



RESEARCH ARTICLE

## Exploration of Potential Bacterial Endophytes against Root Knot Nematode, *Meloidogyne incognita* in Banana

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### ABSTRACT

A field survey was conducted in 12 districts of Assam viz., Jorhat, Golaghat, Nagaon, Marigaon, Goalpara, Dibrugarh, Tinsukia, Lakhimpur, Dhemaji, Sivsagar, Kamrup and Barpeta. A total of 92 root samples were collected and 37 bacterial isolates were isolated from commercial banana cultivars. The culture filtrates extracted from 37 endophytic bacterial isolates, were screened against southern root-knot nematode, *Meloidogyne incognita* in vitro and under pot culture studies. The five bacterial isolates viz., EB4, EB8, BC1, BC11 and BC12 showed 100% inhibition of egg hatching and juvenile mortality of *M. incognita* with an exposure period of 48 and 72h. On seed bacterization, with these five promising isolates, two isolates viz. EB4, BC1 significantly enhanced germination percentage (33.33, 25.31%) and vigour index (75.5, 64.39%) of paddy, receptively. The potential bacterial isolates viz., BC1 and EB4 were identified as *Lysinibacillus* sp. and *Pseudomonas* sp., respectively, based on the morphological phenotypic and biochemical characterization. The pot culture experiment revealed that the bacterial endophytes viz., *Lysinibacillus* sp. (BC1) *Pseudomonas* sp. (EB4) significantly reduced the soil (61.64, 56.71%) and root nematode population (77.29, 68.87%), number of adult females (73.97, 69.89%), egg masses (85.63, 80.11%) and root-knot index (1.33, 1.67) of *M. incognita* compared to untreated control. The bacterial endophytes viz., *Pseudomonas* sp. (EB4), *Lysinibacillus* sp. (BC1) were also significantly increased the growth parameters viz., shoot length (43.33, 39.18%), and root length (78.24, 59.26%) and pseudostem girth (58.38, 52.13%).

**Keywords:** *Lysinibacillus* sp.; *Pseudomonas* sp.; Endophytes; antinematic property; Banana; Root-knot nematode and Growth promotion.

### INTRODUCTION

Root-knot nematode, *Meloidogyne* spp. are obligate, sedentary endoparasites of more than 3000 host plants throughout the world (Sasser, 1979). The southern root-knot nematode, *M. incognita* is one of the major constraints in the production of banana and caused the 15% yield loss in India (Kumar et al., 2020). The main symptom of infestations is typical terminal or tip galls formation and distorted root architecture that attacks the primary and secondary roots (Faske et al., 2018). In recent years, the use of chemical nematicide decreased due to the effective utilization of antagonistic bio-control agents. Plants have complex micro-ecosystems in which different niches are filled by a wide variety of beneficial microorganisms

(Souza et al., 2013). Bacterial endophytes act as biocontrol agents for nematodes (Jie et al., 2009) and promote plant growth in various crops (Kausal et al., 2017). Presently, most commercial biocontrol products on the market contain live microorganisms, such as *Pasteuria penetrans*, *Purpureocillium lilacinum*, and/or their metabolites, which target specific nematodes (Lamovsek et al., 2013).

In general, the phyllosphere region has /more microbial community; however, the root region also has bacterial endophytes, which share same niche with *M. incognita* (Abdel-Salam et al., 2018). Endophytes colonized the plant root tissues may manage sedentary endoparasitic nematodes because both occupy the same ecological niche and are in close association throughout the nematode life

cycle, which makes these bacteria act as excellent biocontrol agent. The endophytes also elicit signals for the induction of defense mechanisms against root-knot nematode. Hence, the present study aims to test the antinemic properties like ovicidal and larvicidal of bacterial endophytes isolated from banana root against root-knot nematode, *M. incognita* and also test their potential for growth enhancement.

## MATERIALS AND METHODS

A field survey was conducted to collect the healthy banana roots for the isolation of endophytes from 12 districts of Assam viz., Jorhat, Golaghat, Nagaon, Marigaon, Goalpara, Dibrugarh, Tinsukia, Lakhimpur, Dhemaji, Sivsagar, Kamrup and Barpeta. A total of 92 root samples were collected from different banana cultivars viz., Malbhog, Seni Champa and Jahaji. The young feeder roots were collected at a depth of 20 to 30cm and collected samples were packed in properly labeled zip lock cover then brought to the laboratory for bacterial isolation. *In vitro* and pot culture studies were conducted at the Department of Nematology, TNAU, Coimbatore.

### Isolation of endophytic bacteria and identification

The collected root samples were washed with tap water and cut into small pieces (1-2 cm) using a sterile blade. Root samples were surface sterilized with 5% NaOCl for 20 minutes, then washed twice with sterile distilled water to remove excess NaOCl. Subsequently washed with 70 per cent ethanol for 30 seconds and then washed 8 times with sterile distilled water, from the 8<sup>th</sup> wash 1 mL was drawn and plated in Nutrient Agar medium as a sterile check. The root samples were homogenized using pestle and mortar by sterile peptone salt (1 g of peptone +1 g of NaCl in 1 litre of sterile distilled water) 10 mL was used for maceration of a sample, allow it to withstand for 20 minutes and 1 mL of supernatant was taken from each sample for serial dilution. Three dilutions were carried out serially ( $10^6$  to  $10^8$ ) for each sample and spread on the Nutrient Agar (NA). Two replications were maintained for each dilution. The NA plates were incubated at 37°C for week 3-5 days for bacterial colony growth. The isolates were initially categorized into two broad groups based on the Gram staining technique described in Hucker's modified method (Rangaswami and Bagyaraj, 1993). Morphological and cultural characters of the isolates were used for further grouping. Based on the results of various biochemical tests viz., starch hydrolysis, KOH test, citrate utilization, catalase, methyl red, gelatin hydrolysis, growth at 4°C, the organisms were identified upto generic level by Bergey's manual and Vetrivelkalai et al. (2010).

### In vitro screening of bacterial endophytes against *M. incognita*

Endophytic bacterial isolates were grown on nutrient broth and allowed for incubation. The culture filtrate was obtained by centrifuging the broth at 10000 rpm for 15 minutes and passed through 0.2 µm bacterial filter to avoid the cells and spores. A total of 37 bacterial isolates were screened against *M. incognita* under laboratory conditions. The effect on egg hatching and juvenile mortality of *M. incognita* were done *in vitro* using 2 mL of cell-free culture filtrate. The eggs and J2 of *M. incognita* were placed in a cavity block (100 nos.) and incubated at  $27 \pm 2^\circ\text{C}$  for studying egg hatching and juvenile mortality, respectively. The broth without bacteria and distilled water were used as control. Observations were recorded on number of egg hatched and immobilized juveniles after 24, 48 and 72h of incubation (Vetrivelkalai and Sivakumar, 2019). Three replications were maintained for each isolate and the experiment was arranged in a completely randomized design.

### Testing of growth promotion

Among the 37 bacterial isolates, five promising bacterial endophytes were used for testing the growth promotion activities. Seed bacterization was done by soaking paddy seeds (25 numbers) in small petri dish (5mL), which contains 3mL of bacterial culture and assessed by a modified roll towel method (ISTA, 1993). The germination percentage, shoot length and root length was recorded at 25 days after germination. The Vigour index (VI) was calculated using the following formula (Abdul Baki and Anderson, 1973).  $VI = \text{Germination percentage} \times \text{Seedling length (shoot length + root length)}$ .

### Evaluation of bacterial endophytes against *M. incognita* in banana

The pot mixtures containing red soil, sand and FYM @ 2:1:1 ratio were prepared and sterilized in an autoclave and was filled in earthen pot. The banana cv. Ney Poovan suckers were trimmed and dipped with bacterial culture @ 10mL/sucker for 15-20 minutes, then planted in 5 kg earthen pots at one sucker per pot. Freshly hatched 5000 J2 of *M. incognita* was inoculated per pot at 15 days after planting. Three replications were maintained for each isolate and the experiment was arranged in a completely randomized design. The observations on growth parameters and nematode multiplication factors were recorded at 90 days after nematode inoculation. The collected soil and root samples were processed by Cobb's decanting and sieving method (Cobb, 1918) and Modified Baermann funnel technique (Schindler, 1961), respectively. The representative 5g root samples of each pot were washed free of soil and stained with 0.1 per cent

acid fuchsin - lactophenol to examine the number of females and egg masses. The root-knot index was graded using 1 to 5 scale rating (Heald *et al.*, 1989) viz., 1: no galls; 2: 1-25% galls; 3: 25-50% galls 4: 50-75% galls; 5; > 75% galls/ root system.

### Statistical analysis

All the experiments were analyzed independently. The treatment means were compared by Duncan's Multiple Range-Test (DMRT) (Gomez and Gomez, 1984). The package used for analysis was IRRISTAT version 92-1 developed by the International Rice Research Institute Biometrics unit, Philippines.

## RESULTS AND DISCUSSION

### Survey, isolation and identification of endophytic bacteria

A total of 37 endophytic bacterial isolates were obtained from different banana cultivars viz., Malbhog, Seni Champa and Jahaji. Isolation of endophytic bacterial strains from various monocots and woody plants has reported by several authors (Ragavi *et al.*, 2019; Kaushal *et al.*, 2020 and Gomez-Lama Cabanas *et al.* (2021).

### In vitro screening against nematodes

Among the bacterial isolates screened, 12

isolates viz., EB4, EB8 EA1, EA2, EA3, EA4, EA5, BC1, BC4, BC9, BC11 and BC12 found that inhibited egg hatching of *M. incognita* by cent per cent (100%) with an exposure period of 24, 48 and 72 h at 100 per cent concentration of culture filtrate. The second stage juvenile of *M. incognita* was dead at 100 per cent in 10 bacterial isolates viz., EB1, EB2, EB3, EB4, EB5, EB8, EB9, BC1, BC11 and BC12 at 48 and 72h exposure period (Table1).

Among these 37 bacterial isolates, five isolates viz., EB4 BC8, BC1, BC11 and BC12 possessed both inhibitions of egg hatching and juvenile mortality of *M. incognita* with an exposure period of 48 and 72h at 100 per cent concentration of cell free culture filtrate. The high degree of ovicidal and larvicidal properties of the endophytic bacterial isolates is attributed due to parasitizing, production of the toxin, secondary metabolites and antibiotics. The findings of Munif *et al.*, (2019) were in accordance with the present study in which they have reported endophytic consortium inhibited *M. incognita* egg hatching up to 81.33% and increased J2 of *M. incognita* mortality up to 85 per cent compared to control. Su *et al.* (2017) *Streptomyces* sp. strain showed an inhibiting rate of >50 per cent *in vitro* and biocontrol efficiency of 70.7 per cent against *M. javanica* compared to the control.

**Table 1. Effect of culture filtrate of various isolates of bacterial endophytes on egg hatching and juvenile mortality of *M. incognita***

Endophytic bacterial isolates	Number of eggs hatched			Number of dead juveniles		
	24 h	48 h	72 h	24 h	48 h	72 h
EB1	1.67 <sup>cd</sup> (1.27)	3.67 <sup>cd</sup> (2.04)	8.33 <sup>c</sup> (2.97)	5.00 <sup>kl</sup> (2.35)	100.00 <sup>a</sup> (10.02)	100.00 <sup>a</sup> (10.02)
EB2	1.33 <sup>bc</sup> (1.35)	2.67 <sup>bc</sup> (1.78)	9.67 <sup>c</sup> (3.19)	1.67 <sup>op</sup> (1.47)	100.00 <sup>a</sup> (10.02)	100.00 <sup>a</sup> (10.02)
EB3	1.00 <sup>b</sup> (1.22)	1.67 <sup>b</sup> (1.47)	5.33 <sup>b</sup> (2.42)	4.67 <sup>klm</sup> (2.27)	100.00 <sup>a</sup> (10.02)	100.00 <sup>a</sup> (10.02)
EB4	0.00 <sup>a</sup> (0.71)	0.00 <sup>a</sup> (0.71)	0.00 <sup>a</sup> (0.71)	100.00 <sup>a</sup> (10.02)	100.00 <sup>a</sup> (10.02)	100.00 <sup>a</sup> (10.02)
EB5	1.00 <sup>b</sup> (1.22)	6.00 <sup>e</sup> (2.55)	12.00 <sup>d</sup> (3.54)	18.67 <sup>e</sup> (4.38)	100.00 <sup>a</sup> (10.02)	100.00 <sup>a</sup> (10.02)
EB6	3.33 <sup>ef</sup> (1.96)	9.33 <sup>f</sup> (3.14)	22.00 <sup>gh</sup> (4.74)	4.33 <sup>klmn</sup> (2.20)	12.33 <sup>k</sup> (3.58)	21.33 <sup>mn</sup> (4.67)
EB7	3.67 <sup>f</sup> (2.04)	5.00 <sup>de</sup> (2.35)	8.00 <sup>c</sup> (2.92)	3.00 <sup>no</sup> (1.87)	7.67 <sup>i</sup> (2.86)	20.00 <sup>mno</sup> (4.53)
BC8	0.00 <sup>a</sup> (0.71)	0.00 <sup>a</sup> (0.71)	0.00 <sup>a</sup> (0.71)	100.00 <sup>a</sup> (10.02)	100.00 <sup>a</sup> (10.02)	100.00 <sup>a</sup> (10.02)
EB9	4.33 <sup>d</sup> (2.20)	12.67 <sup>hi</sup> (3.63)	13.33 <sup>de</sup> (3.72)	1.33 <sup>pq</sup> (1.35)	100.00 <sup>a</sup> (10.02)	100.00 <sup>a</sup> (10.02)
EB10	7.00 <sup>i</sup> (2.74)	14.67 <sup>k</sup> (3.89)	20.33 <sup>e</sup> (4.56)	8.00 <sup>ij</sup> (2.92)	17.33 <sup>j</sup> (4.22)	21.67 <sup>m</sup> (4.71)
EB11	8.33 <sup>j</sup> (2.97)	17.67 <sup>i</sup> (4.26)	28.67 <sup>i</sup> (5.40)	10.67 <sup>h</sup> (3.34)	17.33 <sup>j</sup> (4.22)	23.00 <sup>lm</sup> (4.85)
EB12	4.33 <sup>d</sup> (2.20)	11.33 <sup>gh</sup> (3.44)	25.00 <sup>i</sup> (5.05)	3.33 <sup>m</sup> (1.96)	9.67 <sup>i</sup> (3.19)	17.33 <sup>op</sup> (4.22)
EA1	0.00 <sup>a</sup> (0.71)	0.00 <sup>a</sup> (0.71)	0.00 <sup>a</sup> (0.71)	0.00 <sup>q</sup> (0.71)	3.33 <sup>mn</sup> (1.96)	6.33 <sup>r</sup> (2.61)
EA2	0.00 <sup>a</sup> (0.71)	0.00 <sup>a</sup> (0.71)	0.00 <sup>a</sup> (0.71)	0.00 <sup>q</sup> (0.71)	9.33 <sup>j</sup> (3.14)	17.67 <sup>nop</sup> (4.26)
EA3	0.00 <sup>a</sup> (0.71)	0.00 <sup>a</sup> (0.71)	0.00 <sup>a</sup> (0.71)	0.00 <sup>q</sup> (0.71)	0.33 <sup>o</sup> (0.91)	1.00 <sup>s</sup> (1.22)
EA4	0.00 <sup>a</sup> (0.71)	0.00 <sup>a</sup> (0.71)	0.00 <sup>a</sup> (0.71)	0.00 <sup>q</sup> (0.71)	0.00 <sup>o</sup> (0.71)	1.00 <sup>s</sup> (1.22)
EA5	0.00 <sup>a</sup> (0.71)	0.00 <sup>a</sup> (0.71)	0.00 <sup>a</sup> (0.71)	0.00 <sup>q</sup> (0.71)	0.67 <sup>o</sup> (1.08)	1.33 <sup>s</sup> (1.35)
BC1	0.00 <sup>a</sup> (0.71)	0.00 <sup>a</sup> (0.71)	0.00 <sup>a</sup> (0.71)	100.00 <sup>a</sup> (10.02)	100.00 <sup>a</sup> (10.02)	100.00 <sup>a</sup> (10.02)
BC2	3.67 <sup>f</sup> (2.04)	13.00 <sup>ij</sup> (3.67)	20.00 <sup>e</sup> (4.53)	9.33 <sup>hi</sup> (3.14)	20.67 <sup>i</sup> (4.60)	28.00 <sup>k</sup> (5.34)
BC3	2.67 <sup>de</sup> (1.78)	11.33 <sup>gh</sup> (3.44)	15.00 <sup>ef</sup> (3.94)	16.00 <sup>f</sup> (4.06)	27.67 <sup>f</sup> (5.31)	33.00 <sup>hi</sup> (5.79)

BC4	0.00 <sup>a</sup> (0.71)	0.00 <sup>a</sup> (0.71)	0.00 <sup>a</sup> (0.71)	13.33 <sup>g</sup> (3.72)	22.67 <sup>hi</sup> (4.81)	26.00 <sup>kl</sup> (5.15)
BC5	14.33 <sup>p</sup> (3.85)	22.00 <sup>n</sup> (4.74)	25.00 <sup>i</sup> (5.05)	3.00 <sup>n</sup> (1.87)	9.67 <sup>i</sup> (3.19)	5.67 <sup>p</sup> (4.02)
BC6	3.67 <sup>fg</sup> (2.04)	10.33 <sup>fg</sup> (3.29)	14.33 <sup>e</sup> (3.85)	13.00 <sup>g</sup> (3.67)	16.00 <sup>fg</sup> (5.15)	32.33 <sup>j</sup> (5.73)
BC7	11.67 <sup>kl</sup> (3.49)	20.33 <sup>m</sup> (4.56)	23.67 <sup>hi</sup> (4.92)	3.67 <sup>lmn</sup> (2.04)	13.33 <sup>k</sup> (3.72)	20.67 <sup>q</sup> (4.60)
BC8	16.33 <sup>q</sup> (4.10)	26.67 <sup>p</sup> (5.21)	30.00 <sup>i</sup> (5.52)	1.33 <sup>q</sup> (1.35)	5.00 <sup>m</sup> (2.35)	11.00 <sup>i</sup> (3.39)
BC9	0.00 <sup>a</sup> (0.71)	0.00 <sup>a</sup> (0.71)	0.00 <sup>a</sup> (0.71)	13.33 <sup>g</sup> (3.72)	23.67 <sup>gh</sup> (4.92)	29.33 <sup>jk</sup> (5.46)
BC10	3.00 <sup>ef</sup> (1.87)	10.00 <sup>fg</sup> (3.24)	13.33 <sup>de</sup> (3.72)	5.33 <sup>k</sup> (2.42)	14.67 <sup>jk</sup> (3.89)	22.67 <sup>lm</sup> (4.81)
BC11	0.00 <sup>a</sup> (0.71)	0.00 <sup>a</sup> (0.71)	0.00 <sup>a</sup> (0.71)	100.00 <sup>a</sup> (10.02)	100.00 <sup>a</sup> (10.02)	100.00 <sup>a</sup> (10.02)
BC12	0.00 <sup>a</sup> (0.71)	0.00 <sup>a</sup> (0.71)	0.00 <sup>a</sup> (0.71)	100.00 <sup>a</sup> (10.02)	100.00 <sup>a</sup> (10.02)	100.00 <sup>a</sup> (10.02)
BC13	6.00 <sup>h</sup> (2.55)	13.00 <sup>ji</sup> (3.67)	17.33 <sup>f</sup> (4.22)	38.67 <sup>b</sup> (6.26)	79.67 <sup>b</sup> (8.95)	90.33 <sup>b</sup> (9.53)
BC14	12.00 <sup>lm</sup> (3.54)	23.33 <sup>no</sup> (4.88)	36.33 <sup>i</sup> (6.07)	21.33 <sup>d</sup> (4.67)	38.67 <sup>d</sup> (6.26)	73.67 <sup>d</sup> (8.61)
BC15	11.00 <sup>k</sup> (3.39)	19.00 <sup>lm</sup> (4.42)	36.67 <sup>i</sup> (6.10)	18.67 <sup>e</sup> (4.38)	35.67 <sup>e</sup> (6.01)	66.67 <sup>e</sup> (8.20)
BC16	12.67 <sup>mn</sup> (3.63)	24.00 <sup>o</sup> (4.49)	41.67 <sup>m</sup> (6.49)	4.67 <sup>klm</sup> (2.27)	16.67 <sup>j</sup> (4.14)	36.00 <sup>gh</sup> (6.04)
BC17	12.33 <sup>lmn</sup> (3.58)	18.00 <sup>i</sup> (4.30)	34.00 <sup>k</sup> (5.87)	7.67 <sup>j</sup> (2.86)	20.67 <sup>i</sup> (4.60)	36.33 <sup>gh</sup> (6.04)
BC18	13.67 <sup>op</sup> (3.76)	24.33 <sup>o</sup> (4.98)	30.00 <sup>i</sup> (5.52)	5.00 <sup>kl</sup> (2.35)	20.67 <sup>i</sup> (4.60)	39.00 <sup>g</sup> (6.28)
BC19	8.67 <sup>i</sup> (3.03)	14.33 <sup>jk</sup> (3.85)	21.00 <sup>e</sup> (4.64)	27.33 <sup>c</sup> (5.28)	52.33 <sup>c</sup> (7.27)	83.00 <sup>c</sup> (9.14)
BC20	13.00 <sup>no</sup> (3.67)	19.33 <sup>lm</sup> (4.45)	29.33 <sup>i</sup> (5.46)	13.33 <sup>g</sup> (3.72)	27.33 <sup>f</sup> (5.28)	50.33 <sup>f</sup> (7.13)
Nutrient broth	45.00 <sup>r</sup> (6.75)	76.33 <sup>q</sup> (8.73)	90.00 <sup>r</sup> (9.48)	0.00 <sup>q</sup> (0.71)	0.00 <sup>o</sup> (0.71)	0.00 <sup>s</sup> (0.71)
Distilled water	49.00 <sup>s</sup> (7.04)	80.67 <sup>r</sup> (8.93)	92.83 <sup>r</sup> (9.63)	0.00 <sup>q</sup> (0.71)	0.00 <sup>o</sup> (0.71)	0.00 <sup>s</sup> (0.71)
<b>SEd</b>	0.50	0.73	0.85	0.72	1.26	1.68
<b>CD (P=0.01)</b>	1.33	1.93	2.25	1.92	3.34	4.44

\*Values are mean of three replications

Means followed by a common letter are not significantly different at 1% level by DMRT

Values in parenthesis are square root transformed values

### Morphology and biochemical characterization

Based on the results of egg hatching and juvenile mortality, five isolates were identified using biochemical methods. The Gram stain reaction and cell morphology studies revealed that out of five isolates, three isolates EB4 to EB8 and BC11 belonged to Gram-negative and rod-shaped, non-spore-forming bacteria and two isolates viz., BC1 and BC12 belonged to Gram-positive and rod-shaped, spore-forming bacteria. The results obtained by analyzing primary character and nutrient source utilization of different endophytic bacteria revealed

that three endophytic bacterial isolates viz., EB4 and BC11 belonged to *Pseudomonas* spp., EB8 belonged to *Klebsiella* sp. and two bacterial isolates viz., BC1 belonged to *Lysinibacillus* and BC12 belonged to *Bacillus* spp. (Table 2). This result is in confirmation with the findings of Vetrivelkalai *et al.* (2010), who have recorded that based on the positive and negative results of different biochemical test, production of metabolites and utilization of nutrient sources, ten endophytic bacterial isolates viz., EB9 to EB18 identified as *Bacillus* spp. and nine isolates EB1 to EB8 and EB19 identified as *Pseudomonas* spp.

**Table 2. Biochemical characterization of endophytic bacterial isolates**

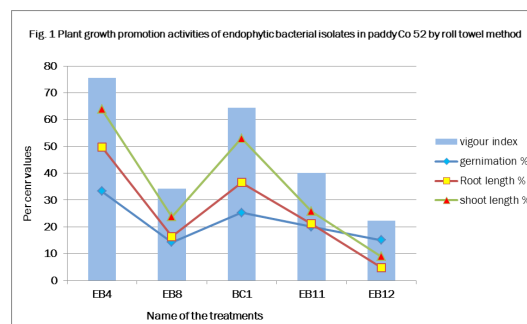
S. No	Endophytic isolates	Gram staining	Catalase test	KOH test	Citrate utilization	Growth in 5% NaCl	Starch hydrolysis	IAA production	Flourescence on KB medium	Genus name
1	EB4	-	+	+	+	+	-	+	+	<i>Pseudomonas</i> sp.
2	EB8	-	+	+	+	-	+	+	+	<i>Klebsiella</i> sp.
3	BC1	+	+	-	+	+	+	+	-	<i>Lysinibacillus</i> sp.
4	BC11	-	+	+	+	-	-	-	+	<i>Pseudomonas</i> sp.
5	BC12	+	+	-	+	+	-	-	-	<i>Bacillus</i> sp.

### Growth promotion activity

The results of the current study revealed that seed bacterization with endophytic bacterial isolates viz., EB4 and BC1 enhanced vigour index in paddy by 75.5 and 64.39 per cent, respectively (Fig. 1).

Germination percentage (33.33; 25.31 %), root length (49.82; 36.55%) and shoot length (63.85; 53%) were increased compared to untreated control. Similarly, various reports have indicated that bacterial endophytes promoted the growth and

health of crop plants (Vetrivelkai and Sivakumar, 2019; Munif *et al.* 2019). The mechanisms by which growth improved may be the production of phytohormones, IAA and enhanced availability of nutrients, reduction of ethylene levels, production of antibiotics and induced systemic resistance. Antagonism bacterial endophyte improves the resistance and exhibited PGPR activities (Liu *et al.*, 2019; Bubici *et al.*, 2019). The present results were also in conformity with the earlier reports.



**Table 3. Influence of endophytes on growth parameters of banana under pot culture conditions**

Endophytic bacteria	Shoot length (cm)	Root length (cm)	Shoot weight (g)	Root weight (g)	Pseudostem grid (cm)
<i>Pseudomonas</i> sp. (EB4)	57.33 <sup>a</sup> (43.33)	128.33 <sup>a</sup> (78.24)	322.67 <sup>a</sup> (40.29)	486.67 <sup>a</sup> (82.27)	12.67 <sup>a</sup> (58.38)
<i>Klebsiella</i> sp. (EB8)	52.67 <sup>b</sup> (24.06)	111.33 <sup>b</sup> (35.33)	265.67 <sup>c</sup> (13.43)	340.67 <sup>b</sup> (21.63)	11.00 <sup>b</sup> (27.27)
<i>Lysinibacillus</i> sp. (BC1)	55.67 <sup>a</sup> (39.18)	114.67 <sup>b</sup> (59.26)	298.67 <sup>b</sup> (29.86)	457.67 <sup>b</sup> (71.41)	12.17 <sup>a</sup> (52.13)
<i>Pseudomonas</i> sp. (BC11)	53.67 <sup>b</sup> (34.18)	108.67 <sup>b</sup> (50.93)	257.33 <sup>c</sup> (29.86)	328.33 <sup>b</sup> (22.97)	10.83 <sup>b</sup> (35.38)
<i>Bacillus</i> sp. (BC12)	49.67 <sup>b</sup> (19.47)	96.33 <sup>c</sup> (25.26)	251.33 <sup>c</sup> (11.88)	314.00 <sup>b</sup> (14.97)	10.17 <sup>c</sup> (21.34)
Nutrient broth	39.00 <sup>c</sup>	72.33 <sup>d</sup>	236.33 <sup>d</sup>	267.67 <sup>c</sup>	8.83 <sup>c</sup>
Distilled water Control	40.33 <sup>c</sup>	74.33 <sup>d</sup>	239.00 <sup>d</sup>	268.67 <sup>c</sup>	8.53 <sup>c</sup>
<b>SEd</b>	1.06	2.80	5.88	7.57	0.25
<b>CD (P=0.01)</b>	3.18	8.33	17.52	22.56	0.75

\*Values are mean of three replications

Means followed by a common alphabet of not significantly different at 1% level by DMRT

Values in parenthesis are per cent increased over control

### Evaluation of bacterial endophytes against *M. incognita* in banana

The best performing five bacterial isolates screened for their nematicidal action against root-knot nematode, *M. incognita* in banana based on the results of growth promotion activities. The

significant reduction in the number of adult females and number of egg masses was observed in banana plants treated with *Lysinibacillus* (BC1) isolate by 73.97 and 85.63. It was followed by *Pseudomonas* (EB4) (69.86; 80.11 %).

**Table 4. Effect of endophytes on root-knot nematode multiplication under pot culture conditions**

Endophytic bacteria	Soil population (200 cc)	Root population (5g)	No. of females (5g)	No. of egg masses (5g)	Root-knot index
<i>Pseudomonas</i> sp. (EB4)	172.00 <sup>b</sup> (56.71)	28.33 <sup>b</sup> (68.87)	22.00 <sup>a</sup> (69.86)	12.00 <sup>b</sup> (80.11)	1.67
<i>Klebsiella</i> sp. (EB8)	203.67 <sup>c</sup> (48.74)	63.33 <sup>d</sup> (30.41)	57.67 <sup>e</sup> (21.00)	29.00 <sup>e</sup> (51.93)	4.0
<i>Lysinibacillus</i> sp. (BC1)	154.00 <sup>a</sup> (61.24)	20.67 <sup>a</sup> (77.29)	19.00 <sup>a</sup> (73.97)	8.67 <sup>a</sup> (85.63)	1.33
<i>Pseudomonas</i> sp. (BC11)	222.00 <sup>d</sup> (44.13)	49.67 <sup>c</sup> (45.42)	45.67 <sup>c</sup> (37.44)	21.00 <sup>c</sup> (65.19)	2.67
<i>Bacillus</i> sp. (BC12)	216.33 <sup>d</sup> (45.55)	61.00 <sup>d</sup> (32.97)	52.00 <sup>d</sup> (28.77)	23.33 <sup>d</sup> (61.33)	3.0
Nutrient broth	387.67 <sup>e</sup>	86.67 <sup>e</sup>	63.67 <sup>f</sup>	53.33 <sup>f</sup>	5.0
Distilled water Control	397.33 <sup>e</sup>	91.00 <sup>f</sup>	73.00 <sup>g</sup>	60.33 <sup>g</sup>	5.0
<b>SEd</b>	5.36	0.97	1.18	0.47	-
<b>CD (P=0.01)</b>	15.97	2.90	3.51	1.40	-

\*Values are mean of three replications

Means followed by a common alphabet of not significantly different at 1% level by DMRT

Values in parenthesis are per cent decreased over control



The reduction in soil and root population observed in *Lysinibacillus* (BC1) treated plants by 61.24 and 77.29 per cent respectively, followed by *Pseudomonas* (EB4), which accounted for 56.71, 68.55 per cent reduction over control, respectively (Table 3). The lowest root gall index (1.33; 1.67) registered in both *Lysinibacillus* (BC1) and *Pseudomonas* (EB4) treated banana plants compared to untreated control (5).

It was also found that banana suckers treated with *Lysinibacillus* (BC1) and *Pseudomonas* (EB4) significantly reduced the number of adult females, egg masses, and root and soil population of *M. incognita* under pot culture conditions. Vetrivelkai (2019) and Munif *et al.* (2019) reported that endophytic bacterial isolates significantly reduced soil and root population, root gall index compared to untreated control. Siddiqui and Shaukat (2003) also found that cell suspension of *P. fluorescens* strains CHAO or CHAO/pME3424 at various inoculum levels  $10^7$ ,  $10^8$ ,  $10^9$  cfu/g significantly reduced root knot development density in tomato under glasshouse conditions.

The mechanisms of nematode population reduction might be due to competition for space, nutrients; premature egg hatching, reduction in viability and mortality of juveniles induced by hydrocyanic acid, cellulase, lipases protease and  $\beta$ -1, 3-glucanase enzymes. Endophytic bacteria also triggered the production of indole acetic acid, enhanced the total nitrogen and available phosphorus (Naureen *et al.*, 2017; Gopalakrishnan *et al.*, 2015). The isolates viz., *Pseudomonas* (EB4) and *Lysinibacillus* (BC1) significantly increased growth parameters viz., pseudostem height, root length and pseudostem girth of banana under pot culture conditions (Table 4). The present results were also in conformity with the earlier reports of Harish (2005) found that application of endophytic bacterial strains significantly increased the growth parameters viz., pseudostem height, girth and number of leaves in banana plants under greenhouse conditions.

## CONCLUSION

A varied group of microorganisms that play a significant role in optimizing plant growth and development inhabits plant roots. Endophytic bacteria viz., *Lysinibacillus* (BC1) and *Pseudomonas* (EB4) of banana have antinemic properties against root-knot nematode, *M. incognita* and growth promotion activities identified in the present study. Further, investigations on production of secondary metabolites and toxin compound, which is responsible for antinemic properties, have to be carried out. This will be helpful for the complete understanding and usage of endophytes as

potential biocontrol agents for the management of nematodes.

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## Consent for publication

All the authors agreed to publish the content.

## Competing interests

There were no conflict of interest in the publication of this content

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