Diverse bacteria associated with entomopathogenic nematode species- Potential sources of insecticidal toxins

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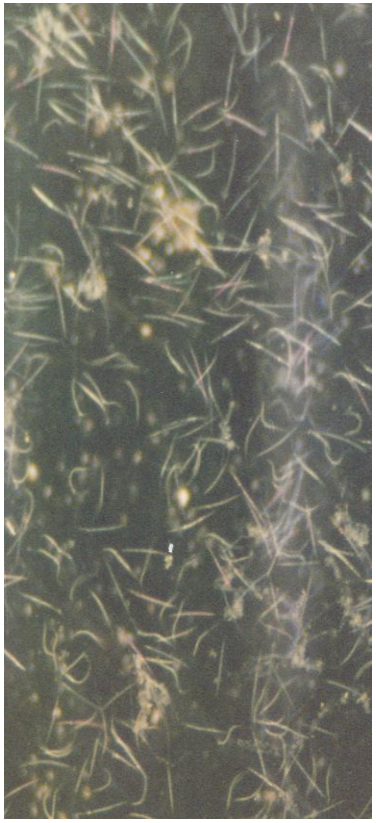
The Entomopathogenic nematodes (EPN) have generated a great deal of interest in recent years as potential bio-control component in IPM of cotton.

Why use EPN

- **Quick insect kill 24-48 h**
- **Wide host range**
- **Nontoxicity to Man**
- **Can actively search insects**



Bacterial Symbiont of EPN

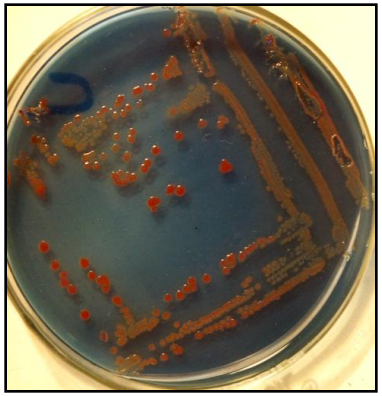
- Bacteria largely responsible for insect death.
- Provide suitable nutritive environment for nematode growth and multiplication.
- Suppress competing organisms by production of antibiotics
- Nematodes protects bacteria in external envt.
- Vectors them into insect haemocoel.
- Inhibits insect immune system.

Bacterial symbionts of *EPN*

Two genera

Photorhabdus spp. With Heterorhabditis spp.

And Xenorhabdus spp. With Steinernema spp.



Secondary Form

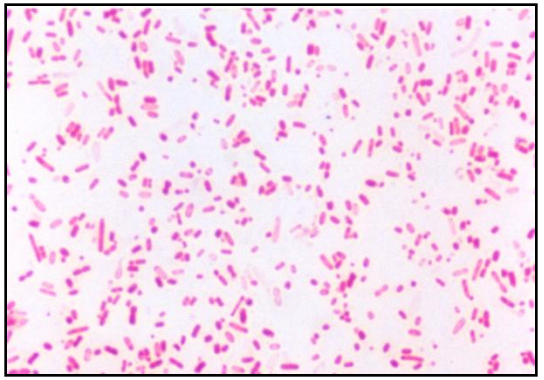
Primary Form

Enterobacteriaceae.

Gram negative facultatively anaerobic rods

Negative for nitrate reductase

***Xenorhabdus* are negative for catalase.**



Gram stained bacteria

Bacteria belonging to genera isolated from*Helicoverpa armigera* and *Spodoptera litura*

Bacillus, Paenibacillus, Cellulomonas, Acinetobacter, Micrococcus, Enterobacter and Providentia, *Enterococcus, Klebsiella* belonging to 16 phylogenetic group affiliated with lactobacillales, Actinobacteria, Proteobacteria, cytophaga-Flavobacterium, Bacteroides, Enterobacteriaceae.

Identification of bacteria

Biochemical

Morphological

Molecular characterization based on 16S ribosomal RNA sequence

Primers (5’GGA GAG TTA GAT CTT GGC TC3’ sense and 5’AAG GAG GTG ATC CAG CCG CA3’)

Some of the Bacterial Sequences with NCBI accession number.

S. No.	Bacterial isolate	Accession Number
1.	*Photorhabdus luminescens* subsp. *akhurstii* strain CICR-BBSc	JX276776
2.	*Providencia vermicola (CICR-SPBB)*	JX645158
3.	*Enterobacter gergoviae* (CICR-GV1)	JX567313
4	*Bacillus cereus CICR X-1*	JN579711
5.	*Bacillus cereus CICR X-2*	JN 579712
6.	*Bacillus nealsoni* (CICR-PKV).	JQ319066
7.	*Xenorhabdus poinarii* (CICR-WR).	JQ284032
8.	*Xenorhabdus indica* (CICR-WG).	JQ284033
9.	*Brevibacterium epidermidis* (CICR-G1).	JQ284034
10.	*Bacillus cereus* (CICR-D6)	JQ284035
11.	*Lysinibacillus sphaericus* isolate CICR-X12	KC759142
12.	*Enterobacter hormaechei* isolate CICR-XA	KC759141
13.	*Ochrobactrum pseudogrignonense* isolate CICR-BBR	KC342237
14.	*Providencia vermicola* strain CICR-SPBB	JX645158

Amplification of toxin genes

Taq polymerase used	Amplicons generated	Size of amplicons (Kb)
Regular	TcaA	3 kb
	TcaC	2.9 kb
	TcaZ	2 kb
	TccC	2.9 kb
	TccA	2.5 kb
	TccZ	1 kb
	Tcb A	1.5 kb
	Txp40	1.5 kb
Long range Taq Polymerase	TcaC	4.2 kb
	TccA	2.7 kb
	TcaA	3.0 kb
	TccC	2.7 kb
	W14 TccC	2.6kb
	Tcc1B	1.6 kb
	Txp 40A	2.8 kb
	Txp 40 D	4.5 kb
	TccC	3.1 kb

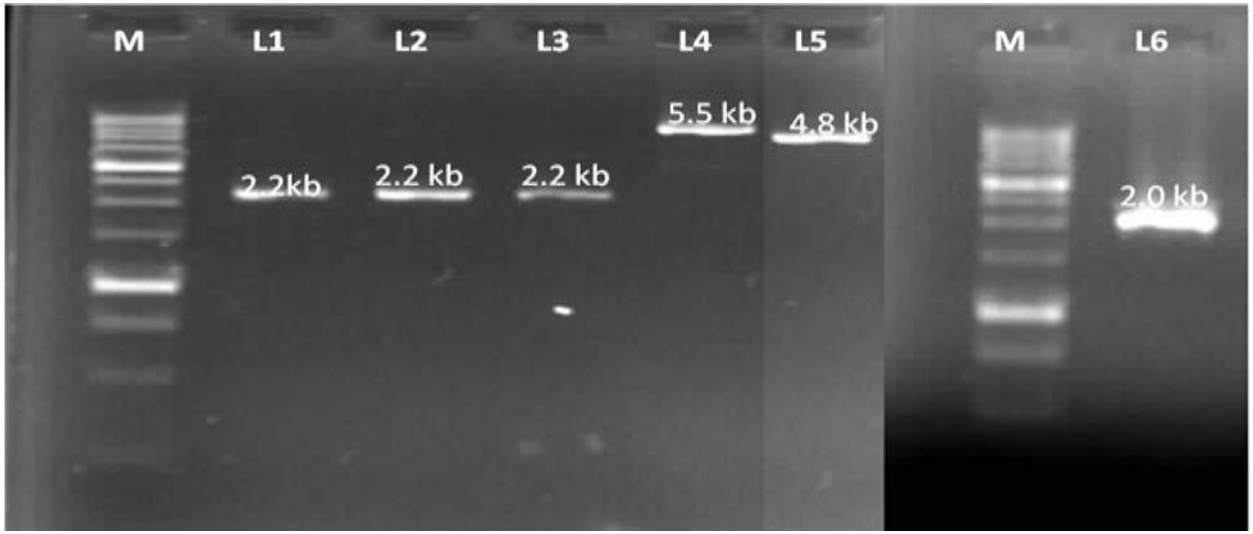
Some of the Insecticidal toxin genes from bacterial symbionts of EPN

Toxin genes from *Photorhabdus spp.*	**Toxin genes from *Xenorhabdus spp.***
Tca, Locus (*tcaA*, *tcaB*, and *tcaC)* Tcb locus (*tcbA)* Tcc locus (*tccA* and *tccB* , *tccC tccC2-tccC5)* Tcd locus (*tcdA1-tcdA4*,*tcdB1-tcdB2)* *Photorhabdus* virulence cassettes" (PVCs TXP 40 PIR AB Mcf1	Xpta1, XptA2, XptB1, and XptC1

Amplification of insecticidal toxin genes from other bacteria associated with Entomopathogenic nematodes species.

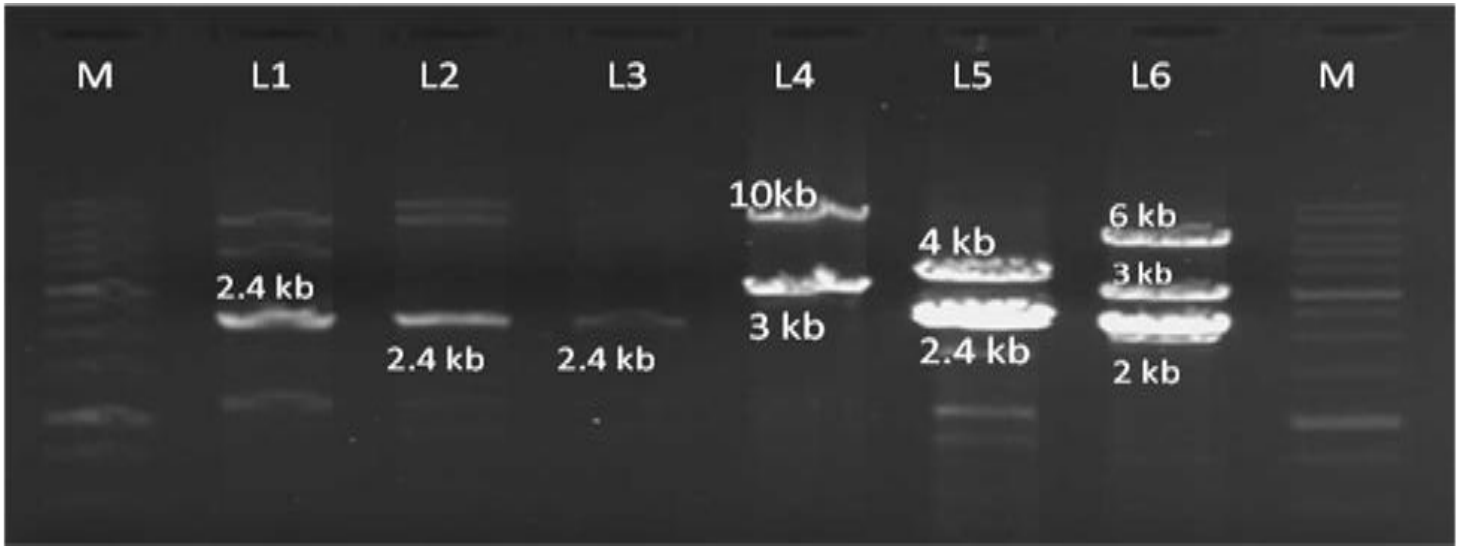
Bacterial species	**Amplicon**	**Size**
Xenorhabdus indica GV4 , GV5isolate	Tcc1b Txp40	2.2 kb 2.0kb
Providencia sp. BB isolate	Tcc1b Txp40	2.2kb 2.0kb
Klebsiella variicola BW isolate	Tcc1b	5.5 kb
Xenorhabdus indica WG isolate	Tca C	4.8kb
Xenorhabdus indica WG isolate	Tcc1a	2.0kb

Amplification of insecticidal toxin genes from other bacteria



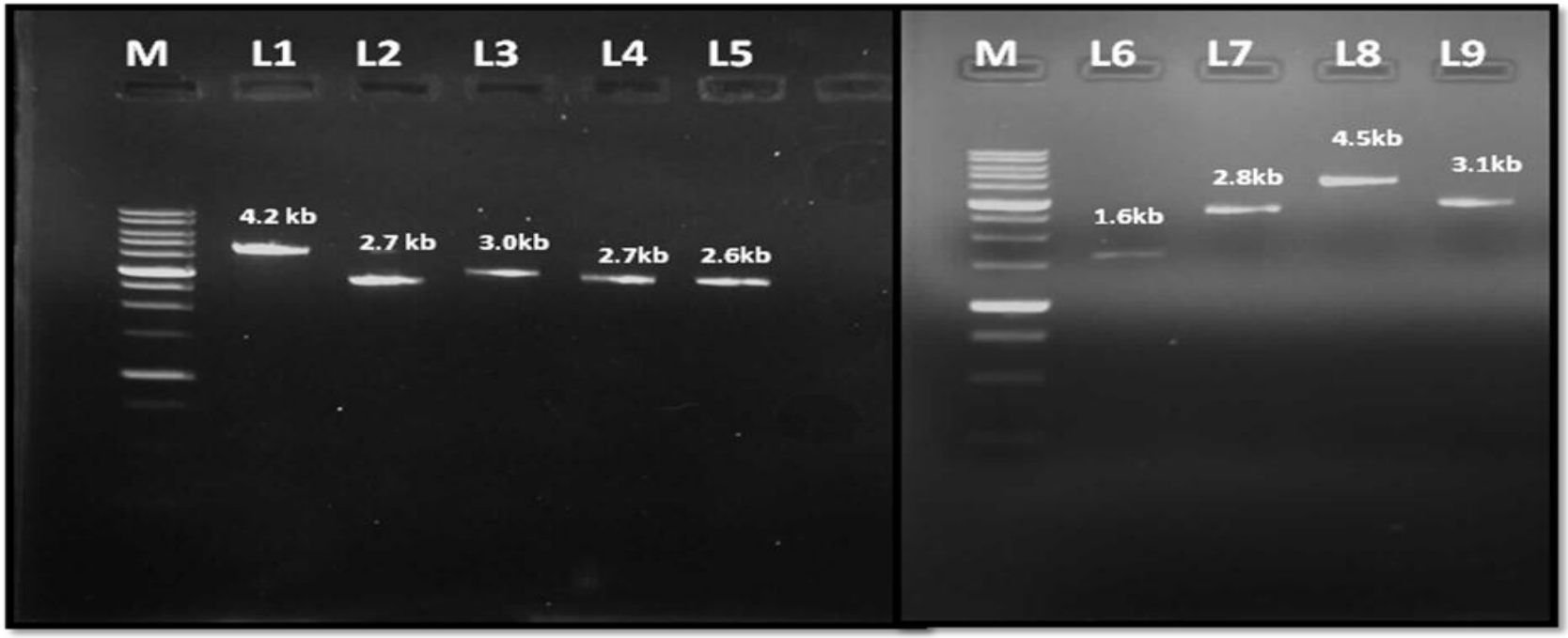
M : 1kb DNA Ladder,L1 : GV4 Tcc1b (2.2kb),L2 : GV5 Tcc1b (2.2kb),L3 : BB Tcc1b (2.2kb), L4 : BW Tcc1b (5.5 kb),L5 : WG Tca C (4.8kb),M : 1kb DNA Ladder,L6 : WG Tcc1a (2.0kb)

Toxin Amplicons with Long Range Taq DNA Polymerase



M: 1 KB DNA Ladder,L1: GV4 TCC1b L2: GV5 TCC1b,L3: BB TCC1b,L4: BW TCC1b,L5: WG TCC1a ,L6: WG Tca C

Photorhabdus luminescens subsp. Akhurstii (BBSc) **Toxin Amplicons**



TcaC (4.2 kb),L2: TccA (2.7kb),L3: TcaA (3.0 kb),L4: TccC (2.7 kb),L5 : W14 TccC (2.6kb),L6: Tcc1B (1.6 kb),L7: Txp40A (2.8 kb),L8: Txp 40 D (4.5 kb),L9: TccC (3.1 kb).

The amplicons were cloned into pGEMT vector systems (Promega) and transformed in chemically competent *E.coli* strain JM109 and plated on LB agar with Ampicillin, X-Gal and IPTG. Amplicons up to 3kb could be cloned and were sent for sequencing through primer walking.

The sequences BBSc TccC on blasting were found to have 98% similarity to *Photorhabdus* TccC while BN Tcc1c was 99% identity with *Xenorhabdus nematophilus* xptA1 gene, and 98% identity with *Xenorhabdus nematophila* Tcc1 gene.

The amplicon BBSc P12 was 97% similar to *Photorhabdus luminescens* tccA Identities while BBSc P3 was similar to *Photorhabdus luminescens* tcaC.

The two amplicons tcc1 and tccC were put in New England Biolabs PURExpress® *In Vitro* Protein Synthesis Kit for synthesis of protein. Yield was 10–200 µg/ml.

Photorhabdus BBSC TccC and WG TCC1 purified proteins displayed a single band and a molecular weight of over 200 kDa determined by SDS-PAGE.

In bioassay both the proteins caused cessation in feeding and reduction in larval weight of *H. armigera*.

When fed to third instar larvae of *H. armigera* in an artificial diet at 7.3 µg/g (w/w) toxin protein, growth reduction was to 81% extent


THANK
YOU!