in the laboratory for their efficacy against egg and adult of *T. truncatus*. Both cell suspension and cell free supernatant of the bacteria was used for the study.

#### 3.3.1 Laboratory rearing of *Tetranychus truncatus*

Mass culture of *T. truncatus* was maintained in the Acarology laboratory of AINPAA on mulberry leaves. Gravid females collected from the nucleus culture maintained in the laboratory were released on the leaves placed with their abaxial surface on wet absorbent sponge in plastic trays ($40 \times 25$ $cm^2$) (Plate 4). The leaves were washed in clean water and wiped dry before placing on absorbent sponge in trays. The leaves were replaced once in every three to four days with fresh leaves, for which old leaf with mites was placed on the fresh leaf allowing the mites to move from old to fresh leaves by themselves.

#### 3.3.2 Preparation of Cell suspension and Cell free suspension (CFS) of bacterial isolates

Sterile liquid broth of NBT (150 ml) was taken in a conical flask and a loop full of fresh bacterial culture was added aseptically in laminar air flow chamber, sealed properly and kept in shaker at 200 rpm for 24-48 h and used as mother culture. In order to check the initial concentration of the mother culture, serial dilution, plating and colony counting method was used (Plate 3). For serial dilution, 1 ml of the mother culture was added to 9 ml of sterile water blank and marked as $10^{-1}$, and from that, 1 ml was taken and added to the next water blank (9 ml), which was marked as $10^{-2}$ and the process continued up to the dilution $10^{-8}$. One ml of the cell suspension was added to the plate followed by pouring the media (10-15 ml), mixed thoroughly and allowed to solidify by incubating in BOD at 27 ºC. Three replications per dilution were plated. After 24-48 h, the grown up single colonies were counted. After reading

the initial concentration, different treatments/concentrations were prepared by serial dilution.

The dilutions (10 ml) were centrifuged carefully at 4000 rpm for 20 min. Cell pellets were formed at the bottom of the tubes. The supernatants of different concentrations were collected separately and used for bioassay as CFS. The cell pellets were again dissolved in water and used as bacterial cell suspension treatments.

### 3.3.3 Laboratory bioassay

Laboratory bioassays were conducted to evaluate the efficacy of both bacterial cells and cell free supernatant (CFS) of five bacterial isolates (1 isolate of *P. luminescens*, 2 isolates of *P. aeruginosa*, and 2 isolates of *Ochrobactrum*) against *T. truncatus* at five different concentrations. Both the cell suspension and cell free supernatant of each isolate were evaluated at concentrations of $10^4$, $10^5$, $10^6$, $10^7$ and $10^8$ Cells/ml separately against egg and adult of *T. truncatus*. Each experiment was laid out in a Completely Randomized Design (CRD) with 26 treatments including control and three replications per treatment (Plate 6).

#### 3.3.3.1 Ovicidal effect of bacterial isolates

The efficacy of cells and CFS of the bacterial isolates on the eggs of *T. truncatus* was studied following topical application method. Ten gravid females of *T. truncatus* were transferred with the help of a camel hair brush on to leaf bit of mulberry (5x5 $cm^2$ each), placed in Petri plate lined with wet cotton and allowed for laying eggs. Sides of the leaf bit were bordered with moistened cotton in order to avoid the escape of mites. After 24 h, the mites were removed and 25 eggs were maintained per leaf bit by removing all the excess eggs. Different concentrations of bacterial cells and CFS were sprayed on leaf bits with eggs of *T. truncatus* using a hand atomizer (2 ml/bit). Hatchability of the eggs was observed under stereo binocular microscope (LEICA EZ4 HD) at 24, 48, 72, 96 h after spraying and the data recorded. Per cent mortality of eggs was calculated as follows

$$\text{Per cent mortality} = \frac{25 - \text{total number of eggs hatched}}{25} \times 100$$

The data on per cent mortality of eggs was subjected to analysis of variance (ANOVA).

#### 3.3.3.2 Adulticidal effect of bacterial isolates

Efficacy of the cell suspension and CFS of the bacterial isolates on the adults of *T. truncatus* was tested by topical application of the treatments. Twenty five healthy gravid female mites were released on to mulberry leaf bit of size 5x5 $cm^2$, placed on moist cotton pad in a Petri plate. The margins of the leaf bit were bordered with wet cotton, to prevent the escape of mites. Three leaf bits with 25 mites each were maintained in a Petri plate as replications (Plate 5). Different treatment concentrations were sprayed on leaf bits with adult mites using a hand atomizer (2 ml/bit). The leaf bits were observed under stereo binocular microscope and mortality of the mites was recorded at 24, 48, 72, and 96 h after treatment. From the data, per cent mortality of mites was calculated and then subjected to analysis of variance.

### 3.4 Molecular basis of virulence in *P. luminescens* isolate against *Tetranychus truncatus*

To identify the molecular mechanism involved for the virulence of *P. luminescens* against *T. truncatus* the toxin complex gene was amplified and characterized.

#### 3.4.1 Amplification of *TcdA* – Toxin complex gene

The amplification of toxin complex (*TcdA*) gene was attempted using internal primers.

##### 3.4.1.1 Reported primers

In order to amplify the full length Toxin complex gene (7.5 Kb), initially three sets of internal primers reported by Mulla *et al*. (2017) were used (Table 6). In this case the whole gene was split into three parts of 2.4, 2.7 and 2.4 Kb length each.

##### 3.4.1.2 Primer Designing

As the reported primers failed to amplify the toxin gene, primers were designed in the study. The entire gene was split into eight regions each with 1100bp with 100 bp of overlapping sequences on both ends. Forward and reverse primers were designed separately for each region by using the online platform Primer 3 (version 0.4.0). All the important parameters of primer designing (optimum size, melting temperatures, GC content and least dimerization capacity) were considered to pick the best ones. The details of the designed primers are described in the Table 7.

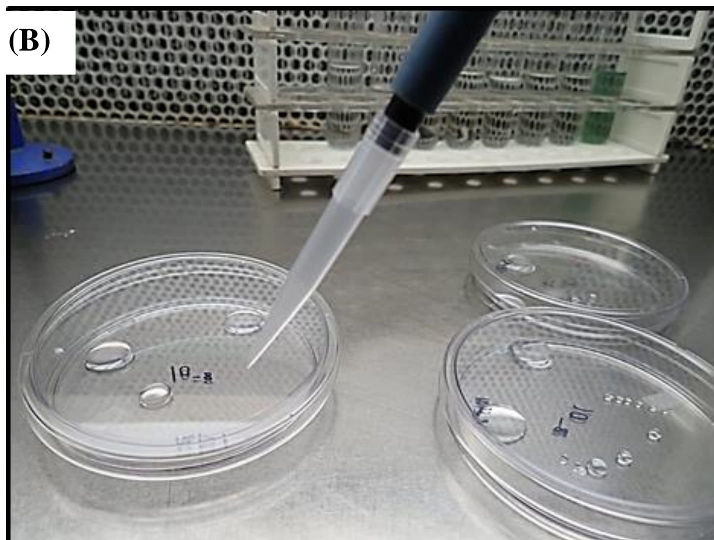



**Plate 3. Serial dilution (A) and plating (B)**

**Plate 4. Laboratory cultures of *Tetranychus truncatus***

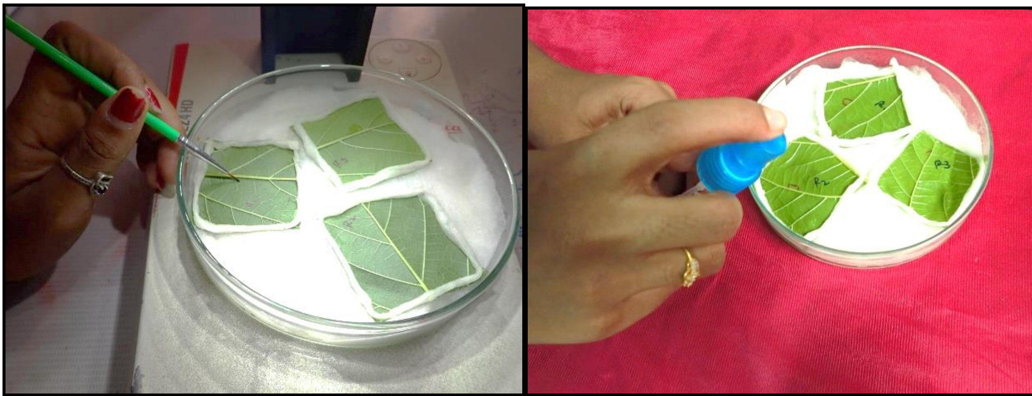


**Plate 5. Releasing of mites and topical application of treatments**

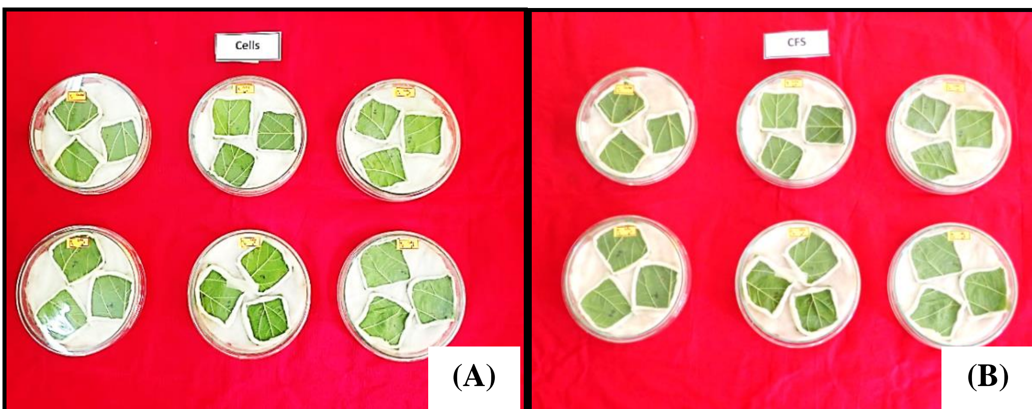



**Plate 6. Layout for the bioassay**

**(A) Cells (B) CFS**

**Table 6. Details of reported primers (*TcdA* gene) used for the study**

| Sl. No. | Name | Locus | Sequence | Reference |
|---|---|---|---|---|
| **1.** | TcdAi | Forward | 5' CGATGCGGATCCATGAACGAGTCTGTAAAAG 3' | Mulla *et al.*, 2017 |
| | | Reverse | 5' TACTTAGCTAGCACCGAGGACGCTTTTTCG 3’ | |
| **2.** | TcdAii | Forward | 5' CGGTGCTAGCGGCATTTGAAGCTAACTCGT 3' | |
| | | Reverse | 5' GTGAGGGCCCCACCAGGTACCATCTGAT 3’ | |
| **3.** | TcdAiii | Forward | 5' GGTGGGGCCCTCACTTTGTTAGAGATGATAAAG 3' | |
| | | Reverse | 5‘ GTGCAGCTCGAGTTATTTAATGGTGTAGCGAATATGC 3’ | |

**Table 7. Details of newly designed primers used for the *TcdA* gene amplification**

| S.l No. | Name | Regions | Locus | Sequence | Length (bp) | Expected product size (bp) |
|---|---|---|---|---|---|---|
| 1. | TcdA one | 1(1-1100 bp) | Forward | 5' AATGAATTTCGCCAGCAAGT 3' | 20 | 965 |
| | | | Reverse | 5' TCACCACCGAAGGGAAATAG 3’ | 20 | |
| 2. | TcdA two | 2(1000-2100 bp) | Forward | 5' AGCTATTTCCCTTCGGTGGT 3' | 20 | 1000 |
| | | | Reverse | 5' CCGTGGTAGACGGTATCCAG 3’ | 20 | |
| 3. | TcdA three | 3(2000-3100 bp) | Forward | 5' CCTATATTGCGGCCACCTT 3' | 19 | 977 |
| | | | Reverse | 5' GATAAAGAATTGGCGGCTGA 3’ | 20 | |
| 4. | TcdA four | 4(3000-4100 bp) | Forward | 5' CCGCCAATTCTTTATCGACT 3' | 20 | 997 |
| | | | Reverse | 5' TGATGCGGCTTTGTAATTGA 3’ | 20 | |
| 5. | TcdA five | 5(4000-5100 bp) | Forward | 5' TTACAAAGCCGCATCAAGTG 3' | 20 | 958 |
| | | | Reverse | 5' CCAACGTTCATCACCATGAG 3’ | 20 | |

Table 7. continued

| | | | | | | |
|---|---|---|---|---|---|---|
| 6. | TcdA six | 6(5000-6100 bp) | Forward | 5' TCAAGATTATCACGCCAAGG 3' | 20 | 957 |
| | | | Reverse | 5' AAACGCCACAGGGACATAAA 3’ | 20 | |
| 7. | TcdA seven | 7(6000-7100 bp) | Forward | 5' CTTCTCAAGGTGGAGGCAAG3' | 20 | 977 |
| | | | Reverse | 5' GCGTTTATCGCGTTTCAGAT 3’ | 20 | |
| 8. | TcdA eight | 8(6595-7551 bp) | Forward | 5' TGAAGCAAATCGATGCTCAG 3' | 20 | 859 |
| | | | Reverse | 5' GCATTTGGGAAGCTCAGTGT 3’ | 20 | |

As the designed primers did not amplify regions 5 and 6 of the toxin gene, new primers were developed for these regions and the details are furnished below (Table 8).

**Table 8. Details of new primers (for the region 5 and 6) used in the study**

| Sl No. | Name | Locus | Sequence | Length in (bp) | Expected product size (bp) |
|---|---|---|---|---|---|
| 1. | TcdA five New | Forward | 5'CCGCTATGCAGAGGATTATGA3' | 21 | 1183 |
| | | Reverse | 5'TGGGACATCATCAAGAGGAA3’ | 20 | |
| **2.** | TcdA six New | Forward | 5'CTCATGGTGATGAACGTTGG 3' | 20 | 1064 |
| | | Reverse | 5' CTTGCCTCCACCTTGAGAAG3’ | 20 | |

**3.4.1.3 PCR amplification of the *TcdA* gene**

Amplification of the toxin gene was carried out following colony PCR. For this, 19 µl of water, 1µl each of forward and reverse primers, 4 µl of bacterial sample (initially denatured at 94 $^{0}$C for 10 min) as a DNA source and 25 µl of Takara master mix (EmeraldAmp GT PCR Master mix- 2x Premix) were added into 0.2 ml PCR tubes (Table 9). All the tubes with reaction mixture were briefly spun by using minicentrifuge to push all the liquid contents to bottom before the PCR. Proflex (Thermo scientific) thermal cycler was used by setting the thermal programme presented in the Table 10.

**3.4.1.3.1 Standardization of optimum conditions for the designed primers**

Two important factors which has to be considered for every PCR are correct proportion of the components in the reaction mixture and the temperature of various steps in the reaction (denaturation, annealing and extension temperatures). Annealing

temperature was optimized using gradient PCR, using the Tm calculated based on length of the primer and its GC content.

**Table 9. Reagents used in the thermal cycling for the amplification of *TcdA* regions**

| Sl. No. | Reagents | Quantity (μl) |
|---|---|---|
| 1. | Water | 19 |
| 2. | Master mix | 25 |
| 3. | Reverse primer | 1 |
| 4. | Forward primer | 1 |
| 5. | Colony (in 100uL water) | 4 |
| | Total | 50 |

**Table 10. The thermal program used for the amplification of *TcdA* regions**

| Stage | Steps | Temperature | Duration | No. of cycles |
|---|---|---|---|---|
| **1** | Initial denaturation | 94 ºC | 4 mins | 1 |
| **2** | Denaturation | 94 ºC | 30 secs | × 35 |
| **3** | Annealing | Based on primer | 45 secs | |
| **4** | Extension | 72 ºC | 45 secs | |
| **5** | Final extension | 72 ºC | 8 mins | 1 |
| **6** | Hold | 4 ºC | ∞ | |

Annealing temperatures for each primmer combination are presented in the Table 11.

**Table 11. Annealing temperatures used for the amplification *TcdA* regions**

| Sl. No. | Primer combination | Annealing temperatures ($^{0}$C ) |
|---|---|---|
| 1. | TcdA one | 54.8 |
| 2. | TcdA two | 55.1 |
| 3. | TcdA three | 55.0 |
| 4. | TcdA four | 54.7 |
| 7. | TcdA seven | 55.1 |
| 8. | TcdA eight | 55.1 |

**3.4.1.4 Sequencing of the amplified products**

After thermal cycling, products were visualized by agarose gel electrophoresis (1.4 %) and sequenced (AgriGenome Labs, Pvt. Ltd., Cochin, Kerala).

**3.4.1.5 Analysis of sequence data**

Trimmed forward and reverse sequences were merged by using CAP3 sequence assembler, to form the contigs. Sequence homology was checked using BLASTn (Basic Local Alignment Search Tool) at NCBI.

**3.4.1.6 Sequence analysis**

To construct the full length gene, the six contigs amplified during the study were merged, leaving the sequence of 5$^{th}$ and 6$^{th}$ regions as gap. Nucleotide variation from the available sequences was assessed by aligning using Clustal Omega (1.2.4) (https://www.ebi.ac.uk/Tools/msa/clustalo/).

Results

# 4. RESULTS

The results of the study "Molecular mechanism of virulence in the bacterium, *Photorhabdus luminescens* (Thomas and Poinar) against *Tetranychus truncatus* Ehara (Prostigmata: Tetranychidae)" are discussed in this chapter.

## 4.1 Isolation of bacterial isolates associated with the entomopthogenic nematodes

### 4.1.1 Isolation of entomopathogenic nematode, *Heterorhabditis*.

Four isolates of entomopathogenic nematodes (EPN), *Heterorhabditis* were isolated from the soil samples collected from different localities of Kerala. The four isolates of EPN obtained during the study and seven isolates being maintained in the Acarology laboratory, AINP on Agricultural Acarology were used for the isolation of EPN associated bacteria (Table 12; Plate 7).

### 4.1.2 Isolation of bacteria

The EPN infected cadaver of *Galleria* larva was used to isolate the bacterial isolates, by streaking the haemolymph of the cadaver on NBTA media in Petri plate. Eleven isolates of bacteria isolated from the EPN, *Heterorhabditis* sp. include MP1, HS1, HQ1, WH1, KL1, KT1, MT1, FR1, EKM1, CF1 and HI1. The bacterial isolates were stored in glycerol stock for further studies.

## 4.2 Identification of the bacteria associated with the EPN, *Heterorhabditis*

To study the cultural characters of the eleven bacterial isolates associated with *Heterorhabditis* sp., they were cultured on NBTA media in Petri plates. Molecular characterisation of the isolates was also carried out by PCR amplification and sequencing the *16S rRNA* region.

**Table 12. Details of the isolates of *Heterorhabditis* sp. used for isolating bacteria**

| Sl. No. | Accession number | Location | No. of isolates | District | GPS | |
|---|---|---|---|---|---|---|
| | | | | | **Longitude** | **Latitude** |
| 1. | KL1 | Kaladi | 1 | Ernakulam | 76$^{o}$42’65”E | 10$^{o}$15’31”N |
| 2. | KT1 | Kottapadam | 1 | Palakkad | 76$^{o}$39’14”E | 10$^{o}$99’79”N |
| 3. | MT1 | Mattathur | 1 | Thrissur | 76$^{o}$19’15”E | 10$^{o}$22’45”N |
| 4. | FR1 | Fruit research station, KAU | 1 | Thrissur | 76$^{o}$28’57”E | 10.54’65”N |
| 5. | EKM1 | Angamaly | 1 | Ernakulam | 76$^{o}$24’0”E | 10$^{o}$11’60”N |
| 6. | CF1 | Coconut farm, KAU | 1 | Thrissur | 76$^{o}$28’57”E | 10.54’65”N |
| 7. | HI1 | Hostel, Vellanikkara | 1 | Thrissur | 76$^{o}$28’57”E | 10.54’65”N |
| 8. | MP1 | Nilambur | 1 | Malappuram | 76$^{o}$13’33”E | 11$^{o}$16’37”N |
| 9. | HS1 | Vellanikkara | 1 | Thrissur | 76$^{o}$28’57”E | 10.54’65”N |
| 10. | HQ1 | Headquarters, KAU | 1 | Thrissur | 76$^{o}$28’57”E | 10.54’65”N |
| 11. | WH1 | Waterhouse, KAU | 1 | Thrissur | 76$^{o}$28’57”E | 10.54’65”N |

### 4.2.1 Cultural characterisation of the bacterial isolates

The cultural characters of the bacterial isolates *viz*., colony shape, edge, margin, elevation, surface and colour of the bacterial isolates were recorded four days after inoculation on NBTA media. The change in the colour of the media was also recorded after four days of bacterial growth. The bacterial smear was observed under microscope to record the cell shape and gram reaction. The results are presented in Table 13 (Plate 8).

The cultural characteristics of the bacterial colonies of isolates KL1 and KT1 were similar on NBTA media. The colonies were circular in shape with irregular edge, opaque, flat and the surface was smooth. In these isolates, the colour of the media changed from yellowish to bluish green. Similarly, the isolates MP1, MT1, FR1, EKM1, HI1, HQ1 and HS1 were similar to each other in cultural characters, 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) and the colonies were opaque and smooth. Elevation varied with the isolates *viz*, flat, low convex and raised. The colour of the media was yellowish green. The isolates WH1 and CF1 were similar which produced dark red colony with white margin and the media turned from yellowish to greenish blue. Circular to irregular, opaque, raised, smooth colonies were produced.

Observation of the bacterial cells under microscope showed that the cells appeared red in colour and were identified as Gram's negative (Plate 9). However, the shape of the cell varied among the isolates from short to long rods.

**Table 13. Cultural characters of the bacterial isolates**

| Sl. No. | Isolates | Shape | Edge | Opacity | Elevation | Surface | Colour of the 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 bluish green | Gram negative | Rod shape |
| 2. | KT1 | Circular | Irregular | Opaque | Flat | Smooth | Red | Yellowish turned to bluish green | 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 Greenish blue | Gram negative | Short rods |
| 7. | HI1 | Circular | Entire | Opaque | Raised | Smooth | Red | Yellowish Green | Gram negative | Short to long rods |

**Table 13. continued**

| 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 |

### 4.2.2 Molecular characterization of bacterial isolates

#### 4.2.2.1 Colony PCR and sequencing of PCR products

Through colony PCR, the *16S rRNA* region of all the isolates were amplified successfully. Quality of the amplified products were checked by running gel electrophoresis, where all the isolates produced distinct bands of nearly 1500 bp size (Plate 10).

Among the eleven Sanger sequence received, only nine had sufficient quality for further studies. The sequence details of nine isolates are furnished in Annexure (1) respectively in FASTA format.

#### 4.2.2.2 Analysis of sequence data by using in silico tools

Contigs developed were used for homology analysis by BLASTn and sequences showing maximum similarity percentage, query coverage and E value of zero were identified (Table 14). Accordingly, the species identity of nine bacterial isolates was established.

The isolates KL1 and KT1 showed maximum similarity with *Pseudomonas aeruginosa* accessions MH7461061 and MK607451.1, respectively. The isolate MP1 showed maximum similarity with *Ochrobactrum* sp. (MN263248.1), while FR1 isolate showed maximum similarity with *Ochrobactrum pseudogrignonensis* (MN889402.1). The isolates EKM1 and HS1 showed maximum similarity with the accessions of *Ochrobactrum* sp., MK351298.1 and MN263248.1, respectively. The isolates HI1, HQ1 and CF1 showed maximum similarity with *Ochrobactrum anthropi* (MG550982.1), *Stenotrophomonas maltophilla* (MK600536.1) and *Photorhabdus luminescens* (JX221723.1) (Figure 1), respectively. The isolates MT1 and WH1 failed to get accession identity as the sequence information was incomplete.

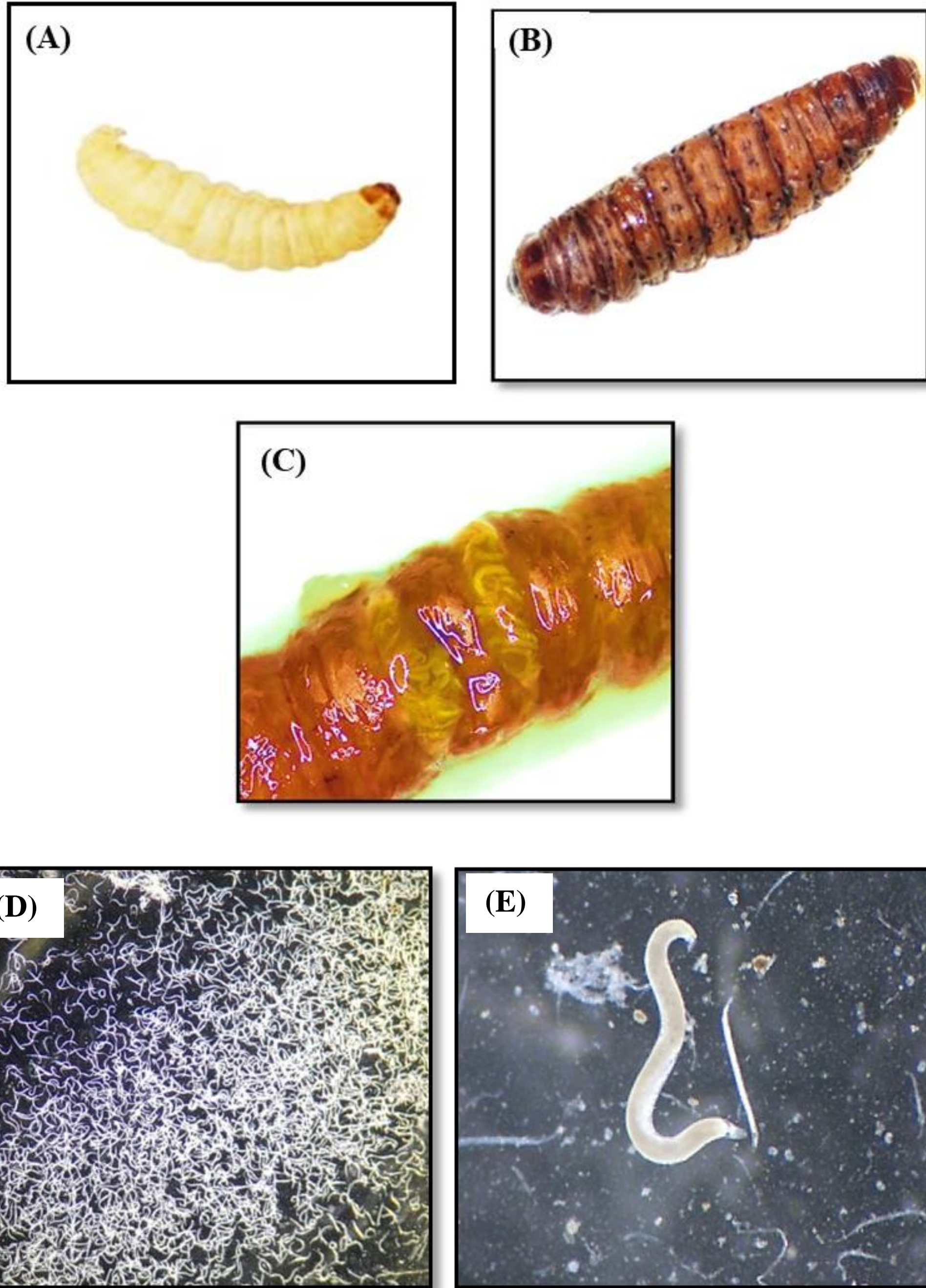



**Plate 7. EPN isolation procedure**

**(A) Healthy *G. mellonella* larva (B) EPN infected cadaver (C) Nematodes inside the host body (D) Nematodes collected in the white's trap ((E) EPN, *Heterorhabditi*s sp. (10X magnification)**

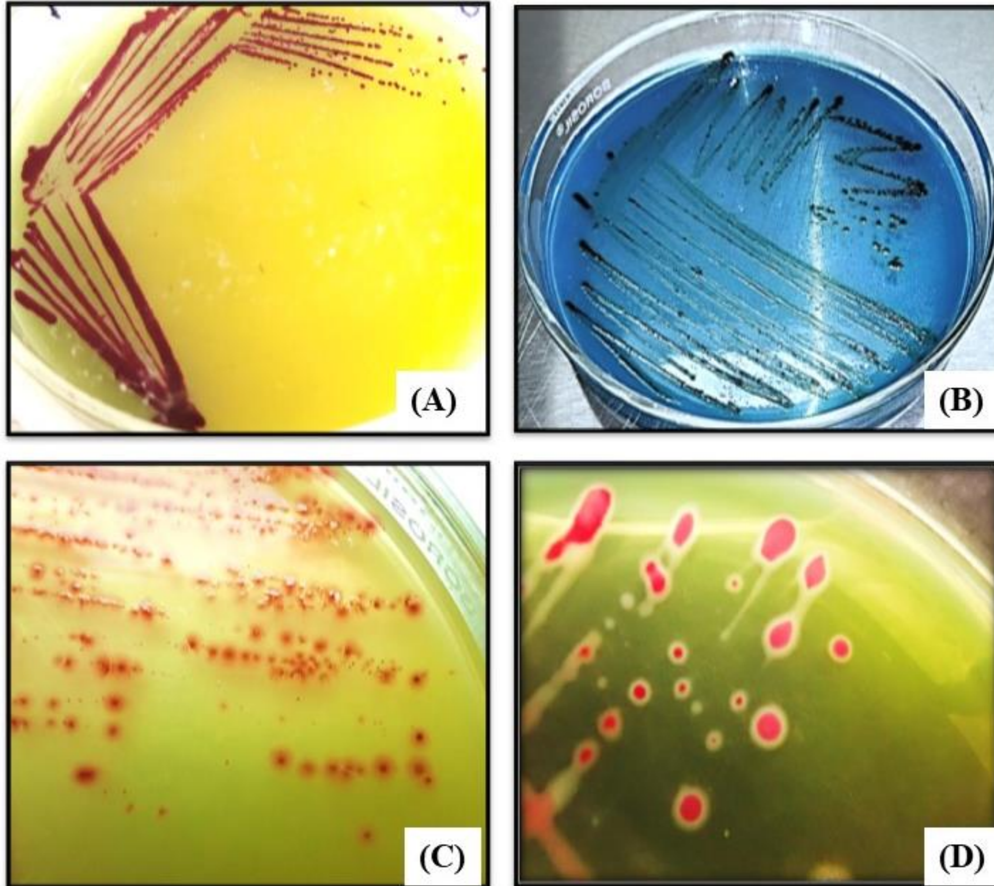



**Plate 8. Bacterial colonies**
**(A) (C) and (D) different types of colonies produced by bacteria on NBTA media**
**(B) Change in media color due to pigmentation by CF1 isolate**

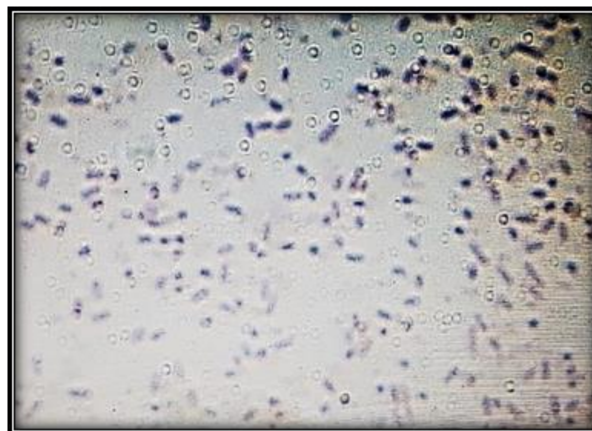


**Plate 9. Gram stained bacterial cells (100X magnification)**

**Table 14. Homology of *16S rRNA* sequences of bacteria**

| Sl. No. | Isolate | NCBI Identity | Per cent Identity | Query coverage | Accession no. of NCBI Identity | E value |
|---|---|---|---|---|---|---|
| 1. | KL1 | *Pseudomonas aeruginosa* strain QK-3 | 99.76 % | 100 % | MH7461061 | 0.0 |
| 2. | KT1 | *Pseudomonas aeruginosa* strain PA0504 | 100 % | 100 % | MK607451.1 | 0.0 |
| 3. | MP1 | *Ochrobactrum* sp. strain QY-1 | 99.85 % | 100 % | MN263248.1 | 0.0 |
| 4. | FR1 | *Ochrobactrum pseudogrignonensis* strain OsEnb HZB H6 | 100 % | 100 % | MN889402.1 | 0.0 |
| 5. | EKM1 | *Ochrobactrum* sp. strain QY-1 | 99.92 % | 100 % | MK351298.1 | 0.0 |
| 6. | HI1 | *Ochrobactrum anthropi* strain DP5 | 100 % | 100 % | MG550982.1 | 0.0 |
| 7. | HQ1 | *Stenotrophomonas maltophilla* | 99.86 % | 100 % | MK600536.1 | 0.0 |
| 8. | HS1 | *Ochrobactrum* sp. strain QY-1 | 99.77 % | 100 % | MN263248.1 | 0.0 |
| 9. | CF1 | *Photorhabdus luminescens* strain SG-HR4 | 99.56 % | 100 % | JX221723.1 | 0.0 |

#### 4.2.2.3 Phylogenetic analysis

Nine *16S rRNA* sequences of bacterial isolates generated in the study, and 25 accessions of *Ochrobactrum* spp., seven accessions of *Photorhabdus luminiscens*, 10 sequences of *Pseudomonas aeruginosa* and five accessions of *Stenotrophomonas* spp. retrieved from NCBI database (Table 5) were used to construct the phylogenetic tree (Figure 2).

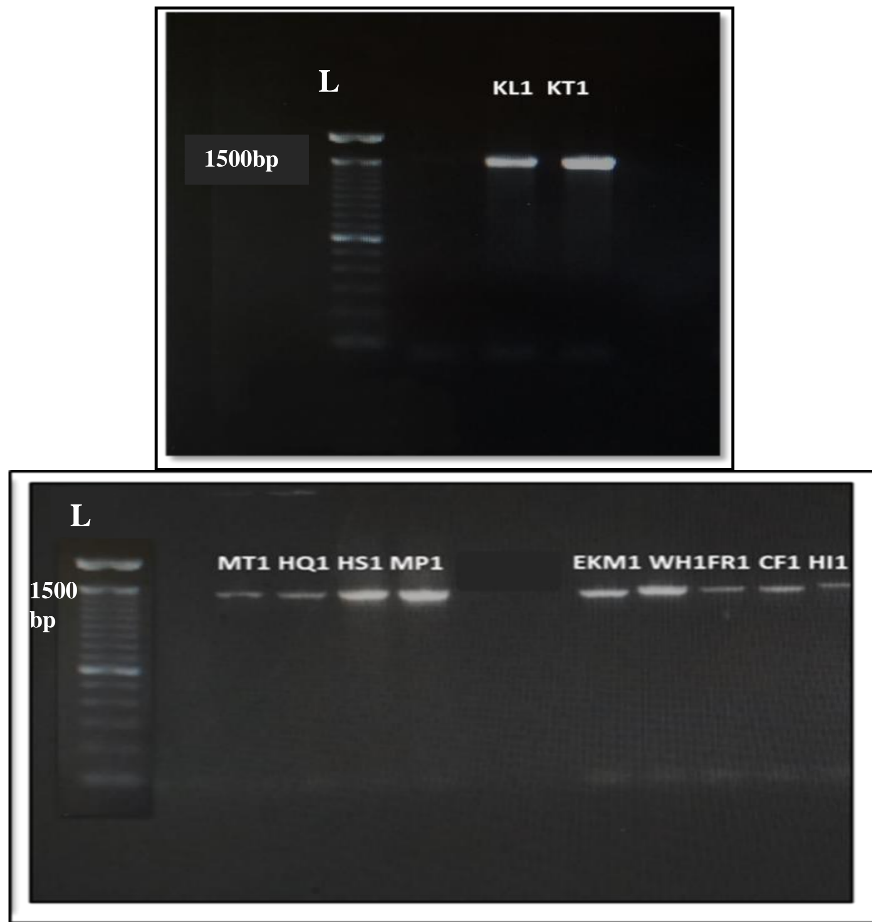



**Plate 10. Gel picture showing the amplified bands of 1.5Kb *16S rRNA* of different isolates**

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| Job Title | Contig1 CF1 |
| RID | H8GSY907014 Search expires on 07-20 20:57 pm Download All |
| Program | BLASTN Citation |
| Database | nt See details |
| Query ID | lcl\|Query_49427 |
| Description | Contig1 CF1 |
| Molecule type | dna |
| Query Length | 1376 |
| Other reports | Distance tree of results MSA viewer |

Filter Results

Organism *only top 20 will appear* exclude

Type common name, binomial, taxid or group name

Add organism

Percent Identity to | E value to | Query Coverage to

Filter | Reset

Descriptions | Graphic Summary | Alignments | Taxonomy

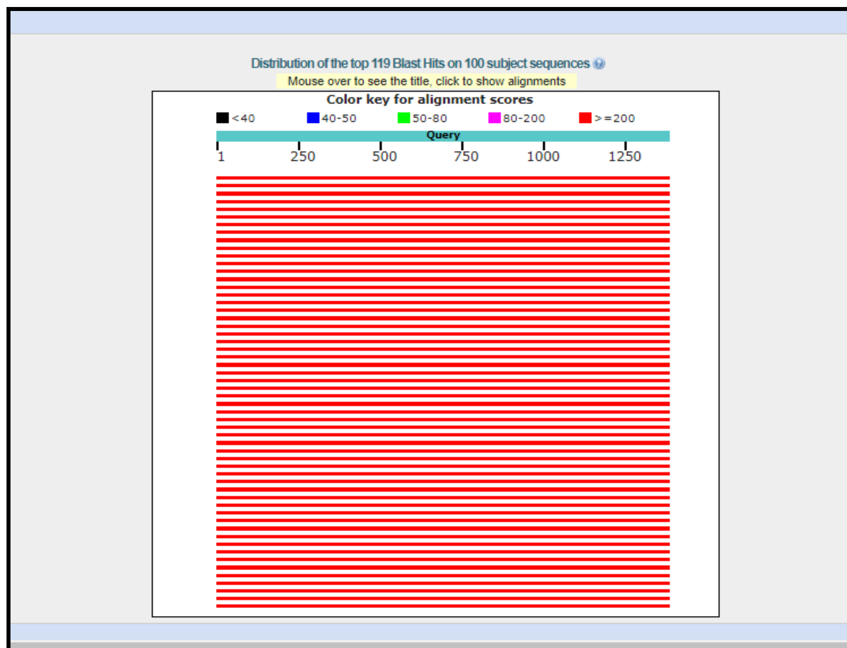
Sequences producing significant alignments | Download | Manage Columns | Show 100

select all *100 sequences selected* | GenBank | Graphics | Distance tree of results

| Description | Max Score | Total Score | Query Cover | E value | Per. Ident | Accession |
|---|---|---|---|---|---|---|
| Photorhabdus luminescens strain SG-HR4 16S ribosomal RNA gene, partial sequence | 2507 | 2507 | 100% | 0.0 | 99.56% | JX221723.1 |
| Photorhabdus luminescens subsp. akhurstii strain EG2 16S ribosomal RNA gene, partial sequence | 2507 | 2507 | 100% | 0.0 | 99.56% | AY278644.1 |
| Photorhabdus luminescens strain NBAII Hb105 16S ribosomal RNA gene, partial sequence | 2501 | 2501 | 100% | 0.0 | 99.49% | KF780170.1 |
| Photorhabdus luminescens subsp. akhurstii strain IND 16S ribosomal RNA gene, partial sequence | 2501 | 2501 | 100% | 0.0 | 99.49% | AY278643.1 |
| Photorhabdus luminescens 16S rRNA gene | 2499 | 2499 | 100% | 0.0 | 99.42% | Y17605.1 |
| Photorhabdus luminescens strain VITICRI 16S ribosomal RNA gene, partial sequence | 2495 | 2495 | 100% | 0.0 | 99.42% | JN834009.1 |
| Photorhabdus luminescens strain LPP30 16S ribosomal RNA gene, partial sequence | 2495 | 2495 | 100% | 0.0 | 99.42% | JN200818.1 |
| Photorhabdus luminescens strain SZL62 16S ribosomal RNA gene, partial sequence | 2481 | 2481 | 100% | 0.0 | 99.13% | MN630636.1 |
| Photorhabdus luminescens subsp. akhurstii strain HiB2N 16S ribosomal RNA gene, partial sequence | 2479 | 2479 | 100% | 0.0 | 99.20% | KX022122.1 |
| Photorhabdus luminescens subsp. akhurstii gene for 16S rRNA, partial sequence, strain: LN2 | 2479 | 2479 | 100% | 0.0 | 99.20% | AB355866.1 |
| Photorhabdus sp. KcTs129 gene for 16S rRNA, partial sequence | 2479 | 2479 | 100% | 0.0 | 99.20% | AB355864.1 |
| Photorhabdus luminescens strain H3 16S ribosomal RNA gene, partial sequence | 2477 | 2477 | 100% | 0.0 | 99.13% | KJ995730.1 |
| Photorhabdus luminescens strain SG-NGP 16S ribosomal RNA gene, partial sequence | 2477 | 2477 | 99% | 0.0 | 99.13% | JX240394.1 |
| Photorhabdus luminescens strain MAP 16S ribosomal RNA gene, partial sequence | 2473 | 2473 | 100% | 0.0 | 99.06% | MT658665.1 |
| Photorhabdus sp. OnKn2 gene for 16S rRNA, partial sequence | 2473 | 2473 | 100% | 0.0 | 99.13% | [illegible] |

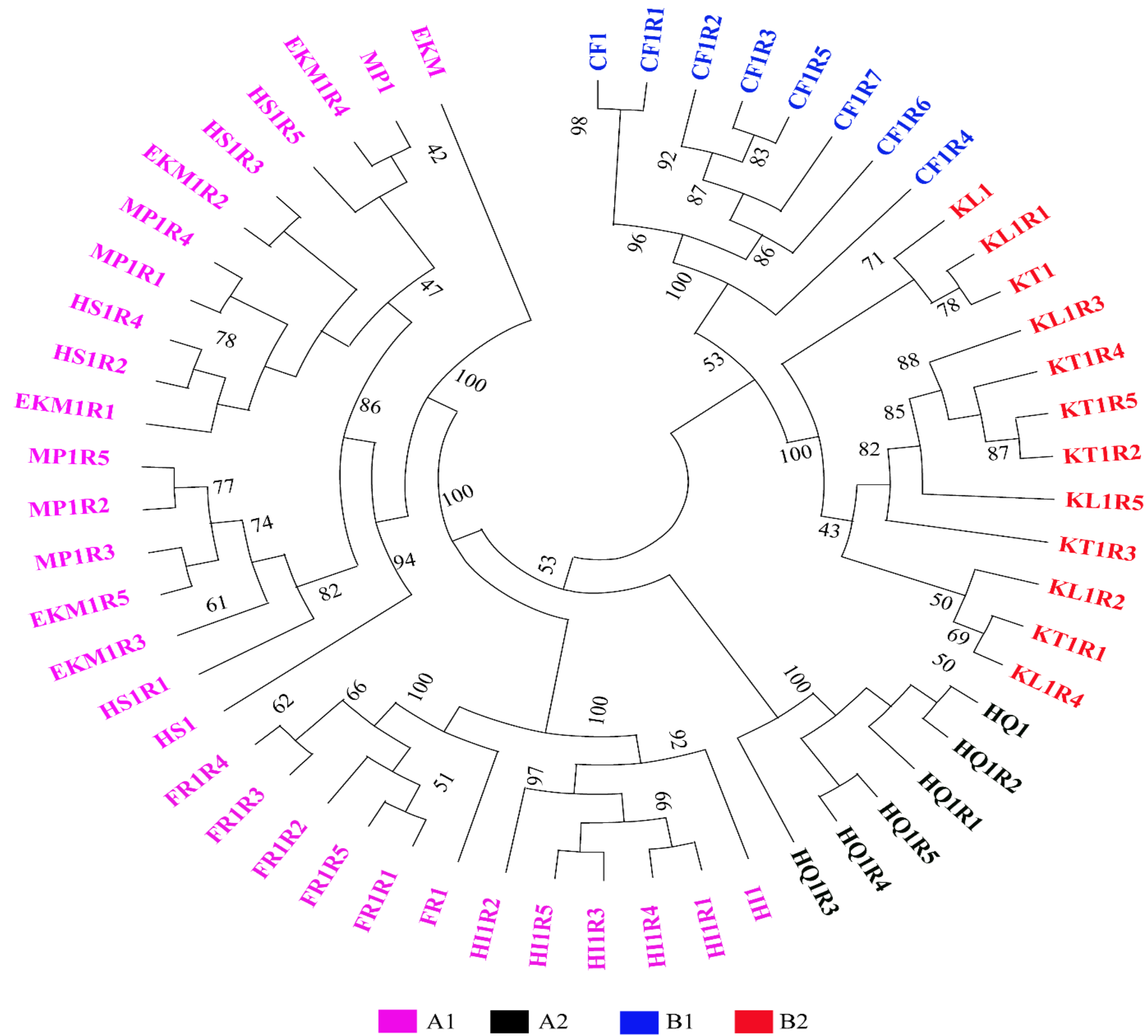
Feedback

**(A)**




**(B)**

**Figure 1. (A) and (B) BLAST search for the isolate CF1**




**A1-** *Ochrobactrum* spp.

**A2**- *Stenotrophomonas* sp.

**B1**- *Photorhabdus luminescens*

**B2**- *Pseudomonas aeruginosa*

**Figure 2. Phylogenetic tree representing the relationship between the bacterial isolates**

**A1, A2, B1 and B2 are representing different bacteria**

**Table 15. Phylogenetic tree description**

| Main cluster | Sub cluster | | Accessions | |
|---|---|---|---|---|
| A | A1 | A1a | EKM1, MP1, HS1, EKM1R4, HS1R5, HS1R3, EKM1R2, MP1R4, MP1R1, HS1R4, HS1R2, EKM1R1, MP1R5, MP1R2, MP1R3, EKM1R5, EKM1R3, HS1R1, | *Ohrobactrum* spp. |
| | | A1b | FR1R4, FR1R3, FR1R2, FR1R5, FR1R1, FR1, HI1R2, HI1R5, HI1R3, HI1R4, HI1R1, HI1 | |
| | A2 | A2a | HQ1, HQ1R2, HQ1R1, HQ1R5, HQ1R4 | *Stenotrophomonas* sp. |
| | | A2b | HQ1R3 | |
| B | B1 | B1a | CF1, CF1R1, CF1R2, CF1R3, CF1R5, CF1R7, CF1R6 | *Photorhabdus luminescens* |
| | | B1b | CF1R4 | |
| | B2 | B2a | KL1, KL1R1, KT1 | *Pseudomonas aeruginosa* |
| | | B2b | KL1R3, KT1R4, KT1R5, KT1R2, KL1R5, KT1R3, KL1R2, KT1R1, KL1R4 | |

The phylogenetic tree had shown two clusters, A and B. Cluster A had A1 subcluster with all the sequences of *Ochrobactrum* clustered and A2 with all the accessions of *Stenotrophomonas.* The sub cluster A1 was further divided into two branches - A1a and A1b. The sequences of three accessions of *Ochrobactrum* generated in the study (EKM1, MP1 and HS1), fifteen retrieved sequences of *Ochrobactrum viz.*, EKM1R4 (JX646632.1), HS1R5 (LN851900.1), HS1R3 (KT992334.1), EKM1R2 (KJ018990.1), MP1R4 (MG008507.1), MP1R1 (KF826289.1), HS1R4 (KX832688.1), HS1R2 (MK351298.1), EKM1R1 (KR149612.1), MP1R5 (MF062571.1), MP1R2 (AM490610.1), MP1R3 (DQ305290.1), EKM1R5 (MT742989.1), EKM1R3 (KF026284.1), and HS1R1 (MN263248.1) formed the A1a group representing the species of *Ochrobactrum* with maximum bootstrap per cent of 100. However, the accession EKM1 (*Ochrobactrum* sp.) formed an out-group within A1a. While A1b (bootstrap per cent- 100) includes two accessions, generated from the study (HI1 and FR1) and ten retrieved accessions, FR1R4 (MN889372.1), FR1R3 (MN889382.1), FR1R2 (MN889385.1), FR1R5 (MK165125.1), FR1R1 (MN889402.1), HI1R2 (MT083950.1), HI1R5 (MN252068.1), HI1R3(MT081283.1), HI1R4 (MG550982.1), and HI1R1(MT534544.1) (*Ochrobactrum pseudogrignonensis, Ochrobactrum anthropi* and some unidentified *Ochrobactrum* sp).

Similarly, cluster B had sub clusters B1 with the accessions of *Photorhabdus luminiscens.* It was branched into B1a with the accessions CF1 (generated in the study), and retrieved sequences of CF1R1 (JX221723.1), CF1R2 (AY278644.1), CF1R3 (KF780170.1), CF1R5 (Y17605.1), CF1R7 (AB355866.1), CF1R6 (JN200818.1) and B1b with the accession, CF1R4 (KT963833.1) as an out-group. Sub cluster B2 had *Pseudomonas aeruginosa,* with B2a having two accessions generated in the study, KL1 and KT1 with one retrieved accession KL1R1 (MH746106.1) and B2b accommodating accessions KL1R3 (MH010896.1), KT1R4 (MT598024.1), KT1R5 (MT598022.1), KT1R2 (MT633047.1), KL1R5 (MT598026.1), KT1R3 (MT626658.1), KL1R2 (KX778119.1), KT1R1 (MT646431.1), and KL1R4 (KF984154.1).

### 4.3 Bioassay of different bacterial isolates against Spider mites

Bio-efficacy of cell suspension and cell free supernatants of the bacterial isolates, (1 isolate of *P. luminescens*, 2 isolates of *P. aeruginosa*, and 2 isolates of *Ochrobactrum* sp.) were evaluated at different concentrations against eggs and adults of *T. truncatus* separately in the laboratory. Bacterial cell count of the mother culture was taken by serial dilution and colony counting method. An average of 250 colonies (*P. luminescens*) were produced from the 3 replications at $10^{-6}$ dilution which indicates presence of $2.5\times10^{8}$ Cells/ml in the dilution. The other dilutions ($10^{7}$, $10^{6}$, $10^{5}$ and $10^{4}$ Cells/ml) were prepared by serial dilution.

#### 4.3.1 Ovicidal effect of bacterial cell suspension of different bacterial isolates against *Tetranychus truncatus*

The effect of cell suspensions of different bacterial isolates on the eggs of *T. truncatus* at various concentrations is furnished in the Table 16. The ovicidal assay showed that the eggs did not hatch in any of the treatments until 24 h. After 48 h, highest hatchability was observed in the treatment $10^{5}$ Cells/ml of *P. luminescens* (T22- 25.33 %). Hatchability varied from 4.00 - 22.67 per cent in other treatments. At 72 h, 92.00 per cent of hatchability was observed in the treatments $10^{5}$ Cells/ml of *Ochrobactrum* sp. strain 1 (T2), $10^{8}$ Cells/ml of *Ochrobactrum* sp. strain 1 (T5) and $10^{6}$ Cells/ml of *Ochrobactrum* sp. strain 2 (T8). These were followed by $10^{7}$ Cells/ml of *Ochrobactrum* sp. strain 1 (T4- 89.33 %), $10^{8}$ Cells/ml of *Ochrobactrum* sp. strain 2 (T10- 88.00 %), $10^{7}$ Cells/ml of *Ochrobactrum* sp. strain 2 (T9- 88.00 %), $10^{5}$ Cells/ml of *Ochrobactrum* sp. strain 2 (T7- 88.00 %), $10^{4}$ Cells/ml of *Ochrobactrum* sp. strain 2 (T6- 86.67 %), $10^{5}$ Cells/ml of *Ochrobactrum* sp. strain 1 (T1- 86.67 %), $10^{6}$ Cells/ml of *Ochrobactrum* sp. strain 1 (T3- 77.33 %), $10^{7}$ Cells/ml of *P. aeruginosa* strain 1 (T14-77.33 %) and $10^{4}$ Cells/ml of *P. aeruginosa* strain 1 (T11- 76.00 %) which were on par with each other. The treatments$10^{5}$ Cells/ml of *P. aeruginosa* strain 1 (T12- 68.00 %), $10^{6}$ Cells/ml of *P. aeruginosa* strain 1 (T13- 66.67 %), $10^{7}$ Cells/ml of *P. aeruginosa* strain 2 (T19- 62.67 %), $10^{6}$ Cells/ml of *P. aeruginosa* strain 2 (T18- 60.00 %), $10^{6}$ Cells/ml of *P. luminescens* (T23- 58.67 %), $10^{7}$ Cells/ml of *P. luminescens* (T24- 57.33 %) and $10^{8}$ Cells/ml of *P. aeruginosa*

strain 2 (T20- 56.00 %) recorded mortality on par with each other. This was followed by $10^8$ Cells/ml of *P. luminescens* (T25- 53.33 %), $10^4$ Cells/ml of *P. luminescens* (T21- 53.33 %), $10^8$ Cells/ml of *P. aeruginosa* strain 1 (T15- 53.33 %), $10^5$ Cells/ml of *P. luminescens* (T22- 52.00 %), $10^5$ Cells/ml of *P. aeruginosa* strain 2 (T17- 52.00 %) and $10^4$ Cells/ml of *P. aeruginosa* strain 2 (T16- 49.33 %).

At 96 h, highest hatchability of 97.33 per cent was recorded in the treatment $10^5$ Cells/ml of *Ochrobactrum* sp. strain 2 (T7). This was followed by $10^5$ Cells/ml of *Ochrobactrum* sp. strain 1 (T2- 96.00 %), $10^6$ Cells/ml of *Ochrobactrum* sp. strain 2 (T8- 96.00 %), $10^4$ Cells/ml of *Ochrobactrum* sp. strain 1 (T1- 94.67 %), $10^8$ Cells/ml of *Ochrobactrum* sp. strain 2 (T10- 94.67 %), $10^7$ Cells/ml of *Ochrobactrum* sp. strain 2 (T9- 93.33 %), $10^8$ Cells/ml of *Ochrobactrum* sp. strain 1 (T5- 93.33 %), $10^7$ Cells/ml of *Ochrobactrum* sp. strain 1 (T4- 92.00 %), $10^4$ Cells/ml of *Ochrobactrum* sp. strain 2 (T6- 92.00 %), $10^6$ Cells/ml of *Ochrobactrum* sp. strain 1 (T3- 86.67 %), $10^7$ Cells/ml of *P. aeruginosa* strain 2 (T19- 82.67 %), $10^7$ Cells/ml of *P. aeruginosa* strain 1 (T14- 82.67 %), $10^4$ Cells/ml of *P. aeruginosa* strain 1 (T11- 80.00 %), $10^7$ Cells/ml of *P. luminescens* (T24- 78.67 %), $10^6$ Cells/ml of *P. aeruginosa* strain 2 (T18- 78.67 %), $10^6$ Cells/ml of *P. aeruginosa* strain 1 (T13-77.33 %), $10^6$ Cells/ml of *P. luminescens* (T23- 76.00 %) which were on par with each other. The treatments $10^4$ Cells/ml of *P. aeruginosa* strain 2 (T16), $10^5$ Cells/ml of *P. aeruginosa* strain 1 (T12), $10^4$ Cells/ml of *P. luminescens* (T21), $10^5$ Cells/ml of *P. luminescens* (T22), $10^8$ Cells/ml of *P. aeruginosa* strain 2 (T20), $10^5$ Cells/ml of *P. aeruginosa* strain 2 (T17), $10^8$ Cells/ml of *P. aeruginosa* strain 1 (T15), $10^8$ Cells/ml of *P. luminescens* (T25) has recorded 76.00, 74.67, 74.67, 72.00, 72.00, 72.00, 70.67, 69.33, per cent of hatchability respectively.

At 96 h, significantly higher mortality of *T. truncatus* eggs was observed in the treatment $10^8$ Cells/ml of *P. luminescens* (T25- 30.67 %) which was on par with the treatments $10^8$ Cells/ml of *P. aeruginosa* strain 1 (T15- 29.33 %), $10^5$ Cells/ml of *P. luminescens* (T22- 28.00 %), $10^8$ Cells/ml of *Pseudomonas aeruginosa* strain 2 (T20- 28.00 %). $10^5$ Cells/ml of *P. aeruginosa* strain 2 (T17- 28.00 %), $10^5$ Cells/ml of *P. aeruginosa* strain 1 (T12- 25.33 %), $10^4$ Cells/ml of *P. luminescens* (T21- 25.33 %), $10^6$ Cells/ml of *P. luminescens* (T23- 24.00 %), $10^4$ Cells/ml of

*P. aeruginosa* strain 2 (T16- 24.00 %), $10^6$ Cells/ml of *P. aeruginosa* strain 1 (T13- 22.67 %), $10^7$ Cells/ml of *P. luminescens* (T24- 21.33 %), $10^6$ Cells/ml of *P. aeruginosa* strain 2 (T18- 21.33 %), $10^4$ Cells/ml of *P. aeruginosa* strain 1 (T11- 20.00 %), $10^7$ Cells/ml of *P. aeruginosa* strain 2 (T19- 17.33 %), $10^7$ Cells/ml of *P. aeruginosa* strain 1 (T14- 17.33 %) and $10^6$ Cells/ml of *Ochrobactrum* sp. strain 1 (T3- 13.33 %). These were followed by the treatment $10^7$ Cells/ml of *Ochrobactrum* sp. strain 1 (T4) and $10^4$ Cells/ml of *Ochrobactrum* sp. strain 2(T6), both recording 8.00 per cent mortality. The treatments $10^7$ Cells/ml of *Ochrobactrum* sp. strain 2 (T9), $10^8$ Cells/ml of *Ochrobactrum* sp. strain 1 (T5), $10^4$ Cells/ml of *Ochrobactrum* sp. strain 1 (T1), $10^8$ Cells/ml of *Ochrobactrum* sp. strain 2 (T10), $10^6$ Cells/ml of *Ochrobactrum* sp. strain 2 (T8) $10^5$ Cells/ml of *Ochrobactrum* sp. strain 1 (T2) and $10^5$ Cells/ml of *Ochrobactrum* sp. strain 2 (T7) recorded 6.67, 6.67, 5.33, 5.33, 4.00, 4.00 and 2.67 per cent mortality, respectively.

**Table 16. Ovicidal effect of cell suspension of bacterial isolates against *Tetranychus truncatus***

| Treatment | | Hatchability (%) | | | | Mortality (%) |
|---|---|---|---|---|---|---|
| | | **24 h** | **48 h** | **72 h** | **96 h** | **96 h** |
| T1 | $10^4$ Cells/ml of *Ochrobactrum* sp. strain 1 | 0.00 (0.00) | 10.67$^{abcde}$ (3.18) | 86.67$^{ab}$ (1.21) | 94.67$^{ab}$ (9.73) | 5.33$^{efg}$ (2.17) |
| T2 | $10^5$ Cells/ml of *Ochrobactrum* sp. strain 1 | 0.00 (0.00) | 10.67$^{abcde}$ (3.33) | 92.00$^{a}$ (1.29) | 96.00$^{ab}$ (9.80) | 4.00$^{efg}$ (2.12) |
| T3 | $10^6$ Cells/ml of *Ochrobactrum* sp. strain 1 | 0.00 (0.00) | 8.00$^{cde}$ (2.45) | 77.33$^{abcd}$ (1.08) | 86.67$^{abcd}$ (9.30) | 13.33$^{abcdefg}$ (3.45) |
| T4 | $10^7$ Cells/ml of *Ochrobactrum* sp. strain 1 | 0.00 (0.00) | 16.00$^{abcde}$ (4.05) | 89.33$^{ab}$ (1.23) | 92.00$^{abc}$ (9.59) | 8.00$^{bcdefg}$ (2.85) |
| T5 | $10^8$ Cells/ml of *Ochrobactrum* sp. strain 1 | 0.00 (0.00) | 8.00$^{abcde}$ (2.85) | 92.00$^{a}$ (1.29) | 93.33$^{ab}$ (9.66) | 6.67$^{cdefg}$ (2.66) |
| T6 | $10^4$ Cells/ml of *Ochrobactrum* sp. strain 2 | 0.00 (0.00) | 8.00$^{abcde}$ (2.85) | 86.67$^{ab}$ (1.21) | 92.00$^{abc}$ (9.59) | 8.00$^{defg}$ (2.45) |
| T7 | $10^5$ Cells/ml of *Ochrobactrum* sp. strain 2 | 0.00 (0.00) | 9.33$^{abcde}$ (3.04) | 88.00$^{ab}$ (1.22) | 97.33$^{a}$ (9.86) | 2.67$^{g}$ (1.65) |
| T8 | $10^6$ Cells/ml of *Ochrobactrum* sp. strain 2 | 0.00 (0.00) | 4.00$^{e}$ (1.92) | 92.00$^{a}$ (1.29) | 96.00$^{ab}$ (9.80) | 4.00$^{fg}$ (1.92) |
| T9 | $10^7$ Cells/ml of *Ochrobactrum* sp. strain 2 | 0.00 (0.00) | 13.33$^{abcde}$ (3.54) | 88.00$^{ab}$ (1.22) | 93.33$^{ab}$ (9.66) | 6.67$^{cdefg}$ (2.66) |
| T10 | $10^8$ Cells/ml of *Ochrobactrum* sp. strain 2 | 0.00 (0.00) | 6.67$^{bcde}$ (2.66) | 88.00$^{ab}$ (1.23) | 94.67$^{ab}$ (9.72) | 5.33$^{efg}$ (2.12) |

**Table 16. continued**

| | | | | | | |
|---|---|---|---|---|---|---|
| T11 | $10^4$ Cells/ml of *P. aeruginosa* strain 1 | 0.00 (0.00) | 8.00$^{cde}$ (2.57) | 76.00$^{abcde}$ (1.07) | 80.00$^{abcd}$ (8.94) | 20.00$^{abcde}$ (4.44) |
| T12 | $10^5$ Cells/ml of *P. aeruginosa* strain 1 | 0.00 (0.00) | 12.00$^{abcde}$ (3.33) | 68.00$^{bcdef}$ (0.97) | 74.67$^{bcd}$ (8.63) | 25.33$^{ab}$ (5.05) |
| T13 | $10^6$ Cells/ml of *P. aeruginosa* strain 1 | 0.00 (0.00) | 9.33$^{abcde}$ (3.12) | 66.67$^{bcdef}$ (0.99) | 77.33$^{abcd}$ (8.76) | 22.67$^{abcde}$ (4.47) |
| T14 | $10^7$ Cells/ml of *P. aeruginosa* strain 1 | 0.00 (0.00) | 13.33$^{abcde}$ (3.66) | 77.33$^{abcd}$ (1.09) | 82.67$^{abcd}$ (9.09) | 17.33$^{abcdef}$ (4.18) |
| T15 | $10^8$ Cells/ml of *P. aeruginosa* strain 1 | 0.00 (0.00) | 6.67$^{de}$ (2.30) | 53.33$^{def}$ (0.82) | 70.67$^{d}$ (8.27) | 29.33$^{a}$ (4.18) |
| T16 | $10^4$ Cells/ml of *P. aeruginosa* strain 2 | 0.00 (0.00) | 6.67$^{bcde}$ (2.66) | 49.33$^{f}$ (0.77) | 76.00$^{bcd}$ (8.66) | 24.00$^{abcd}$ (4.59) |
| T17 | $10^5$ Cells/ml of *P. aeruginosa* strain 2 | 0.00 (0.00) | 16.00$^{abcde}$ (4.05) | 52.00$^{ef}$ (0.80) | 72.00$^{cd}$ (8.46) | 28.00$^{ab}$ (5.20) |
| T18 | $10^6$ Cells/ml of *P. aeruginosa* strain 2 | 0.00 (0.00) | 20.00$^{abcd}$ (4.46) | 60.00$^{cdef}$ (0.89) | 78.67$^{abcd}$ (8.87) | 21.33$^{abcd}$ (4.65) |
| T19 | $10^7$ Cells/ml of *P. aeruginosa* strain 2 | 0.00 (0.00) | 20.00$^{abc}$ (4.51) | 62.67$^{cdef}$ (0.91) | 82.67$^{abcd}$ (9.09) | 17.33$^{abcdef}$ (4.20) |
| T20 | $10^8$ Cells/ml of *P. aeruginosa* strain 2 | 0.00 (0.00) | 20.00$^{abcd}$ (4.38) | 56.00$^{cdef}$ (0.85) | 72.00$^{cd}$ (8.46) | 28.00$^{a}$ (5.28) |
| T21 | $10^4$ Cells/ml of *P. luminescens* | 0.00 (0.00) | 18.67$^{abcd}$ (4.34) | 53.33$^{def}$ (0.82) | 74.67$^{bcd}$ (8.62) | 25.33$^{abc}$ (4.98) |

**Table 16. continued**

| T22 | $10^5$ Cells/ml of *P. luminescens* | 0.00 (0.00) | 25.33$^{a}$ (4.98) | 52.00$^{ef}$ (0.81) | 72.00$^{cd}$ (8.48) | 28.00$^{a}$ (5.31) |
|---|---|---|---|---|---|---|
| T23 | $10^6$ Cells/ml of *P. luminescens* | 0.00 (0.00) | 16.00$^{abcde}$ (3.98) | 58.67$^{cdef}$ (0.88) | 76.00$^{abcd}$ (8.69) | 24.00$^{abcd}$ (4.80) |
| T24 | $10^7$ Cells/ml of *P. luminescens* | 0.00 (0.00) | 21.33$^{abc}$ (4.54) | 57.33$^{cdef}$ (0.86) | 78.67$^{abcd}$ (8.87) | 21.33$^{abcd}$ (4.65) |
| T25 | $10^8$ Cells/ml of *P. luminescens* | 0.00 (0.00) | 22.67$^{ab}$ (4.78) | 53.33$^{def}$ (0.81) | 69.33$^{d}$ (8.39) | 30.67$^{a}$ (5.41) |
| T26 | Control | 0.00 (0.00) | 14.67$^{abcde}$ (3.66) | 78.67$^{abc}$ (1.10) | 92.00$^{abc}$ (9.59) | 8.00$^{bcdefg}$ (2.85) |
| **SE(m)** | | | **0.63** | **0.08** | **0.34** | **0.59** |

Post hoc analysis was carried out by DMRT
Figures in the parenthesis of columns
48 and 96 hours mortality are square root transformed means($\sqrt{X = 0.5}$)
72 hours are arc sin transformed means
96 hours is square root transformed means ($\sqrt{X}$)

### 4.3.2 Adulticidal effect of cell suspension of bacterial isolates against *Tetranychus truncatus*

The effect of cell suspension of different bacterial isolates on the gravid females of *T. truncatus* is furnished in the Table 17. No significant mortality was observed in any of the treatments at 24 h of treatment. At 48 h, the treatments $10^8$ Cells/ml (T25), $10^7$ Cells/ml (T24) and $10^6$ Cells/ml (T23) of *P. luminescens* recorded significantly higher mortality of 34.67, 34.67 and 34.00 per cent respectively and were on par with $10^4$ Cells/ml of *P .luminescens* (T21- 22.67 %), $10^8$ Cells/ml of *P. aeruginosa* strain 1 (T15- 20.00 %), $10^6$ Cells/ml of *P. aeruginosa* strain 1 (T13- 20.00 %), $10^6$ Cells/ml of *P. aeruginosa* strain 2 (T18- 18.67 %), and $10^4$ Cells/ml of *P. aeruginosa* strain 1 (T11- 18.67 %). This was followed by $10^5$ Cells/ml of *P. luminescens* (T22), $10^7$ Cells/ml of *P. aeruginosa* strain 2 (T19), $10^7$ Cells/ml of *P. aeruginosa* strain 1 (T14), $10^5$ Cells/ml of *P. aeruginosa* strain 2 (T17), $10^4$ Cells/ml of *P. aeruginosa* strain 2 (T16) which were on par with each other by showing 18.67, 18.67, 17.33, 12.00 and 10.67 per cent of mortality respectively.

At 72 h, $10^8$ Cells/ml of *P. luminescens* (T25- 58.00 %) recorded significantly higher mortality, followed by $10^5$ Cells/ml of *P. luminescens* (T22- 48.00 %), $10^4$ Cells/ml of *P. luminescens* (T21- 46.67 %), $10^6$ Cells/ml of *P. luminescens* (T23- 41.33 %) and $10^7$ Cells/ml of *P. luminescens* (T24- 33.33 %) which were on par with each other. This was followed by $10^8$ Cells/ml of *P. aeruginosa* strain 1 (T15) and $10^7$ Cells/ml of *P. aeruginosa* strain 2 (T19) recording mortality of 29.33 and 26.67 per cent, respectively and on par with each other. The treatments $10^6$ Cells/ml of *P. aeruginosa* strain 1 (T13- 25.33 %), $10^6$ Cells/ml of *P. aeruginosa* strain 2 (T18- 25.33 %), $10^5$ Cells/ml of *P. aeruginosa* strain 2 (T17- 22.67 %), $10^7$ Cells/ml of *P. aeruginosa* strain 1 (T14- 22.67 %), $10^4$ Cells/ml of *P. aeruginosa* strain 1(T11- 22.67 %), $10^8$ Cells/ml of *P. aeruginosa* strain 2 (T20- 21.33 %) and $10^4$ Cells/ml of *P. aeruginosa* strain 2 (T16- 20.00 %) recorded mortality on par with each other. All the remaining treatments recorded mortality inferior to the above treatments, though showed significantly higher mortality than control.

At 96 h also the treatment $10^8$ Cells/ml of *P. luminescens* (T25) recorded significantly higher mortality (60.67 %). The treatments $10^7$ Cells/ml of *P. luminescens* (T24- 52.00 %), $10^5$ Cells/ml of *P. luminescens* (T22- 50.67 %), $10^6$ Cells/ml of *P. luminescens* (T23- 49.33 %), and $10^4$ Cells/ml of *P. luminescens* (T21- 49.33 %) were found to be the next best and were on par with each other. This was followed by$10^8$ Cells/ml of *P. aeruginosa* strain 2 (T20- 33.33 %), $10^6$ Cells/ml of *P. aeruginosa* strain 2 (T18- 32.00 %), $10^8$ Cells/ml of *P. aeruginosa* strain 1 (T15- 32.00 %), $10^4$ Cells/ml of *P. aeruginosa* strain 2 (T16- 30.67 %), $10^7$Cells/ml of *P. aeruginosa* strain 2 (T19- 29.33 %), $10^5$ Cells/ml of *P. aeruginosa* strain 2 (T17- 25.33 %), $10^6$ Cells/ml of *P. aeruginosa* strain 1 (T13- 25.33 %), $10^4$ Cells/ml of *P. aeruginosa* strain 1 (T11- 24.00 %) and $10^7$ Cells/ml of *P. aeruginosa* strain 1 (T14- 22.67 %) which were on par with each other. The remaining treatments recorded significantly lower mortality than the above treatments, though found superior to control.

**Table 17. Adulticidal effect of cell suspension of bacterial isolate against *Tetranychus truncatus***

| **Treatment** | | **Mortality** (%) | | | |
|---|---|---|---|---|---|
| | | **24 h** | **48 h** | **72 h** | **96 h** |
| T1 | $10^4$ Cells/ml of *Ochrobactrum* sp. strain 1 | 1.33 (2.65) | $6.67^{defg}$ (2.66) | $12.00^{ghij}$ (0.36) | $12.00^{efg}$ (0.36) |
| T2 | $10^5$ Cells/ml of *Ochrobactrum* sp. strain 1 | 0.00 (2.86) | $8.00^{cdefg}$ (2.85) | $8.00^{ijk}$ (0.29) | $8.00^{fgh}$ (0.29) |
| T3 | $10^6$ Cells/ml of *Ochrobactrum* sp. strain 1 | 0.00 (1.91) | $4.00^{efg}$ (1.92) | $5.33^{jk}$ (0.23) | $6.67^{gh}$ (0.25) |
| T4 | $10^7$ Cells/ml of *Ochrobactrum* sp. strain 1 | 0.00 (1.65) | $2.67^{fg}$ (1.65) | $5.33^{jk}$ (0.21) | $5.33^{gh}$ (0.21) |
| T5 | $10^8$ Cells/ml of *Ochrobactrum* sp. strain 1 | 0.00 (1.91) | $4.00^{efg}$ (1.92) | $6.67^{ijk}$ (0.25) | $6.67^{gh}$ (0.25) |
| T6 | $10^4$ Cells/ml of *Ochrobactrum* sp. strain 2 | 0.00 (2.39) | $5.33^{efg}$ (2.38) | $8.00^{ijk}$ (0.29) | $10.67^{fg}$ (0.32) |
| T7 | $10^5$ Cells/ml of *Ochrobactrum* sp. strain 2 | 2.67 (1.65) | $4.00^{fg}$ (1.65) | $5.33^{jk}$ (0.21) | $8.00^{fgh}$ (0.29) |
| T8 | $10^6$ Cells/ml of *Ochrobactrum* sp. strain 2 | 5.33 (2.77) | $8.00^{cdefg}$ (2.76) | $10.67^{hijk}$ (0.32) | $10.67^{fgh}$ (0.32) |
| T9 | $10^7$ Cells/ml of *Ochrobactrum* sp. strain 2 | 1.33 (1.65) | $2.67^{fg}$ (1.65) | $12.00^{ghij}$ (0.35) | $12.00^{efg}$ (0.35) |
| T10 | $10^8$ Cells/ml of *Ochrobactrum* sp. strain 2 | 0.00 (2.18) | $5.33^{efg}$ (2.17) | $12.00^{fghij}$ (0.36) | $12.00^{efg}$ (0.36) |
| T11 | $10^4$ Cells/ml of *P. aeruginosa* strain 1 | 0.00 (4.34) | $18.67^{abcd}$ (4.34) | $22.67^{defgh}$ (0.49) | $24.00^{cd}$ (0.50) |

**Table 17. continued**

| T12 | $10^5$ Cells/ml of *P. aeruginosa* strain 1 | 2.67<br>(2.86) | $8.00^{cdefg}$<br>(2.85) | $16.00^{efghi}$<br>(0.41) | $17.33^{def}$<br>(0.42) |
|---|---|---|---|---|---|
| T13 | $10^6$ Cells/ml of *P. aeruginosa* strain 1 | 5.33<br>(4.51) | $20.00^{abc}$<br>(4.51) | $25.33^{de}$<br>(0.52) | $25.33^{cd}$<br>(0.52) |
| T14 | $10^7$ Cells/ml of *P. aeruginosa* strain 1 | 1.33<br>(4.13) | $17.33^{bcd}$<br>(4.14) | $22.67^{defgh}$<br>(0.49) | $22.67^{cde}$<br>(0.49) |
| T15 | $10^8$ Cells/ml of *P. aeruginosa* strain 1 | 0.00<br>(4.51) | $20.00^{abc}$<br>(4.51) | $29.33^{cde}$<br>(0.57) | $32.00^{c}$<br>(0.59) |
| T16 | $10^4$ Cells/ml of *P. aeruginosa* strain 2 | 0.00<br>(3.19) | $10.67^{bcdef}$<br>(3.18) | $20.00^{defgh}$<br>(0.46) | $30.67^{c}$<br>(0.59) |
| T17 | $10^5$ Cells/ml of *P. aeruginosa* strain 2 | 0.00<br>(3.50) | $12.00^{bcde}$<br>(3.50) | $22.67^{defg}$<br>(0.50) | $25.33^{cd}$<br>(0.52) |
| T18 | $10^6$ Cells/ml of *P. aeruginosa* strain 2 | 0.00<br>(4.34) | $18.67^{abcd}$<br>(4.34) | $25.33^{def}$<br>(0.52) | $32.00^{c}$<br>(0.61) |
| T19 | $10^7$ Cells/ml of *P. aeruginosa* strain 2 | 1.33<br>(4.23) | $18.67^{bcd}$<br>(4.22) | $26.67^{cde}$<br>(0.54) | $29.33^{cd}$<br>(0.57) |
| T20 | $10^8$ Cells/ml of *P. aeruginosa* strain 2 | 2.67<br>(2.86) | $8.00^{cdefg}$<br>(2.85) | $21.33^{defgh}$<br>(0.48) | $33.33^{c}$<br>(0.61) |
| T21 | $10^4$ Cells/ml of *P. luminescens* | 1.33<br>(4.78) | $22.67^{ab}$<br>(4.78) | $46.67^{b}$<br>(0.76) | $49.33^{b}$<br>(0.77) |
| T22 | $10^5$ Cells/ml of *P. luminescens* | 0.00<br>(4.16) | $18.67^{bcd}$<br>(4.16) | $48.00^{b}$<br>(0.77) | $50.67^{b}$<br>(0.79) |
| T23 | $10^6$ Cells/ml of *P. luminescens* | 2.67<br>(6.04) | $34.00^{a}$<br>(6.05) | $33.33^{bcd}$<br>(0.62) | $49.33^{b}$<br>(0.78) |

**Table 17. continued**

| | | | | | |
|---|---|---|---|---|---|
| T24 | $10^7$ Cells/ml of *P. luminescens* | 4.00 (6.04) | 34.67$^{a}$ (6.05) | 41.33$^{bc}$ (0.70) | 52.00$^{b}$ (0.80) |
| T25 | $10^8$ Cells/ml of *P. luminescens* | 5.33 (6.04) | 34.68$^{a}$ (6.05) | 58.00$^{a}$ (0.87) | 60.67$^{a}$ (0.89) |
| T26 | Control | 0.00 (1.18) | 1.33$^{g}$ (1.18) | 2.67 (0.17) | 2.67 (0.17) |
| **SE(m)** | | **0.46** | **0.53** | **0.05** | **0.05** |

Post hoc analysis was carried out by DMRT

Figures in the parenthesis of first two durations (24 and 48 hours) are square root transformed means($\sqrt{X = 0.5}$) and the next two (72 and 96hours) are arc sin transformed means

**4.3.3 Ovicidal effect of cell free supernatant of different bacterial isolates against *Tetranychus truncatus***

The effect of cell free supernatant of different bacterial isolates on the eggs of *T. truncatus* at various concentrations is furnished in the Table 18. No hatchability was observed in any of the treatments until 24 h. Eggs started hatching only after 48 h but there was no significant differences among the treatments.

After 72 h, the treatments $10^5$ CFS/ml of *Ochrobactrum* sp. strain 2 (T7- 93.33 %) and $10^7$ CFS/ml of *Ochrobactrum* sp. strain 2 (T9- 92.00 %) recorded higher hatchability and were on par with $10^8$ CFS/ml of *Ochrobactrum* sp. strain 2 (T10- 88.00 %), $10^8$ CFS/ml of *Ochrobactrum* sp. strain 1 (T5- 86.67 %), $10^6$ CFS/ml of *Ochrobactrum* sp. strain 1 (T3- 86.67 %), $10^4$ CFS/ml of *Ochrobactrum* sp. strain 1 (T1- 86.67 %), $10^6$ CFS/ml of *Ochrobactrum* sp. strain 2 (T8- 84.00 %) $10^7$ CFS/ml of *Ochrobactrum* sp. strain 1 (T4- 82.67 %), $10^4$ CFS/ml of *Ochrobactrum* sp. strain 2 (T6- 81.33 %) and $10^5$ CFS/ml of *Ochrobactrum* sp. strain 1 (T2- 81.33 %), $10^6$ CFS/ml of *P. aeruginosa* strain 1 (T13- 76.00 %) and control (86.67 %). This was followed by $10^4$ CFS/ml of *P. aeruginosa* strain (T11) and$10^5$ CFS/ml of *P. aeruginosa* strain 2(T17) with 73.33 and 72.00 per cent of hatchability. The treatments $10^4$ CFS/ml of *P. aeruginosa* strain 2 (T16) and $10^7$ CFS/ml of *P. aeruginosa* strain 2 (T19) recorded 69.33 per cent hatchability, followed by $10^7$ CFS/ml of *P. luminescens* (T24) with 66.67 per cent hatchability. However, the treatments $10^6$ CFS/ml of *P. aeruginosa* strain 2 (T18), $10^5$ CFS/ml of *P. aeruginosa* strain 1 (T12), $10^8$ CFS/ml of *P. luminescens* (T25), $10^5$ CFS/ml of *P. luminescens* (T22), $10^7$ CFS/ml of *P. aeruginosa* strain 1 (T14), $10^6$ CFS/ml of *P. luminescens* (T23),$10^8$ CFS/ml of *P. aeruginosa* strain 2 (T20), $10^4$ CFS/ml of *P. luminescens* (T21), and $10^8$ CFS/ml of *P. aeruginosa* strain 1 (T15) recorded 65.33, 62.67, 61.33, 61.33, 56.00, 52.00, 48.00 and 44.00 per cent hatchability, respectively.

At 96 h the treatment $10^8$ Cells/ml of *Ochrobactrum* sp. strain 2 (T10) showed higher hatchability of 97.33 per cent, which was on par with the treatments $10^8$ CFS/ml of *Ochrobactrum* sp. strain 1 (T5- 96.00 %), $10^7$ CFS/ml of *Ochrobactrum* sp. strain 2 (T9- 94.67 %), $10^5$ CFS/ml of *Ochrobactrum* sp. strain 1 (T2- 94.67 %),

$10^5$ CFS/ml of *Ochrobactrum* sp. strain 2 (T7- 94.67 %), $10^7$ CFS/ml of *Ochrobactrum* sp. strain 1 (T4- 93.33 %), $10^4$ CFS/ml of *Ochrobactrum* sp. strain 1 (T1- 93.33 %), $10^4$ CFS/ml of *Ochrobactrum* sp. strain 2 (T6- 93.33 %), $10^6$ CFS/ml of *Ochrobactrum* sp. strain 1 (T3- 92.00 %) and $10^6$ CFS/ml of *Ochrobactrum* sp. strain 2 (T8- 90.67 %). This was followed by $10^5$ CFS/ml of *P. aeruginosa* strain 2 (T17- 84.00 %). The treatment $10^6$ CFS/ml of *P. aeruginosa* strain 1(T13- 81.33 %) followed the above and was followed by $10^4$ CFS/ml of *P. aeruginosa* strain 2 (T16- 80.00 %). The treatments $10^4$ CFS/ml of *P. aeruginosa* strain 1 (T11- 78.67 %), $10^5$ CFS/ml of *P. aeruginosa* strain 1 (T12- 76.00 %), $10^5$ CFS/ml of *P. luminescens* (T22- 76.00 %), $10^4$ CFS/ml of *P. luminescens* (T21- 74.67 %) and $10^7$ CFS/ml of *P. aeruginosa* strain 2 (T19- 73.33 %) were on par with each other. Remaining treatments $10^8$ CFS/ml of *P. aeruginosa* strain 2 (T20), $10^6$ CFS/ml of *P. luminescens* (T23), $10^7$ CFS/ml of *P. luminescens* (T24), $10^6$ CFS/ml of *P. aeruginosa* strain 2 (T18), $10^7$ CFS/ml of *P. aeruginosa* strain 1 (T14),$10^8$ CFS/ml of *P. aeruginosa* strain 1 (T15) and $10^8$ CFS/ml of *P. luminescens* (T25) recorded 72.00, 72.00, 72.00, 70.67, 69.33, 69.33 and 66.67 per cent mortality, respectively.

Per cent mortality of eggs at 96 h was also worked out. $10^8$ CFS/ml of *P. luminescens* (T25) recorded significantly higher mortality of 33.33 per cent on par with the treatments $10^7$ CFS/ml of *P. aeruginosa* strain 1 (T14- 30.67 %), $10^8$ CFS/ml of *P. aeruginosa* strain 1 (T15- 30.67 %), $10^6$ CFS/ml of *P. aeruginosa* strain 2 (T18- 29.33 %), $10^8$ CFS/ml of *P. aeruginosa* strain 2 (T20- 28.00 %), $10^6$ CFS/ml of *P. luminescens* (T23- 28.00 %), $10^7$ CFS/ml of *P. luminescens* (T24- 28.00 %), $10^7$ CFS/ml of *P. aeruginosa* strain 2 (T19- 26.67 %), $10^4$ CFS/ml of *P. luminescens* (T21- 25.33 %), $10^5$ CFS/ml of *P. aeruginosa* strain 1 (T12- 24.00 %), $10^5$ CFS/ml of *P. luminescens* (T22- 24.00 %), $10^4$ CFS/ml of *P. aeruginosa* strain 1 (T11- 21.33 %), $10^4$ CFS/ml of *P. aeruginosa* strain 2 (T16- 20.00 %) and $10^6$ CFS/ml of *P. aeruginosa* strain 1 (T13- 18.67 %). This was followed by the treatment $10^5$ CFS/ml of *P. aeruginosa* strain 2 (T17) with 16.00 per cent mortality which was followed by $10^6$ CFS/ml of *Ochrobactrum* sp. strain 2 (T8- 9.33 %). The remaining treatments, $10^6$ CFS/ml of *Ochrobactrum* sp. strain 2 (T3), $10^7$ CFS/ml of *Ochrobactrum* sp. strain 1 (T4), $10^4$ CFS/ml of *Ochrobactrum* sp. strain 1 (T1), $10^4$

CFS/ml of *Ochrobactrum* sp. strain 2 (T6), $10^7$ CFS/ml of *Ochrobactrum* sp. strain 2 (T9), $10^5$ CFS/ml of *Ochrobactrum* sp. strain 1 (T2), $10^5$ CFS/ml of *Ochrobactrum* sp. strain 2 (T7), $10^8$ CFS/ml of *Ochrobactrum* sp. strain 1(T5) and $10^8$ CFS/ml of *Ochrobactrum* sp. strain 2 (T10) recorded 8.00, 6.67, 6.67, 6.67, 5.33, 5.33, 5.33, 4.00, 2.67 per cent mortality respectively, and were on par with control (5.33 %).

**Table 18. Ovicidal effect of cell free supernatant of bacterial isolates against *Tetranychus***

| Treatment | | Hatchability (%) | | | | Mortality (%) |
|---|---|---|---|---|---|---|
| | | 24 h | 48 h | 72 h | 96 h | 96 h |
| T1 | $10^4$ CFS/ml of *Ochrobactrum* sp. strain 1 | 0.00 (0.00) | 4.00 (1.92) | $86.67^{ab}$ (9.31) | $93.33^{abc}$ (9.66) | $6.67^{def}$ (2.60) |
| T2 | $10^5$ CFS/ml of *Ochrobactrum* sp. strain 1 | 0.00 (0.00) | 8.00 (2.91) | $81.33^{abcd}$ (9.00) | $94.67^{ab}$ (9.72) | $5.33^{ef}$ (2.38) |
| T3 | $10^6$ CFS/ml of *Ochrobactrum* sp. strain 1 | 0.00 (0.00) | 12.00 (3.50) | $86.67^{ab}$ (9.31) | $92.00^{abc}$ (9.59) | $8.00^{def}$ (2.85) |
| T4 | $10^7$ CFS/ml of *Ochrobactrum* sp. strain 1 | 0.00 (0.00) | 12.00 (3.33) | $82.67^{abc}$ (9.09) | $93.33^{abc}$ (9.66) | $6.67^{def}$ (2.66) |
| T5 | $10^8$ CFS/ml of *Ochrobactrum* sp. strain 1 | 0.00 (0.00) | 10.67 (3.24) | $86.67^{ab}$ (9.30) | $96.00^{ab}$ (9.80) | $4.00^{ef}$ (1.92) |
| T6 | $10^4$ CFS/ml of *Ochrobactrum* sp. strain 2 | 0.00 (0.00) | 9.33 (3.12) | $81.33^{abcd}$ (9.01) | $93.33^{abc}$ (9.65) | $6.67^{ef}$ (2.38) |
| T7 | $10^5$ CFS/ml of *Ochrobactrum* sp. strain 2 | 0.00 (0.00) | 6.67 (2.66) | $93.33^{a}$ (9.65) | $94.67^{ab}$ (9.73) | $5.33^{ef}$ (2.17) |
| T8 | $10^6$ CFS/ml of *Ochrobactrum* sp. strain 2 | 0.00 (0.00) | 6.67 (2.66) | $84.00^{abc}$ (9.15) | $90.67^{abcd}$ (9.52) | $9.33^{cde}$ (3.12) |
| T9 | $10^7$ CFS/ml of *Ochrobactrum* sp. strain 2 | 0.00 (0.00) | 10.67 (3.30) | $92.00^{a}$ (9.59) | $94.67^{ab}$ (9.72) | $5.33^{ef}$ (2.38) |
| T10 | $10^8$ CFS/ml of *Ochrobactrum* sp. strain 2 | 0.00 (0.00) | 9.33 (3.12) | $88.00^{ab}$ (9.37) | $97.33^{a}$ (9.87) | $2.67^{f}$ (1.44) |
| T11 | $10^4$ CFS/ml of *P. aeruginosa* strain 1 | 0.00 (0.00) | 8.00 (2.85) | $73.33^{bcde}$ (8.56) | $78.67^{efg}$ (8.86) | $21.33^{abc}$ (4.61) |
| T12 | $10^5$ CFS/ml of *P. aeruginosa* strain 1 | 0.00 (0.00) | 13.33 (3.54) | $62.67^{efg}$ (7.91) | $76.00^{efgh}$ (8.72) | $24.00^{ab}$ (4.94) |

**Table 18. continued**

| | | | | | | |
|---|---|---|---|---|---|---|
| T13 | $10^6$ CFS/ml of *P. aeruginosa* strain 1 | 0.00 (0.00) | 17.33 (4.14) | $76.00^{abcde}$ (8.72) | $81.33^{cdef}$ (9.01) | $18.67^{abc}$ (4.36) |
| T14 | $10^7$ CFS/ml of *P. aeruginosa* strain 1 | 0.00 (0.00) | 14.67 (3.40) | $61.33^{efg}$ (7.81) | $69.33^{gh}$ (8.32) | $30.67^{ab}$ (5.56) |
| T15 | $10^8$ CFS/ml of *P. aeruginosa* strain 1 | 0.00 (0.00) | 13.33 (3.66) | $44.00^{h}$ (6.61) | $69.33^{gh}$ (8.31) | $30.67^{ab}$ (5.56) |
| T16 | $10^4$ CFS/ml of *P. aeruginosa* strain 2 | 0.00 (0.00) | 8.00 (2.85) | $69.33^{cdef}$ (8.33) | $80.00^{defg}$ (8.94) | $20.00^{abc}$ (4.51) |
| T17 | $10^5$ CFS/ml of *P. aeruginosa* strain 2 | 0.00 (0.00) | 14.67 (3.83) | $72.00^{bcde}$ (8.46) | $84.00^{bcde}$ (9.16) | $16.00^{bcd}$ (4.05) |
| T18 | $10^6$ CFS/ml of *P. aeruginosa* strain 2 | 0.00 (0.00) | 18.67 (3.76) | $65.33^{efg}$ (8.06) | $70.67^{fgh}$ (8.40) | $29.33^{ab}$ (5.42) |
| T19 | $10^7$ CFS/ml of *P. aeruginosa* strain 2 | 0.00 (0.00) | 16.00 (3.93) | $69.33^{cdef}$ (8.31) | $73.33^{efgh}$ (8.56) | $26.67^{ab}$ (5.18) |
| T20 | $10^8$ CFS/ml of *P. aeruginosa* strain 2 | 0.00 (0.00) | 18.67 (4.14) | $52.00^{gh}$ (7.21) | $72.00^{fgh}$ (8.48) | $28.00^{ab}$ (5.33) |
| T21 | $10^4$ CFS/ml of *P. luminescens* | 0.00 (0.00) | 16.00 (3.86) | $48.00^{h}$ (6.89) | $74.67^{efgh}$ (8.63) | $25.33^{ab}$ (5.05) |
| T22 | $10^5$ CFS/ml of *P. luminescens* | 0.00 (0.00) | 30.67 (5.13) | $61.33^{efg}$ (7.82) | $76.00^{efgh}$ (8.70) | $24.00^{ab}$ (4.77) |
| T23 | $10^6$ CFS/ml of *P. luminescens* | 0.00 (0.00) | 18.67 (4.31) | $56.00^{fgh}$ (7.46) | $72.00^{fgh}$ (8.48) | $28.00^{ab}$ (5.31) |
| T24 | $10^7$ CFS/ml of *P. luminescens* | 0.00 (0.00) | 10.67 (2.38) | $66.67^{def}$ (8.16) | $72.00^{fgh}$ (8.47) | $28.00^{ab}$ (5.26) |
| T25 | $10^8$ CFS/ml of *P. luminescens* | 0.00 (0.00) | 16.00 (4.05) | $61.33^{efg}$ (7.84) | $66.67^{h}$ (8.16) | $33.33^{a}$ (5.78) |

| | | | | | | |
|---|---|---|---|---|---|---|
| T26 | Control | 0.00 (0.00) | 12.00 (3.50) | 86.67$^{ab}$ (9.31) | 94.67$^{ab}$ (9.72) | 5.33$^{ef}$ (2.38) |
| **SE(m)** | | | **0.79** | **0.28** | **0.20** | **0.48** |

Post hoc analysis was carried out by DMRT
Figures in the parenthesis for the durations:

- 48 hours and 96 hours (mortality rate) are square root transformed means $(\sqrt{X = 0.5})$ and
- 72 and 96 hours are square root transformed means$(\sqrt{X})$

### 4.3.4 Adulticidal effect of cell free supernatant of bacterial isolates against *Tetranychus truncatus*

The effect of cell free supernatant of different bacterial isolates on gravid females of *T. truncatus* is presented below (Table 19). No significant mortality was observed at 24 h. At 48 h, the treatment $10^8$ CFS/ml of *P. luminescens* (T25) recorded the higher mortality of 41.33 per cent which was on par with $10^7$ CFS/ml of *P. luminescens* (T24) with 24.00 per cent mortality. This was followed by the treatments $10^5$ CFS/ml of *P. luminescens* (T22- 22.67 %), $10^6$ CFS/ml of *P. luminescens* (T23- 20.00 %), $10^6$ CFS/ml of *P. aeruginosa* strain 1 (T13- 18.67 %), $10^4$ CFS/ml of *P. luminescens* (T21- 16.00 %), $10^8$ CFS/ml of *P. aeruginosa* strain 2 (T20- 14.67 %), $10^4$ CFS/ml of *P. aeruginosa* 1 (T11- 14.67 %), $10^6$ CFS/ml of *P. aeruginosa* strain 2 (T18- 13.33 %), $10^5$ CFS/ml of *P. aeruginosa* strain 1 (T12- 13.33 %), $10^5$ CFS/ml of *P. aeruginosa* strain 2 (T17- 12.00 %), $10^4$ CFS/ml of *P. aeruginosa* strain 2 (T16- 12.00 %) and $10^7$ CFS/ml of *P. aeruginosa* strain 1 (T14- 10.67 %), which were on par with each other. The treatments $10^4$ CFS/ml of *Ochrobactrum* sp. strain 2 (T6- 9.33 %), $10^7$ CFS/ml of *P. aeruginosa* strain 2 (T19- 8.00 %), $10^8$ CFS/ml of *P. aeruginosa* strain 1 (T15- 8.00 %), $10^4$ CFS/ml of *Ochrobactrum* sp. strain 1 (T1- 8.00 %) and $10^8$ CFS/ml of *Ochrobactrum* sp. strain 1 (T5- 6.67 %) followed the above treatment and were on par with each other. These were followed by the treatment $10^6$ CFS/ml of *Ochrobactrum* sp. strain 2 (T8- 5.33 %). The remaining treatments $10^8$ CFS/ml of *Ochrobactrum* sp. strain 2 (T10), $10^7$ CFS/ml of *Ochrobactrum* sp. strain 1 (T4), $10^7$ CFS/ml of *Ochrobactrum* sp. strain 2(T9), $10^6$ CFS/ml of *Ochrobactrum* sp. strain 1 (T3) and $10^5$ CFS/ml of *Ochrobactrum* sp. strain 1 (T2) recorded 4.00, 4.00, 5.33, 4.00 and 2.67 per cent mortality, respectively.

At 72 h, treatment, $10^8$ CFS/ml of *P. luminescens* (T25) has recorded the higher mortality rate 62.67 per cent which was on par with $10^5$ CFS/ml of *P. luminescens* (T22), $10^6$ CFS/ml of *P. luminescens* (T23), $10^7$ CFS/ml of *P. luminescens* (T24) with the mortality 50.66, 49.33 and 48.00 per cent respectively. This was followed by $10^4$ CFS/ml of *P. luminescens* (T21) with 40.00 per cent

mortality. The $10^8$ CFS/ml of *P. aeruginosa* strain 1 (T15- 29.33 %), $10^4$ CFS/ml of *P. aeruginosa* strain 2 (T16- 26.67 %), $10^7$ CFS/ml of *P. aeruginosa* strain 1 (T14- 26.67 %), $10^6$ CFS/ml of *P. aeruginosa* strain 1 (T13- 25.33 %), $10^7$ CFS/ml of *P. aeruginosa* strain 2 (T19- 25.33 %), $10^4$ CFS/ml of *P. aeruginosa* 1 (T11- 24.00 %), $10^5$ CFS/ml of *P. aeruginosa* strain 1 (T12- 24.00 %) and $10^6$ CFS/ml of *P. aeruginosa* strain 2 (T18- 24.00 %) were recorded as the next best treatments. Remaining treatments recorded mortality inferior to above treatments though superior to control.

At 96 h, the treatments $10^8$ CFS/ml of *P. luminescens* (T25) and $10^7$ CFS/ml of *P. luminescens* (T24) recorded significantly higher mortality of 64.00 and 56.00 per cent respectively and were on par with the treatments $10^5$ CFS/ml of *P. luminescens* (T22- 53.33 %), $10^6$ CFS/ml of *P. luminescens* (T23- 52.00 %) and $10^4$ CFS/ml of *P. luminescens* (T21- 48.00 %). The treatment $10^8$ CFS/ml of *P. aeruginosa* strain 2 (T20) with 38.67 per cent mortality followed the above treatments and was followed by $10^8$ CFS/ml of *P. aeruginosa* strain 1 (T15) with 36.00 per cent mortality. The treatments $10^6$ CFS/ml of *P. aeruginosa* strain 2 (T18- 34.67 %), $10^5$ CFS/ml of *P. aeruginosa* strain 2 (T17- 32.00 %) and $10^4$ CFS/ml of *P. aeruginosa* strain 2 (T16- 32.00 %), were recorded as the next best treatments and were on par with each other. These were followed by the on par $10^7$ CFS/ml of *P. aeruginosa* strain 2 (T19), $10^6$ CFS/ml of *P. aeruginosa* strain 1 (T13), $10^7$ CFS/ml of *P. aeruginosa* strain 1 (T14), $10^5$ CFS/ml of *P. aeruginosa* strain 1 (T12), and $10^4$ CFS/ml of *P. aeruginosa* 1 (T11) with 29.33, 26.67, 26.67, 24.00 and 24.00 per cent of mortality, respectively. The remaining treatments $10^7$ CFS/ml of *Ochrobactrum* sp. strain 2 (T9- 21.33 %), $10^8$ CFS/ml of *Ochrobactrum* sp. strain 2 (T10- 18.67 %), $10^6$ CFS/ml of *Ochrobactrum* sp. strain 2 (T8- 14.67 %), $10^4$ CFS/ml of *Ochrobactrum* sp. strain 2 (T6- 12.00 %), $10^8$ CFS/ml of *Ochrobactrum* sp. strain 1 (T5- 10.67 %), $10^4$ CFS/ml of *Ochrobactrum* sp. strain 1(T1- 10.67 %), $10^6$ CFS/ml of *Ochrobactrum* sp. strain 1 (T3- 8.00 %), $10^7$CFS/ml of *Ochrobactrum* sp. strain 1 (T4- 5.33 %) and $10^5$ CFS/ml of *Ochrobactrum* sp. strain 1 (T2- 5.33 %) recorded the mortality superior to control.

**Table 19. Adulticidal effect of cell free supernatant of bacterial isolates against *Tetranychus truncatus***

| Treatment | | Mortality (%) | | | |
|---|---|---|---|---|---|
| | | 24 h | 48 h | 72 h | 96 h |
| T1 | $10^4$ CFS/ml of *Ochrobactrum* sp. strain 1 | 0.00 (0.70) | $8.00^{cdefg}$ (2.85) | $9.33^{ghij}$ (3.06) | $10.67^{ijkl}$ (0.32) |
| T2 | $10^5$ CFS/ml of *Ochrobactrum* sp. strain 1 | 0.00 (0.70) | $2.67^{gh}$ (1.44) | $4.00^{jk}$ (1.65) | $5.33^{kl}$ (0.21) |
| T3 | $10^6$ CFS/ml of *Ochrobactrum* sp. strain 1 | 0.00 (0.70) | $4.00^{fgh}$ (1.92) | $8.00^{hijk}$ (2.85) | $8.00^{jkl}$ (0.29) |
| T4 | $10^7$ CFS/ml of *Ochrobactrum* sp. strain 1 | 0.00 (0.70) | $4.00^{efgh}$ (2.12) | $5.33^{ijk}$ (2.38) | $5.33^{kl}$ (0.23) |
| T5 | $10^8$ CFS/ml of *Ochrobactrum* sp. strain 1 | 0.00 (0.70) | $6.67^{cdefg}$ (2.66) | $9.33^{fghij}$ (3.12) | $10.67^{ijkl}$ (0.33) |
| T6 | $10^4$ CFS/ml of *Ochrobactrum* sp. strain 2 | 0.00 (0.70) | $9.33^{cdefg}$ (2.92) | $12.00^{fghi}$ (3.33) | $12.00^{ijkl}$ (0.33) |
| T7 | $10^5$ CFS/ml of *Ochrobactrum* sp. strain 2 | 0.00 (0.70) | $0.00^{h}$ (0.70) | $2.67^{k}$ (1.44) | $2.67^{l}$ (0.16) |
| T8 | $10^6$ CFS/ml of *Ochrobactrum* sp. strain 2 | 0.00 (0.70) | $5.33^{defgh}$ (2.38) | $13.33^{efghi}$ (3.66) | $14.67^{hijk}$ (0.39) |
| T9 | $10^7$ CFS/ml of *Ochrobactrum* sp. strain 2 | 0.00 (0.70) | $5.33^{efgh}$ (2.12) | $20^{defgh}$ (4.40) | $21.33^{fghi}$ (0.47) |
| T10 | $10^8$ CFS/ml of *Ochrobactrum* sp. strain 2 | 0.00 (0.70) | $4.00^{efgh}$ (2.12) | $18.67^{defgh}$ (4.34) | $18.67^{ghij}$ (0.44) |
| T11 | $10^4$ CFS/ml of *P. aeruginosa* strain 1 | 0.00 (0.70) | $14.67^{bcde}$ (3.80) | $24.00^{cde}$ (4.94) | $24.00^{efgh}$ (0.51) |

**Table 19. continued**

| | | | | | |
|---|---|---|---|---|---|
| T12 | $10^5$ CFS/ml of *P. aeruginosa* strain 1 | 0.00 (0.70) | 13.33$^{bcdef}$ (3.66) | 24.00$^{cde}$ (4.94) | 24.00$^{efgh}$ (0.51) |
| T13 | $10^6$ CFS/ml of *P. aeruginosa* strain 1 | 0.00 (0.70) | 18.67$^{bc}$ (4.28) | 25.33$^{cde}$ (5.06) | 26.67$^{efgh}$ (0.54) |
| T14 | $10^7$ CFS/ml of *P. aeruginosa* strain 1 | 0.00 (0.70) | 10.67$^{bcdef}$ (3.33) | 26.67$^{cde}$ (5.20) | 26.67$^{efgh}$ (0.55) |
| T15 | $10^8$ CFS/ml of *P. aeruginosa* strain 1 | 0.00 (0.70) | 8.00$^{cdefg}$ (2.85) | 29.33$^{cd}$ (5.42) | 36.00$^{cdef}$ (0.63) |
| T16 | $10^4$ CFS/ml of *P. aeruginosa* strain 2 | 5.33 (1.82) | 12.00$^{bcdef}$ (3.33) | 26.67$^{cde}$ (5.20) | 32.00$^{defg}$ (0.60) |
| T17 | $10^5$ CFS/ml of *P. aeruginosa* strain 2 | 0.00 (0.70) | 12.00$^{bcdef}$ (3.50) | 21.33$^{def}$ (4.66) | 32.00$^{defg}$ (0.61) |
| T18 | $10^6$ CFS/ml of *P. aeruginosa* strain 2 | 0.00 (0.70) | 13.33$^{bcdef}$ (3.66) | 24.00$^{cde}$ (4.90) | 34.67$^{def}$ (0.62) |
| T19 | $10^7$ CFS/ml of *P. aeruginosa* strain 2 | 0.00 (0.70) | 8.00$^{cdefg}$ (2.91) | 25.33$^{cde}$ (5.05) | 29.33$^{efgh}$ (0.56) |
| T20 | $10^8$ CFS/ml of *P. aeruginosa* strain 2 | 2.67 (1.44) | 14.67$^{bcde}$ (3.80) | 20$^{defg}$ (4.51) | 38.67$^{bcde}$ (0.67) |
| T21 | $10^4$ CFS/ml of *P. luminescens* | 0.00 (0.70) | 16.00$^{bcd}$ (4.05) | 40.00$^{bc}$ (6.34) | 48.00$^{abcd}$ (0.77) |
| T22 | $10^5$ CFS/ml of *P. luminescens* | 0.00 (0.70) | 22.67$^{b}$ (4.77) | 50.67$^{ab}$ (7.11) | 53.33$^{ab}$ (0.82) |
| T23 | $10^6$ CFS/ml of *P. luminescens* | 0.00 (0.70) | 20.00$^{bc}$ (4.38) | 49.33$^{ab}$ (7.03) | 52.00$^{abc}$ (0.81) |
| T24 | $10^7$ CFS/ml of *P. luminescens* | 1.33 (1.18) | 24.00$^{ab}$ (4.94) | 48.00$^{ab}$ (6.94) | 56.00$^{a}$ (0.85) |

**Table 19. continued**

| | | | | | |
|---|---|---|---|---|---|
| T25 | $10^8$ CFS/ml of *P. luminescens* | 5.33 (2.12) | 41.33[a] (6.38) | 62.67[a] (7.95) | 64.00[a] (0.93) |
| T26 | Control | 0.00 (0.70) | 1.33 (1.18) | 2.67 (1.44) | 4.00 (0.19) |
| **SE(m)** | | **0.32** | **0.52** | **0.48** | **0.05** |

Post hoc analysis was carried out by DMRT
Figures in the parenthesis of first three durations (24, 48 and 72 hours) are square root transformed means($\sqrt{X = 0.5}$) and the last one (96hours) is arc sin transformed means

## 4.4 Molecular basis of virulence in *P. luminescens* isolate against *Tetranychus truncatus*

### 4.4.1 PCR Amplification and sequencing of *TcdA* – Toxin complex gene

Three sets of reported internal primers, TcdAi, TcdAii and TcdAiii (Mulla *et al*., 2017) were used for amplification of full length Toxin complex gene. As none of the primer sets amplified the gene, the whole gene was split into eight regions (each of 1100 bp) and primers were designed separately for each regions. Care was taken while designing the primers so that the orientation of the gene sequence should not get altered. The quality of the amplified products was checked on 1.4 per cent of agarose gel electrophoresis. Among eight sets of internal primers, only six sets produced the bands in the range of 850 – 1000 bp on agarose gel. Six amplified regions of the gene were sequenced.

### 4.4.2 Analysis of the sequence data

Homology of sequences obtained by BLASTn for each regions are presented in the Figure (3-8). The full length sequence of the gene, *TcdA* except regions 5 and 6 was constructed by merging the six contigs (Annexure II).

The variation in the contigs of toxin gene of the isolated *P. luminescens* from the reference sequence (AF188483.1) was analyzed through multiple sequence alignment. The sequences had 98.89 per cent similarity. However, there were one deletion, three insertions, 12 transversions and a number of transitions (Table 20 and Figures 9-14).

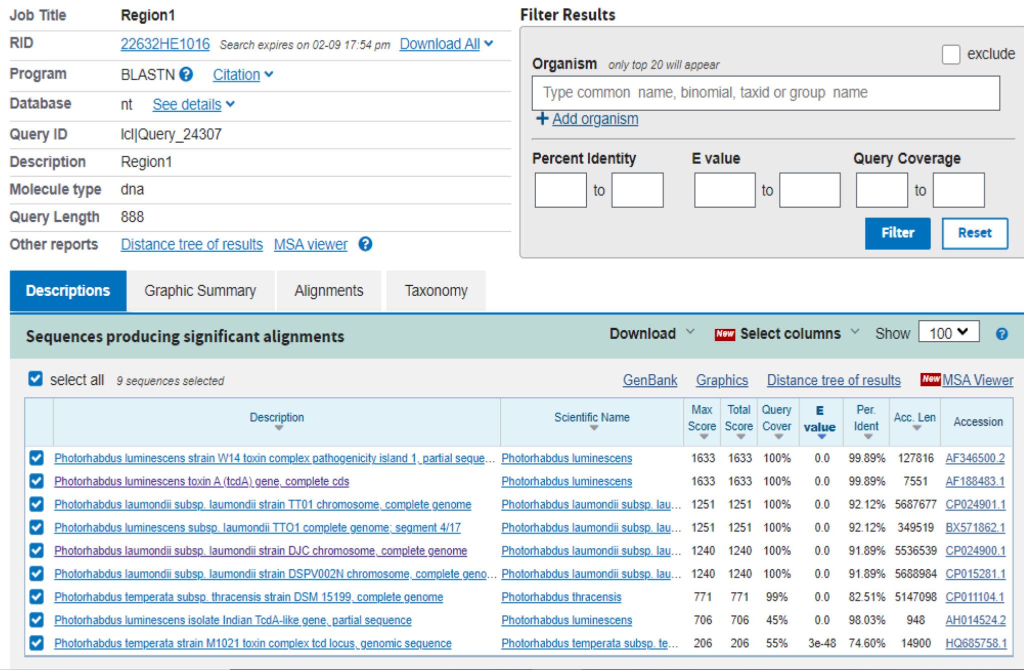
**Table 20. Positions of variation in the contigs of toxin complex gene**

| Sl. No. | Regions | Type of variation | Position of variation (bp) |
|---|---|---|---|
| 1. | Region 1 | Deletion | 137 |
| 2. | | Insertion | 974 |
| 3. | Region 2 | Transversion | 1228 |
| 4. | | Transition | 1500 |
| 5. | | Transition | 1503 |
| 6. | | Transition | 1524 |
| 7. | | Transition | 1602 |
| 8. | | Transition | 1632 |
| 9. | | Transversion | 1641 |
| 10. | | Transition | 1647 |
| 11. | | Transition | 1647 |
| 12. | | Transition | 1648 |
| 13. | | Transition | 1671 |
| 14. | | Transition | 1688 |
| 15. | | Transversion | 1692 |
| 16. | | Transition | 1725 |
| 17. | | Transition | 1728 |
| 18. | | Transversion | 1773 |
| 19. | | Transition | 1813 |
| 20. | | Transition | 1828 |
| 21. | | Transition | 1855 |
| 22. | | Transition | 1875 |
| 23. | | Transition | 1878 |
| 24. | | Transversion | 1891 |
| 25. | | Transition | 1915 |
| 26. | | Transition | 1921 |

**Table 20. continued**

| | | | |
|---|---|---|---|
| 27. | | Transition | 1932 |
| 28. | Region 3 | Transition | 2209 |
| 29. | | Transition | 2211 |
| 30. | | Transition | 2222 |
| 31. | | Transversion | 2235 |
| 32. | | Transition | 2298 |
| 33. | | Transversion | 2365 |
| 34. | | Transition | 2377 |
| 35. | | Transition | 2382 |
| 36. | | Transition | 2406 |
| 37. | | Transition | 2415 |
| 38. | | Transversion | 2485 |
| 39. | | Transition | 2623 |
| 40. | | Transition | 2898 |
| 41. | | Transition | 2899 |
| 42. | | Transition | 2907 |
| 43. | | Transition | 2910 |
| 44. | | Transition | 2934 |
| 45. | Region 4 | Transversion | 3096 |
| 46. | | Transition | 3240 |
| 47. | | Transition | 3243 |
| 48. | | Transition | 3261 |
| 49. | | Transition | 3288 |
| 50. | | Transition | 3336 |
| 51. | | Transition | 3384 |
| 52. | | Transition | 3395 |
| 53. | | Transition | 3378 |
| 54. | | Transition | 3536 |
| 55. | | Transition | 3594 |
| 56. | | Transition | 3603 |
| 57. | | Transition | 3618 |

| 58. | | Transition | 3618 |
|---|---|---|---|
| 59. | | Transition | 3621 |
| 60. | | Transition | 3655 |
| 61. | | Transition | 3667 |
| 62. | | Transition | 3673 |
| 63. | | Transversion | 3674 |
| 64. | | Transversion | 3680 |
| 65. | | Transition | 3689 |
| 66. | | Transition | 3756 |
| 67. | | Transition | 3798 |
| 68. | | Transition | 3812 |
| 69. | | Transition | 3821 |
| 70. | | Transition | 3837 |
| 71. | Region 7 | Transition | 6105 |
| 72. | | Transition | 6146 |
| 73. | | Transition | 6264 |
| 74. | | Transition | 6405 |
| 75. | | Transition | 6465 |
| 76. | | Transition | 6468 |
| 77. | | Transition | 6498 |
| 78. | | Transition | 6501 |
| 79. | | Transversion | 6522 |
| 80. | | Transition | 6552 |
| 81. | Region 8 | Transition | 6723 |
| 82. | | Transition | 6760 |
| 83. | | Insertion | 6927 |
| 84. | | Transition | 7101 |
| 85. | | Transition | 7224 |
| 86. | | Transition | 7254 |
| 87. | | Insertion | 7458 |

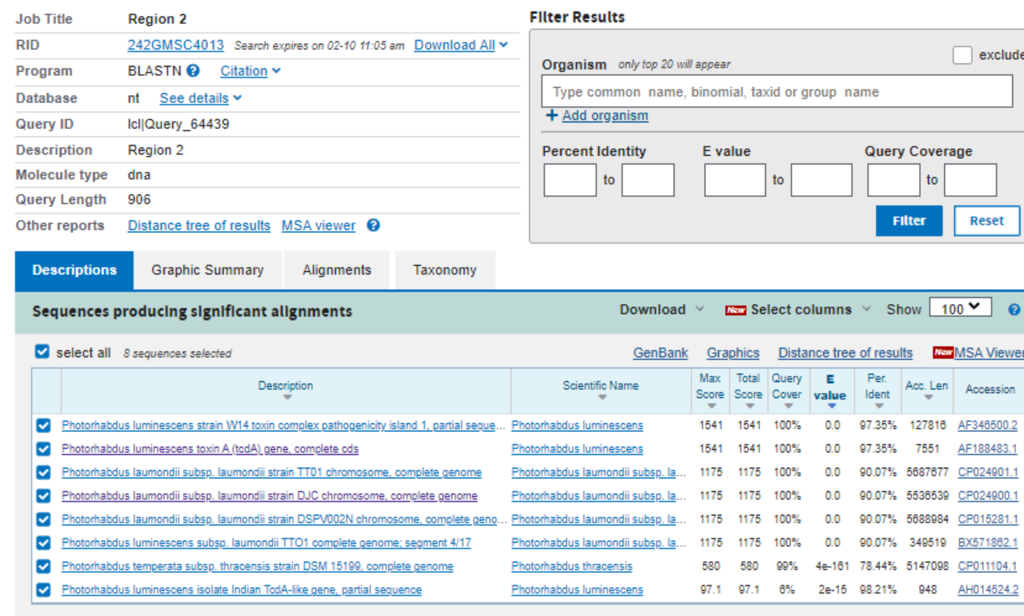


| Job Title | Region1 |
|---|---|
| RID | 22632HE1016 Search expires on 02-09 17:54 pm Download All |
| Program | BLASTN Citation |
| Database | nt See details |
| Query ID | lcl\|Query_24307 |
| Description | Region1 |
| Molecule type | dna |
| Query Length | 888 |
| Other reports | Distance tree of results MSA viewer |

Sequences producing significant alignments — select all 9 sequences selected

| Description | Scientific Name | Max Score | Total Score | Query Cover | E value | Per. Ident | Acc. Len | Accession |
|---|---|---|---|---|---|---|---|---|
| Photorhabdus luminescens strain W14 toxin complex pathogenicity island 1, partial seque... | Photorhabdus luminescens | 1633 | 1633 | 100% | 0.0 | 99.89% | 127816 | AF346500.2 |
| Photorhabdus luminescens toxin A (tcdA) gene, complete cds | Photorhabdus luminescens | 1633 | 1633 | 100% | 0.0 | 99.89% | 7551 | AF188483.1 |
| Photorhabdus laumondii subsp. laumondii strain TT01 chromosome, complete genome | Photorhabdus laumondii subsp. lau... | 1251 | 1251 | 100% | 0.0 | 92.12% | 5687677 | CP024901.1 |
| Photorhabdus luminescens subsp. laumondii TTO1 complete genome; segment 4/17 | Photorhabdus laumondii subsp. lau... | 1251 | 1251 | 100% | 0.0 | 92.12% | 349519 | BX571862.1 |
| Photorhabdus laumondii subsp. laumondii strain DJC chromosome, complete genome | Photorhabdus laumondii subsp. lau... | 1240 | 1240 | 100% | 0.0 | 91.89% | 5536539 | CP024900.1 |
| Photorhabdus laumondii subsp. laumondii strain DSPV002N chromosome, complete geno... | Photorhabdus laumondii subsp. lau... | 1240 | 1240 | 100% | 0.0 | 91.89% | 5688984 | CP015281.1 |
| Photorhabdus temperata subsp. thracensis strain DSM 15199, complete genome | Photorhabdus thracensis | 771 | 771 | 99% | 0.0 | 82.51% | 5147098 | CP011104.1 |
| Photorhabdus luminescens isolate Indian TcdA-like gene, partial sequence | Photorhabdus luminescens | 706 | 706 | 45% | 0.0 | 98.03% | 948 | AH014524.2 |
| Photorhabdus temperata strain M1021 toxin complex tcd locus, genomic sequence | Photorhabdus temperata subsp. te... | 206 | 206 | 55% | 3e-48 | 74.60% | 14900 | HQ685758.1 |

**Figure 3. BLASTn result *TcdA* region 1**

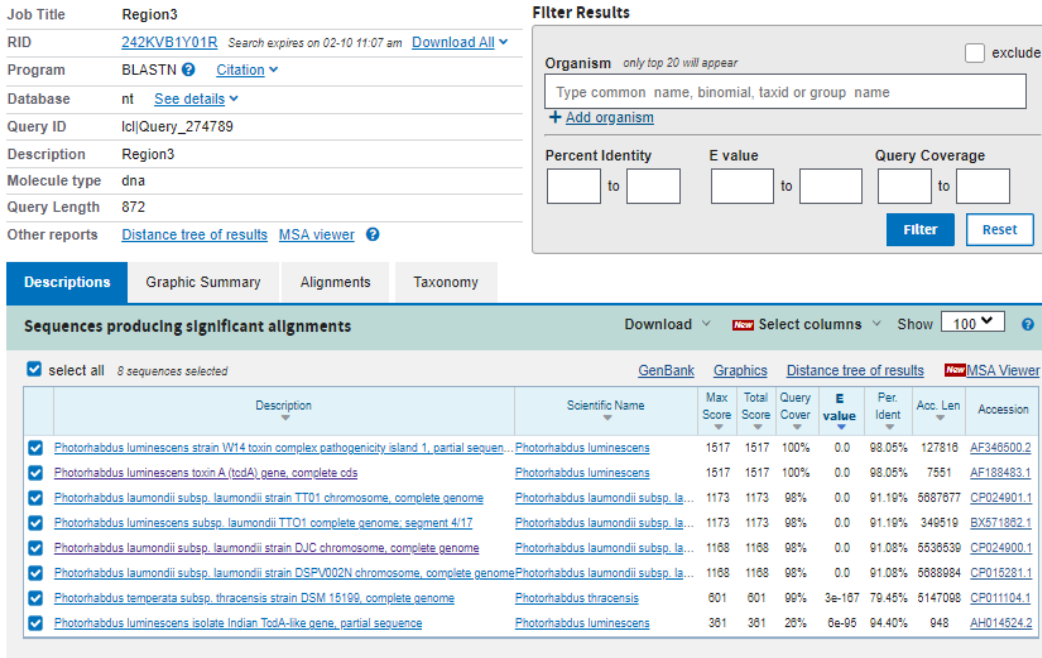


| Job Title | Region 2 |
|---|---|
| RID | 242GMSC4013 Search expires on 02-10 11:05 am Download All |
| Program | BLASTN Citation |
| Database | nt See details |
| Query ID | lcl\|Query_64439 |
| Description | Region 2 |
| Molecule type | dna |
| Query Length | 906 |
| Other reports | Distance tree of results MSA viewer |

Sequences producing significant alignments — select all 8 sequences selected

| Description | Scientific Name | Max Score | Total Score | Query Cover | E value | Per. Ident | Acc. Len | Accession |
|---|---|---|---|---|---|---|---|---|
| Photorhabdus luminescens strain W14 toxin complex pathogenicity island 1, partial seque... | Photorhabdus luminescens | 1541 | 1541 | 100% | 0.0 | 97.35% | 127816 | AF346500.2 |
| Photorhabdus luminescens toxin A (tcdA) gene, complete cds | Photorhabdus luminescens | 1541 | 1541 | 100% | 0.0 | 97.35% | 7551 | AF188483.1 |
| Photorhabdus laumondii subsp. laumondii strain TT01 chromosome, complete genome | Photorhabdus laumondii subsp. la... | 1175 | 1175 | 100% | 0.0 | 90.07% | 5687677 | CP024901.1 |
| Photorhabdus laumondii subsp. laumondii strain DJC chromosome, complete genome | Photorhabdus laumondii subsp. la... | 1175 | 1175 | 100% | 0.0 | 90.07% | 5536539 | CP024900.1 |
| Photorhabdus laumondii subsp. laumondii strain DSPV002N chromosome, complete geno... | Photorhabdus laumondii subsp. la... | 1175 | 1175 | 100% | 0.0 | 90.07% | 5688984 | CP015281.1 |
| Photorhabdus luminescens subsp. laumondii TTO1 complete genome; segment 4/17 | Photorhabdus laumondii subsp. la... | 1175 | 1175 | 100% | 0.0 | 90.07% | 349519 | BX571862.1 |
| Photorhabdus temperata subsp. thracensis strain DSM 15199, complete genome | Photorhabdus thracensis | 580 | 580 | 99% | 4e-161 | 78.44% | 5147098 | CP011104.1 |
| Photorhabdus luminescens isolate Indian TcdA-like gene, partial sequence | Photorhabdus luminescens | 97.1 | 97.1 | 6% | 2e-15 | 98.21% | 948 | AH014524.2 |

**Figure 4. BLASTn result *TcdA* region 2**

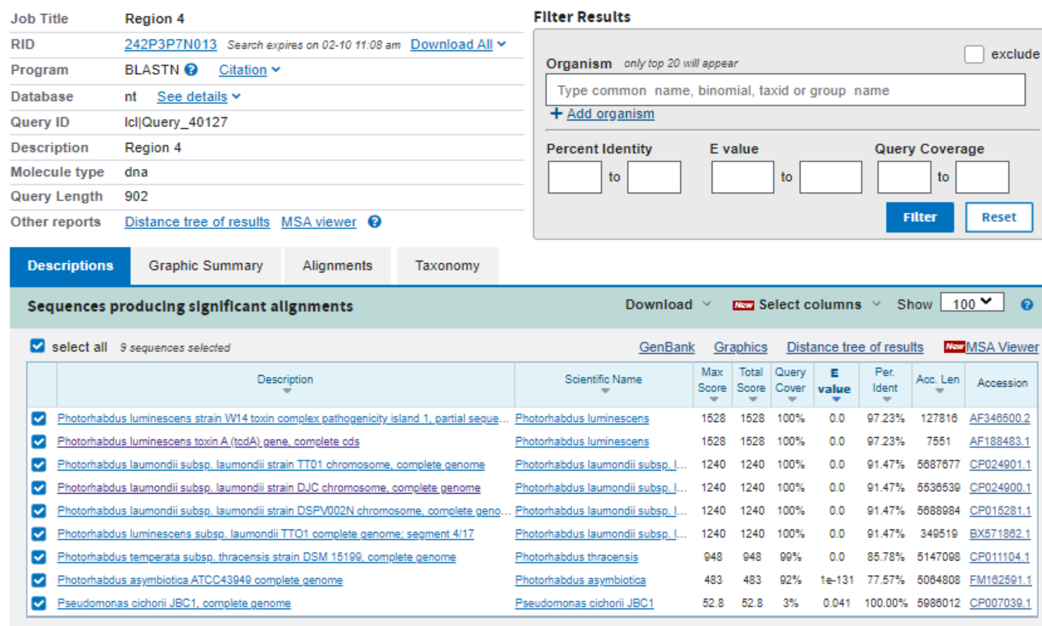


| Field | Value |
|---|---|
| Job Title | Region3 |
| RID | 242KVB1Y01R Search expires on 02-10 11:07 am Download All |
| Program | BLASTN Citation |
| Database | nt See details |
| Query ID | lcl\|Query_274789 |
| Description | Region3 |
| Molecule type | dna |
| Query Length | 872 |
| Other reports | Distance tree of results MSA viewer |

Sequences producing significant alignments — 8 sequences selected

| Description | Scientific Name | Max Score | Total Score | Query Cover | E value | Per. Ident | Acc. Len | Accession |
|---|---|---|---|---|---|---|---|---|
| Photorhabdus luminescens strain W14 toxin complex pathogenicity island 1, partial sequen... | Photorhabdus luminescens | 1517 | 1517 | 100% | 0.0 | 98.05% | 127816 | AF346500.2 |
| Photorhabdus luminescens toxin A (tcdA) gene, complete cds | Photorhabdus luminescens | 1517 | 1517 | 100% | 0.0 | 98.05% | 7551 | AF188483.1 |
| Photorhabdus laumondii subsp. laumondii strain TT01 chromosome, complete genome | Photorhabdus laumondii subsp. la... | 1173 | 1173 | 98% | 0.0 | 91.19% | 5687677 | CP024901.1 |
| Photorhabdus luminescens subsp. laumondii TTO1 complete genome; segment 4/17 | Photorhabdus laumondii subsp. la... | 1173 | 1173 | 98% | 0.0 | 91.19% | 349519 | BX571862.1 |
| Photorhabdus laumondii subsp. laumondii strain DJC chromosome, complete genome | Photorhabdus laumondii subsp. la... | 1168 | 1168 | 98% | 0.0 | 91.08% | 5536539 | CP024900.1 |
| Photorhabdus laumondii subsp. laumondii strain DSPV002N chromosome, complete genome | Photorhabdus laumondii subsp. la... | 1168 | 1168 | 98% | 0.0 | 91.08% | 5688984 | CP015281.1 |
| Photorhabdus temperata subsp. thracensis strain DSM 15199, complete genome | Photorhabdus thracensis | 601 | 601 | 99% | 3e-167 | 79.45% | 5147098 | CP011104.1 |
| Photorhabdus luminescens isolate Indian TcdA-like gene, partial sequence | Photorhabdus luminescens | 361 | 361 | 28% | 6e-95 | 94.40% | 948 | AH014524.2 |

**Figure 5. BLASTn result *TcdA* region 3**

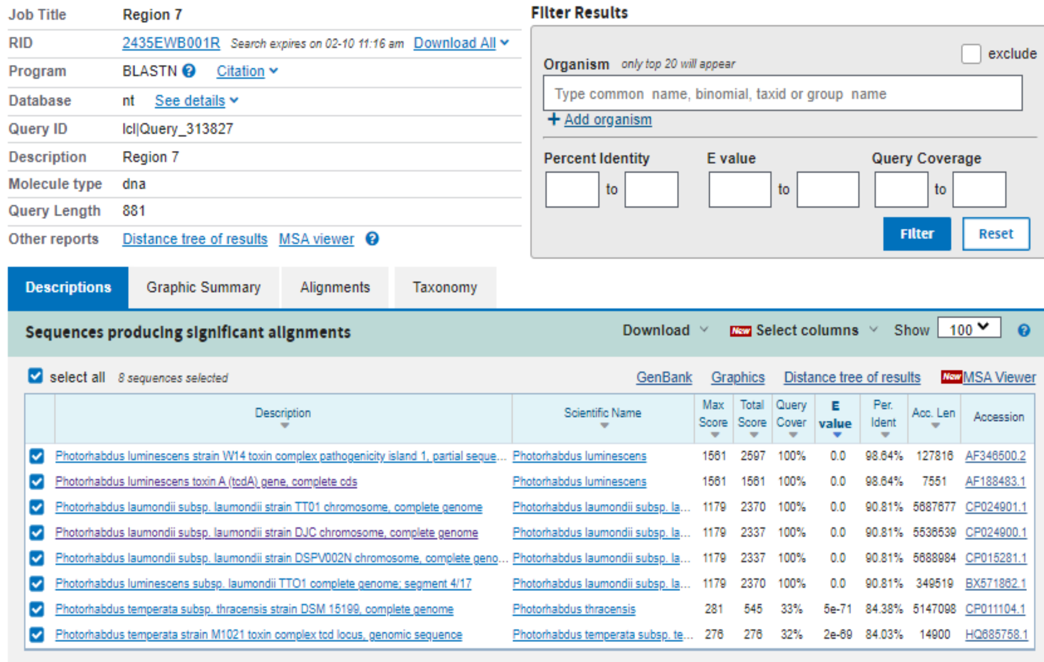


| Field | Value |
|---|---|
| Job Title | Region 4 |
| RID | 242P3P7N013 Search expires on 02-10 11:08 am Download All |
| Program | BLASTN Citation |
| Database | nt See details |
| Query ID | lcl\|Query_40127 |
| Description | Region 4 |
| Molecule type | dna |
| Query Length | 902 |
| Other reports | Distance tree of results MSA viewer |

Sequences producing significant alignments — 9 sequences selected

| Description | Scientific Name | Max Score | Total Score | Query Cover | E value | Per. Ident | Acc. Len | Accession |
|---|---|---|---|---|---|---|---|---|
| Photorhabdus luminescens strain W14 toxin complex pathogenicity island 1, partial seque... | Photorhabdus luminescens | 1528 | 1528 | 100% | 0.0 | 97.23% | 127816 | AF346500.2 |
| Photorhabdus luminescens toxin A (tcdA) gene, complete cds | Photorhabdus luminescens | 1528 | 1528 | 100% | 0.0 | 97.23% | 7551 | AF188483.1 |
| Photorhabdus laumondii subsp. laumondii strain TT01 chromosome, complete genome | Photorhabdus laumondii subsp. l... | 1240 | 1240 | 100% | 0.0 | 91.47% | 5687677 | CP024901.1 |
| Photorhabdus laumondii subsp. laumondii strain DJC chromosome, complete genome | Photorhabdus laumondii subsp. l... | 1240 | 1240 | 100% | 0.0 | 91.47% | 5536539 | CP024900.1 |
| Photorhabdus laumondii subsp. laumondii strain DSPV002N chromosome, complete geno... | Photorhabdus laumondii subsp. l... | 1240 | 1240 | 100% | 0.0 | 91.47% | 5688984 | CP015281.1 |
| Photorhabdus luminescens subsp. laumondii TTO1 complete genome; segment 4/17 | Photorhabdus laumondii subsp. l... | 1240 | 1240 | 100% | 0.0 | 91.47% | 349519 | BX571862.1 |
| Photorhabdus temperata subsp. thracensis strain DSM 15199, complete genome | Photorhabdus thracensis | 948 | 948 | 99% | 0.0 | 85.78% | 5147098 | CP011104.1 |
| Photorhabdus asymbiotica ATCC43949 complete genome | Photorhabdus asymbiotica | 483 | 483 | 92% | 1e-131 | 77.57% | 5064808 | FM162591.1 |
| Pseudomonas cichorii JBC1, complete genome | Pseudomonas cichorii JBC1 | 52.8 | 52.8 | 3% | 0.041 | 100.00% | 5986012 | CP007039.1 |

**Figure 6. BLASTn result *TcdA* region 4**



Job Title: Region 7
RID: 2435EWB001R — Search expires on 02-10 11:16 am — Download All
Program: BLASTN — Citation
Database: nt — See details
Query ID: lcl|Query_313827
Description: Region 7
Molecule type: dna
Query Length: 881
Other reports: Distance tree of results — MSA viewer

Filter Results
Organism (only top 20 will appear) — exclude
Type common name, binomial, taxid or group name
+ Add organism
Percent Identity ... to ... E value ... to ... Query Coverage ... to ...
Filter Reset

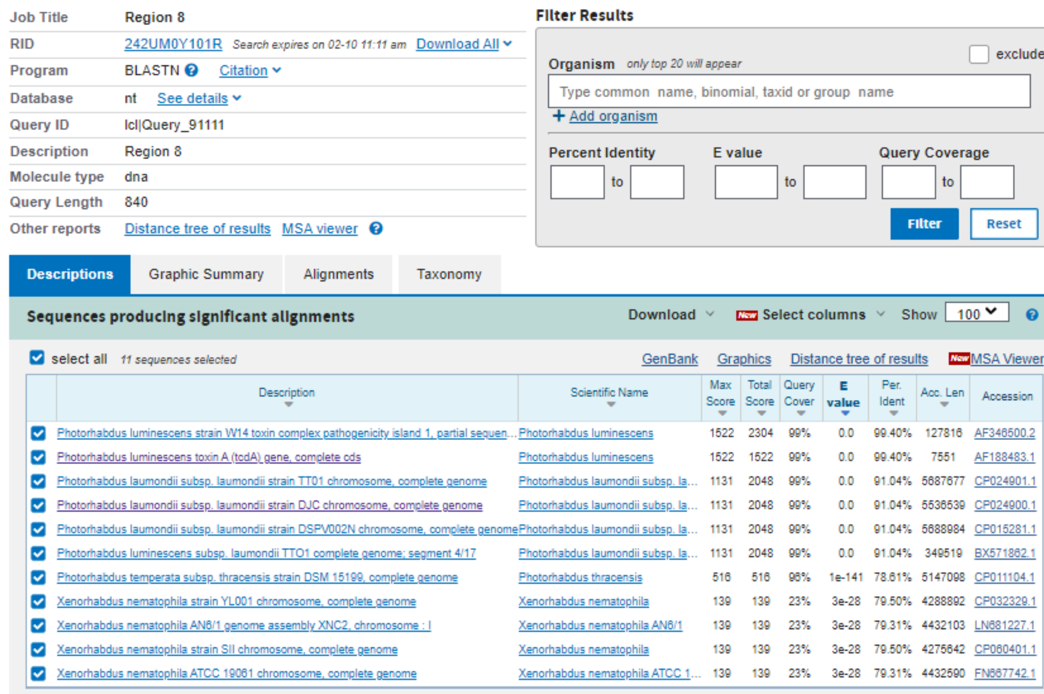
Descriptions | Graphic Summary | Alignments | Taxonomy

Sequences producing significant alignments — Download — Select columns — Show 100

select all — 8 sequences selected — GenBank — Graphics — Distance tree of results — MSA Viewer

| Description | Scientific Name | Max Score | Total Score | Query Cover | E value | Per. Ident | Acc. Len | Accession |
|---|---|---|---|---|---|---|---|---|
| Photorhabdus luminescens strain W14 toxin complex pathogenicity island 1, partial seque... | Photorhabdus luminescens | 1561 | 2597 | 100% | 0.0 | 98.64% | 127816 | AF346500.2 |
| Photorhabdus luminescens toxin A (tcdA) gene, complete cds | Photorhabdus luminescens | 1561 | 1561 | 100% | 0.0 | 98.64% | 7551 | AF188483.1 |
| Photorhabdus laumondii subsp. laumondii strain TT01 chromosome, complete genome | Photorhabdus laumondii subsp. la... | 1179 | 2370 | 100% | 0.0 | 90.81% | 5687677 | CP024901.1 |
| Photorhabdus laumondii subsp. laumondii strain DJC chromosome, complete genome | Photorhabdus laumondii subsp. la... | 1179 | 2337 | 100% | 0.0 | 90.81% | 5536539 | CP024900.1 |
| Photorhabdus laumondii subsp. laumondii strain DSPV002N chromosome, complete geno... | Photorhabdus laumondii subsp. la... | 1179 | 2337 | 100% | 0.0 | 90.81% | 5688984 | CP015281.1 |
| Photorhabdus luminescens subsp. laumondii TTO1 complete genome; segment 4/17 | Photorhabdus laumondii subsp. la... | 1179 | 2370 | 100% | 0.0 | 90.81% | 349519 | BX571862.1 |
| Photorhabdus temperata subsp. thracensis strain DSM 15199, complete genome | Photorhabdus thracensis | 281 | 545 | 33% | 5e-71 | 84.38% | 5147098 | CP011104.1 |
| Photorhabdus temperata strain M1021 toxin complex tcd locus, genomic sequence | Photorhabdus temperata subsp. te... | 276 | 276 | 32% | 2e-69 | 84.03% | 14900 | HQ685758.1 |

**Figure 7. BLASTn result *TcdA* region 7**



Job Title: Region 8
RID: 242UM0Y101R — Search expires on 02-10 11:11 am — Download All
Program: BLASTN — Citation
Database: nt — See details
Query ID: lcl|Query_91111
Description: Region 8
Molecule type: dna
Query Length: 840
Other reports: Distance tree of results — MSA viewer

Filter Results
Organism (only top 20 will appear) — exclude
Type common name, binomial, taxid or group name
+ Add organism
Percent Identity ... to ... E value ... to ... Query Coverage ... to ...
Filter Reset

Descriptions | Graphic Summary | Alignments | Taxonomy

Sequences producing significant alignments — Download — Select columns — Show 100

select all — 11 sequences selected — GenBank — Graphics — Distance tree of results — MSA Viewer

| Description | Scientific Name | Max Score | Total Score | Query Cover | E value | Per. Ident | Acc. Len | Accession |
|---|---|---|---|---|---|---|---|---|
| Photorhabdus luminescens strain W14 toxin complex pathogenicity island 1, partial sequen... | Photorhabdus luminescens | 1522 | 2304 | 99% | 0.0 | 99.40% | 127816 | AF346500.2 |
| Photorhabdus luminescens toxin A (tcdA) gene, complete cds | Photorhabdus luminescens | 1522 | 1522 | 99% | 0.0 | 99.40% | 7551 | AF188483.1 |
| Photorhabdus laumondii subsp. laumondii strain TT01 chromosome, complete genome | Photorhabdus laumondii subsp. la... | 1131 | 2048 | 99% | 0.0 | 91.04% | 5687677 | CP024901.1 |
| Photorhabdus laumondii subsp. laumondii strain DJC chromosome, complete genome | Photorhabdus laumondii subsp. la... | 1131 | 2048 | 99% | 0.0 | 91.04% | 5536539 | CP024900.1 |
| Photorhabdus laumondii subsp. laumondii strain DSPV002N chromosome, complete genome | Photorhabdus laumondii subsp. la... | 1131 | 2048 | 99% | 0.0 | 91.04% | 5688984 | CP015281.1 |
| Photorhabdus luminescens subsp. laumondii TTO1 complete genome; segment 4/17 | Photorhabdus laumondii subsp. la... | 1131 | 2048 | 99% | 0.0 | 91.04% | 349519 | BX571862.1 |
| Photorhabdus temperata subsp. thracensis strain DSM 15199, complete genome | Photorhabdus thracensis | 516 | 516 | 96% | 1e-141 | 78.61% | 5147098 | CP011104.1 |
| Xenorhabdus nematophila strain YL001 chromosome, complete genome | Xenorhabdus nematophila | 139 | 139 | 23% | 3e-28 | 79.50% | 4288892 | CP032329.1 |
| Xenorhabdus nematophila AN6/1 genome assembly XNC2, chromosome : I | Xenorhabdus nematophila AN6/1 | 139 | 139 | 23% | 3e-28 | 79.31% | 4432103 | LN681227.1 |
| Xenorhabdus nematophila strain SII chromosome, complete genome | Xenorhabdus nematophila | 139 | 139 | 23% | 3e-28 | 79.50% | 4275642 | CP060401.1 |
| Xenorhabdus nematophila ATCC 19061 chromosome, complete genome | Xenorhabdus nematophila ATCC 1... | 139 | 139 | 23% | 3e-28 | 79.31% | 4432590 | FN667742.1 |

**Figure 8. BLASTn result *TcdA* region 8**

## Region 1

```
CF1tcdA         ATGAACGAGTCTGTAAAAGAGATACCTGATGTATTAAAAAGCCAGTGTGGTTTTAATTGT     60
AF188483.1      ATGAACGAGTCTGTAAAAGAGATACCTGATGTATTAAAAAGCCAGTGTGGTTTTAATTGT     60
                ************************************************************

CF1tcdA         CTGACAGATATTAGCCACAGCTCTTTTAATGAATTTCGCCAGCAAGTATCTGAGCACCTC     120
AF188483.1      CTGACAGATATTAGCCACAGCTCTTTTAATGAATTTCGCCAGCAAGTATCTGAGCACCTC     120
                ************************************************************

CF1tcdA         TCCTGGTCCGAAACAC-CGACTTATATCATGATGCACAACAGGCACAAAAGGATAATCGC     179
AF188483.1      TCCTGGTCCGAAACACACGACTTATATCATGATGCACAACAGGCACAAAAGGATAATCGC     180
                **************** *******************************************

CF1tcdA         CTGTATGAAGCGCGTATTCTCAAACGCGCCAATCCCCAATTACAAAATGCGGTGCATCTT     239
AF188483.1      CTGTATGAAGCGCGTATTCTCAAACGCGCCAATCCCCAATTACAAAATGCGGTGCATCTT     240
                ************************************************************

CF1tcdA         GCCATTCTCGCTCCCAATGCTGAACTGATAGGCTATAACAATCAATTTAGCGGTAGAGCC     299
AF188483.1      GCCATTCTCGCTCCCAATGCTGAACTGATAGGCTATAACAATCAATTTAGCGGTAGAGCC     300
                ************************************************************

CF1tcdA         AGTCAATATGTTGCGCCGGGTACCGTTTCTTCCATGTTCTCCCCCGCCGCTTATTTGACT     359
AF188483.1      AGTCAATATGTTGCGCCGGGTACCGTTTCTTCCATGTTCTCCCCCGCCGCTTATTTGACT     360
                ************************************************************

CF1tcdA         AGTGATGGCACGGGTTAAGGTATATCGGATCACCCGCGAATATACAACCAATGCTTATCA    1019
AF188483.1      AGTGATGGCACGG-TTAAGGTATATCGGATCACCCGCGAATATACAACCAATGCTTATCA    1019
                ************* **********************************************

CF1tcdA         AATGGATGTGGAGCTATTTCCCTTCGGTGGTGAGAATTATCGGTTAGATTATAAATTCAA    1079
AF188483.1      AATGGATGTGGAGCTATTTCCCTTCGGTGGTGAGAATTATCGGTTAGATTATAAATTCAA    1079
                ************************************************************
```

**Figure 9. SNPs in *TcdA* region 1 compared to reference sequence**

## Region 2

```
CF1tcdA       AAATTTTTATAATGCCTCTTATTTATCCATCAAGTTAAATGATAAAAGAGAACTTGTTCG    1139
AF188483.1    AAATTTTTATAATGCCTCTTATTTATCCATCAAGTTAAATGATAAAAGAGAACTTGTTCG    1139
              ************************************************************

CF1tcdA       AACTGAAGGCGCTCCTCAAGTCAATATAGAATACTCCGCAAATATCACATTAAATACCGC    1199
AF188483.1    AACTGAAGGCGCTCCTCAAGTCAATATAGAATACTCCGCAAATATCACATTAAATACCGC    1199
              ************************************************************

CF1tcdA       TGATATCAGTCAACCTTTTGAAATTGGCGTGACACGAGTACTTCCTTCCGGTTCTTGGGC    1259
AF188483.1    TGATATCAGTCAACCTTTTGAAATTGGCCTGACACGAGTACTTCCTTCCGGTTCTTGGGC    1259
              **************************** *******************************

CF1tcdA       ATATGCCGCCGCAAAATTTACCGTTGAAGAGTATAACCAATACTCTTTTCTGCTAAAACT    1319
AF188483.1    ATATGCCGCCGCAAAATTTACCGTTGAAGAGTATAACCAATACTCTTTTCTGCTAAAACT    1319
              ************************************************************


CF1tcdA       TGCACCTATTTCACAACGTTCATACGATAATCAACCTAGCCAATTTGATCGCCTGTTTAA    1559
AF188483.1    CGCGCCTATTTCACAACGTTCATATGATAATCAACCTAGCCAATTTGATCGCCTGTTTAA    1559
               **.******************** ***********************************


CF1tcdA       TACGCCATTACTGAACGGACAATATTTTTCTACCGGCGATGAAGAGATTGATTTAAATTC    1619
AF188483.1    TACGCCATTACTGAACGGACAATATTTTTCTACCGGCGATGAGGAGATTGATTTAAATTC    1619
              ******************************************.*****************

CF1tcdA       AGGTAGCACCGGTGATTGGCGTAAAACTGTACTTAAGCGTGCATTTAATATCGATGATGT    1679
AF188483.1    AGGTAGCACCGGCGATTGGCGAAAAACCATACTTAAGCGTGCATTTAATATTGATGATGT    1679
              ************ ********:***** .********************** ********

CF1tcdA       CTCGCTCTACCGGCTGCTTAAAATTACCGACCATGATAATAAAGACGGGAAAATTAAAAA    1739
AF188483.1    CTCGCTCTTCCGCCTGCTTAAAATTACCGACCATGATAATAAAGATGGAAAAATTAAAAA    1739
              ********:*** ******************************** **.***********

CF1tcdA       TAACCTAAAGAATCTTTCCAATTTATATATTGGTAAATTACTGGCAGATATTCATCAATT    1799
AF188483.1    TAACCTAAAGAATCTTTCCAATTTATATATTGGAAAATTACTGGCAGATATTCATCAATT    1799
              *********************************:**************************

CF1tcdA       AACCATTGATGAATTGGATTTATTACTGGTTGCCGTAGGTGAAGGAAAAACTAATCTATC    1859
AF188483.1    AACCATTGATGAACTGGATTTATTACTGATTGCCGTAGGTGAAGGAAAAACTAATTTATC    1859
              ************* **************.************************** ****

CF1tcdA       CGCTATCAGTGATAAACAGTTGGCTACCCTGCTCAGAAAACTCAATACTATTACCGGCTG    1919
AF188483.1    CGCTATCAGTGATAAGCAATTGGCTACCCTGATCAGAAAACTCAATACTATTACCAGCTG    1919
              ***************.**.************.***********************.****

CF1tcdA       GTTACATACACAAAAGTGGAGTGTATTCCAGCTATTTATCATGACCTCCACCAGCTATAA    1979
AF188483.1    GCTACATACACAGAAGTGGAGTGTATTCCAGCTATTTATCATGACCTCCACCAGCTATAA    1979
              * **********.***********************************************
```

**Figure 10. SNPs in TcdA region 2 compared to reference sequence**

**Region3**

```
CF1tcdA         CGGCGACGGCGCAATGACAGCAGAAAAATTCTGGGACTGGTTGAATACTCAATATACGCC    2219
AF188483.1      CGGCGACGGCGCAATGACAGCAGAAAAATTCTGGGACTGGTTGAATACTAAGTATACGCC    2219
                ************************************************* * ********

CF1tcdA         GGATTCATCGGAAGCAGTAGAAACGCAGGAACATATCGTTCAGTATTGTCAGGCTCTGGC    2279
AF188483.1      GGGTTCATCGGAAGCCGTAGAAACGCAGGAACATATCGTTCAGTATTGTCAGGCTCTGGC    2279
                ** ************ ********************************************

CF1tcdA         ACAATTGGAAATGGTTTATCATTCCACCGGCATCAACGAAAACGCCTTCCGTCTATTTGT    2339
AF188483.1      ACAATTGGAAATGGTTTACCATTCCACCGGCATCAACGAAAACGCCTTCCGTCTATTTGT    2339
                ****************** *****************************************

CF1tcdA         GACAAAACCAGAGATGTTTGGCGCTTCAACTGGAGCAACGCCTGCGCATGATGCCCTTTC    2399
AF188483.1      GACAAAACCAGAGATGTTTGGCGCTGCAACTGGAGCAGCGCCCGCGCATGATGCCCTTTC    2399
                ************************* *********** ***** ****************

CF1tcdA         ACTGATCATGCTGACGCGTTTTGCGGATTGGGTGAACGCACTAGGCGAAAAAGCGTCCTC    2459
AF188483.1      ACTGATTATGCTGACACGTTTTGCGGATTGGGTGAACGCACTAGGCGAAAAAGCGTCCTC    2459
                ****** ******** ********************************************

CF1tcdA         GGTGCTAGCGGCATTTGAAGCTAACACGTTAACGGCAGAACAACTGGCTGATGCCATGAA    2519
AF188483.1      GGTGCTAGCGGCATTTGAAGCTAACTCGTTAACGGCAGAACAACTGGCTGATGCCATGAA    2519
                ************************* **********************************


CF1tcdA         CCCAGTAACTCCAGAAAATGCGTTCTCCTGTTGGACATCTATCGATACTATCCTGCAATG    2639
AF188483.1      CCCAGTAACTCCAGAAAATGCGTTCTCCTGTTGGACATCTATCAATACTATCCTGCAATG    2639
                ******************************************* ****************

CF1tcdA         GGTTAATGTCGCACAACAATTGAATGTCGCCCCACAGGGCGTTTCCGCTTTGGTCGGGCT    2699
AF188483.1      GGTTAATGTCGCACAACAATTGAATGTCGCCCCACAGGGCGTTTCCGCTTTGGTCGGGCT    2699
                ************************************************************

CF1tcdA         GGATTATATTCAATCAATGAAAGAGACACCGACCTATGCCCAGTGGGAAAACGCGGCAGG    2759
AF188483.1      GGATTATATTCAATCAATGAAAGAGACACCGACCTATGCCCAGTGGGAAAACGCGGCAGG    2759
                ************************************************************

CF1tcdA         CGTATTAACCGCCGGGTTGAATTCACAACAGGCTAATACATTACACGCTTTTCTGGATGA    2819
AF188483.1      CGTATTAACCGCCGGGTTGAATTCACAACAGGCTAATACATTACACGCTTTTCTGGATGA    2819
                ************************************************************

CF1tcdA         ATCTCGCAGTGCCGCATTAAGCACCTACTATATCCGTCAAGTCGCCAAGGCAGCGGCGGC    2879
AF188483.1      ATCTCGCAGTGCCGCATTAAGCACCTACTATATCCGTCAAGTCGCCAAGGCAGCGGCGGC    2879
                ************************************************************

CF1tcdA         TATTAAAAGCCGTGATGATCTGTATCAGTATTTACTGATTGATAATCAGGTTTCCGCGGC    2939
AF188483.1      TATTAAAAGCCGTGATGACTTGTATCAATACTTACTGATTGATAATCAGGTTTCTGCGGC    2939
                ******************  ******* ** *********************** *****
```

**Figure 11. SNPs in *TcdA* region 3 compared to reference sequence**

**Region 4**

```
CF1tcdA        CTGGGACAAATACAATAAACGCTACAGCACTTGGGCTGGTGTTTCTCAATTAGTTTACTA    3119
AF188483.1     CTGGGACAAATACAATAAACGCTACAGCACTTGGGCGGGTGTTTCTCAATTAGTTTACTA    3119
               ************************************ ***********************

CF1tcdA        CCCGGAAAACTATATTGATCCGACCATGCGTATCGGACAAACCAAAATGATGGACGCATT    3179
AF188483.1     CCCGGAAAACTATATTGATCCGACCATGCGTATCGGACAAACCAAAATGATGGACGCATT    3179
               ************************************************************

CF1tcdA        ACTGCAATCCGTCAGCCAAAGCCAATTAAACGCCGATACCGTCGAAGATGCCTTTATGTC    3239
AF188483.1     ACTGCAATCCGTCAGCCAAAGCCAATTAAACGCCGATACCGTCGAAGATGCCTTTATGTC    3239
               ************************************************************

CF1tcdA        CTACCTGACATCGTTTGAACAGGTGGCTAATCTTAAAGTTATTAGCGCGTATCACGATAA    3299
AF188483.1     TTATCTGACATCGTTTGAACAAGTGGCTAATCTTAAAGTTATTAGCGCATATCACGATAA    3299
                ** *****************.**************************.***********

CF1tcdA        TATTAATAACGATCAAGGGCTGACCTATTTTATCGGGCTCAGTGAAACTGATGCCGGTGA    3359
AF188483.1     TATTAATAACGATCAAGGGCTGACCTATTTTATCGGACTCAGTGAAACTGATGCCGGTGA    3359
               ************************************.***********************

CF1tcdA        ATATTATTGGCGCAGTGTCGATCATAGTAAATTCAGCGATGGTAAATTCGCGGCTAATGC    3419
AF188483.1     ATATTATTGGCGCAGTGTCGATCACAGTAAATTCAACGACGGTAAATTCGCGGCTAATGC    3419
               ************************ **********.*** ********************

CF1tcdA        AGTGATATATAAATCCCGCCTGTATCTGCTCTGGTTGGAACAAAAGGAGATAACCAAACA    3539
AF188483.1     AGTGATATATAAATCCCGCCTGTATCTGCTCTGGTTGGAACAAAAGGAGATCACCAAACA    3539
               ***************************************************.********

CF1tcdA        GACAGGAAATAGTAAAGATGGCTATCAAACTGAAACGGATTATCGTTATGAACTGAAATT    3599
AF188483.1     GACAGGAAATAGTAAAGATGGCTATCAAACTGAAACGGATTATCGTTATGAACTAAAATT    3599
               ******************************************************.*****

CF1tcdA        GGCACATATCCGCTATGACGGTACTTGGAATACGCCAATCACCTTTGATGTCAATGAAAA    3659
AF188483.1     GGCGCATATCCGCTATGATGGCACTTGGAATACGCCAATCACCTTTGATGTCAATAAAAA    3659
               ***.************** ** *********************************.****

CF1tcdA        AATATCCAAGCTAGCACTGGCAAAAAATAAAGCGCCCGGACTCTATTGTGCCGGTTATCA    3719
AF188483.1     AATATCCGAGCTAAAACTGGAAAAAAATAGAGCGCCCGGACTCTATTGTGCCGGTTATCA    3719
               *******.*****..*****.********.******************************

CF1tcdA        AGGTGAAGATACGTTGCTGGTGATGTTTTATAACCAGCAAGACACACTAGATAGTTATAA    3779
AF188483.1     AGGTGAAGATACGTTGCTGGTGATGTTTTATAACCAACAAGACACACTAGATAGTTATAA    3779
               ************************************.***********************

CF1tcdA        AAACGCTTCAATGCAAGGGCTATATATCTTTGCCGATATGGTATCCAAAGATATGACTCC    3839
AF188483.1     AAACGCTTCAATGCAAGGACTATATATCTTTGCTGATATGGCATCCAAAGATATGACCCC    3839
               ******************.************** ******* *************** **
```

**Figure 12. SNPs in *TcdA* region 4 compared to reference sequence**

**Region 7**

```
CF1tcdA         GCACATGCTGGAAAATGCGCGCGGCATGGTTAGCCAGCTCACCCAATTCGGCTCCACGTT    6119
AF188483.1      GCACATGCTGGAAAATGCGCGCGGCATGGTTAGCCAGCTCACCCAGTTCGGCTCCACGTT    6119
                *********************************************.**************

CF1tcdA         ACAAAATATTATCGAACGTCAGGACGTGGAAGCGCTCAATGCGTTATTACAAAATCAGGC    6179
AF188483.1      ACAAAATATTATCGAACGTCAGGACGCGGAAGCGCTCAATGCGTTATTACAAAATCAGGC    6179
                ************************** *********************************

CF1tcdA         CGCCGAGCTGATATTGACTAACCTGAGCATTCAGGACAAAACCATTGAAGAATTGGATGC    6239
AF188483.1      CGCCGAGCTGATATTGACTAACCTGAGCATTCAGGACAAAACCATTGAAGAATTGGATGC    6239
                ************************************************************

CF1tcdA         CGAGAAAACGGTGTTGGAAAAATCCAAAGCGGGAGCACAATCGCGCTTTGATAGCTACAG    6299
AF188483.1      CGAGAAAACGGTGTTGGAAAAATCCAAAGCGGGAGCACAATCGCGCTTTGATAGCTACGG    6299
                **********************************************************.*

CF1tcdA         CAAACTGTACGATGAGAATATCAACGCCGGTGAAAACCAAGCCATGACGCTACGAGCGTC    6359
AF188483.1      CAAACTGTACGATGAGAATATCAACGCCGGTGAAAACCAAGCCATGACGCTACGAGCGTC    6359
                ************************************************************

CF1tcdA         CGCCGCCGGGCTTACCACGGCAGTTCAGGCATCCCGTCTGGCCGGCGCGGCGGCTGATCT    6419
AF188483.1      CGCCGCCGGGCTTACCACGGCAGTTCAGGCATCCCGTCTGGCCGGTGCGGCGGCTGATCT    6419
                ********************************************* **************

CF1tcdA         GGTGCCTAACATCTTCGGCTTTGCCGGTGGCGGCAGCCGTTGGGGAGCCATCGCTGAGGC    6479
AF188483.1      GGTGCCTAACATCTTCGGCTTTGCCGGTGGCGGCAGCCGTTGGGGGGCTATCGCTGAGGC    6479
                *********************************************.** ***********

CF1tcdA         GACAGGTTATGTGATGGAGTTTTCCGCGAATGTTATGAACACAGAAGCGGATAAAATTAG    6539
AF188483.1      GACAGGTTATGTGATGGAATTCTCCGCGAATGTTATGAACACCGAAGCGGATAAAATTAG    6539
                ******************.** ********************.*****************

CF1tcdA         CCAATCTGAAACTTACCGTCGTCGCCGTCAGGAGTGGGAGATCCAGCGGAATAATGCCGA    6599
AF188483.1      CCAATCTGAAACCTACCGTCGTCGCCGTCAGGAGTGGGAGATCCAGCGGAATAATGCCGA    6599
                ************ ***********************************************

CF1tcdA         AGCGGAATTGAAGCAAATCGATGCTCAGCTCAAATCACTCGCTGTACGCCGCGAAGCCGC    6659
AF188483.1      AGCGGAATTGAAGCAAATCGATGCTCAGCTCAAATCACTCGCTGTACGCCGCGAAGCCGC    6659
                ************************************************************

CF1tcdA         CGTATTGCAGAAAACCAGTCTGAAAACCCAACAAGAACAGACCCAATCTCAATTGGCCTT    6719
AF188483.1      CGTATTGCAGAAAACCAGTCTGAAAACCCAACAAGAACAGACCCAATCTCAATTGGCCTT    6719
                ************************************************************

CF1tcdA         CCTACAACGTAAGTTCAGCAATCAGGCGTTATACAACTGGTTGCGTGGTCGACTGGCGGC    6779
AF188483.1      CCTGCAACGTAAGTTCAGCAATCAGGCGTTATACAACTGGCTGCGTGGTCGACTGGCGGC    6779
                ***.************************************ *******************
```

**Figure 13. SNPs in *TcdA* region 7 compared to reference sequence**

**Table 20. continued**

## Region 8

```
CF1tcdA        CTATGCCGGTCTGCTTGCAGGTGAAACCCTTGATGCTGAGTCTGGCACAAATGGAAGACG   6959
AF188483.1     CTATGCCGGTCTGCTTGCAGGTGAAAC-CTTGATGCTGAGTCTGGCACAAATGGAAGACG   6958
               *************************** ********************************

CF1tcdA        CTCATCTGAAACGCGATAAACGCGCATTAGAGGTTGAACGCACAGTATCGCTGGCCGAAG   7019
AF188483.1     CTCATCTGAAACGCGATAAACGCGCATTAGAGGTTGAACGCACAGTATCGCTGGCCGAAG   7018
               ************************************************************

CF1tcdA        TTTATGCAGGATTACCAAAAGATAACGGTCCATTTTCCCTGGCTCAGGAAATTGACAAGC   7079
AF188483.1     TTTATGCAGGATTACCAAAAGATAACGGTCCATTTTCCCTGGCTCAGGAAATTGACAAGC   7078
               ************************************************************

CF1tcdA        TGGTGAGTCAAGGTTCAGGCAGCGCCGGCAGTGGTAATAATAATTTGGCGTTCGGCGCCG   7139
AF188483.1     TGGTGAGTCAAGGTTCAGGCAGTGCCGGCAGTGGTAATAATAATTTGGCGTTCGGCGCCG   7138
               ********************** *************************************

CF1tcdA        GCACGGACACTAAAACCTCTTTGCAGGCATCAGTTTCATTCGCTGATTTGAAAATTCGTG   7199
AF188483.1     GCACGGACACTAAAACCTCTTTGCAGGCATCAGTTTCATTCGCTGATTTGAAAATTCGTG   7198
               ************************************************************

CF1tcdA        AAGATTACCCGGCATCGCTTGGCAAGATTCGACGTATCAAACAGATCAGCGTCACCTTGC   7259
AF188483.1     AAGATTACCCGGCATCGCTTGGCAAAATTCGACGTATCAAACAGATCAGCGTCACTTTGC   7258
               *************************.***************************** ****
```

**Figure 14. SNPs in *TcdA* region 8 compared to reference sequence**

Discussion

# 5. DISCUSSION

The results of the study entitled "Molecular mechanism of virulence in the bacterium, *Photorhabdus luminescens* (Thomas and Poinar) against *Tetranychus truncatus* Ehara (Prostigmata: Tetranychidae)" are discussed critically by using the available literature.

## 5.1 Isolation of bacteria associated with the entomopthogenic nematodes

### 5.1.1 Isolation of entomopathogenic nematode, *Heterorhabditis*

In the first part of the study, four isolates of entomopathogenic nematode (EPN), *Heterorhabditis* were isolated from the soil samples collected from the cropped areas of different localities (Kerala) by using *Galleria* baiting method explained by Bedding and Akhurst (1975). The baiting method was successfully employed by several scientists for isolation of entomopathogenic nematodes from soil (Stuart and Gaugler, 1994; Hsieh *et al.,* 2009; Kumar *et al*., 2011; Orozco *et al*., 2014; Pervez *et al*., 2014; Devi *et al*., 2016; Kalita *et al*., 2019; Bhat *et al.,* 2019). Entomopathogenic nematodes are cosmopolitan in nature but their distribution in the soil depends on soil moisture, soil type, physical and chemical parameters of the soil. It may also vary with the seasons and many other unknown factors. Bhat *et al*. (2020) used *Galleria* baiting method for the isolation of 29 isolates of *Steinernema* spp. and 12 isolates of *Heterorhabditis indica* from the agricultural soils of Western Uttar Pradesh, India. Final instar larvae of *Corcyra cephalonica* were used by Seenivasan and co-workers (2012) for the isolation of EPN as a modification to the standard baiting methodand isolated 27 isolates of EPN from the cotton fields of 10 different districts of Tamil Nadu in India.

### 5.1.2 Isolation and identification of bacterial isolates from the entomopathogenic nematode *Heterorhabditis* spp.

*Photorhabdus luminescens* (Enterobacteriaceae), is the main entomopathogenic bacteria which exists in association with the EPN *Heterorhabditis.* The EPN infected cadavers obtained from the *Galleria* baiting method were used for

the isolation of the bacterial isolates. The *Galleria* larvae killed by *Heterorhabditis*, became brick red and the tissues turned to a gummy consistency (Shahina *et al*., 2004; Orozco *et al.,* 2014; Paschapur *et al.,* 2018; Manickam *et al.,* 2017). In this study, cadavers which turned brick red, indicating infection by *Heterorhabditis* were used for isolation of bacteria associated with the EPN. A drop of haemolymph from the aseptically dissected infected cadavers were streaked on Petri plates containing NBTA media for the bacterial isolation and incubated in BOD. The standard procedure followed by earlier workers for isolation of bacteria from the infected *Galleria* cadaver was followed in this study (Fukruksa *et al.,* 2017; Uma *et al*., 2010a; Kumar *et al*., 2014; Kumar and Mohan 2013). In this study, eleven bacterial isolates (KL1, KT1, MT1, FR1, EKM1, CF1, HI1, MP1, HS1, HQ1 and WH1) were isolated in association with *Heterorhabditis* infected cadaver.

### 5.1.2.1 Cultural characteristics of bacterial isolates on NBTA media

Eleven isolates of bacteria associated with the EPN (*Heterorhabditis* spp.) were identified based on morphological and molecular characteristics belonging to four genera namely, *Photorhabdus*, *Pseudomonas*, *Ochrobactrum* and *Stenotrophomonas*

In this study, the isolates of *Pseudomonas aeruginosa* (KL1 and KT1) produced opaque, flat, circular colonies with irregular edge. Yellowish media turned to bluish green due to pigmentation. The morphology and colony characteristics are in agreement with the reports of Gaby (1946) and Raja *et al*. (2006). *P. aeruginosa* produces an antimicrobial soluble pigment, pyocyan which gives characteristic bluish green colour to the media (Hassan and Fridovich, 1980; El-Fouly *et al*., 2015)

The isolates MP1, FR1, EKM1, HI1, HQ1 and HS1 also produced similar colonies but differed from KL1 and KT1 in colour. The cells were Gram-negative, short to long rods, and the colonies were opaque, smooth, flat and the colour varied from red (FR1, HI1) to pinkish red (HS1, MP1, EKM1, HQ1). The isolates were identified as *Ochrobactrum* spp. based on molecular studies. The cultural characters of *Ochrobactrum* spp. isolated in the study are in agreement with the reports of Abouelhag and El-Sadawy (2012), Razia *et al*. (2011) and Upadhyay *et al*. (2019). Fu

and Liu (2019) also observed opaque, mucoid, smooth, protuberant and rapidly confluent *Ochrobactrum* colonies on NBTA media.

The isolate of *P. luminescens* (CF1) produced smooth, circular, entire, opaque, raised, dark red colonies with white margin in NBTA media. The media colour changed from yellowish to greenish blue after three days. The cells were found to be Gram negative, short rods. The isolates of *P. luminescens* were reported to produce small, circular, raised, opaque, light red colonies with white margin (Pervez *et al*., 2015). Similar observations were recorded by Pervez *et al*. (2020); Peel *et al*. (1999); Glaeser *et al.* (2017); An and Grewal (2010); and Nagesh *et al.* (2002). However, Kumar *et al*. (2014) reported that colonies produced by *P. luminescens* were white and shiny, small, smooth, circular, glistering, with entire margin.

#### 5.1.2.2 Molecular characterisation

Drancourt *et al*. (2000) used *16S rRNA* gene based analysis for the identification of large number of unidentifiable bacterial isolates and reported it as an excellent method for bacterial identification where the phenotypic methods for the same is difficult. Tran and co-workers (2017) reported that short reads from *16S rRNA* genes retain sufficient information for the identification of unknown bacteria in the oral microbial communities. The same method was followed by a number of researchers for the easier identification of bacterial isolates (Brunel *et al*., 1997; Hsieh *et al*., 2009; An and Grewal, 2010; Aly and Mona, 2009). Pervez *et al.* (2020) isolated symbiotically associated *P. luminescens* (one isolate) and some associated bacterial isolates (five) from *Heterorhabditis* spp. (IISR-EPN) and characterized by using *16S rRNA* gene analysis by confirming the amplification by visualizing the single dark distinct bands produced by all the six isolates around 1500 bp on agarose gel electrophoresis. They also reported that the accuracy of bacterial identification through *16S rRNA* analysis increases in combination with the conventional methods (morphological and biochemical characterization).

In the study, universal primers were used for the amplification of the *16S rRNA* gene to identify the bacteria at molecular level. Colony PCR method was used for the amplification of the gene which is an easy and quick process for DNA sequence

identification (Woodman, 2008). The amplified products were confirmed on agarose gel. The position of single intense band around 1500 bp was same for all the eleven isolates, which confirmed the successful amplification of *16S rRNA* gene. As the sequence information was incomplete for two isolates, remaining nine isolates were used for further study.

The morphological observations on nine bacterial isolates obtained in the study were validated by using molecular methods, by comparing the sequences of *16S rRNA* gene with the existing sequences in NCBI and sequence homology was analysed by using BLAST programme. Altschul *et al*. (1990) suggested that the homology searching tool, BLAST, which can detect significant sequence similarity can be used for rapid sequence comparison of genes.In BLAST analysis, the accession CF1 showed maximum similarity with the accession JX221723.1 representing the species *P. luminescens* reported by Somvanshi *et al*. (2012) from India confirming the morphological identification on the study. Aly and Mona (2009) isolated three new isolates of *P. luminescens* from *Heterorhabditis bacteriophora* and one isolate of *X. nematophila* from *Steinernema carpocapsae* and characterized by using conventional methods (cultural and morphological characterization) and later confirmed by comparing the *16S rRNA* sequence in GenBank using the BLAST programme. The accessions KL1 and KT1 in this study were identified as *Pseudomonas aeruginosa*, while the accessions MP1, FR1, EKM1, HI1 and HS1 showed sequence homology with the bacterial genus *Ochrobactrum* (FR1- *Ochrobactrum pseudogrignonense*; HI1- *Ochrobactrum anthropi,* MP1, EKM1 and HS1- unidentified species of *Ochrobactrum*). The isolate HQ1 was identified as *Stenotrophomonas* sp. Morphological observations also confirmed the same.

The bioluminescent, entomopathogenic bacteria, *Photorhabdus luminescens* is a natural symbiont of the entomopathogenic nematode, *Heterorhabditis* spp. The bacteria supports growth of the nematode in the association, but is a deadly pathogen to most of the insect pests (Clarke, 2008). These are present in the gut of the nematode and released in to the insect haemocoel soon after the EPN infects the insect. Within 24-48 h of infection, the bacteria kill the host by rapid multiplication

and septicaemia (Forst *et al*. 1997). These are the predominant group of bacteria which are associated with the EPN, *Heterorhabditis* spp.

A number of other bacteria also were reported to be associated with the EPN, *Heterorhabditis* spp. For instance, Ruiu *et al*. (2017) isolated different bacterial species viz., *Alcaligenes aquatilis, Alcaligenes faecalis, Enterococcus mundtii, Pseudomonas protegens, Serratia nematodiphila, Serratia marcescens* and *Stenotrophomonas maltophilia* from the *Heterorhabditis* infected *Galleria* cadaver. Upadhyay *et al.* (2019) isolated the non-symbiotic bacterial contaminants from *Heterorhabditis indica* and infected *Galleria mellonella* and *Dorysthenes huegelii* cadavers and were identified (using *16S rRNA* analysis) as *Bacillus subtilis*, *B*. *licheniformis*, *Staphylococcus cohnii* and *Acinetobacter rhizosphaerae* (*D*. *huegelii*), *Bacillus bombysepticus*, *Ochrobactrum anthropi*, *Ochrobactrum* sp. (*G.* *mellonella*) and *Sphingomonas koreensis*, *Sphingobacterium* sp., *Ochrobactrum anthropi* (*H. indica*)

*Pseudomonas* is also an important genus of bacteria containing many significant disease causing bacterial species as an opportunistic pathogen. *P. aeruginosa* is a free living, gram negative bacteria having extensive metabolic diversity/flexibility (one of the reasons for its opportunistic nature).

Alnor *et al.* (1994) and Velasco *et al*. (1998) reported the similarity of *O. anthropi and O. Intermedium* with *Photorhabdus luminescens* subsp. *akhurstii* (from *Heterorhabditis indica*). The isolates of different species of *Ochrobactrum* isolated from EPN infected *Galleria* cadaver in this study also showed similarity in morphological characters with the isolate of *Photorhabdus luminescens.*

*Stenotrophomonas* spp. formerly isolated as *Pseudomonas maltophilia* is a Gram negative, emerging multidrug-resistant opportunistic pathogen. *Stenotrophomonas maltophilia* was isolated from the *Heterorhabditis* infected *Galleria* cadaver by Ruiu *et al*. (2017).

#### 5.1.2.3 Phylogenetic analysis

For phylogenetic analysis of *16S rRNA* sequences, tree can be constructed by neighbor joining method using (MEGA X software). In the present study, tree was constructed for 56 *16S rRNA* sequences representing four species with 500 bootstrap replications. The tree formed two clades with all sequences of *Ochrobactrum* spp. and *Stenotrophomonas* sp. grouping in to a single clade. In the other clade, the sequences of *Photorhabdus* spp. and *Pseudomonas* sp. were grouped.

In clade 1 the sequences of *Ochrobactrum* spp .and *Stenotrophomonas* spp. formed two subclades indicating the divergence between the two genera of bacteria which are having common ancestory. In clade 2 the sequences of *Photorhabdus* and *Pseudomonas* were sub clustered which indicates the evolutionary relationship among the two species. Ruffner *et al*. (2015) studied the evolutionary events which is responsible for the different versions of the Mcf/Fit toxin in *Photorhabdus* and *Pseudomonas* through phyologenetic analyses. They suggested a common ancestry for the insecticidal toxin genes in the two bacteria.

## 5.2 Bioassay of different bacterial isolates against spider mites

In the present study, the effect cell suspension and cell free supernatant of five bacterial isolates were evaluated at various concentrations against eggs and adults of *T. truncatus* separately.

### 5.2.1 Laboratory evaluation of cell suspension and cell free supernatant

Among the five different bacterial isolates evaluated in the present study, none of the isolate's cell suspension showed significant ovicidal action against *T. truncatus* eggs at all the five different concentrations evaluated viz., $10^4$, $10^5$, $10^6$, $10^7$ and $10^8$ Cells/ml. The entomopathogenic bacteria *P. luminescens,* though recorded significant mortality of adult mite, could cause only 29.33 per cent mortality of egg at 96 h of treatment, even at the highest concentration of $10^8$ Cells/ml which is superior to other bacterial isolates. In adulticidal assay, the isolate of *P. luminescens* could cause 60.67 per cent mortality of adult mites within 96 h in the laboratory, when applied at the

concentration of $10^8$ Cells/ml. This clearly indicates that the cell suspension of *P. luminescens* isolates is more effective as adulticidal than ovicidal.

Adult mortality rate was highest with *P. luminescens* treatment, in comparison to *P. aeruginosa* and *Ochrobactrum*. At highest concentration of $10^8$ Cells/ml, *P. luminescens* recorded 60.67 per cent mortality. Among the treatments of *P. aeruginosa*, highest mortality of 33.33 per cent was recorded in the treatment $10^8$ Cells/ml of *P. aeruginosa* strain 2 (KT1 isolate). No significant mortality was observed in any of the treatments of *Ochrobactrum* spp. This clearly indicates that *P. luminescens* is more effective against *T. truncatus* than the other two bacteria (Figure 15). The efficacy of the symbiotic bacteria *P. luminescens* against different insect pests has been reported by several workers. However studies on efficacy of *P. luminescens* on mites are limited.

Bussaman *et al*. (2012) assessed the pathogenicity of five strains of *Xenorhabdus* and one strain of *Photorhabdus* against mushroom mite, *Lucia phorusperniciosus* and observed the highest mortality of 85 and 83 per cent with the strains *X. nematophila* (X1) and *P. luminescens* (P1) respectively, at $1\times10^8$ Cells/ml after 3 days of treatment. They also reported the increased mortality with increased concentration of bacterial cells from $1\times10^4$ Cells/ml to $1\times10^8$ Cells/ml and no significant difference in further high concentrations. Kumar *et al*. (2014) studied the bioefficacy of broth cultures of different isolates of *P. luminescens* against sucking pest (*Aphis gossypii* and *Tetranychus macfarlanei*) and defoliators (*Plutella xylostella* and *Spodoptera litura*). The isolate Z-8-1 was found to be effective against *Aphis gossypii* after 24 h with 100 per cent of nymphal mortality and the isolate Z-3-1 was effective against *T. macfarlanei* with 100 per cent mortality after 36 h. None of the isolates were pathogenic to defoliators. Abdel-Razek *et al*. (2003) observed 40 percent mortality of diamondback moth, *Plutella xylostella* pupae with *X. nematophilus* (LC50 values - $5.5\times10^5$) and 60 per cent mortality of the same with *P. luminescens (*$5\times10^4$ Cells/ml) and thus reported that the *P. luminescens* strains were more effective than *X. nematophilus*.

Fu and Liu (2019) evaluated the entomopathogenecity of one of the *Ochrobactrum* species (*Ochrobactrum tritici*) isolated from the EPN *Oscheius chongmingensis* against *Galleria mellonella* and observed 93.33 per cent mortality within 144 h at $2 \times 10^9$ CFU/ml concentration. In contrast to this, Babic *et al.* (2000) reported that *Ochrobactrum* spp, showed no pathogenicity to the Lepidopteran insects, *G. mellonella* and *S. littoralis*, which were treated with the the bacterium. Ryan and Pembroke (2020) also reported the low virulence of *Ochrobactrum* due to limited number of virulence factors. Present study reveals that *Ochrobactrum* is less virulent against the mite pest, *T. truncatus* as indicated by the low mortality (12.00 % in Cell suspension and 18.67 % in CFS) exhibited in the laboratory bioassays.

Salgado-Morales and co-workers (2019) assessed the pathogenicity of two bacterial species *(P. luminescens* HIM3 and *P. aeruginosa* NA04) which were isolated from the EPN *H. indica* against *G. mellonella* and some other insect pests. Both the isolates were found to be highly virulent to *G. mellonella* with almost 100 per cent of mortality after 24 h. *Photorhabdus luminescens* was virulent to all the insect hosts but the pathogenicity *P. aeruginosa* varied with the hosts. Higher dose of *P. aeruginosa* was required for the infection of *Tenebrio molitor.* Only 10 per cent of mortality was observed in case of *Diatraea magnifactella* Dyar after 36 h of treatment, however 100 per cent mortality was shown with *P. luminescescens.* In the present study mortality of *T. truncatus* was less when treated with *P. aeruginosa* compared to *P. luminescens*.

Oral toxicity of *Xenorhabdus* and *Photorhabdus* bacteria against *Aedes* spp. was confirmed by Vitta *et al*. (2018) as they produced 80-99 as well as 50-78 per cent of larval mortality, respectively after 96 h. Two species of *Aedes viz*., *Aedes aegypti* and *Aedes albopictus* were treated with 2 ml of a suspension at $10^7$-$10^8$ CFU/ml concentration

Uma *et al*. (2010a) evaluated the efficacy of *P. luminescens* against *Thrips palmi* by using different concentrations of cells and cells free supernatant, from $10^1$ to $10^{10}$ Cells/ CFS/ml. At the highest concentration of $10^{10}$ Cells/CFS/ml

highest mortality of 67.50 per cent (Cell suspension) and 85.00 per cent (CFS) was observed after 48 h of treatment. By considering the results they suggested that the CFS was more virulent than the cell suspension. In this study, no significant ovicidal effect was observed in any of the treatments. Even at higher concentration of $10^8$ CFS/ml, *P. luminescens* caused only 33.33 per cent mortality of eggs. However, the isolate showed significant adulticidal effect recording 64.00 per cent of mortality at the concentration of $10^8$ CFS/ml (Figure 16)

Efficacy of both cell suspension and cell free supernatants of the entomopathogenic bacteria *P. luminescens* isolated from the EPN, *Heterorhabditis indica* was evaluated against *Aphis gossypii* by Uma *et al*. (2010b) at different concentrations ($10^1$ to $10^{10}$ Cells/CFS/ml). Almost 100 per cent mortality was observed in the highest concentration of CFS ($10^{10}$ CFS/ml - LC50 - $2.02 \times 10^2$ CFS) and a mortality of 77.50 per cent was recorded in the treatments cell suspension at $10^{10}$ Cells/ml concentration. They reported efficacy of CFS is more in comparison with the cell suspension. In contrast to this, Shahina *et al.* (2011) reported higher mortality of *Galleria mellonella* larva and subterranean termite, *Macrotermis* sp. in the treatments of cell suspension of *P. luminescens* compared to CFS. Maximum cell penetration was observed with the concentration $4.0 \times 10^4$ bacterial cell/ml. A mortality of 65 percent for *G. mellonella* and 80 percent for *Macrotermis* spp. was observed in the cell free supernatant study, while 98 per cent mortality was observed in the cell suspension (palletes) treatments. However, in the present study, CFS recorded higher mortality of *T. truncatus* than the cell suspension treatments.

### 5.3 Molecular basis of virulence in *P. luminescens* isolate against *Tetranychus truncatus*

The toxin complex gene, *TcdA* is a big gene of 7.5 kb size and hence amplification of full length gene is difficult. Mulla *et al.* (2017) though attempted amplifying the whole *TcdA* gene of *P. luminescens* could not succeed. However, they could successfully amplify the whole gene by splitting the gene into three regions using three sets of internal primers. In this study, amplification of *TcdA* gene using the internal primers which were reported by Mulla *et al.* (2017) has failed. Later, eight

primer sets were designed to amplify the toxin gene (*TcdA*) by splitting the entire gene to eight regions. Among the eight, six regions have got successfully amplified. The contigs from the six regions were aligned and the sequence for the regions generated. The toxin gene of *P. luminescens* isolate showed 98.89 per cent similarity with the reference gene (AF188483.1) in NCBI database.

The Toxin complexes (TCs) produced by the bacteria *P. luminescens* are orally active and possess insecticidal property. These are encoded by different loci *viz*., *tca, tcb, tcc,* and *tcd*. (Kushwah and Somvanshi, 2015). The loci *tcd* and *tca* produce most of the orally active toxins, while the *tcc* genes are responsible for the putative virulence functions (Blackburn *et al*., 1998; Yang *et al*., 2006). The *tcd* locus is by far the largest of the four *tc* loci, and it consists of many subgroups of genes *viz*., a group of four genes (*tcdA1-tcdA4)*, a group of two genes, (*tcdB1-tcdB2*), and again a grou pof four genes (*tccC2-tccC5*) (Hinchliffe *et al.,* 2010).

The toxin gene expression studies carried out by Waterfield *et al.* (2001) by transforming *E. coli* with the toxin complex genes of *P. luminescens* W14 showed that, coexpression of all the three genes (*tcdA, tcdB*, and *tccC*) is essential for oral toxicity of the bacteria against *Manduca sexta*. Silva *et al.* (2002) used *Manduca sexta* as an infection model to study the pathology of *Photorhabdus* infection, wherein soon after the infection, the bacteria releases the toxins, which begins to destroy the midgut epithelium. The destruction leads to a cessation in feeding behaviour of the insect and ultimately results in death due to starvation. Liu *et al*. (2003) reported the efficacy of the toxin, toxin A (283 KDa), encoded by the gene *TcdA* from *P. luminescens* against variety of insects in *Arabidopsis thaliana* using transformation experiments. Waterfield *et al.* (2005) suggested that, further studies are required to understand the full biology of this gene so that, it can be used as a potential alternative toxins to those of *Bacillus thuringiensis* (Bt). Though some studies on pathogenicity of *P. luminescens* against spider mites were carried out earlier (Kumar *et al*., 2014; Uma *et al*., 2010a), the exact mode of action of toxins and the genes encoding the toxin complex in bacteria has not been invrstigated so far.

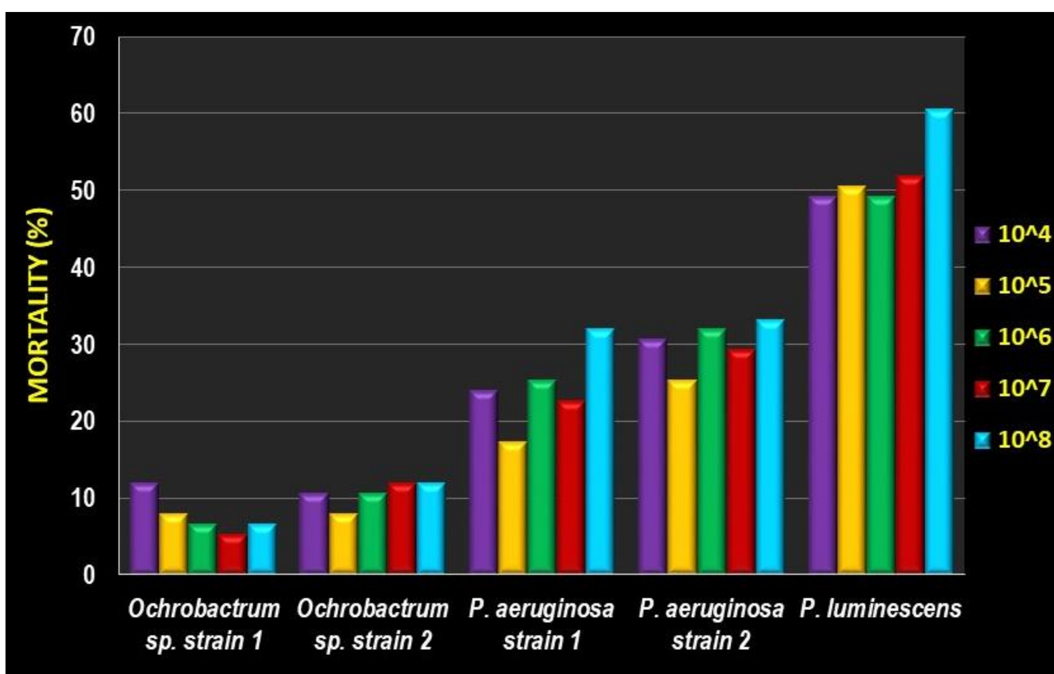



**Figure 15. Adulticidal effect of cell suspension of different bacterial isolates against *Tetranychus truncatus* at 96 h**

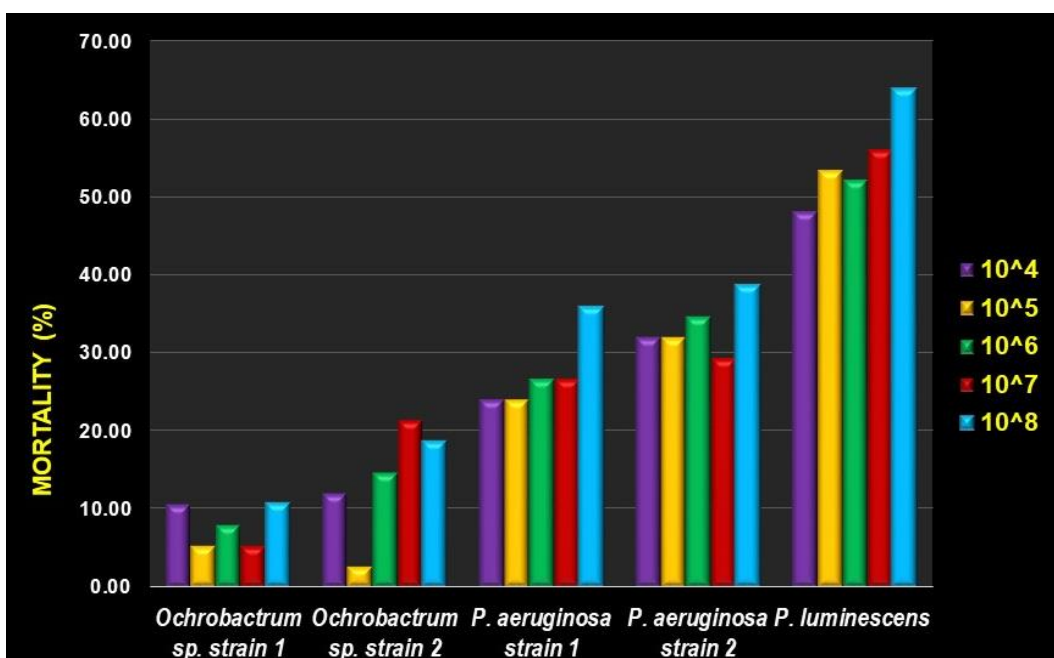



**Figure 16. Adulticidal effect of cell free supernatant of bacterial isolates against *Tetranychus truncatus* at 96 h**

Summary

# 6. SUMMARY

The study entitled "Molecular mechanism of virulence in the bacterium, *Photorhabdus luminescens* (Thomas and Poinar) against *Tetranychus truncatus* Ehara (Prostigmata: Tetranychidae) was carried Centre for Plant Biotechnology and Molecular Biology and All India Network Project on Agricultural Acarology, College of Agriculture, Kerala Agricultural University, Vellanikkara, during 2018-2020. The objectives of the study were to identify the virulent strains of *P luminescens* and to elucidate the molecular mechanism of virulence against *T. truncatus*. Totally eleven bacterial isolates associated with the entamopathogenic nematodes were isolated in the study, of which seven were isolated from the EPN collected from the cultures being maintained in the Acarology laboratory and remaining four isolates were from the EPNs isolated during the study. Soil samples randomly collected from different localities of Kerala were used to collect the EPNs. The bacterial isolates were coded as MP1, HS1, HQ1, WH1, KL1, KT1, MT1, FR1, EKM1, CF1 and HI1 based on the locality from which they were isolated and were identified through morphological and molecular characterization.

Salient findings of the study are as follows

1. All the eleven isolates were subjected to morphological characterization in which they produced circular to irregular, entire, opaque, smooth colonies. Color varied from dark red to pinkish red based on the isolates. All the isolates were found to be Gram negative and rod shaped
2. By using universal primers, the *16S rRNA* gene of the eleven isolates were amplified and sent for sequencing to AgriGenome Labs. Pvt. Ltd., Cochin upon confirmation of amplification. Single intense band produced by all the isolates around 1500 bp on the agarose gel electrophoresis
3. Contigs were developed by merging the forward and reverse sequences obtained from the sequencing results and were used for homology search through BLASTn (NCBI) for the bacterial identification. Of the eleven, only nine isolates were identified, as the sequencing information for the remaining two isolates were incomplete. The isolates KL1 and KT1 were identified as

*Pseudomonas aeruginosa*. The isolate HQ1 was identified as *Stenotrophomonas* sp. The isolate CF1 showed sequence homology with *Photorhabdus luminescens*, while the remaining isolates MP1, FR1, EKM1, HI1 and HS1 were identified as the bacterial genus *Ochrobactrum* (FR1- *Ochrobactrum pseudogrignonense*; EKM1- *O. pseudointermedium*; HI1- *Ochrobactrum anthropi,* MP1 and HS- unidentified species of *Ochrobactrum*)

4. Laboratory bioassays with the cell suspension and cell free supernatants (CFS) of five bacterial isolates (1 isolate of *P. luminescens*, 2 isolates of *P. aeruginosa*, and 2 isolates of *Ochrobactrum sp.*) were carried out separately to evaluate the efficacy against the eggs and adults of *T. truncatus* and the results indicated that none of the bacterial isolates showed significant ovicidal effect, however the significant adulticidal effects were found. The *P. luminescens* isolate was more effective (64.00 % mortality in CFS and 60.67 % mortality in cell suspension treatments at 96 h) compared to the other two bacterial isolates
5. The virulent *P. luminescens* isolate was used for the characterization of toxin complex (*TC* gene)
6. The full length gene (7.5 kb) was split into eight regions and the primers were designed to amplify each regions separately. Among the eight regions, only six got amplified and sequenced
7. Contigs were developed for the six regions and the sequence homology was analysed using BLAST (NCBI)
8. Full length gene was constructed by filling the remaining sequence information from the reference gene
9. Toxin gene sequence of the isolated *P. luminescens* bacteria which involved in the virulence against *T. truncatus* was partially sequenced and the variation in the sequence was analyzed

- The study identified the potential isolate of *P. luminescens* with significant adulticidal action against *T. truncatus*
- The major gene *TcdA* encoding the toxin complexes which possess appreciable insectidal activity was sequenced and characterized

Reference

## 7. REFERENCE

Abdel-Razek, A. S. 2003. Pathogenic effects of *Xenorhabdus nematophilus* and *Photorhabdus luminescens* (Enterobacteriaceae) against pupae of the Diamondback Moth, *Plutella xylostella* (L.). *J. Pest Sci.* 76: 108-111.

Abouelhag, H. A. and El-Sadawy, H. A. 2012. Natural associations between symbionts *Photorhabdus* spp. and *Xenorhabdus* spp. and bacteria related to *Ochrobactrum anthropi*, *Bacillus pumilus* and *Enterobacter cloacae. Life Sci. J.* 9(4): 4783-4790.

Adams, B. J., Fodor, A., Koppenhofer, H. S., Stackebrandt, E., Stock, S. P., and Klein, M. G. 2006. Reprint of biodiversity and systematics of nematode- bacterium entomopathogens. *Biol. Control* 38(1): 4-21.

Akhurst, R. J. 1980. Morphological and functional dimorphism in *Xenorhabdus* spp., bacteria symbiotically associated with the insect pathogenic nematodes *Neoaplectana* and *Heterorhabditis*. *Microbiology* 121(2): 303-309.

Akhurst, R. J. 1982. Antibiotic activity of *Xenorhabdus* spp., bacteria symbiotically associated with insect pathogenic nematodes of the families Heterorhabditidae and Steinernematidae. *Microbiology* 128(12): 3061-3065.

Akhurst, R. J. and Boemare, N. E. 1988. A numerical taxonomic study of the genus *Xenorhabdus* (Enterobacteriaceae) and proposed elevation of the subspecies of *X. nematophilus* to species. *J. Gen. Microbiol.* 134: 1835-1845.

Akhurst, R. J., Mourant, R. G., Baud, L., and Boemare, N. E. 1996. Phenotypic and DNA relatedness study between nematode symbionts and clinical strains of the genus *Photorhabdus* (Enterobacteriaceae). *Int. J. Syst. Bacteriol.* 46: 1034-1041.

Alnor, D., Frimodt-Moller, N., Espersen, F., and Frederiksen, W. 1994. Infections with the unusual human pathogens *Agrobacterium* species and *Ochrobactrum anthropi*. *Clin. Infect. Dis.* 18(6): 914-920.

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. 1990. Basic local alignment search tool. *J. Mol. Biol.* 215(3): 403-410.

Aly, N. A. and Mona, H. A. 2009. Novel characteristics of local *Xenorhabdus* and *Photorhabdus* isolates with phenotypic heterogeneity, *16S rRNA* sequence variation and high toxicity to *Galleria mellonella*. *Genes, Genomes Genomics* 3(1): 72-79.

An, R. and Grewal, P. S. 2010. *Photorhabdus temperata* subsp. *stackebrandtii* subsp. nov. (Enterobacteriales: Enterobacteriaceae). *Curr. Microbiol.* 61(4): 291-297.

Ansari, M. A., Adhikari, B. N., Ali, F., and Moens, M. 2008. Susceptibility of *Hoplia philanthus* (Coleoptera: Scarabaeidae) larvae to entomopathogenic nematodes (Rhabditida: Steinernematidae, Heterorhabditidae). *Biol. Control* 47(3): 315-321.

Arunima, V. 2017. DNA barcoding of spider mites (Prostigmata: Tetranychidae) on major crop plants of Kerala. M.Sc. (Ag) thesis, Kerala Agricultural University, Thrissur, 59p.

Au, C., Dean, P., Reynolds, S. E., and Ffrench-Constant, R. H. 2004. Effect of the insect pathogenic bacterium *Photorhabdus* on insect phagocytes. *Cell Microbiol*. 6(1): 89-95.

Aujoulat, F., Pages, S., Masnou, A., Emboule, L., Teyssier, C., Marchandin, H., Gaudriault, S., Givaudan, A., and Jumas-Bilak, E. 2019. The population structure of *Ochrobactrum* isolated from entomopathogenic nematodes indicates interactions with the symbiotic system. *Infect. Genet. Evol*. 70: 131-139.

Babic, I., Fischer-LeSaux, M., Giraud, E., and Boemare, N. 2000. Occurrence of natural dixenic associations between the symbiont *Photorhabdus luminescens* and bacteria related to *Ochrobactrum* spp. in tropical entomopathogenic *Heterorhabditis* spp. (Nematoda, Rhabditida). *Microbiology* 146(3): 709-718.

Bachhar, A., Bhaskar, H., Pathrose, B., and Shylaja, M. R. 2019. Resistance to acaricides in *Tetranychus truncatus* Ehara on vegetables. *Indian J. Entomol.* 81(1): 130-133.

Bedding, R. A. and Akhurst, R. J. 1975. A simple technique for the detection of insect parasitic rhabditid nematodes in soil. *Nematologica* 21(1): 109-110.

Bennett, H. P. J. and Clarke, D. J. 2005. The *pbgPE* operon in *Photorhabdus luminescens* is required for pathogenicity and symbiosis. *J. Bacteriol*. 187(1): 77-84.

Bennur, S., Abida, P. S., Valsala, P. A., Mathew, D., and Bhaskar, H. 2015. DNA barcoding of spider mites (Prostigmata: Tetranychidae) in vegetables using *COI* and *ITS2* marker. *Genome* 58(5): 195.

Bhat, A. H., Askary, T. H., Ahmad, M. J., and Chaubey, A. K. 2019. Description of *Heterorhabditis bacteriophora* (Nematoda: Heterorhabditidae) isolated from hilly areas of Kashmir Valley. *Egypt. J. Biol. Pest Control* 29(1): 1-7.

Bhat, A. H., Chaubey, A. K., Shokoohi, E., and Machado, R. A. 2020. Molecular and phenotypic characterization of *Heterorhabditis indica* (Nematoda: Rhabditida) nematodes isolated during a survey of agricultural soils in Western Uttar Pradesh, India. *Acta Parasitol*. 2(2): 1-17.

Blackburn, D., Burk, T. J., and Adams, B. J. 2016a. Virulence modulation in *Photorhabdus* spp. *Nematology* 18(6): 711-717.

Blackburn, D., Wood Jr, P. L., Burk, T. J., Crawford, B., Wright, S. M., and Adams, B. J. 2016b. Evolution of virulence in *Photorhabdus* spp. entomopathogenic nematode symbionts. *Syst. Appl. Microbiol.* 39(3): 173-179.

Blackburn, M. B., Martin, P. A. W., Kuhar, D., Farrar, R. R. J., and Gundersen-Rindal, D. E. 2011. The occurrence of *Photorhabdus*-Like toxin complexes in *Bacillus thuringiensis*. *PLoS ONE* 6(3): e18122.

Blackburn, M., Golubeva, E., Bowen, D., and Ffrench-Constant, R. H. 1998. A novel insecticidal toxin from *Photorhabdus luminescens*, toxin complex a (*Tca*), and its histopathological effects on the midgut of *Manduca sexta*. *Appl. Environ. Microbiol.* 64(8): 3036-3041.

Bleakley, B. and Nealson, K. H. 1988. Characterization of primary and secondary forms of *Xenorhabdus luminescens* strain Hm. *FEMS Microbiol. Ecol.* 4(5): 241-249.

Boemare, N. E., Akhurst, R. J., and Mourant, R. G. 1993. DNA relatedness between *Xenorhabdus* sp. (Enterobacteriaceae) symbiotic bacteria of entomopathogenic nematodes, and a propoasal to transfer *Xenorhabdus lumenescens* to new genus, *Photorhabdus. Int. J. Syst. Bacteriol.* 43: 249-255.

Bondi, M., Messi, P., Sabia, C., Contri, M. B., and Manicardi, G. 1999. Antimicrobial properties and morphological characteristics of two *Photorhabdus luminescens* strains. *New Microbiol.* 22(2): 117-127.

Bowen, D. J. and Ensign, J. C. 1998. Purification and characterization of a high-molecular-weight insecticidal protein complex produced by the entomopathogenic bacterium *Photorhabdus luminescens*. *Appl. Environ. Microbiol*. 64(8): 3029-3035.

Bowen, D. J. and Ensign, J. C. 2001. Isolation and characterization of intracellular protein inclusions produced by the entomopathogenic bacterium *Photorhabdus luminescens. Appl. Environ. Microbiol.* 67: 4834-4841.

Bowen, D., Blackburn, M., Rocheleau, T., and Grutzmacher, C. 2000. Secreted proteases from *Photorhabdus luminescens*: separation of the extracellular proteases from the insecticidal Tc toxin complexes. *Insect Biochem. Mol. Biol.* 30(1): 69-74.

Bowen, D., Rocheleau, T. A., Blackburn, M., Andreev, O., Golubeva, E., and Bhartia, R. 1998. Insecticidal toxins from the bacterium *Photorhabdus luminescens*. *Science* 280(5372): 2129-2132.

Brunel, B., Givaudan, A., Lanois, A., Akhurst, R. J., and Boemare, N. 1997. Fast and accurate identification of *Xenorhabdus* and *Photorhabdus* species by restriction analysis of PCR-amplified *16S rRNA* genes. *Appl. Environ. Microbiol.* 63(2): 574-580.

Bussaman, P., Sa-Uth, C., Rattanasena, P., and Chandrapatya, A. 2012. Acaricidal activities of whole cell suspension, cell-free supernatant, and crude cell extract of *Xenorhabdus stokiae* against mushroom mite (*Luciaphorus* sp.) *J. Zhejiang Univ. Sci. B.* 13(4): 261-266.

Chaston, J. M, Suen, G., Tucker, S. L., Andersen, A. W., and Bhasin, A. 2011. The entomopathogenic bacterial endosymbionts *Xenorhabdus* and *Photorhabdus*: convergent lifestyles from divergent genomes. *PLoS ONE* 6(11): e27909.

Ciche, T. A. and Ensign, J. C. 2003. For the insect pathogen *Photorhabdus luminescens*, which end of a nematode is out. *Appl. Environ. Microbiol.* 69(4): 1890-1897.

Clarke, D. J. 2008. *Photorhabdus*: a model for the analysis of pathogenicity and mutualism. *Cell. Microbiol.* 10(11): 2159-2167.

Daborn, P. J., Waterfield, N., Au, C. P. Y., Sharma, S., and Ffrench-Constant, R. H. 2002. A single *Photorhabdus* gene, makes caterpillars floppy (*mcf*), allows *Escheria coli* to persist within and kill insects. *Proc. Natl. Acad. Sci.* USA 99: 10742-10747.

Devi, G., Mishra, H., Bhattacharyya, B., and Nath, D. J. 2016. Occurrence of entomopathogenic nematode (Rhabditida: Heterorhabditidae, Steinernematidae) in white grub infested areas of Majuli, Assam, India. *J. Biopesticides* 9(2): 148-156.

Drancourt, M., Bollet, C., Carlioz, A., Martelin, R., Gayral, J. P., and Raoult, D. 2000. *16s* ribosomal DNA sequence analysis of a large collection of environmental and clinical unidentifiable bacterial isolates. *J. Clin. Microbiol.* 38(10): 3623-3630.

Duchaud, E., Rusniok, C., Frangeul, L., Buchrieser, C., Givaudan, A., Taourit, S., Bocs, S., Boursaux-Eude, C., Chandler, M., Charles, J. F., Dassa, E., Derose, R., Derzelle, S., Freyssinet, G., Gaudriault, S., Medigue, C., Lanois, A., Powell, K., Siguier, P., Vincent, R., Wingate, V., Zouine, M., Glaser, P., Boemare, N., Danchin, A., and Kunst, F. 2003. The genome sequence of the entomopathogenic bacterium *Photorhabdus luminescens*. *Nat. Biotechnol*. 21: 1307-1313.

El-Fouly, M. Z., Sharaf, A. M., Shahin, A. A. M., El-Bialy, H. A., and Omara, A. M. A. 2015. Biosynthesis of pyocyanin pigment by *Pseudomonas aeruginosa*. *J. Radiat. Res. Appl. Sci.* 8(1): 36-48.

Emelianoff, V., LeBrun, N., Pages, S., Stock, S. P., Tailliez, P., Moulia, C., and Sicard, M. 2008. Isolation and identification of entomopathogenic nematodes and their symbiotic bacteria from Herault and Gard (Southern France). *J. Invertebr. Pathol.* 98(2): 211-217.

Enright, M. R., McInerney, J. O., and Griffin, C. T. 2003. Characterization of endospore-forming bacteria associated with entomopathogenic nematodes, *Heterorhabditis* spp., and description of *Paenibacillus nematophilus* sp. nov. *Int. J. Syst. Evol. Microbiol.* 53(2): 435-441.

Eroglu, C., Cimen, H., Ulug, D., Karagoz, M., Hazir, S., and Cakmak, I. 2019. Acaricidal effect of cell-free supernatants from *Xenorhabdus* and *Photorhabdus* bacteria against *Tetranychus urticae* (Acari: Tetranychidae). *J. Invertebr. Pathol*. 160: 61-66.

Ferreira, T. and Malan, A. P. 2014. *Xenorhabdus* and *Photorhabdus*, bacterial symbionts of the entomopathogenic nematodes *Steinernema* and *Heterorhabditis* and their in vitro liquid mass culture: a review. *Afr. Entomol.* 22(1): 1-14.

Ferreira, T., Van-Reenen, C. A., Endo, A., Tailliez, P., Pages, S., Sproer, C., Malan, A. P., and Dicks, L. M., 2014. *Photorhabdus heterorhabditis* sp. nov, a

symbiont of the entomopathogenic nematode *Heterorhabditis zealandica. Int. J. Syst. Evol. Microbiol.* 64(5): 1540-1545.

Ferreira, T., Van-Reenen, C., Pages, S., Tailliez, P., Malan, A. P., and Dicks, L. M., 2013. *Photorhabdus luminescens* subsp. *noenieputensis* subsp. nov., a symbiotic bacterium associated with a novel *Heterorhabditis* species related to *Heterorhabditis indica*. *Int. J. Syst. Evol. Microbiol.* 63(5): 1853-1858.

Ffrench-constant and Bowen D. 1999. *Photorhabdus* toxins: novel biological insecticides. *Curr. Opin. Microbiol.* 2(3): 284-288.

Ffrench-constant, R. H., Waterfield, N., Burland, V., Perna, N. T., Daborn, P. J., Bowen, D. J., and Blattner, F. R. 2000. A Genomic sample sequence of the entomopathogenic bacterium *Photorhabdus luminescens* W14: Potential implications for virulence. *Appl. Environ. Microbiol.* 66**:** 3310-3329.

Ffrench-Constant, R., Dowling, A., and Waterfield, N. 2007. Insecticidal toxins from *Photorhabdus* bacteria and their potential use in agriculture. *Toxicon* 49(4): 436-451.

Ffrench-constant, R., Waterfield, N., and Daborn, P. 2003. *Photorhabdus*: towards a functional genomics analysis of a symbiont and pathogen. *FEMS Microbiol. Rev.* 26**:** 433-456**.**

Fischer-LeSaux, M., Viallard, V., Brunel, B., Normand, P., and Boemare, N. 1999. Polyphasic classification of the genus *Photorhabdus* and proposal of new taxa: *P. luminescens* subsp. *luminescens* subsp. nov., *P. luminescens* subsp. *akhurstii* subsp. nov., *P. luminescens* subsp. *laumondii* subsp. nov., *P. temperate* sp. nov., *P. temperata* subsp. *temperata* subsp. nov., and *P. asymbiotica* sp. nov. *Int. J. Syst. Bacteriol.* 49: 1645-1656.

Forst, S., Dowds, B., Bowmare, N., and Stackebrandt, E. 1997. *Xenorhabdus* and *Photorhabdus* spp.: bugs that kill bugs. *Annu. Rev. Microbiol.* 5: 47-72.

Fu, J. R. and Liu, Q. Z. 2019. Evaluation and entomopathogenicity of gut bacteria

associated with dauer juveniles of *Oscheius chongmingensis* (Nematoda: Rhabditidae). *Microbiology* 8(9): e00823.

Fukruksa, C., Yimthin, T., Suwannaroj, M., Muangpat, P., Tandhavanant, S., Thanwisai, A., and Vitta, A. 2017. Isolation and identification of *Xenorhabdus* and *Photorhabdus* bacteria associated with entomopathogenic nematodes and their larvicidal activity against *Aedes aegypti*. *Parasites Vectors* 10(1)**:** 440-452.

Gaby, W. L. 1946. Study of dissociative behavior of *Pseudomonas aeruginosa*. *J. Bacteriol.* 51(2): 217-222.

Gatsogiannis C, Lang, A. E., Meusch, D., Pfaumann, V., Hofnagel, O, Benz, R., Aktories, K., Raunser, S. 2013. A syringe-like injection mechanism in *Photorhabdus luminescens* toxins. *Nature* 495**:** 520-523.

Geldenhuys, J., Malan, A. P., and Dicks, L. M. T. 2016. First report of the isolation of the symbiotic bacterium *Photorhabdus luminescens* subsp. *laumondii* associated with *Heterorhabditis safricana* from South Africa. *Curr. Microbiol.* 73(6): 790-795.

Gerdes, E., Upadhyayl, D., Mandjiny, S., Bullard- Dillard, R., Storms, M., Menefee, M., and Holmesi, L. D. 2015. *Photorhabdus luminescens:* virulent properties and agricultural applications. *J. Agric. Forestry* 3(5): 171-177.

Gerritsen, L. J. M., Georgieva, J., and Wiegers, G. L. 2004. Oral toxicity of bacterial toxins against thrips species. *J. Invertebr Pathol.* 88(3): 207-211.

Gerritsen, L. J., De Raay, G., and Smits, P. H. 1992. Characterization of form variants of *Xenorhabdus luminescens*. *Appl. Environ. Microbiol*. 58(6): 1975-1979.

Gerritsen, L. J., Georgieva, J., and Wiegers, G. L. 2005. Oral toxicity of *Photorhabdus* toxins against thrips species. *J. Invertebr. Pathol*. 88(3): 207-211.

Glaeser, S. P., Tobias, N. J., Thanwisai, A., Chantratita, N., Bode, H. B., and Kampfer, P. 2017. *Photorhabdus luminescens* subsp. *namnaonensis* subsp. nov.,

isolated from *Heterorhabditis baujardi* nematodes. *Int. J. Syst. Evol. Microbiol.* 67(4): 1046-1051.

Han, R. and Ehlers, R. 2001. Effect of *Photorhabdus luminescens* phase variants on the in vivo and in vitro development and reproduction of the entomopathogenic nematodes *Heterorhabditis bacteriophora* and *Steinernema carpocapsae*. FEMS *Microbiol. Ecol.* 35: 239-247.

Hassan, H. M. and Fridovich, I. 1980. Mechanism of the antibiotic action pyocyanine. *J. Bacteriol.* 141(1): 156-163.

Hazir, S., Kaya, H. K., Stock, S. P., and Keskin, N. 2004. Entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) for biological control of soil pests. *Turk. J. Biol.* 27(4): 181-202.

Heermann, R. and Fuchs M. T. 2008. Comparative analysis of the *Photorhabdus luminescens* and the *Yersinia enterocolitica* genomes: uncovering candidate genes involved in insect pathogenicity. *BMC genomics* 9(40): 410-415.

Hinchliffe, S. J., Hares, M. C., and Dowling, A. J. 2010. Insecticidal toxins from the *Photorhabdus* and *Xenorhabdus* bacteria. *J. Toxicol.* 3(1): 83-100.

Hsieh, F. C., Tzeng, C. Y., Tseng, J. T., Tsai, Y. S., Meng, M., and Kao, S. S. 2009. Isolation and characterization of the native entomopathogenic nematode, *Heterorhabditis brevicaudis*, and its symbiotic bacteria from Taiwan. *Curr. Microbial.* 58(6): 564-570.

Jackson, T. J., Wang, H., Nugent, M. J., Griffin, C. T., Burnell, A. M., and Dowds, B. C. 1995. Isolation of insect pathogenic bacteria, *Providencia rettgeri*, from *Heterorhabditis* spp. *J. Appl. Bacteriol.* 78(3): 237-244.

Kalita, R. M., George, J., Devi, G., and Bhattacharyya, B. 2019. Isolation of entomopathogenic nematodes from Assam Agricultural University, Jorhat, Assam. *J. Entomol. Zool. Stud.* 7(5): 208-214.

Kazimierczak, W., Skrzypek, H., Sajnaga, E., Skowronek, M., Wasko, A., and Kreft, A. 2017. Strains of *Photorhabdus* spp. associated with polish *Heterorhabditis* isolates: their molecular and phenotypic characterization and symbiont exchange. *Arch. Microbiol*. 199(7): 979-989.

Khan, A. and Brooks, W. M. 1977. A chromogenic bioluminescent bacterium associated with the entomophilic nematode *Chromonema heliothidis. J. Invertebr. Pathol.* 29: 253-261.

Krasomil-Osterfel, K. C. 1995. Influence of osmolarity on phase shift in *Photorhabdus luminescens*. *Appl. Environ. Microbiol.* 61(10): 3748-3749.

Kulkarni, R. A., Prabhuraj, A., Ashoka, J., Hanchinal, S. G., and Hiregoudar, S. 2017. Generation and evaluation of nanoparticles of supernatant of *Photorhabdus luminescens* (Thomas and Poinar) against mite and aphid pests of cotton for enhanced efficacy. *Curr. Sci.* 112(11): 2312-2316.

Kumar, K. K. and Mohan, S. 2013. Insecticidal activity of primary, secondary and maternally linked mutant form variants of *Photorhabdus luminescens* cells on *Galleria mellonella*. *Indian J. Nematol.* 43(2): 195-199.

Kumar, K. V., Vendan, K. T., and Nagaraj, S. B. 2014. Isolation and characterization of entomopathogenic symbiotic bacterium, *Photorhabdus luminescens* of *Heterorhabditis indica* from soils of five agro climatic zones of Karnataka. *Biosci. Biotechnol. Res. Asia* 11: 129-139.

Kumar, S. V., Mulla, S. R., Sandeep, C., and Suresh, C. K. 2011. Pathogenicity of symbiotic bacteria associated with entomopathogenic nematodes on larvae of *Galleria mellonella. J. Phytol.* 3(11): 30-32.

Kushwah, J. and Somvanshi, V. S. 2015. *Photorhabdus*: A microbial factory of insect-killing toxins. In: Kalia, V. C. (Ed), *Microbial Factories*, Springer, New Delhi, pp. 235-240.

Kushwah, J., Kumar, P., Garg, V., and Somvanshi, V. S. 2017. Discovery of a highly virulent strain of *Photorhabdus luminescens* ssp. *akhurstii* from Meghalaya, India. *Indian J. Microbiol.* 57(1): 125-128.

Liu, D., Burton, S., Glancy, T., Li, Z. S., Hampton, R., Meade, T., and Merlo, D. J. 2003. Insect resistance conferred by 283-kDa *Photorhabdus luminescens* protein *TcdA* in *Arabidopsis thaliana*. *Nat*. *Biotechnol*. 21(10): 1222-1228.

Machado, R. A., Bruno, P., Arce, C. C., Liechti, N., Kohler, A., Bernal, J., Bruggmann, R., and Turlings, T. C. 2019. *Photorhabdus khanii* subsp. *guanajuatensis* subsp. nov., isolated from *Heterorhabditis atacamensis*, and *Photorhabdus luminescens* subsp. *mexicana* subsp. nov., isolated from *Heterorhabditis mexicana* entomopathogenic nematodes. *Int. J. Syst. Evol. Microbiol.* 69(3): 652-661.

Mandadi, N., Hendrickson, C., Handanahal, S., Rajappa, T., Pai, N., Javeed, S., Verghese, A., Rai, A., Pappu, A., Nagaraj, G., and Dhingra, A. 2015. Genome sequences of *Photorhabdus luminescens* strains isolated from entomopathogenic nematodes from southern India. *Genom. Data* 6: 46-47.

Manickam, S., Kumar, S. S., Reddy, P. N., and Reddy, P. S. 2017. Isolation, identification and pathogenicity of native entomopathogenic nematode strains collected from Hyderabad, Telangana State in India. *Asian J. Adv. Agril. Res.* 4(4): 1-9.

Marokhazi, J., Waterfield, N., LeGoff, F., Feil, E., Stabler, R., Hinds, J., Fodor, A., Richard, H., and Ffrench-Constant, R. C. 2003. Using a DNA microarray to investigate the distribution of insect virulence factors in strains of *Photorhabdus* bacteria. *J. Bacteriol*. 185(15): 4648-4656.

Meusch, D., Gatsogiannis, C., Efremov, R. G., Lang, A. E., Hofnagel, O., Vetter, I. R., Aktories, K., and Raunser, S. 2014. Mechanism of Tc toxin action revealed in molecular detail. *Nature* 508(7494): 61-65.

Mohan, S., Raman, R., and Gaur, H. S. 2003. Foliar application of *Photorhabdus luminescens*, symbiotic bacteria from entomopathogenic nematode *Heterorhabditis indica*, to kill cabbage butterfly *Pieris brassicae*. *Curr. Sci.* 84(11): 1397-1397.

Mulla, S. R., Joshi, S., and Das, J. 2017. Isolation, cloning and expression of insecticidal-protein-encoding gene *tcdA* from *Photorhabdus luminescens* in *Escherichia coli. Int. J. Curr. Microbiol. App. Sci.* 6(9): 1718-1724.

Munch, A., Stingl, L., Jung, K., and Heermann, R. 2008. *Photorhabdus luminescens* genes induced upon insect infection. *BMC genomics* 9(1): 229-234.

Nagesh, M., Hussaini, S. S., and Singh, S. P. 2002. Isolation and characterization of symbiotic bacteria from *Heterorhabditis* spp. and *S. carpocapsae* Weiser. *Pest Manag. Hort. Ecos*. 8: 38-42.

Orozco, R. A., Lee, M. M., and Stock, S. P. 2014. Soil sampling and isolation of entomopathogenic nematodes (Steinernematidae, Heterorhabditidae). *J. Visualized Exp.* 89: e52083.

Orozco-Hidalgo, M. T., Quevedo-Hidalgo, B., and Saenz-Aponte, A. 2019. Growth kinetics and pathogenicity of *Photorhabdus luminescens* subsp. *akhurstii* SL0708. *Egypt. J. Biol. Pest Control* 29: 11-17.

Palma, L., DelValle, E. E., Frizzo, L., Berry, C., and Caballero, P. 2016. Draft genome sequence of *Photorhabdus luminescens* strain DSPV002N isolated from Santa Fe, Argentina. *Genome Announc.* 4(4): e00744-16.

Paschapur A. U., VijayaLakshmi, K., Sunanda, B. S., and Pawar, V. 2018. Survey for the presence of entomopathogenic nematodes (EPNs) in sugarcane growing regions of Telangana state. *J. Entomol. Zool. Stud*. 6(6): 320-326.

Peat, S. M., Ffrench-Constant, R. H., Waterfield, N. R., Marokhazi, J., Fodor, A., and Adams, B. J. 2010. A robust phylogenetic framework for the bacterial genus *Photorhabdus* and its use in studying the evolution and maintenance of

bioluminescens: a case for *16S*, *gyrB*, and *glnA*. *Mol. Phylogenet. Evol.* 57: 728-774.

Peel, M. M., Alfredson, D. A., Gerrard, J. G., Davis, J. M., Robson, J. M., McDougall, R. J., Scullie, B. L., and Akhurst, R. J. 1999. Isolation, identification, and molecular characterization of strains of *Photorhabdus luminescens* from infected humans in Australia. *J. Clin. Microbiol.* 37(11): 3647-3653.

Pervez, R., Eapen, S. J., Devasahayam, S., and Jacob, T. K. 2014. Natural occurrence of entomopathogenic nematodes associated with ginger (*Zingiber officinale* Rosc.) ecosystem in India. *Indian J. Nematol*. 42(2): 238-245.

Pervez, R., Lone, S. A., and Pattnaik, S. 2020. Characterization of symbiotic and associated bacteria from entomopathogenic nematode *Heterorhabditis* sp. (nematode: Heterorhabditidae) isolated from India. *Egypt. J. Biol. Pest Control* 30(1): 1-9.

Pervez, R., Revathi, J., Eapen, S. J., Devasahayam, S., and Jacob, T. K. 2015. Isolation and identification of symbiotic bacterium associated with the entomopathogenic nematode, *Heterorhabditis* sp. (IISR-EPN 01) from India. *Res. J. Pharm. Biol. Chem. Sci.* 6(2): 339-343.

Pinheiro, V. B. and Ellar, D. J. 2007. Expression and insecticidal activity of *Yersinia pseudotuberculosis* and *Photorhabdus luminescens* toxin complex proteins. *Cell Microbiol*. 9(10): 2372-2380.

Quiroz-Castaneda, R. E., Mendoza-Mejia, A., Obregon-Barboza, V., Martinez-Ocampo, F., Hernandez-Mendoza, A., Martinez-Garduno, F., Guillen-Solis, G., Sanchez-Rodriguez, F., Pena-Chora, G., Ortiz-Hernandez, L., and Gaytan-Colin, P. 2015. Identification of a new *Alcaligenes faecalis* strain MOR02 and assessment of its toxicity and pathogenicity to insects. *Biomed. Res. Int.* 2015: 459-510.

Raja, C. E., Anbazhagan, K., and Selvam, G. S. 2006. Isolation and characterization of

a metal-resistant *Pseudomonas aeruginosa* strain. *World J. Microbiol. Biotechnol.* 22(6): 577-585.

Razia, M., Raja, R. K., Padmanaban, K., Chellapandi, P. and Sivaramakrishnan, S., 2011. *16S rDNA*-based phylogeny of non-symbiotic bacteria of entomopathogenic nematodes from infected insect cadavers. *Genomics, Proteomics Bioinf.* 9(3): 104-112.

Richardson, W. H., Schmidt, T. M., and Nealson, K. H. 1988. Identification of a Danthraquinone pigment and a hydroxystilbene antibiotic from *Xenorhabdus luminescens*. *Appl. Environ. Microbiol.* 54(6): 1602-1605.

Rodou, A., Ankrah, D. O., and Stathopoulos, C. 2010. Toxins and secretion systems of *Photorhabdus luminescens*. *Toxins* 2(6): 1250-1264.

Ruffner, B., Pechy-Tarr, M., Hofte, M., Bloemberg, G., Grunder, J., Keel, C., and Maurhofer, M. 2015. Evolutionary patchwork of an insecticidal toxin shared between plant-associated pseudomonads and the insect pathogens *Photorhabdus* and *Xenorhabdus*. *BMC Genomics* 16(1): 1-14.

Ruiu, L., Virdis, B., Mura, M. E., Floris, I., Satta, A., and Tarasco, E. 2017. Oral insecticidal activity of new bacterial isolates against insects in two orders. *Biocontrol Sci. Technol.* 27(7): 886-902.

Ryan, M. P. and Pembroke, J. T. 2020. The Genus *Ochrobactrum* as major opportunistic pathogens. *Microorganisms* 8: 1797-1803.

Saitou, N. and Nei, M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* 4(4): 406-425.

Salazar-Gutierrez, J. D., Castelblanco, A., Rodriguez-Bocanegra, M. X., Teran, W., and Saenz-Aponte, A. 2017. *Photorhabdus luminescens* subsp. *akhurstii* SL0708 pathogenicity in *Spodoptera frugiperda* (Lepidoptera: Noctuidae) and *Galleria mellonella* (Lepidoptera: Pyralidae). *J. Asia-Pac. Entomol.* 20(4): 1112-1121.

Salgado-Morales, R., Martinez-Ocampo, F., Obregon-Barboza, V., Vilchis-Martinez, K., Jimenez-Perez, A., and Dantan-Gonzalez, E. 2019. Assessing the pathogenicity of two bacteria isolated from the entomopathogenic nematode *Heterorhabditis indica* against *Galleria mellonella* and some pest insects. *Insects* 10(3): 83-88.

Salgado-Morales, R., Rivera-Gomez, N., Martinez-Ocampo, F., Lozano-Aguirre Beltran, L. F., Hernandez-Mendoza, A., and Dantan-Gonzalez, E. 2017. Draft genome sequence of *Photorhabdus luminescens* HIM3 isolated from an entomopathogenic nematode in agricultural soils. *Genom. Announc.* 5: e00745-817.

Schnepf, E., Crickmore, N., VanRie, J., Lereclus, D., Baum, J., Feitelson, J., et al. (1998) *Bacillus thuringiensis* and its pesticidal crystal proteins. *Microbiol. Mol. Biol. Rev.* 62: 775- 806.

Seenivasan, N., Prabhu, S., Makesh, S., and Sivakumar, M. 2012. Natural occurrence of entomopathogenic nematode species (Rhabditida: Steinernematidae and Heterorhabditidae) in cotton fields of Tamil Nadu, India. *J. Nat. Hist.* 46(45): 2829-2843.

Sergeant, M., Jarrett, P., Ousley, M., and Morgan, J. A. W., 2003. Interactions of insecticidal toxin gene products from *Xenorhabdus nematophilus* PMFI296. *Appl. Environ. Microbiol.* 69(6): 3344-3349.

Shahina, F., Manzar, H., and Tabassum, K. A. 2004. Symbiotic bacteria *Xenorhabdus* and *Photorhabdus* associated with entomopathogenic nematodes in Pakistan. *Pak. J. Nematol.* 22(2): 117-122.

Shahina, F., Tabassum, K. A., Salma, J., and Mahreen, G. 2011. Biopesticidal affeVittact of *Photorhabdus luminescens* against *Galleria mellonella* larvae and subterranean termite (Termitidae: Macrotermis). *Pak. J. Nematol*. 29(1): 35-43.

Shawer, R., Donati, I., Cellini, A., Spinelli, F., and Mori, N. 2018. Insecticidal activity of *Photorhabdus luminescens* against *Drosophila suzukii*. *Insects* 9(4): 148-153.

Sheets, J. J., Hey, T. D., Fencil, K. J., Burton, S. L., Ni, W., Lang, A. E., Benz, R., and Aktories, K. 2011. Insecticidal toxin complex proteins from *Xenorhabdus nematophilus*: structure and pore formation. *J. Biol. Chem.* 286(26): 22742-22749.

Silva, C. P., Waterfield, N. R., Daborn P. J., Dean, P., Chilver, T., Au, C. P. Y., Sharma, S., Potter, U., Reynolds, S. E., and Ffrench-Constant, R H. 2002. Bacterial infection of a model insect: *Photorhabdus luminescens* and *Manduca sexta. Cell Microbiol*. 4(6): 329-339.

Silva, L. R. J., Schwalm, U. F., Silva, E. C., daCosta, M., Heermann, R., and Santosdasilva, O. 2017. Larvicidal and growth-inhibitory activity of entomopathogenic bacteria culture fluids against *Aedes aegypti* (Diptera: Culicidae). *J. Econ. Entomol.* 110(2): 378-385.

Singh, S., Eric, M., Floyd, I., and Leonard, H. D. 2012. Characterization of *Photorhabdus luminescens* growth for the rearing of the beneficial nematode *Heterorhabditis bacteriophora. Indian J. Microbiol.* 52(3): 325-331.

Skowronek, M., Sajnaga, E., Pleszczynska, M., Kazimierczak, W., Lis, M., and Wiater, A. 2020. Bacteria from the midgut of common cockchafer (*Melolontha melolontha* L.) larvae exhibiting antagonistic activity against bacterial symbionts of entomopathogenic nematodes: isolation and molecular identification. *Int. J. Mol. Sci.* 21(2): 580-585.

Smigielski, A. J. and Akhurst, R. J. 1994. Megaplasmids in *Xenorhabdus* and *Photorhabdus* spp., bacterial symbionts of Entomopathogenic nematodes (Families Steirnernematidae and Heterorhabditidae). *J. Invertebr. Pathol*. 64(3): 214-220.

Somvanshi, V. S., Sloup, R. E., Crawford, J. M., Martin, A. R., Heidt, A. J., Kim, K. S., Clardy, J., and Ciche, T. A. 2012. A single promoter inversion switches *Photorhabdus* between pathogenic and mutualistic states. *Science* 337(6090): 88-93.

Srinivasan, R., Karaoz, U., Volegova, M., MacKichan, J., Kato-Maeda, M., and Miller, S. 2015. Use of *16S rRNA* Gene for identification of a broad range of clinically relevant bacterial pathogens. *PLoS ONE* 10(2): e0117617.

Steiner, G. 1923. *Aplectana kraussei* n. sp., one in the sawfly *Lyda* sp. parasitic nematode form, along with remarks about the side organ of the parasitic nematode *Nature* 2(59): 14-18.

Stuart, R. J. and Gaugler, R. 1994. Patchiness in populations of entomopathogenic nematodes. *J. Invertebr. Pathol.* 64: 39-45.

Szallas, E., Koch, C., Fodor, A., Burghardt, J., Buss, O., Szentirmai, A., Nealson, K.H., and Stackebrandt, E. 1997. Phylogenetic evidence for the taxonomic heterogeneity of *Photorhabdus luminescens*. *Int. J. Syst. Evol. Microbiol.* 47(2): 402-407.

Tailliez, P., Laroui, C., Ginibre, N., Paule, A., Pages, S., and Boemare, N. 2010. Phylogeny of *Photorhabdus* and *Xenorhabdus* based on universally conserved protein-coding sequences and implications for the taxonomy of these two genera. Proposal of new taxa: *X. vietnamensis* sp. nov., *P. luminescens* subsp. *caribbeanensis* subsp. nov., *P. luminescens* subsp. *hainanensis* subsp. nov., *P. temperata* subsp. *khanii* subsp. nov., *P. temperata* subsp. *tasmaniensis* subsp. nov., and the reclassification of *P. luminescens* subsp. *thracensis* as *P. temperata* subsp. *thracensis* comb. nov. *Int. J. Syst. Evol. Microbiol.* 60(8): 1921-1937.

Thomas, G. M. and Poinar, Jr. G. O. 1979. *Xenorhabdus* a genus of entomopathogenic nematophilic bacteria of the family Enterobacteriaceae. *Int. J. Syst. Evol. Microbiol.* 29: 352-360.

Toth, T. and Lakatos, T. 2008. *Photorhabdus temperata* subsp. *cinerea* subsp. nov., isolated from *Heterorhabditis* nematodes. *Int. J. Syst. Evol. Microbiol.* 58(11): 2579-2581.

Tran, Q., Pham, D. T., and Phan, V. 2017. Using *16S rRNA* gene as marker to detect unknown bacteria in microbial communities. *BMC Bioinf.* 18(14): 155-161.

Turlin, E., Pascal, G., Rousselle, J. C., Lenormand, P., Ngo, S., Danchin, A., and Derzelle, S., 2006. Proteome analysis of the phenotypic variation process in *Photorhabdus luminescens. Proteomics* 6(9): 2705-2725.

Uma, G. P., Prabhuraj, A., and Vimala. 2010a. Effect of the entomopathogenic bacterium *Photorhabdus luminescens* to *Aphis gossypii* Glover. *J. Biopestic*. 3(1): 100-104.

Uma, G. P., Prabhuraj, A., and Vimala. 2010b. Bio-efficacy of *Photorhabdus luminescens*, a symbiotic bacterium against *Thrips palmi* Karny (Thripidae: Thysanoptera). *J. Biopestic*. 3(2): 458-462.

Upadhyay, A., Banakar, P., and Mohan, S. 2019. *16S rDNA* based identification of non-symbiotic bacteria isolated from *Heterorhabditis indica* cuticle and infected *Galleria mellonella* and *Doresthenes huegelii* cadavers. *Indian J. Nematol.* 49: 91-96.

Vassiliou, V. A. and Kitsis, P. 2013. Acaricide resistance in *Tetranychus urticae* (Acari: Tetranychidae) populations from Cyprus. *J. Econ. Entomol*. 106(4): 1848-1854.

Velasco, J., Romero, C., Lopez-Goni, I., Leiva, J., Díaz, R., and Moriyon, I. 1998. Evaluation of the relatedness of *Brucella* spp. and *Ochrobactrum anthropi* and description of *Ochrobactrum intermedium* sp. nov., a new species with a closer relationship to *Brucella* spp. *Int. J. Syst. Bacteriol.* 48(3): 759-768.

Vitta, A., Thimpoo, P., Meesil, W., Yimthin, T., Fukruksa, C., Polseela, R., Mangkit, B., Tandhavanant, S., and Thanwisai, A. 2018. Larvicidal activity of *Xenorhabdus* and *Photorhabdus* bacteria against *Aedes aegypti* and *Aedes albopictus*. *Asian Pac. J. Trop. Biomed.* 8(1): 31-38.

Wang, H. and Dowds, B. C. A. 1993. Phase variation in *Xenorhabdus luminescens*:

cloning and sequencing of the lipase gene and analysis of its expression in primary and secondary phases of the bacterium. *J. Bacteriol*. 175: 1665-1673.

Waterfield, N. R. and Daborn, P. J. 2002. Genomic islands in *Photorhabdus*. *Trends Microbiol.* 10(12): 541-545.

Waterfield, N. R., Ciche, T., and Clarke, D. 2009. *Photorhabdus* and a host of hosts. *Annu. Rev. Microbiol.* 63: 557-574.

Waterfield, N., Dowling, A., Sharma, S., Daborn, P. J., Potter, U., and FrenchConstant, R. H. 2001. Oral toxicity of *Photorhabdus luminescens* W14 toxin complexes in *Escherichia coli*. *Appl. Environ. Microbiol.* 67(11):5017-5024.

Waterfield, N., Hares, M., Yang, G., Dowling, A., and Ffrench-Constant, R. 2005. Potentiation and cellular phenotypes of the insecticidal Toxin complexes of *Photorhabdus* bacteria. *Cell Microbiol*. 27(3): 373-82.

Woodman, M. E. 2008. Direct PCR of intact bacteria (colony PCR). *Curr. Protoc. Microbiol.* 9(1): 122-128.

Yang, G. and Waterfield, N. R., 2013. The role of *TcdB* and *TccC* subunits in secretion of the *Photorhabdus Tcd* toxin complex. *PLoS Pathog*. 9(10): e1003644.

Yang, G., Dowling, A. J., Gerike, U., and Waterfield, N. R. 2006. *Photorhabdus* virulence cassettes confer injectable insecticidal activity against the wax moth. *J. Bacteriol.* 188(6): 2254-2261.

Yooyangket, T., Muangpat, P., Polseela, R., Tandhavanant, S., Thanwisai, A., and Vitta, A. 2018. Identification of entomopathogenic nematodes and symbiotic bacteria from Nam Nao National Park in Thailand and larvicidal activity of symbiotic bacteria against *Aedes aegypti* and *Aedes albopictus*. *PLoS ONE* 13(4): e0195681.

Annexure

# ANNEXURE I

**List of contigs formed for *16S rRNA* sequences of EPN associated bacteria**

>CF1- *Photorhabdus luminescens*
CTTTTGCAACCCACTCCCATGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACG
TATTCACCGTAGCATGCTGATCTACGATTACTAGCGATTCCGACTTCATGGAGTC
GAGTTGCAGACTCCAATCCGGACTACGACAGACTTTGTGTGTTCCGCTTGCTCTC
GCGAGGTCGCTTCACTTTGTATCCGCCATTGTAGCACGTGTGTAGCCCTACTCGTA
AGGGCCATGATGACTTGACGTCATCCCCACCTTCCTCCGGTTTATCACCGGCAGT
CTCCTTTGAGTTCCCGCCATCACGCGCTGGCAACAAAGGATAAGGGTTGCGCTCG
TTGCGGGACTTAACCCAACATTTCACAACACGAGCTGACGACAGCCATGCAGCAC
CTGTCTCTCAGTTCCCGAAGGCACTTCGCTGTCTCCAGCAAATTCTGAGGATGTCA
AGAGTAGGTAAGGTTCTTCGCGTTGCATCGAATTAAACCACATGCTCCACCGCTT
GTGCGGGCCCCCGTCAATTCATTTGAGTTTTAACCTTGGGCCGTACTCCCCAGGC
GGTCGATTTAACGCGTTAGCTTCGGAAGCCACAGCTCAGGGCCGCAACCTCCAAA
TCGACATCGTTTACAGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACG
CTTTCGCACCTGAGCGTCAGTCTTCGTCCAGGGGGCCGCCTTCGCCACCGGTATTC
CTCCACATCTCTACGCATTTCACCGCTACACATGGAATTCTACCCCCCTCTACGAG
ACTCCAGTCAACCAGTCTTAGATGCCGTTCCCAGGTTGAGCCCGGGGATTTCACA
TCTAACTTAATTTGACCGCCTGCGTGCGCTTTACGCCCAGTCATTCCGATTAACGC
TCGCACCCTCCGTATTACCGCGGCTGCTGGCACGGAGTTAGCCGGTGCTTCTTTTG
CGGGTAACGTCAAGGCCCAGCCCTGTTCAGGCTCAACCCTTCCTCCCCGCTGAAA
GTACTTTACAACCCGAAGGCCTTCTTCATACACGCGGCATGGCTGCATCAGGCTT
GCGCCCATTGTGCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGT
CTCAGTCCCAGTGTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAG
GTAGGCCATTACCCTACCTACCAGCTAATCCCATCTGGGTTCATCCGACGGCGTG
AGGCCCTTTCAGGTCCCCCACTTTGGTCTCGCGACATTATGCGGTATTAGCCACCG
TTTCCAGTGGTTATCCCCCGCCCTCGGGCAGATCCCCAGACATTACTCACCCGTCC
GCCGCTCGTCAGCAAAAGCGCAAGCGCTTTCCTGTTACCGCCCGAC

>HI1- *Ochrobactrum* spp.
TCGGGTAAAACCAACTCCCATGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAA
CGTATTCACCGCGGCATGCTGATCCGCGATTACTAGCGATTCCAACTTCATGCAC
TCGAGTTGCAGAGTGCAATCCGAACTGAGATGGCTTTTGGAGATTAGCTCACACT
CGCGTGCTCGCTGCCCACTGTCACCACCATTGTAGCACGTGTGTAGCCCAGCCCG
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AGTCCCCTTAGAGTGCCCAACTAAATGCTGGCAACTAAGGGCGAGGGTTGCGCTC
GTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGCAGC
ACCTGTATCCGGTCCAGCCGAACTGAAAGACACATCTCTGTGTCCGCGACCGGTA
TGTCAAGGGCTGGTAAGGTTCTGCGCGTTGCTTCGAATTAAACCACATGCTCCAC
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CAGGCGGAATGTTTAATGCGTTAGCTGCGCCACCGAAGAGTAAACTCCCCAACG
GCTAACATTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCC
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GTGTTCCTCCGAATATCTACGAATTTCACCTCTACACTCGGAATTCCACTCACCTC
TACCATACTCAAGACTTCCAGTATCAAAGGCAGTTCCGGGGTTGAGCCCCGGAT
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CCTTCATCACTCACGCGGCATGGCTGGATCAGGCTTGCGCCCATTGTCCAATATTC
CCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGA
TCATCCTCTCAGACCAGCTATGGATCGTCGCCTTGGTGAGCCTTTACCTCACCAAC
TAGCTAATCCAACGCGGGCCGATCCTTTGCCGATAAATCTTTCCCCCGAAGGGCA
CATACGGTATTAGCACAAGTTTCCCTGAGTTATTCCGTAGCAAAAGGTACGTTCC
CACGCGTTACTCACCCGTCTGCCGC

>HQ1- *Stenotrophomonas maltophilla*
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TGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCAGCAATGCTGA
TCTGCGATTACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACTCCAATCCG
GACTGAGATAGGGTTTCTGGGATTGGCTTACCGTCGCCGGCTTGCAGCCCTCTGT
CCCTACCATTGTAGTACGTGTGTAGCCCTGGCCGTAAGGGCCATGATGACTTGAC
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TACGTGCTGGCAACTAAGGACAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACA
TCTCACGACACGAGCTGACGACAGCCATGCAGCACCTGTGTTCGAGTTCCCGAAG
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CGCGGCTGCTGGCACGAAGTTAGCCGGTGCTTATTCTTTGGGTACCGTCATCCCA
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CGAAAAAGTAGATTCCGATGTATTCCTCACCCGTCCGCCACTCGCCACCCAGAGA
GCAAGCTCTCCTGTGCTGCC

>KL1- *Pseudomonas aeruginosa*
ACGATTACTAGCGATTCCGACTTCACGCAGTCGAGTTGCAGACTGCGATCCGGAC
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ACCATTGTAGCACGTGTGTAGCCCTGGCCGTAAGGGCCATGATGACTTGACGTCA
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GCCACTAAGATCTCAAGGATCCCAACGGCTAGTCGACATCGTTAACGGCGTGGAC
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ACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGACTGATCA
TCCTCTCAGACCAGTTACGGATCGTCGCCTTGGTAGGCCTTTACCCCACCAACTA
GCTAATCCGACCTAGGCTCATCTGATAGCGTGAGGTCCGAAGATCCCCCACTTTC
TCCCTCAGGACGTATGCGGTATTAGCGCCCGTTTCCGGACGTTATCCCCCACTACC
AGGCAGATTCCTAGGCATTACTCACCCGTCCGCCG

>HS1- *Ochrobactrum* spp.
CTTGCGGTTAGCACAGCGCCTTCGGGTAAAACCAACTCCCATGGTGTGACGGGCG
GTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCATGCTGATCCGCGATTACTA
GCGATTCCAACTTCATGCACTCGAGTTGCAGAGTGCAATCCGAACTGAGATGGCT
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CTGACGACAGCCATGCAGCACCTGTCTCCGATCCAGCCGAACTGAAGGAAAGTG
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CCGAAGAGTAAACTCCCCAACGGCTAACATTCATCGTTTACGGCGTGGACTACCA
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CAGTGAGCCGCCTTCGCCACTGGTGTTCCTCCGAATATCTACGAATTTCACCTCTA
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TGGTAGGCCTTTACCCCACCAACTAGCTAATCCAACGCGGGCTCATCCATCACCG
ATAAATCTTTCACCTCTCGGTCGTATAGGGTATTAGCACAAGTTTCCCTGAGTTAT
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>KT1- *Pseudomonas aeruginosa*
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>EKM- *Ochrobactrum* spp.
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>MP1- *Ochrobactrum* spp.
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TACGC

>FR1- *Ochrobactrum* spp.
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GGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCATGCTGATCCGCGA
TTACTAGCGATTCCAACTTCATGCACTCGAGTTGCAGAGTGCAATCCGAACTGAG
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TCCGTAGCAAAAGGTAGATTCCCACGCGTTACTCACCCGTCTGCCGCTCCCCTTG
CGGGGCGCTCGA

# ANNEXURE II

## List of contigs formed for different regions of *TcdA* gene

>Region1
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TCAGTCAAATAAGCGGCGGGGGAGAACATGGAAGAAACGGTACCCGGCGCAAC
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GAGCGAGAATGGCAAGATGCACCGCATTTTGTAATTGGGGATTGGCGCGTTTGAG
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AGTCG

>Region 2
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>Region3
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GTCCAACAGGAGAACGCATTTTCTGGAGTTACTGGGGGAAGATGTTGATGATTTT
GTGCTTGAATACTGGCTTGCAACAGCAAATTAGCATCAAGATTCATGGCATCAGC
CAGTTGTTCTGCCGTTAACGTGTTAGCTTCAAATGCCGCTAGCACCGAGGACGCT
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>Region 4
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GCCATTCACTCCAGGCATTAGCCGCGAATTTACCATCGCTGAATTTACTATGATC
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GTCAGCCCTTGATCGTTATTAATATTATCGTGATACGCGCTAATAACTTTAAGATT
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GCGTTTAATTGGCTTTGGCTGACGGATTGCAGTAATGCGTCCATCATTTTGGTTTG
TCCGATACGCATGGTCGGATCAATATAGTTTTCCGGGTAGTAAACTAATTGAGAA
ACACCAGCCCAAGT

```
>Region 7
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# Molecular mechanism of virulence in the bacterium, *Photorhabdus luminescens* (Thomas and Poinar) against *Tetranychus truncatus* Ehara (Prostigmata: Tetranychidae)

*by*

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**ABSTRACT OF THE THESIS**

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# ABSTRACT

Spider mites (Tetranychidae) are a major group of sucking pests of many agricultural and horticultural crops worldwide. *Tetranychus truncatus* Ehara is the predominant mite species infesting economically important crops of Kerala. Novel acaricides are being extensively used for managing mite infestation, which has led to development of significant level of resistance in mite populations to these acaricides. This, along with other adverse effects of synthetic acaricides to the environment, calls for alternative strategies in mite pest management.

The entomopathogenic bacteria *Photorhabdus luminescens* (Thomas and Poinar), a natural symbiont of the entomopathogenic nematode, *Heterorhabditis* spp. has potential virulent properties and the ability to infect a wide range of insect pests and a few mite pests. An array of toxins produced by the bacteria are responsible for this appreciable insecticidal activity. Most of the orally active toxins are encoded by *tca* and *tcd* loci of *TC* genes. These toxins might also be responsible for the acaricidal activity of the bacterium. In this context, the present study was undertaken to identify the virulent strain of *P. luminescens* against *T. truncatus* and to elucidate the molecular mechanism of virulence against *T. truncatus*.

Eleven bacterial isolates associated with the entomopathogenic nematodes, *Heterorhabditis* spp. were isolated in the study. Cultural characterization of the bacterial isolates showed that the bacterial colonies were circular to irregular, entire, opaque, smooth colonies on NBTA medium. All the isolates were found to be Gram negative and rod shaped. For molecular characterization, the *16S rRNA* gene of the isolates was amplified, sequenced and subjected to BLASTn for homology search. One species of EPN symbiotic bacteria, *P. luminescens* and eight nonsymbiotic/ associated bacteria namely, two isolates of *Pseudomonas aeruginosa*, five isolates of *Ochrobactrum* and one isolate of *Stenotrophomonas maltophila* were identified. Phylogenetic tree was constructed based on the gene sequences of *16S rRNA* to validate the bacterial identification.

Laboratory bioassays were carried out to evaluate the efficacy of the both cell suspension and cell free supernatant (CFS) of five bacterial isolates (1 isolate of *P. luminescens*, 2 isolates of *P. aeruginosa*, and 2 isolates of *Ochrobactrum* sp.) separately against the eggs and adults of *T. truncatus.* Though the isolates did not show any significant ovicidal action, they recorded significant adulticidal action against the mite. The *P. luminescens* isolate was superior over other isolates recording 64.67 and 60.67 per cent mortality for CFS and cell suspension, respectively at 96 h of treatment

The virulent *P. luminescens* isolate was subjected to characterization of the toxin complex (*TcdA* gene). The full length gene (7.5 kb) was split into eight regions and the primers were designed to amplify each regions separately. Among the eight regions, only six got amplified and were sequenced. Toxin gene sequence of the isolated *P. luminescens* bacteria involved in the virulence against *T. truncatus* was partially sequenced and the variations in the sequence were analyzed.

The study identified a potential isolate of *P. luminescens* with significant adulticidal action against *T. truncatus* and the major gene *TcdA* encoding the toxin complexes which possess appreciable insecticidal activity was sequenced and characterized. The *P. luminescens* isolate obtained in the study can be evaluated for efficacy against major insect pests in the region in order to identify the potential in pest management.