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Enhanced Plant Growth and Biomass Accumulation via *Bacillus* spp. Consortia: A Functional Study for Wheat Growth

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Thirty-four distinct bacillus strains were isolated and identified from various districts of the Marathwada region. These strains of *Bacillus sp.* were morphologically and biochemically characterised, such as shape, Gram's nature, and endospores forming ability, arginine hydrolysis, catalase test, hydrolysis of lipid, hydrolysis of casein, liquefaction of gelatin, starch hydrolysis, and urease test. The different plant growth-promoting properties of isolated Bacillus species investigated included IAA and GAƒ production, phosphate solubilisation, siderophore production, and HCN production. Isolates producing the highest amount of IAA, GA_3, HCN, and phosphate solubilizers were selected for the plant growth promotion, including those isolates that show the highest antifungal activity. When a consortium of *Bacillus species* was used instead of individual strains, the roots and shoots showed greater length and dry weight. After 25 days of treatment, the consortium B4 produced the longest roots and shoots. After 25 days of treatment, consortia B3 showed the highest amount of dry weight of roots, while consortium B4 showed the highest amount of dry weight of shoots.

Keywords: Consortium, GA_3, HCN, IAA, Plant growth promotion.

The soil layer around plant roots, known as the rhizosphere, is critical to plant growth and development.[1, 2] Rhizobacteria are bacterial communities that live in this region, and some of them, known as plant growth-promoting rhizobacteria (PGPR), have a favourable impact on plant growth. These PGPR promote plant growth directly or indirectly.[3] These helpful rhizobacteria are classified into various genera, including *Bacillus, Azotobacter, and Pseudomonas*.

Bacillus is a genus of gram-positive bacteria commonly found in soil, with species such as *Bacillus subtilis.* [4] being diazotrophic. Extensive research has been conducted on the ability of *Bacillus* and *Paenibacillus* species to promote plant growth. [5, 6] It is well established that *B. cereus*, *B. subtilis*, and *B. megaterium* can serve as effective biocontrol agents against plant diseases. Additionally, antifungal peptides produced by species of *Bacillus* have the ability to regulate fungal growth.[7]

Paenibacillus and *Bacillus* are widely found in the rhizosphere. Very little information is available about this genus, but it is impossible that only a single species will dominate and outnumber other species in most soils.[8] Many PGPR possess great potential as inoculants, and they can be used for agricultural purposes and environmental protection. These PGPR help to maintain the sustainability of agroecosystems. However, currently, PGPR are not effectively used in agriculture despite many positive reports

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in laboratory conditions. The use of a consortium of PGPR with different plant growth-boosting features can have a greater favourable impact on plant growth than using a single strain of PGPR. Inoculation of plants with mixed strains was found to be more effective than using a single strain.[9] As a result, the purpose of this study was to determine the influence of a *Bacillus* sp. consortium on plant growth promotion.

MATERIALS AND METHODS

Isoaltion and Characterization of *Bacillus sp*

Bacillus sp. from the rhizospheric zone were isolated as previously described.[10] Colonies isolated were examined for their cell shape, gram reaction, and ability to form endospores.[11] *Bacillus* species were identified as previously described.[12] Different biochemical tests were performed, such as arginine hydrolysis, catalase test, lipid hydrolysis, casein hydrolysis, gelatin liquefaction, starch hydrolysis, and urease test.

Determination of plant growth-promoting traits of *Bacillus sp.*

Isolated *Bacillus* species were investigated for their diverse plant growth-promoting characteristics, including as IAA production [13], gibberellic acid production [14], phosphate solubilisation, siderophore production [15], and HCN production.[16]

Antifungal Activity

The antifungal properties of *Bacillus sp.* was assessed as described.[17] *Fusarium oxysporum* was cultured on plates containing PDA (potato dextrose agar) and was inoculated at the center of the plates. A bacterial culture of 24 hours old was streaked parallel 3-4 cm away from the disc on both sides. The incubation of these plates was done at 30°C for 96 hours.

Plant growth promotion experiment

The highest producers of IAA, GA, HCN, and Phosphate solubilizers were employed for the growth promotion experiment in selected plants, including those isolates that show the highest antifungal activity. A Plant growth promotion experiment was conducted as previously described.[18] Wheat seeds were dipped in 0.02% sodium hypochlorite for two to three minutes and were then washed thrice with sterilized distilled water. Carboxymethylcellulose (CMC) 1% solution was used as an adhesive. The seeds were treated with a broth that contained 1 $\times 10^9$ CFU/ml. Seeds

Table 1. Isolates of *Bacillus* Selected for Plant Growth Promotion Experiment

Sr.No	Isolate Code	Plant growth promoting trait
1	Yb8	IAA production
2	Yb1	GA production
3	Yb20	Phosphate Solubilization
4	Yb34	HCN production
5	Yb27	Antifungal activity

Fig. 1. Urease Test

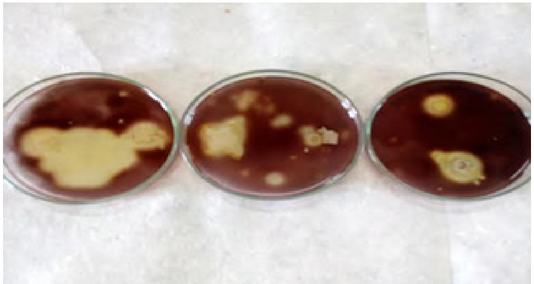

Fig. 2. Starch Hydrolysis Test

Fig. 3. Arginine hydrolysis Test

were treated with each bacterial culture for 30 min and dried overnight. The four treated seeds were sown in soil containing pot and the length of the root, the shoot and the dry weight of the root and the shoot was noted on the eighth day, the fifteenth day and the twenty fifth day of treatment.

Plant growth promotion experiment using consortium of microorganisms

A plant growth promotion experiment using a consortium was conducted as previously described.[19] The highest producers of IAA, GA, HCN, and Phosphate solubilizers were selected for the plant growth promotion experiment, including those isolates that show the highest antifungal activity. Wheat seeds were soaked for 2-3 minutes in 0.02% sodium hypochlorite, then washed three times with sterilised distilled water. The glue was made of 1% CMC (carboxymethylcellulose). Seeds were treated with broth containing 1×10^9 CFU/ml. The consortium had a 1:1 (v/v) ratio of each broth culture. Seeds were treated with each consortium culture for 30 minutes and then dried overnight. The four treated seeds were sown in soil containing a pot, and the length and dry weight of the root and shoot were measured on the eighth, fifteenth, and twenty-fifth days of treatment.

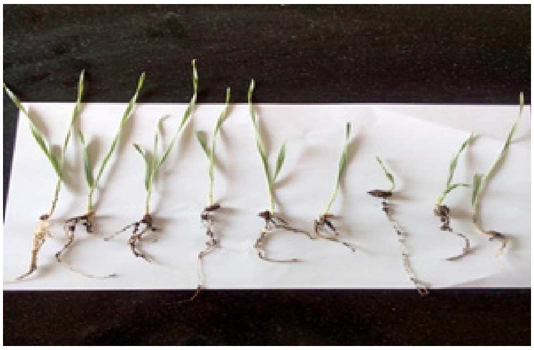

Fig. 4. Effect of *Bacillus* isolates on wheat seeds

RESULTS

A total of 34 Bacillus strains were isolated and identified from seven districts of Marathwada. These strains were characterized morphologically and biochemically. All isolates were gram-positive, rod-shaped, and motile, with the presence of endospores being a common characteristic. The isolates were tested for their ability to promote plant growth by producing siderophores,

Table 2. Consortium of *Bacillus* used for the experiment

Sr. No	Plant growth promoting trait	Consortium Code
2	IAA producer+ GA producer	B1
3	IAA producer+ GA producer+ Phosphate Solubilizer	B2
4	IAA producer+ GA producer+ Phosphate Solubilizer + HCN producer	B3
5	IAA producer+ GA producer+ Phosphate Solubilizer + HCN producer+ Antifungal activity	B4

Table 3. Effect of *Bacillus* isolates on root length and shoot length of wheat seeds

Sr. No	Isolate Code	Root Length (cm)			Shoot Length (cm)		
		After 8 days	After 15 days	After 25 days	After 8 days	After 15 days	After 25 days
1	Yb1	4.27	6.10	11.48	6.25	12.78	16.55
2	Yb8	4.11	5.21	10.45	5.45	11.41	15.20
3	Yb20	3.20	6.85	12.20	6.44	12.20	15.21
4	Yb27	4.20	7.45	10.56	5.21	10.60	15.85
5	Yb34	3.20	6.40	10.87	6.40	10.46	14.50
6	Control	3.10	6.10	9.80	5.20	9.89	12.10
7	SE±	0.09	0.27	0.39	0.15	0.27	0.37
8	CD@ 5%	0.29	0.81	1.10	0.45	0.82	1.11

gibberellic acid, phosphate solubilization, IAA, and HCN. The strains producing the highest amounts of IAA, gibberellic acid, HCN, and those exhibiting the largest zones of phosphate solubilization and antifungal activity were selected for further investigation. Five Bacillus strains were chosen for the plant growth promotion experiment (Table 1). In this experiment, wheat seeds treated with Yb20 exhibited the longest root length after 15 days, while the highest shoot length of 16.55 cm was observed with Yb1 after 25 days of treatment (Table 3). After 25 days of treatment, the B4 consortium (IAA producer + GA producer + phosphate solubilizer + HCN producer + antifungal activity) had the best results in terms of root length, shoot length, and dry root and shoot weight for wheat seeds. The B4 consortium's root and shoot lengths were 11.80 cm and 14.63 cm, respectively (Table 5). The highest dry weight of the root was seen with consortia B3, while the highest dry weight of the shoot was obtained by B4 after 25 days. This study found that the use of Bacillus sp. consortia was more effective in boosting plant growth, in terms of root and shoot length and root and shoot dry weight, than the use of individual *Bacillus*.

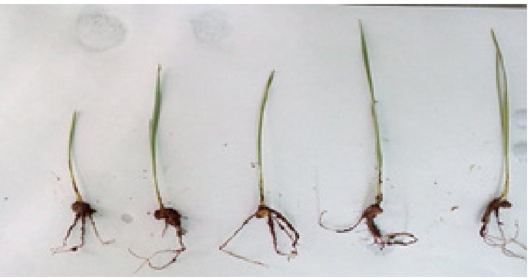

Fig. 5. Effect of consortium of *Bacillus sp* on wheat seeds

DISCUSSION

PGPR exert beneficial effects on plants by different mechanisms such as the production of phytohormones, phosphate solubilization, nitrogen fixation, and biocontrol.[20] *Bacillus* is a common

Table 4. Effect of *Bacillus* isolates on dry root weight and dry shoot weight of wheat seeds.

Sr. No	Isolate Code	Dry root weight(mg)			Dry shoot weight (mg)		
		After 8 days	After 15 days	After 25 days	After 8 days	After 15 days	After 25 days
1	Yb1	0.20	0.40	0.70	0.30	0.54	0.82
2	Yb8	0.26	0.49	0.75	0.41	0.58	0.94
3	Yb20	0.28	0.46	0.76	0.40	0.61	0.99
4	Yb27	0.28	0.50	0.80	0.44	0.69	1.01
5	Yb34	0.30	0.51	0.82	0.39	0.51	0.80
6	Control	0.20	0.45	0.70	0.30	0.45	0.75
7	SE±	0.007	0.017	0.030	0.014	0.020	0.032
8	CD@ 5%	0.021	0.55	0.092	0.042	0.062	0.095

Table 5. Effect of consortium of *Bacillus* isolates on root length and shoot length of wheat seeds

Sr. No	Isolate Code	Root Length (cm)			Shoot Length (cm)		
		After 8 days	After 15 days	After 25 days	After 8 days	After 15 days	After 25 days
1	B1	4.01	7.12	11.44	6.01	11.98	15.21
2	B2	5.01	8.25	12.24	5.08	11.24	14.10
3	B3	5.54	7.54	12.20	5.98	11.66	15.20
4	B4	4.45	7.14	12.30	6.10	12.64	16.25
6	Control	4.01	7.44	11.06	5.54	10.66	12.85
7	SE±	0.10	0.18	0.40	0.11	0.26	0.41
8	CD@ 5%	0.30	0.57	1.22	0.35	0.80	1.24

Table 6. Effect of consortium of *Bacillus* isolates on dry root weight and dry shoot weight of wheat seeds

Sr. No	Isolate Code	Dry Root Weight(mg)			Dry Shoot Weight(mg)		
		After 8 days	After 15 days	After 25 days	After 8 days	After 15 days	After 25 days
1	B1	0.21	0.45	0.71	0.35	0.56	0.85
2	B2	0.26	0.51	0.80	0.45	0.60	1.11
3	B3	0.30	0.56	0.86	0.40	0.69	1.02
4	B4	0.28	0.52	0.89	0.44	0.79	1.35
6	Control	0.21	0.44	0.65	0.35	0.51	0.81
7	SE±	0.011	0.018	0.029	0.015	0.024	0.037
8	CD@ 5%	0.035	0.055	0.087	0.045	0.074	0.11

bacterium genus in the rhizosphere, and the plant growth-promoting properties of numerous of its strains have been well researched, revealing the mechanisms involved.[21] Field investigations with microbial consortia of *Pseudomonas, Azospirillum*, and *Bacillus* on *Withania somnifera* showed significant increases in plant height and root length compared to individual strains.[22] This suggests that combining soil microbial diversity to form consortia of two or more plant growth-promoting rhizobacteria (PGPR) can improve plant development.

The findings of this study highlight the significant potential of *Bacillus* spp. consortia for increasing wheat growth by improving nutrient uptake, stress tolerance, and overall plant health. The observed increases in root and shoot biomass, length, and yield metrics demonstrate the efficacy of these beneficial bacteria in promoting sustainable agriculture methods.[23] This technique, which takes advantage of the plant growth-promoting capabilities of *Bacillus* spp., offers a possible alternative to artificial fertilisers, contributing to environmentally friendly and resilient wheat agriculture. Additional study is required to optimise application methods and assess the long-term impacts on soil health and crop output.

CONCLUSION

For promoting plant growth, a consortium of microorganisms may be more effective than a single strain. Numerous *Bacillus sp*. isolates have several characteristics that promote plant development. These isolates can be applied to agriculture with success. Utilizing these bacteria for sustainable agriculture can be aided by research on their variety.

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This study did not involve human participants, and therefore, informed consent was not required.

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This research does not involve any clinical trials.

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Author Contributions

Aniruddha Ratnakar Apastambh: Methodology, Data Collection, Writing – Original Draft, Review; Mirza Mushtaq Vaseem Baig: Analysis, Editing, Supervision, Conceptualization.

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