

Novel bacteria for biological control of root-knot nematode, *Meloidogyne incognita*

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Introduction

Root knot nematode (RKN) causes serious crop losses worldwide and are among the most important agricultural pests. The search for novel, environmentally friendly alternatives with which to manage RKN populations has therefore become increasingly important. Bacteria are numerically the most abundant organisms in soil, and some of them, have shown great potential for the biological control of nematodes. This research gives an overview of some new types of bacteria which were isolated from soil using *Caenorhabditis elegans* as baits. Bacteria can be used as biological control agents against RKN. Many experiments have given positive results for their application under *In vitro* and greenhouse conditions to control RKN by their secondary metabolites.

Results

10 bacterial isolates were obtained from collected samples, after purification, DNA was extracted from all isolates and molecular characterization was done by PCR amplification of 16S marker (amplicon size was 1300 bp) and after sequencing and BLAST the results showed

| Isolated code | Identification | State | Planted Crop |
|---------------|---|---------------|--------------|
| B1 | <i>Pseudomonas entomophila</i> | Hyderabad | Rice |
| B2 | <i>Burkholderia cepacia</i> | Delhi | Grass |
| B3 | <i>Alcaligenes faecalis</i> | Jammu Kashmir | Mongra |
| B4 | <i>Alcaligenes aquatilis</i> | Delhi | Tomato |
| B5 | <i>Alcaligenes faecalis</i> | Odisha | Rice |
| B6 | <i>Pseudomonas mosselii</i> | Odisha | Asparagus |
| B7 | <i>Burkholderia ambifaria</i> | Punjab | Rice |
| B8 | <i>Burkholderia cepacia</i> | Assam | BorDhan |
| B9 | <i>Burkholderia cepacia</i> | Uttar Pradesh | Papaya |
| B10 | <i>Pseudochrobactruma saccharolyticum</i> | Uttar Pradesh | Brinjal |

Objectives

Isolation, purification and characterization of some new bacteria using nematodes as baits.

In vitro evaluation against *Meloidogyne incognita* by using different dilutions of bacterial filtrates, then check the bacterial metabolites using GC-MS

In vivo evaluation against *Meloidogyne incognita* by previous applying of the bacteria in tomato seedlings

Methodology

Soil collection: Collection of rhizosphere soil samples from different crops from different states of India: Delhi, AP, Punjab, Haryana, Manipur, Assam, TN, Maharashtra, UP, Jammu & Kashmir, Nagaland, Odisha and Kerala.

Maintenance of *M. incognita* and *C. elegans*: Egg mass of *M. incognita* was multiplied on susceptible tomato Pusa Ruby were used for hatching juveniles. Besides, monoxenic culture of *C. elegans* was maintained in the laboratory on nematode growth medium agar using *E. coli* strain OP50 as food source

Isolation, purification and characterization of bacteria: The bacterial isolates were obtained from soil employing "sprinkle plate method" using *C. elegans* as a bait, followed by purification in LB medium characterized using 16S molecular markers

Evaluation of Isolated bacteria: *In vitro* evaluation against *Meloidogyne incognita* by using different dilutions of bacterial filtrates, then *In vivo* evaluation against *Meloidogyne incognita* by previous applying of the bacteria in tomato seedlings

Gas chromatography-mass spectrometry (GC-MS) analysis Volatile organic compounds (VOCs) were identified by library matching from NIST (National Institute of Standards and Technologies) Mass Spectra Library

Conclusion

1- 10 bacterial isolates were obtained from the collected soil samples belonged to 4 genera, they were identified using molecular marker.

2- *In vitro* evaluation of the different dilutions of bacterial filtrates showed that 3 isolates gave mortality percentages against *M. incognita* juveniles

3- *In vivo* evaluation of the best isolates in pots planted with tomato showed that B3 *Alcaligenes faecalis* isolate gave best results in controlling nematode burden comparing with control

4- Myristinoyl pantetheine, Dimethyl disulfide, Ethyl iso-allocholate and Milbemycin B compounds were found in the best three bacterial isolates by using GC-MS analysis. But Heneicosane, Cyclobarbitol and Octadecana compounds were found only in B3 *Alcaligenes faecalis* isolate, which can be responsible in controlling RKN

