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Isolation of rhizobacteria belonging to *Bacillus* spp. from different agro-climatic zones of India and their evaluation as plant stimulants

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Abstract

The plant-growth promoting potency of 161 native *Bacillus* spp. isolates was evaluated at Prof. TNA Innovation Center, Hyderabad, India during 2020-22. In preliminary screening, 19 isolates exhibited superior plant-growth promoting activities. High activities of amylase (>1.3 cm; 8 isolates), protease (>1.2 cm; 9 isolates), cellulase (>1.2 cm; 15 isolates), ammonia (9 isolates), IAA (>10 µg/mL; 9 isolates), and exopolysaccharide production (>0.05 w/v; 7 isolates) were recorded among these isolates. Nutrient solubilization *viz.,* phosphate (10 isolates ranging, 0.30-57.3 µg/mL), zinc (8 isolates ranging, 0.07-0.30 µg/mL), potash (9 isolates ranging, 5.5-45.7 µg/mL), silica (6 isolates ranging, 0.10-0.40 µg/mL) was recorded. None of the isolates showed sulphur mobilization activity. *In vitro* pot studies using *Vigna radiata* as a test plant recorded maximum shoot length (19.47 cm), root length (9.42 cm), and plant dry mass (2.11 gm) when the seeds were treated with isolate B7. The seed germination was high in the B7, B79 and B88 inoculation treatments. Based on the *in vitro* and *in vivo* results, 5 of the best performing *Bacillus* isolates were shortlisted and evaluated for seed and seedling vigour in *Sorghum biocolor* and *V. radiata,* and field bio-efficacy studies using *Capsicum annuum*. Results demonstrated significantly high seed and seedling vigor, and yield (7300 kg/ha) when B7 isolate was used as seed dress and soil application. The isolated B7 was identified as *Aneurinibacillus migulanus* with accession no: NR_112214.1. Efforts are being made to carry out multi-location bio-efficacy studies using the *A. migulanus* on *C. annuum* during the rainy season 2022.

Keywords: *Bacillus*, Enzymatic activity, Nutrient solubilization, Plant growth promoting rhizobacteria, *Capsicum annuum*

1. Introduction

The aerobic, Gram positive, motile, rod-shaped *Bacillus* spp. was reported to be the promising plant growth promoting Rhizobacteria [1]. They colonize the rhizosphere of the plants and promote plant growth [2] through various mechanisms including solubilization of phosphorus, potassium, zinc, silica, iron, and other essential plant nutrients [3]; production of hydrolytic enzymes such as amylase, protease, and cellulase [2]; ammonia [4]; plant hormones like Indole acetic acid, gibberellic acid; and production of antibiotics, salicylic acid, and antifungal metabolites [5]. They are also reported to reduce the biotic and abiotic stresses in plants.

Bacillus spp. are the most attractive among the plant growth-promoting rhizobacteria (PGPR) due to their spore-forming ability, diversity in action and storage stability. Their commercial significance as bio-fertilizers, bio-pesticides and plant stimulants has been well documented [6]. The successful use of *Bacillus* sp. PG-8, *B. polymixa* [7], *B. licheniformis* [8], *B. pumilis* [9], *Bacillus subtilis* [10], *B. amyloliquefaciens* [11], *B. megaterium*, and *Bacillus megaterium var. phosphaticum*, across the globe indicate their importance as a potential plant growth promoter.

India has a rich microbial bio-diversity due to its diverse climatic conditions. However, the microbial diversity has been less explored for their PGP activities. The current study therefore was undertaken to collect various *Bacillus* isolates from varied agro-climatic zones in India, study their bio-efficacy in the laboratory and field in

order to select the best *Bacillus* PGP isolate.The perspective of the study could also serve as guidance for the plant growth promotion research and development.

Soil samples collected from select states of India were brought to the laboratory of Professor Technology needs assessment Innovation Center, Hyderabad, India and stored at 4˚C. The isolation of the microbes was carried out by following the standard operating protocol. About 161 *Bacillus* isolates were isolated from the soil and coded as B1 to B161.

With this background, studies were carried out to characterize isolates B1 to B161 in terms of their ability to promote plant growth. This includes the efficiency to solubilize various plant macro and micro-nutrients; production of hydrolytic enzymes and ammonia; and facilitate seed germination, seedling vigour and yield. The research outcome focuses on the growth promoting potential of 161 *Bacillus* isolates in comparison with *Pseudomonas fluorescens* and *Bacillus pumilus* with the aim to develop a microbial bio-stimulant formulation of the efficient novel *Bacillus* isolate(s).

2. Materials and methods

2.1 Soil sample collection, Bacillus isolation and characterization

Soil samples collected from the plant rhizospheric area in different agro-climatic zones of India were brought to the laboratory and stored at 4°C. The samples were sieved, subjected to heat shock at 100°C for 5 min and the heat stable microbes were isolated. Based on the Gram's staining, hydrolytic tests and morphology of the microbes, the *Bacillus* isolates were established [12], and were coded as B1 to B161. The isolates were stored at 4°C for further use. The sources of 161 *Bacillus* isolates from the different zones of India are provided in Table 1.

Table 1 Details of location, host plant and soil type.

Locations	Soil characteristics		Host Plant	No. of isolates	Isolates
	Soil type	pH			
Vikarabad, Telangana State	Sandy loam	6.8	*Daucus carota subsp. Sativus*	3	1-3
Anthamagudem, Yadadri, Telangana State	Clay	7.3	*Solanum lycopersicum*	5	4-8
Talab, Assam	Alluvial	5.1	*Camellia sinensis*	19	9-27
Khammam, Telangana State	Loamy	6.8	*Psidium guajava*	4	28-31
Kadapa, Andhra Pradesh	Loamy	6.9	*Curcuma longa*	22	32-53
Chevella, Telangana State	Loamy	6.9	*Psidium guajava*	42	54-95
Yellagiri, Telangana State	Ultisols	6.8	*Eleusine coracana*	8	96-103
Rajendra Nagar, Hyderabad	Ultisols	6.8	*Solanum lycopersicum*	20	104-123
Vizianagaram, Andhra Pradesh	Loamy	6.9	*capsicum annum*	10	124-133
Nagarkata, West Bengal	Alluvial	5.3	*Camellia sinensis*	15	134-161
Tindivanam, Tamil Nadu	Clay	7.3	*Oryza sativa*	12	150-161

2.2 Qualitative assay of hydrolytic enzymes, ammonia and exopolysaccharide production

The qualitative enzymatic activity of amylase [13], cellulase, and protease [14], were determined under *in vitro* conditions. The detection of ammonia was assayed using Nessler’s reagent [14], estimation of exopolysacharides by using Anthrone method and indole-3-acetic acid (IAA) production by using Salkowski reagent method [5,15].

2.3 Nutrient solubilization studies

The ability of the *Bacillus* isolates to solubilize important plant nutrients such as phosphorus, potash, zinc, silica, and sulphur was evaluated using standard qualitative and quantitative assays. The nutrient specific media recommended for qualitative and quantitative estimation of potash (Aleksandrow's medium), phosphate (Pikovaskaya's medium); [16], silica (Magnesium tri silicate enriched medium); [17], zinc (Bunt and Rovira medium); [18], and sulphur (Thiosulphate broth) were used. The other vital parameters such as pH and temperature were maintained as per the prescribed protocol.

The qualitative nutrient solubilization efficiency of the respective test organisms was computed using the formula:

$$\text{Nutrient Solubilizing efficiency} = \frac{\text{colony diameter + clear zone diameter)}}{dx\text{colony diameter}} \quad (1)$$

The quantitative estimation of zinc, potash, sulphur and silica was carried out using an atomic absorption spectrophotometer (ELICO make), [17] and phosphorus using UV-Spectrophotometer (ELICO make) [16]. *Bacillus* isolates grown in the respective specific medium were used to estimate the percentage of the nutrient availability.

2.4 Seed & seedling vigour

The select isolates were evaluated to study the vigour of seed [19], and seedling [20] in *Vigna radiata* and *Sorghum bicolor* by using the following formula:

$$\text{Seed vigor index} = \text{Shoot length (cm)} \times \text{Germination (\%)} \quad (2)$$

$$\text{Seedling vigor index} = (\text{Root length} + \text{Shoot length}) / (\text{Germination \%}) \quad (3)$$

2.5 Pot culture assay to evaluate the PGPR activities

The pot culture experiment was conducted using plastic pots (10 cm × 20 cm). Each pot was filled with 9-kg pre-sterilized soil. The seeds of *Vigna radiata* were surface-sterilized and coated with the *Bacillus* isolates @10 mL/kg seed. Ten replications were maintained for each treatment. The pots were kept in green house for 30 days. Sterile water was used to irrigate the pots once in two days. Percent germination was recorded on day 7. The root and shoot length, wet and dry mass were recorded from the plant material collected on 31st day as described [21].

2.6 Field bio-efficacy studies

The field bio-efficacy study was carried out at the farm of Varsha Bioscience and Technology India Pvt. Ltd., during rainy season, 2021 on *Capsicum annuum* L. The experiment was conducted using randomized block design (RBD), with a plot size of 30 square feet per replication. Nine treatments were maintained by keeping three replications per treatment. The soil was drenched with the desired treatments by diluting the culture broth of 5 mL in one-litre water. The drenching was carried out at 25 days and 75 days after the transplantation. The *Capsicum annuum* L yield was computed by pooling the yield data obtained from each picking.

2.7 Statistical analysis

The pooled data from 3 replicates pertaining to on-field data were subjected to two-way ANOVA. The means were compared with each other using Fisher's LSD test at significance level of $p< 0.005$.

3. Results and discussion

3.1 In vitro production of enzymes, ammonia, exopolysaccharides, and Indole acetic acid

A total 161 *Bacillus* isolates were screened for their plant growth promoting activities, and seed and seedling vigour enhancement. Among the isolates screened, 19 *Bacillus* isolates showed multiple plant growth promoting activities such as production of ammonia, IAA and exopolysaccharides and hydrolytic enzyme activities.

Amylase, an important starch degrading enzyme, plays a vital role in promoting plant growth by disintegrating organic matters into starch [13]. Choubane et al. [22] reported better amylase activities with promising plant growth promoting ability by several *Bacillus* spp. In the present study, all the 19 isolates exhibited

amylase activities ranging from 0.8 to 1.5 cm. Among the isolates, B7, B22, B28, B37, B43, B53, B65, B73, B79 and B88 have exhibited high enzymatic activity of amylase ranging from 1.3 to 1.5cm as shown in Figure 1(A).

Several *Bacillus* spp. were reported to be the important protease producing organisms [23]. By maintaining the stability and survival of the plant, protease catalyzes the proteins for the better growth and reproduction [24]. It also regulates the biotic and abiotic stresses in plants. Among the 19 isolates, 8 isolates showed protease activity ranging from 1.2 to 1.7 cm. While *Pseudomonas fluorescens* showed moderate activity, no protease activity was observed in 10 *Bacillus* isolates as shown in Figure 1 (B).

Cellulase enzyme degrades cellulose, a polysaccharide into simple sugars [25]. Cellulase activity ranging from 1.2 to 1.4 cm was observed in *Bacillus* isolates *viz*., B7, B37, B43, B53, B62, B73, B79, B88 and B98. Four isolates and *P. fluorescens* did not show cellulase activity as shown in Figure 1 (C).

IAA plays a vital role in promoting root growth of the plant. Several *Bacillus* sp., are known for their plant growth promoting activity by producing indole acetic acid and other essential plant growth beneficial hormones [15]. In this study, high Indole acetic acid production was recorded in *Bacillus* isolates *viz*., B7, B28, B73, B79 and B88 compared to test control *P. fluorescens.*

Exopolysaccharides (EPS) stimulate plant growth and make them tolerant of drought by producing phytohormones, siderophores and biofilms [26]. Under drought conditions, some of the EPS producing *Bacillus* isolates exhibit accumulation of proline, glucose, and free amino acids [27]. In the current study, among 19 *Bacillus* isolates, 8 isolates have shown exopolysaccharide production activity ranging from 0.021 to 0.074 % w/v (Table 2). Isolates B7 and B73 recorded highest EPS activity as compared to the other evaluated isolates 0.072-0.074 (% w/v).

Supplementation of nitrogen through ammonia production in plant roots by beneficial *Bacillus* spp. has been well documented [14]. The available data suggests that *Bacillus cereus, B. circulans, B. firmus, B. pumilus*, *B. licheniformis*, *B. megaterium*, *B. subterraneous*, *B. aquimaris*, *B. vietnamensis* and, *B. aerophilus* are known to fix atmospheric nitrogen. In the present study, 9 *Bacillus* isolates were found to be positive producer of ammonia ranging from low (+), to high (++++) activity. The highest ammonia activity was recorded in isolates B62, B65, B73 and B79. *Pseudomonas fluorescens* was found to be a poor producer of ammonia as shown in Table 2 and Figure 1 (D).

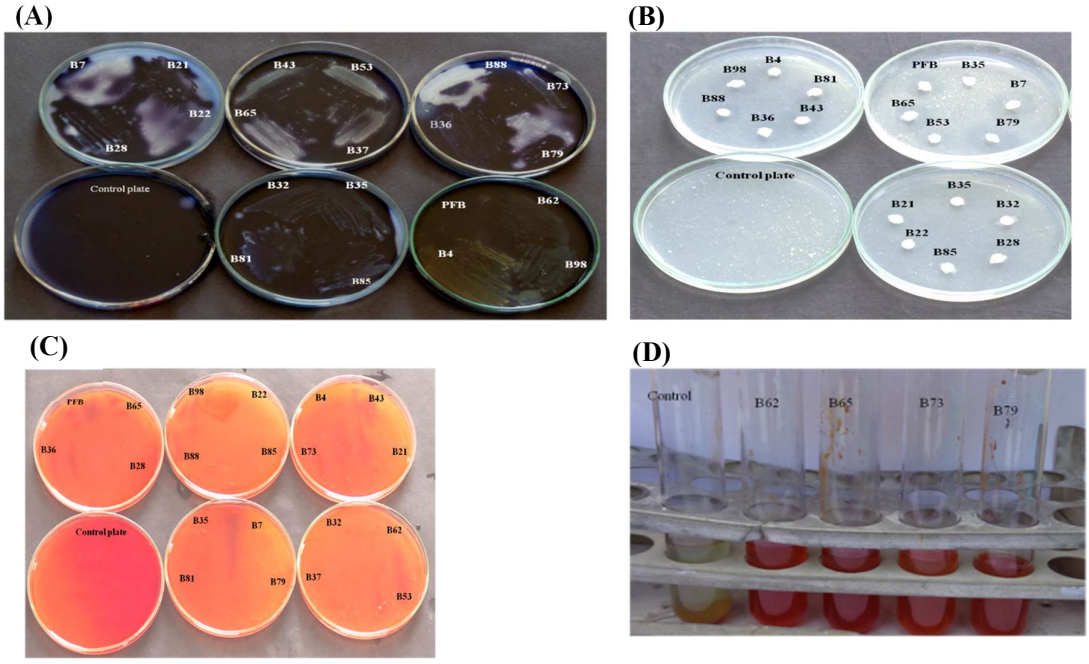

Figure 1 (A) Production of amylase by *Bacillus* isolates (B7, B21, B22, B28, B43, B53, B65, B37, B88, B73, B36, B79, B32, B35, B81, B85, B62, B4 and B98); *Pseudomonas fluorescens bacteria* (PFB), (B) Production of Protease by *Bacillus* isolates (B4, B81, B43, B36, B88, B98, B35, B7, B79, B53, B65, B35, B32, B28, B85, B22 and B21); PFB, (C) Production of Cellulase by *Bacillus* isolates (B65, B28, B36, B98, B22, B85, B88, B4, B43, B73, B21, B35, B7, B81, B79, B32, B62, B37 and B53); PFB, (D) Production of Ammonia by *Bacillus* isolates (B62, B65, B73 and B79).

Table 2 Estimation of exopolysaccharides, production of IAA and enzymatic activity.

Isolates No.	Production of					Estimation of Exopolysaccharide content (%w/v)
	Amylase (cm)	Protease (cm)	Cellulase (cm)	Ammonia	IAA (µg/mL)	
B4	1.2	0	0	-	9.7	0.043
B7	1.4	1.7	1.4	+++	24.6	0.072
B21	1.2	0	1.1	-	0	0
B22	1.4	0	0	-	0	0
B28	1.4	1.4	1.2	+++	13.9	0.064
B32	1.2	0	0.8	-	0	0
B35	1.2	0	1.1	-	0	0
B36	0.8	0	1.0	-	0	0
B37	1.5	0	1.3	-	8.8	0.021
B43	1.4	1.1	1.2	-	10.08	0
B53	1.3	1.2	1.3	-	5.4	0.05
B62	1.2	0	1.4	++++	6.7	0
B65	1.4	1.2	0	++++	7.3	0.055
B73	1.3	0	1.2	++++	16.7	0.074
B79	1.5	1.5	1.3	++++	22.8	0
B81	1.2	0	1.1	-	12.3	0
B85	1.2	0	0	+	9.6	0
B88	1.3	1.0	1.2	++	15.2	0
B98	1.2	1.3	1.2	+	0	0.055
PFB	0	1.2	0	+	13.0	0

Ammonia activity; --No activity; + = low activity; ++= average activity; +++ = Moderate activity; ++++ = High activity.

3.2 Nutrient Solubilization study

Bacillus spp. are reported to maintain the plant health directly/indirectly through nutrient solubilization/mobilization [6]. Phosphate is one of the essential macro-nutrients for plant growth. Most of the phosphorus is present in the soil in insoluble form, *Bacillus* spp. are known phosphate solubilizers. The qualitative and quantitative estimation of phosphate among the 19 isolates indicated phosphate solubilization ranging from 0.4 to 1.9 cm and its availability ranging from 0.30 to 57.3 µg/mL in B53, B36, B35 and B37 isolates (Figure 2 and Table 3 & 4). However, the solubilization zone of 2.2 cm and available 'P' content of 60 µg/mL were found to be superior in the treatment control, *Bacillus megatherium* var. *phosphaticum* as compared to the evaluated *Bacillus* isolates as shown in Figure 2(A).

Bacillus subtilis, *Paenibacillus kribensis* and *Bacillus circulans* show potash solubilization. Theyt manage acidity, alkalinity, salinity, and drought conditions by making insoluble phosphates into available form. The qualitative and quantitative studies of potassium solubilization indicated that isolates B79, B81, and B85 showed better efficiency ranging from 0.6 to 1.6 cm as compared to the other tested isolates. The available form of potassium ranged from 5.5 to 45.7 µg/mL. However, the solubilization and availability of potassium is much higher in treatment control, *Fraturea aurantia* as compared to rest of the isolates as shown in Figure 2 (B).

Zinc is one of the key micro-nutrients required for plants. It increases the plant growth and yield. Several *Bacillus* spp. Have been shown to solubilize zinc by chelating ligands, production of organic acids and amino acids [28]. The qualitative and quantitative estimation of zinc solubilization reported in B43, B4, B7 and B98 was ranging from 0.6 to 1.9 cm. The availability of zinc was ranging from 0.07 to 0.30 µg/mL as shown in Figure 2 (C).

Lee et al. [29] reported that silica is one of the most important elements on earth. *Bacillus* spp. exhibited their ability to convert unavailable form of silica into available form by solubilization. Among 19 *Bacillus* isolates, 6 isolates namely, B32, B35, B37, B53, B79 and, B81 have shown silica solubilization. The qualitative estimation of silica solubilization ranges from 0.5 to 2.5 cm. The availability form of silica is ranging from 0.10 to 0.40 µg/mL. The treatment control *Pseudomonas fluorescens* failed to show silica solubilization Figure 2 (D).

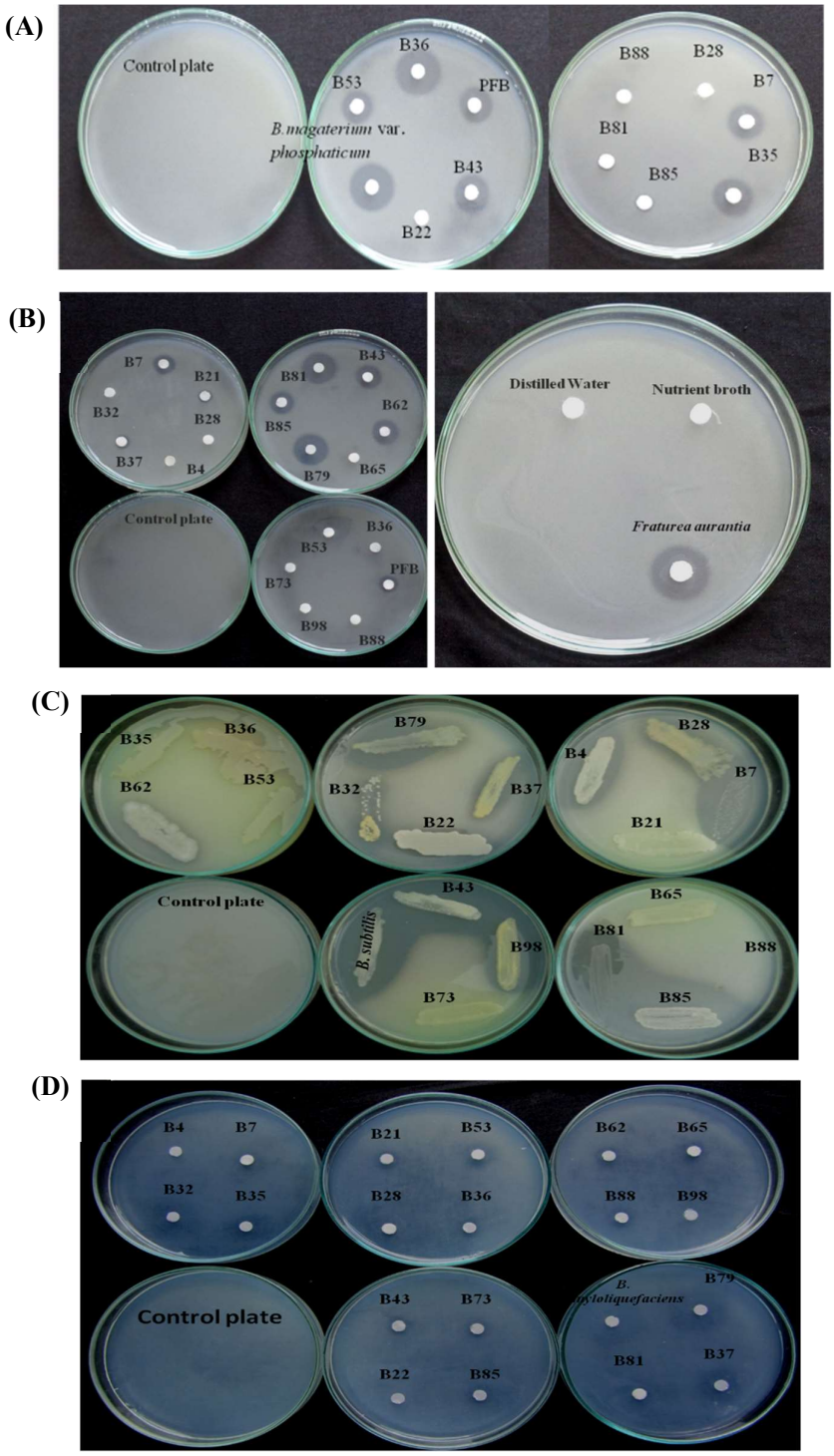

Figure 2 (A) Solubilization of tri-calcium phosphate by *Bacillus* isolates (B36, B53, B22, B43, B88, B28, B7, B35, B85, B81 and *B. magaterium var. phosphaticum*); PFB, (B) Solubilization of potash from *Bacillus* isolates (B7, B21, B28, B4, B37, B32, B81, B43, B62, B65, B79, B85, B36, B88, B53, B73 and B98); *Fraturea aurantia*; PFB, (C) Solubilization of zinc from *Bacillus* isolates (B35, B36, B62, B53, B79, B37, B22, B32, B28, B7, B21, B4, B43, B98, B73, B65, B88, B85, B81 and *B. subtilis*), (D) Solubilization of Magnesium tri-silicate from *Bacillus* isolates (B4, B7, B32, B35, B21, B53, B28, B36, B62, B65, B88, B98, B43, B73, B22, B85, B79, B81, B37 and *B.amyloliquefaciens*).

Table 3 Nutrient Solubilization efficiency of *Bacillus* isolates.

Culture code	Qualitative analysis (Solubilization in 7 days (cm)				
	PSB	ZSB	KSB	SiSB	SSB
B4	0	1.9	0	0	0
B7	1.3	1.6	0.7	0	0
B21	0.4	0	0.8	0	0
B22	0	0	0	0	0
B28	0	1.0	0	0	0
B32	0	0.6	0	0.8	0
B35	1.2	0	0	0.8	0
B36	1.9	0	0	0	0
B37	1.1	0.8	0.6	1.2	0
B43	1.4	1.9	1.3	0	0
B53	1.2	0	1.1	0.5	0
B62	0	0	1.2	0	0
B65	0	0	0	0	0
B73	0.6	0	0	0	0
B79	0.4	0.8	1.6	2.5	0
B81	0	0	1.6	0.5	0
B85	0	0	1.4	0	0
B88	0	0	0	0	0
B98	0.8	1.4	0	0	0
PFB	1.2	0	1.0	0	0
VBT Standard**	2.2	1.8	1.2	2.2	1.7

Table 4 Efficacy of select *Bacillus* isolates on available nutrient quantity.

Culture Code	Quantitative Estimation (µg/mL)				
	PSB	ZSB	KSB	SiSB	SSB
B4	0	0.18	0	0	0
B7	8.8	0.30	10.5	0	0
B21	3.6	0	5.5	0	0
B22	0	0	0	0	0
B28	0	0.23	0	0	0
B32	0	0.14	0	0.14	0
B35	49.5	0	0	0.14	0
B36	55.4	0	0	0	0
B37	53.8	0.14	14.3	0.10	0
B43	0.94	0.07	24.7	0	0
B53	57.3	0	22.1	0.10	0
B62	0	0	33.3	0	0
B65	0	0	0	0	0
B73	6.7	0	0	0	0
B79	0.3	0.24	45.7	0.40	0
B81	0	0	45.7	0.10	0
B85	0	0	43.2	0	0
B88	0	0	0	0	0
B98	18.2	0.21	0	0	0
PFB*	40	0	20.2	0	0
VBT Standard**	60	0.16	30.3	0.30	0.9

**VBT standards for PSB:*Bacillus magaterium* var. *phosphaticum*; ZSB: *B. subtilis*; KSB: *Fraturea aurantia*; Silica: *Bacillus amyloliquefaciens* and Sulphur: *Thiobacillus* spp. SiSB: Silica solubilizing bacteria SSB: Sulphur solubilizing bacteria.

3.3 Pot culture assay to evaluate the PGPR activities

The biological contribution of *Bacillus* isolates on the growth of *Vigna radiata* was studied using pot culture assay and the results were compared with the treatment control (Table 5). *Bacillus* isolates B7, B73, B79, B88 and B65 exhibited superior plant growth promotion traits on *V. radiata*. It was observed that treated plants had longer roots and shoots with higher dry biomass than the untreated control plants (Figure 3).

V. radiata seeds treated with *Bacillus* isolate 7 showed improvement in shoot (19.47 cm), root (9.42 cm) and dry biomass (2.11 g) as compared to other isolates as well as treatment control (Figure 3). Similar results *viz.,* nutrient solubilization and phytohormone production were reported in *B. subtilis*, *B. amyloliquoefaciens*, *B. cereus*, *B. pumilus* and *B. polymyxa*. They are known to solubilize nutrients and produce phytohormones [30].

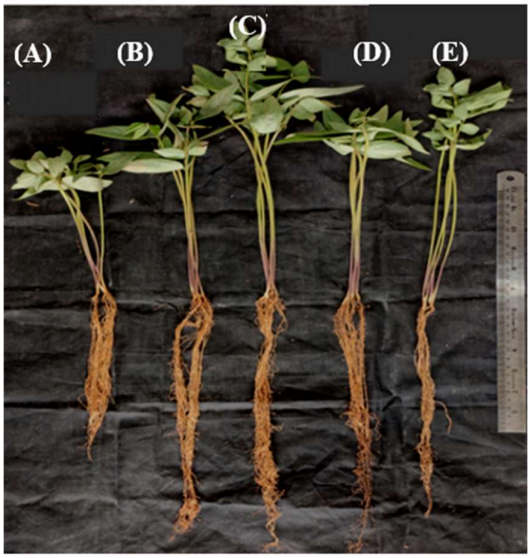

Figure 3 Plant growth promotion traits of (A) PFB, and *Bacillus* isolates (B) B79, (C) B7, (D) B79 (E) *B. pumilus*); evaluated on *Vigna radiata*.

Table 5 Plant growth promoting traits of *Bacillus* isolates evaluated on *Vigna radiata*.

Culture code	Shoot length in (cm)	Root length in (cm)	Dry mass in (g)	Germination (%)
B4	13.4^{bc} (±5.17)	9.09^{ab} (±0.77)	1.83^{ab} (±0.16)	76.08^{cd} (±6.009)
B7	19.47^{a} (±2.14)	9.42^{a} (±1.02)	2.11^{a} (±0.045)	100^{a} (±0)
B21	6.35^{de} (±0.71)	8.96^{ab} (±0.61)	1.48^{bc} (±0.092)	92.21^{ab} (±4.90)
B22	11.005^{c} (±1.25)	7.36^{c} (±1.33)	1.40^{bc} (±0.11)	78.30^{c} (±6.30)
B28	13.69^{bc} (±0.75)	9.20^{a}(±0.68)	1.91^{ab} (±0.14)	85.5^{bc} (±3.89)
B32	15.73^{b} (±1.60)	7.73^{bc} (±1.19)	1.34^{c} (±0.18)	81.63^{bc} (±7.73)
B35	6.18^{de} (±0.47)	7.52^{bc} (±1.003)	1.22^{cd} (±0.14)	87.76^{b} (±4.42)
B36	6.49^{de} (±0.49)	7.53^{bc} (±1.002)	1.22^{cd} (±0.11)	82.75^{bc} (±7.49)
B37	7.67^{d} (±0.98)	7.25^{c} (±0.80)	1.34^{c} (±0.16)	78.28^{c} (±6.32)
B43	11.06^{c} (±2.28)	5.06^{e} (±0.92)	1.34^{c} (±0.12)	63.28^{f} (±5.28)
B53	16.74^{ab} (±1.82)	8.71^{ab} (±0.39)	1.39^{bc} (±0.09)	78.13^{c} (±6.19)
B62	5.79^{e} (±1.74)	5.97^{d} (±0.59)	1.48^{bc} (±0.085)	74.9^{d} (±5.14)
B65	11.48^{c} (±0.52)	6.48^{cd} (±1.02)	1.90^{ab} (±0.18)	94.43^{ab} (±3.89)
B73	18.48^{ab} (±3.97)	6.32^{cd} (±1.002)	2.01^{a} (±0.059)	91.1^{ab} (±5.84)
B79	18.25^{ab} (±2.73)	8.42^{b} (±0.82)	2.005^{a} (±0.052)	97.76^{a} (±1.72)
B81	13.45^{bc} (±2.44)	7.91^{b} (±0.49)	1.84^{ab} (±0.17)	89.98^{b} (±5.48)
B85	7.62^{d} (±1.44)	8.21^{b} (±0.52)	1.61^{b} (±0.13)	89.98^{b} (±4.07)
B88	16.74^{ab} (±1.82)	6.61^{cd} (±1.09)	1.84^{ab} (±0.18)	99.33^{a} (±0.51)
B98	10.97^{c} (±2.61)	5.91^{d} (±0.53)	1.58^{b} (±0.24)	72.18^{de} (±6.39)
V37	11.47^{c} (±0.53)	6.2^{cd} (±0.84)	1.49^{bc} (±0.10)	74.4^{d} (±7.65)
PFB	7.63^{d} (±0.62)	6.77^{cd} (±1.006)	1.34^{c} (±0.11)	74.3^{d} (±7.64)
B. pumilus	8.83^{cd} (±1.07)	7.14^{c} (±1.36)	1.60^{b} (±0.17)	79.41^{c} (±6.18)
Nutrient broth	5.82^{e} (±2.06)	5.55^{de} (±1.09)	1.34^{c} (±0.10)	68.85^{ef} (±4.91)
water	5.58^{e} (±0.19)	3.89^{f} (±0.64)	1.14^{d} (±0.12)	67.16^{ef} (±6.27)
LSD	4.014	1.579	0.245	9.274
CV%	55.323	30.442	24.087	17.306

Values are the mean of 10 determents (±SE) within a column different letters (^{a,b}) after values indicate that there is a significant difference at *p* value of 0.05 as determined by Fischer's least significant difference test.

3.4 Effects of Bacillus isolates on the seed and seedling vigour index

The efficacy of *Bacillus* isolates in enhancing seed and seedling vigour in *V. radiata* and *S. bicolor* is shown in Figure 4. Their efficacy was compared with the commercial standard, *P. fluorescens* and *B. pumilus*. The maximum seedling vigour index was recorded in *V. radiata* (262.6) and *S. bicolor* (266.6) when the seeds were treated with *Bacillus* isolate B7. Similarly, the maximum seed vigour was recorded in *V. radiata* (93.3) and in *S. bicolor* (95.0). The seed vigour and seedling vigour were considerably low when the seeds were treated with *P. fluorescens*, *B. pumilus* and nutrient broth.

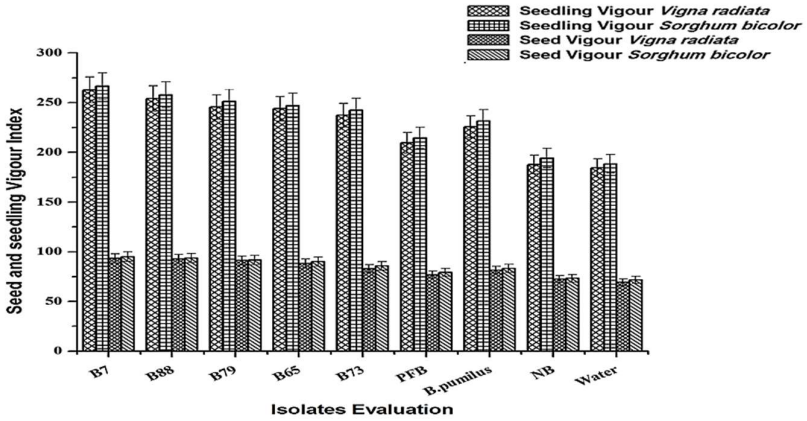

Figure 4 Seedling and seed vigour in *Vigna radiata* and *Sorghum bicolor.*

3.5 Field Bio-efficacy Studies

Based on the laboratory and screen house studies, *Bacillus* isolates B7, B88, B79, B65 and B73 were shortlisted for evaluating their bio-efficacy on *Capsicum annuum* L. These isolates were evaluated in field in comparison with commercial standards, *P. fluorescens* and *B. pumilus* on *Capsicum annuum* L using randomized block design. The crop yield ranged from 6342 to 7300 kg/ha (Table 6). Among the treatments, the maximum yield was recorded in plots treated with isolate B7 (7300 kg/ha) followed by isolate B79 (7091 kg/ha).

Table 6 Field bio efficacy studies on *Capsicum annuum* L.

Culture code	Mean (kg/S.ft)	Yield (kg/ha)
B7	4.19^{a} (±0.061)	7300
B79	4.07^{ab} (±0.063)	7091
B73	3.9^{b} (±0.026)	6847
B65	3.91^{b} (±0.089)	6812
B88	3.85^{bc} (±0.124)	6708
B. pumilus	3.82^{bc} (±0.036)	6655
P. fluorescens	3.81^{bc} (±0.034)	6638
Nutrient broth	3.71^{c} (±0.041)	6464
Untreated control	3.64^{c} (±0.033)	6342
LSD	0.2468	-
CV%	6.506122	-

Values are the mean of 3 determents (±SE) within a column different letters ($^{a,b)}$ after values indicate that there is a significant difference at *p*-value of 0.05 as determined by Fischer's least significant difference test.

4. Conclusion

Out of 161 *Bacillus* isolates evaluated for their plant growth promoting activities, 19 isolates were shortlisted for their PGP activities based on the level of production of indole acetic acid, ammonia, exopolysaccharides, hydrolytic enzymes in addition to phosphate, potash, zinc and silica solubilization and germination capability. Among the 19 isolates, B7, B65, B73, B79 and B88 were shortlisted for their better PGP activities as compared to *P. fluorescens* and *B. pumilus.* Laboratory and field bio-efficacy studies using the shortlisted 5 isolates recorded superior seed and seedling vigour in *V. radiata* and *S. bicolor*. The on-farm study recorded significant yield increase when *Capsicum annuum* L was treated with *Bacillus* isolate B7. Based on the genetic identification, isolate B7 was identified as *Aneurinibacillus migulanus* (Accession no: NR_112214.1).

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