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Evaluation of Bacterial Endophytes for their Plant Growth Promotion *in vitro* Condition

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A B S T R A C T

Endophytes are the micro-organism which shares the life long relation with the host. The bacterial endophytes promote the growth directly and indirectly by several mechanisms. Keeping in mind, the present investigation has been carried out to evaluate the bacterial endophytes for their plant growth promotion in vitro condition. Four screened bacterial antagonists viz., SVC 11, BE 1, NGB 21 and M1W1 were evaluated for the germination and the vigour of French bean, brinjal and rapeseed mustard seeds. The bacteria were used individual as well as in consortium mode on the respective seeds. The result revealed that the seeds of French bean and brinjal treated with isolate NGB 21 as best in terms of germination (91.11% and 84.44% respectively) and vigour (3270.88 and 1094.96 respectively) followed by BE 1. Isolate SVC 11 showed the highest vigour (630.51) at 21st day and germination (84.44%) followed by the isolates NGB 21 and BE 1 in rapeseed mustard. The root length and shoot length were significantly higher in French bean as compared to brinjal and rapeseed mustard. Statistically the germination percentage and the vigour index were found to be significant. The result showed that all the screened potential bacteria have the best plant growth promoting ability as compared to the other crop seed tested. Hence, the microbial consortia could be evaluated further under field conditions to developed consortia against major vegetable pathogens of Meghalaya.

Introduction

Endophytes are the group of microbes may be fungi or bacteria which asymptomatically inhabit the internal tissues of plants; they colonize the same ecological niches as disease-causing organisms. Endophytes are are known to control the plant diseases by suppressing the pathogens but they also helps in plant growth promotion**.** Some of the mechanisms in plant growth promotion are biological nitrogen fixation, ACC deaminase synthesis, siderophore production, IAA production, phosphate solubilization, induction of host plant resistance mechanisms, phytohormone production and synthesis of pathogen inhibiting volatile compounds (Glick, 2015). The seed play an important role in agriculture **.** The overall development of the plant depends upon the seed germination and vigour of seeds.

Malfanova *et al.,* (2011) tested the plant growth promoting abilities of the bacterial endophytes in tomato, wherein he isolated 30

bacterial endophytes from different plant species and evaluated against tomato foot and root rot (TFRR). Of which, *B. subtilis* (HC8), which was isolated from the giant hogweed**,** significantly promoted the plant growth along with giving protection against TFRR.

He indicated the production of metabolites like gibberellin hormone, lipopeptide antibiotics as possible mechanism for plant growth promotion and disease suppression respectively. Wahyudi *et al.,* (2011) reported the ability of *Bacillus* sp. to root length, shoot length of seedlings (seed germination biosssay) *in vitro*. Agrawal and Agrawal, (2013) also isolated *Bacillus* strains from the rhizosphere of tomato plants and screened for their plant growth promoting activities. Five isolates were selected and they showed increased shoot and root length as well as increased seed germination and seedling vigour. The ability of *Bacillus* spp. on the growth promotion *in-vitro* has been reported by several workers (Wahyudi *et al.,* 2011; Agrawal and Agrawal, 2013; Malleshwari *et al.,* 2013)

Materials and Methods

Inoculam preparation

Four endophytic bacteria used in this study (SVC 11, BE 1, NGB 21 AND M1W1) were isolated in the laboratory from different plant tissues and stored at –80 °C in 20% (v/v) glycerol. To prepare inoculam, 1 ml aliquot of a bacterial culture grown overnight (approximately OD600=1.0) were inoculated in 20 ml nutrient broth (3 g beef extract, 10 g proteose peptone, 5 g NaCl in one liter of dH2O, pH 7.2) at 30 °C for 24 h with 150 rpm shaking in rotary shaker. The individual isolates were subjested for compatibilty test and the microbial consortia was prepared by mixing the bacterial growth in the aliquot.

Germination assay

Germination assay was performed on the petri dishes (150mm diameter). The seed is surface sterilized with 0.1% mercuric chloride for 3 min and washed with distilled water for 4-5 times. The seeds were soaked for 3h in 48 h old bacterial broth cultures containing at least 10^{-6} cells/ml and placed in the Petri dish containing the paper towel moistened with water at the rate of 15 seeds per plate and replicated 3 times. One treatment was carried out by mixing the 4 bacterial cultures (Microbial consortium). The seeds treated with sterile water were served as control. The plates were incubated at 28 ± 2^{o} C and moisture of the petri dishes was maintained regularly.

Selection-9 variety of beans, KSP- 1164 Devansh variety of brinjal and variety of Rapeseed Mustard were used for the present investigation. The observations for percentage seed germination were taken at 7 days after incubation. The observations for root, length, shoot length and vigour length was taken at 7, 14 and 21 days after incubation.

Vigour index (Vi) = (RL + SL) × GP Where RL = mean root length (cm) SL = mean shoot length (cm) GP = germination percentage (Gopalakrishnan *et al*., 2012).

Experimental design and Statistical analysis

The above experiment was designed in completely randomized design (CRD).The data were analyzed using one-way analysis of variance (ANOVA). Statistical significance between the treatments was compared using LSD (Least significant difference) and a $P < 0.01$ was considered as significantly different from the other treatments (Gomez and Gomez, 1984).

Results and Discussion

PGPB may affect plant growth either directly or indirectly. The attributes contributing to the promotion of plant growth includes production of phytohormones like IAA (Pattern and Glick, 1996), Giberrelic acid (Mahmoud *et al.,* 1984), Cytokinin (Tien *et al.,* 1979), production of ACC deaminase enzyme, nitrogen fixation, solubilisation of phosphate. The ability of *Bacillus* spp. on the growth promotion *in-vitro* has been reported by several workers (Wahyudi *et al.,* 2011; Agrawal and Agrawal, 2013; Malleshwari *et al.,* 2013). Four screened bacterial antagonists were subjected for the compatibility among themselves and no lysis was observed at the juncture of SVC 11, BE 1, NGB 21 and M1W1 combinations, which indicated the compatibility among the isolates. It was found that seeds of French bean treated with isolate NGB 21 recorded as best in terms of germination (91.11%) and vigour (3270.88 atv 21 st day) followed by M1W1 (85.22% and 2809.25, germination percentage and vigour index respectively) and BE 1 (82.22% and 2864.07, germination percentage and vigour index respectively) (FIg. 1 and 2; Table 1–3).

Table.1 Evaluation of screened endophytes for PGP activities in French bean seeds *in vitro*

Table.1.1 Observation on 7th day

setalosI	Germination (%)	Shoot length (cm)	Root length (cm)	Vigour
SVC 11	77.78±2.23	1.33±0.11	10.6±0.05	928.14
BE 1	82.22±1.67	1.40±0.42	11.0±0.07	1019.55
NGB 21	91.11±1.55	1.70±0.12	11.7±0.12	1217.85
MIW 1	85.22±1.88	1.37±0.23	10.33±0.17	956.51
MC	97.78±1.33	2.03±0.19	12.2±0.17	1394.96
Control	73.33±1.28	1.13±0.11	9.5±0.14	777.339
eulav DC	5.56	0.43	0.25	22.24

Table.1.2 Observation on 14th day

setalosI	Germination (%)	Shoot length (cm)	Root length (cm)	Vigour
SVC 11	77.78±2.23	9.53±0.04	14.4±0.23	1861.481
BE 1	82.22±1.67	9.6±0.07	14.8±0.45	1897.778
NGB 21	91.11±1.55	9.83±0.14	15.76±0.14	2332.444
MIW 1	85.22±1.88	9.53±0.18	14.26±0.17	1904
MC	97.78±1.33	10.27±0.12	16.23±0.13	2591.111
Control	73.33±1.28	9.33±0.42	13.6±0.55	1630.815
eulav DC	5.56	0.34	0.35	22.24

Table.1.3 Observation on 21st day

setalosI	Germination (%)	Shoot length (cm)	Root length (cm)	Vigour
SVC 11	77.78±2.23	18.9±0.06	15.5±0.06	2675.556
BE 1	82.22±1.67	18.93±0.09	15.9±0.09	2864.074
NGB 21	91.11±1.55	19.±0.18	16.8±0.18	3270.889
MIW 1	85.22±1.88	18.83±0.37	15.36±0.37	2809.259
MC	97.78±1.33	19.26±0.19	17.33±0.19	3578.667
Control	73.33±1.28	19.1±0.15	14.7±0.15	2478.667
eulav DC	5.56	0.41	0.32	22.24

Table.2 Evaluation of Screened isolates for PGP activities in brinjal seeds *In vitro*

Table.2.1 Observation on 7th day

setalosI	Germination (%)	Shoot length (cm)	Root length (cm)	Vigour
SVC 11	75.56±1.33	1.30±0.05	3.4±0.13	355.1111
BE 1	78.56±1.40	1.50±0.16	3.6±0.17	362.6667
NGB 21	84.44±1.58	3.90±0.03	6±0.21	836
MIW 1	80.00±1.33	1.83±0.07	3.9±0.34	456
MC	88.89±2.67	2.207±0.04	4.3±0.19	577.7778
Control	57.78±0.33	0.80±0.09	2.9±0.11	230.2222
eulav DC	4.33	0.21	0.35	22.24

Table.2.2 Observation on 14th day

setalosI	Germination (%)	Shoot length (cm)	Root length (cm)	Vigour
SVC 11	75.56±1.33	4.97±0.17	3.4±0.22	632.1481
BE 1	78.56±1.40	4.23±0.12	3.53±0.17	586.8148
NGB 21	84.44±1.58	4.97±0.09	6±0.81	926.0741
MIW 1	80.00±1.33	4.27±0.45	3.9±0.11	653.3333
MC	88.89±2.67	4.77±0.06	4.3±0.05	805.9259
Control	57.78±0.33	4.07±0.74	2.±0.12	402.5185
eulav DC	4.33	0.28	0.41	33.43

Table.2.3 Observation on 21st day

setalosI	Germination (%)	Shoot length (cm)	Root length (cm)	Vigour
SVC 11	75.56±1.33	5.87±0.33	4.57±0.16	783.2593
BE 1	78.56±1.40	5.13±0.19	4.7±0.22	699.2593
NGB 21	84.44±1.58	5.87±0.16	7.1±0.07	1094.963
MIW 1	80.00±1.33	5.17±0.11	5±0.25	813.3333
MC	88.89±2.67	5.67±0.29	5.43±0.15	986.6667
Control	57.78±0.33	4.97±0.41	4±0.17	557.9259
eulav DC	4.33	0.31	0.37	45.11

Table.3 Evaluation of screened isolates for PGP activities in rapeseed mustard seeds *In vitro*

Table 3.1. no noitavresbO7th day				
setalosI	**Germination (%)**	**Shoot length (cm)**	**Root length (cm)**	**Vigour**
SVC 11	84.44±1.33	1.07±0.09	1.37±0.06	301.1852
BE 1	71.11±1.88	0.70±0.06	1.13±0.09	199.1111
NGB 21	78.11±1.58	0.80±0.03	1.57±0.18	227.5556
MIW 1	66.11±1.33	0.93±0.07	1.03±0.37	237.037
MC	88.89±1.67	2.17±0.09	1.00±0.19	459.2593
Control	68.88±1.33	0.57±0.09	1.23±0.15	190.5926
eulav DC	3.63	0.25	0.47	35.25

Table.3.2 Observation on 14th day

setalosI	Germination (%)	Shoot length (cm)	Root length (cm)	Vigour
SVC 11	84.44±1.33	2.3±0.13	2.5±0.11	405.3333
BE 1	71.11±1.88	1.9±0.88	2.1±0.04	275.5556
NGB 21	78.11±1.58	2±0.18	2.4±0.21	312.8889
MIW 1	66.11±1.33	2.1±0.13	2.4±0.34	322.3704
MC	88.89±1.67	3.37±01.7	3±0.19	565.9259
Control	68.88±1.33	1.77±0.33	2.2±0.12	273.2593
eulav DC	3.63	0.22	0.39	39.39

Table.3.3 Observation on 21st day

setalosI	Germination (%)	Shoot length (cm)	Root length (cm)	Vigour
SVC 11	84.44±1.33	3.7±0.13	3.76±0.17	630.5185
BE 1	71.11±1.88	3.3±0.17	3.2±0.12	462.2222
NGB 21	78.11±1.58	3.4±0.01	3.53±0.07	493.037
MIW 1	66.11±1.33	3.53±0.31	3.5±0.21	500.1481
MC	88.89±1.67	4.77±0.05	4.1±0.15	788.1481
Control	68.88±1.33	3.17±0.12	3.3±0.12	445.4815
eulav DC	3.63	0.17	0.22	42.33

Fig.1 Germination percentage

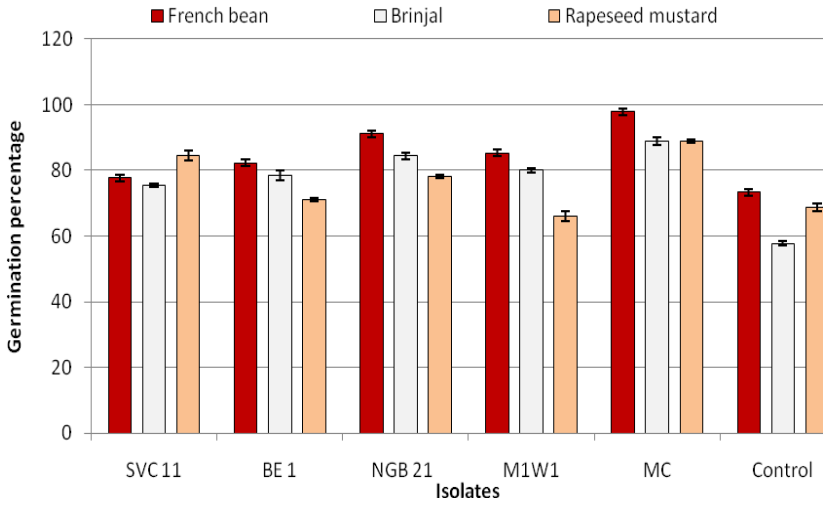


Fig.2 Vigour index of the screened isolates

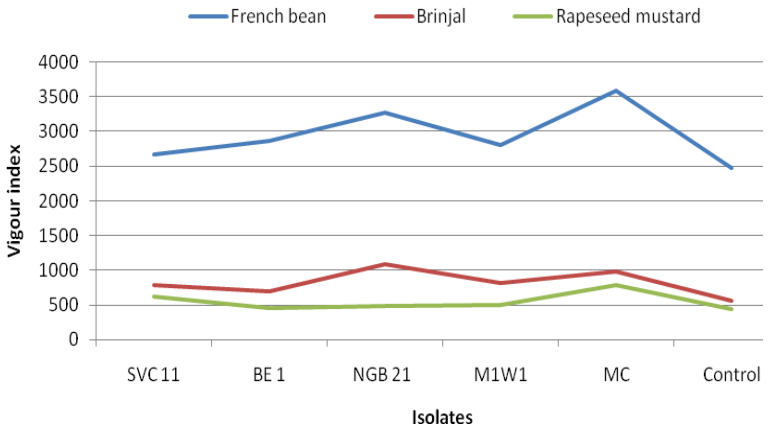
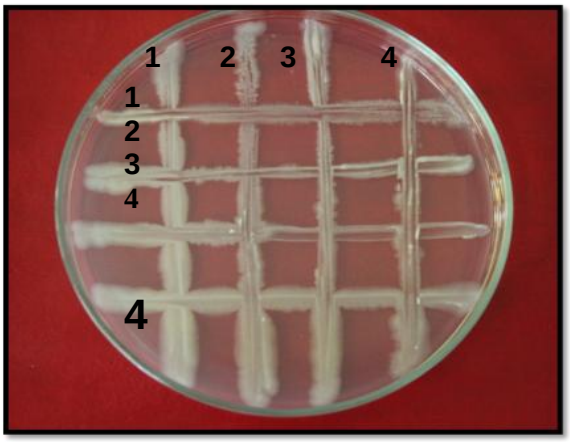
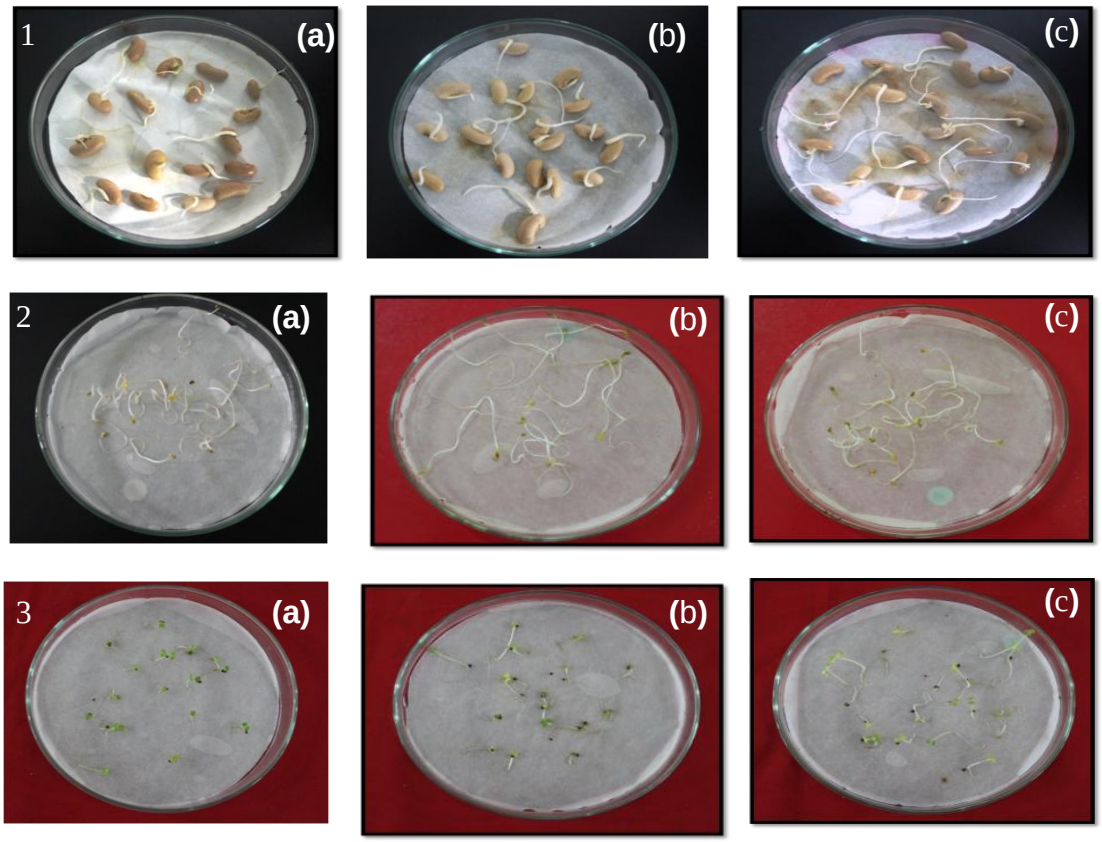


Plate.1 Compatibility test of bacterial isolates



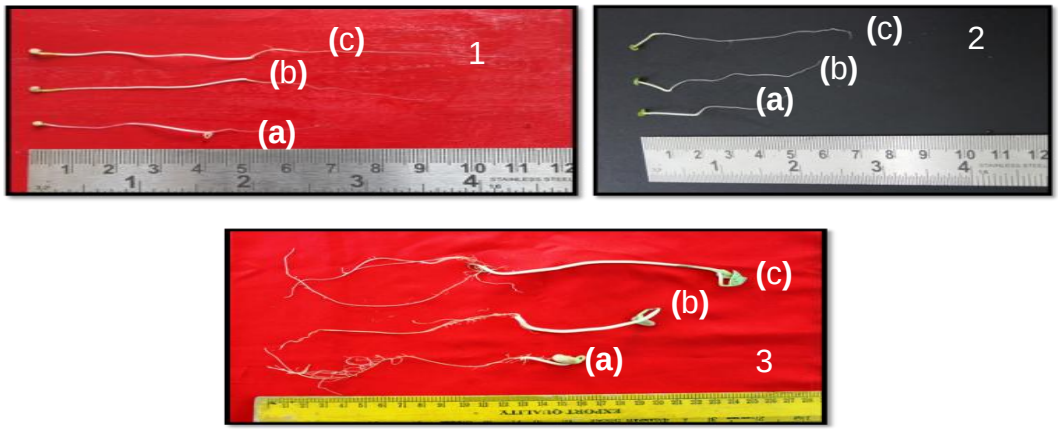
1.BG B4 **2.** BE-1 **3.** NGB 21 **4.**M**1**W**1**

Plate.2 Evaluation of the plant growth promoting (PGP) activities of screened bacterial endophytes in vitro



a) Control b) Treatment with individual isolate c) Treatment with microbial consortia
1. French bean 2. Brinjal 3.Rapeseed mustard

Plate.3 Root and shoot length after 21 days



a) Control b)Treatment with individual isolate c) Treatment with microbial consortia
1. Brinjal 2. Rapeseed mustard 3. French bean

In conclusion, isolate NGB 21 as best in terms of germination and vigour. All the screened potential bacteria has the best plant growth promoting ability as compared to the other crop seed tested. Hence, the microbial consortia could be evaluated further under field conditions to developed consortia against major vegetable pathogens of Meghalaya.

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