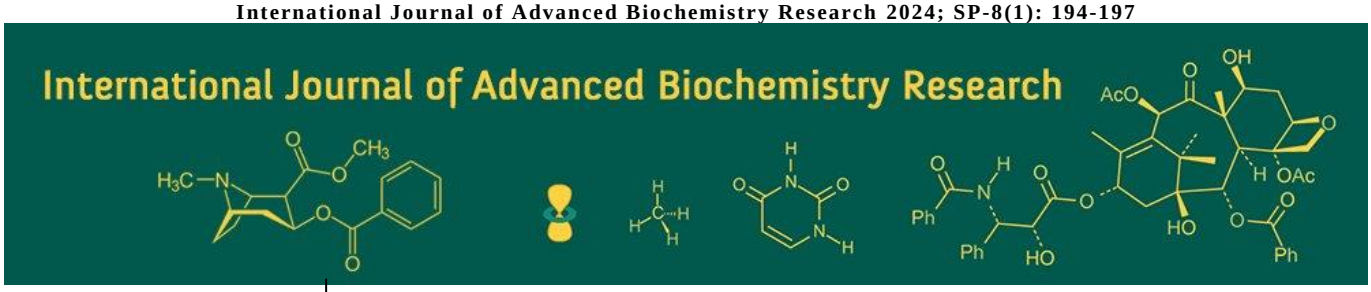

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Screening of *Bacillus* spp. for its plant growth promoting potential

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Abstract
Bacillus spp. are known to influence the crop plants by variety of direct and indirect mechanisms. A total of 20 bacteria were isolated from rhizosphere region of Raichur and Gulbarga districts and characterized morphologically and biochemically. Further, the isolates were screened for the plant growth promoting activities like Indole Acetic Acid production, P solubilization, Siderophore production. By the production of these traits the result shows that rhizosphere *Bacillus* spp are promising source for the plant growth promotion.

Keywords: *Bacillus*, Rhizosphere, IAA, Siderophore, P solubilizaton

Introduction
Intensive agriculture has not benefited from the use of inorganic fertilizer since it is frequently linked to decreased crop yield, acidic soil, and nutrient imbalance. Repeated application of inorganic fertilizers contributes to soil degradation caused by the loss of organic matter that comes with continuous cropping. It has been proven that inoculating various crop plants with phosphate-solubilizing microorganisms increases their P-uptake and yield (Asea *et al*., 1988) [2]. It is reported that the addition of organic matter increases the efficacy of phosphate solubilizers (Banik and Dey, 1985) [6].
Therefore, we need to create microbial consortia that will improve nutrient uptake and enhance Ragi's growth and yield in order to reduce our dependence on chemical fertilizers.
Plant Growth Promoting Rhizobacteria (PGPR) are a diverse group of soil bacteria that invade plant roots and, via various mechanisms, increase the activity of plant growth promotion. Direct and indirect mechanisms can be used to categorize these various mechanisms. PGPR either directly affect plant growth by promoting nutrient cycling processes like biological nitrogen fixation, siderophore production, phosphorus solubilization, and phytohormone synthesis, or indirectly by producing biocontrol compounds that inhibit phytopathogens.
Bacillus species promote plant growth and control plant pathogens through a combination of strategies including antibiosis, competition, mycoparasitism, and systemic resistance in the host plant. Due to their involvement in the synthesis of defense-related enzymes like peroxidase, polyphenol oxidase, and phenylalanine ammonia lyase, which promote systemic resistance, these mechanisms may function alone or in combination (Bais *et al*., 2004) [5]. Furthermore, it is known that they secrete extracellular lytic enzymes, such as β1, 3 glucanase, chitinase, amylase, protease, lipase, and Xylanase, which exhibit antagonistic properties due to the breakdown of bacterial and fungal cell walls (Aliye *et al*., 2008) [1].
Although *Bacillus* species are widely distributed in nature, they can be found more in soil, water, and plant-based food products. *Bacillus* strains make excellent candidates for usage as probiotics. *Bacillus* species are highly metabolically active, and past studies have found that they produce a wide range of useful enzymes as well as antibiotics. Because *Bacillus* can form endospores, they stay stable in probiotic products for a longer period of time than conventional probiotics. This is in addition to their secreted products.

Materials and Methods

The rhizosphere soil of various cereals was used to collect soil samples. We used the serial dilution method to isolate from soil. After being incubated for 24 to 48 hours, the *Bacillus* species that grew on nutrient agar medium were isolated. The isolated *Bacillus* cultures were kept on slants of the appropriate medium in a refrigerator at 4 °C after being purified using the streak plate method.

Screening of *Bacillus* isolates for Plant growth promoting characters

Indole acetic acid (IAA) production

The ability of the bacterial isolates to produce IAA was examined at 28 °C in nutritional broth that was supplemented with 0.1 concentration tryptophan. After three days of incubation, the culture broth's IAA concentration was measured using a spectrophotometric technique with Salkowaski's reagent by centrifuging it for five minutes at 5,000 rpm. The results are shown below:

Salkowaski's reagent (2 ml of 0.5 M FeCl3 + 98 ml of 35% HCl) was combined with 1 ml of the supernatant, and the intensity of the red colour that developed within 30 minutes was measured using a spectrophotometer at 530 nm. Using a standard curve made from an indole acetic acid standard solution, the concentration was ascertained (Mali and Bodhankar, 2009) [9].

Phosphate solubilization (Pikovskaya, 1948)

A single colony of *Bacillus* culture grown from NA plate was streaked onto Pikovskaya's plate containing tricalcium phosphate for the phosphate solubilization study, and it was then incubated at 28 °C for seven to ten days. The colonies' surrounding clear zones of phosphate solubilization were noted on the plates.

Solubilization Index = Zone diameter (cm) + colony diameter (cm)/colony diameter (cm)

Siderophore production

Chromo azurol sulfonate (CAS) agar medium was used to cultivate the PSB isolates in order to screen for siderophores. And these were incubated for twenty-four hours at 37 °C. The medium's colour shift from blue to reddish yellow was interpreted as a sign that the siderophore production was proceeding well. The CAS shuttle assay developed by Payne (1994) [10] was used as the basis for the siderophores assay.

Result and Discussion

Isolation and purification of *Bacillus* spp from rhizosphere soil: *Bacillus* spp. were isolated from collected cereal rhizosphere soils by serial dilution plating on nutrient agar medium. The plates were kept for incubation under 28 ± 2 °C for 7 days in inverted position. A total of forty *Bacillus* spp. isolated from forty different rhizosphere soils of cereals of Raichur and Gulbarga district.

Bacillus subtilis was isolated from the soil of the cotton rhizosphere by Gajbhiye *et al*. (2010) [8], Based on morphological and physiological characteristics, thirty-nine isolates have been identified to be *Bacillus* spp. (Avsar C *et al*., 2017) [4]. Isolated and characterized 28 bacterial cultures from Korea, of these, *Bacillus subtilis* was identified in the remaining isolates, while *Pseudomonas* spp. was found in four of them. Bacterial cultures were isolated by Sandeep C *et al*. (2011) [12] from ten Karnataka agroclimatic zones.

Biochemical characterisation and tentative identification of *Bacillus* isolates

The biochemical tests pertaining to chemical reaction were conducted on each of the forty isolates. The isolates were tentatively identified up to the generic level based on the results of the biochemical tests.

Plant growth promoting traits of *Bacillus* isolates

Plant growth promoting rhizobacteria (PGPR) colonize dicot and monocot plant roots and promote plant growth through both direct and indirect means. They enhance the plant growth by fixation of atmospheric nitrogen to the plants, solubilizing the unavailable form of P to available form, production of siderophore that helps to chelate iron and make it available to the plants.

Indole Acetic Acid production by *Bacillus* isolates

The potential of the *Bacillus* isolates for the production of IAA was estimated by growing the isolates in the tryptophan supplemented culture media. On reaction with Salkowaski's reagent there is a development of pink colour which shows positive result for the IAA production by *Bacillus* isolates and the result was quantified using spectrophotometer. The variability in the production of IAA by 10 isolates was varied from 5.24 µg/ml to 8.12µg/ml as shown (Table 1). BCRS 3 shows the highest IAA production with 8.12 µg/ml followed by BCRC 2 with IAA production of 7.23 µg/ml. and lowest IAA production was recorded by BCGP 1 with 5.24 µg/ml and represented (Table 1) (Fig 1). Wahyudi A T (2011) [14]. They revealed 118 isolates were able to produce IAA in various concentrations. All these tested *Bacillus* spp. positives for the IAA production are supplemented with tryptophan in their culture media.

The activity of *Bacillus* species in the soil of the rhizosphere is responsible for increase in IAA production. Ultimately, IAA production improved plant metabolism.

IAA is considered as the most important phytohormone, functioning as a signal molecule in the processes of development and regulation of plant growth. Seven isolates in the study were able to produce IAA when tryptophan was present, and the amount of IAA produced ranged from 56 to 97 µg/ml (Ashish T *et al*., 2016) [3].

Table 1: Indole Acetic Acid production by efficient *Bacillus* spp. isolates of cereals

S. No.	*Bacillus* isolates	IAA Production (µg/ml)
1.	BCR 1	6.28
2.	BCR 5	5.93
3.	BCRM 2	7.01
4.	BCRM 3	5.93
5.	BCRM 6	5.84
6.	BCRS 3	8.85
7.	BCRC 2	7.23
8.	BCRC 4	5.35
9.	BCRC 5	6.05
10.	BCGP 1	5.24
	Reference strain	8.12
	Control	0.00
	S.Em±	0.11
	CD at 1%	0.43

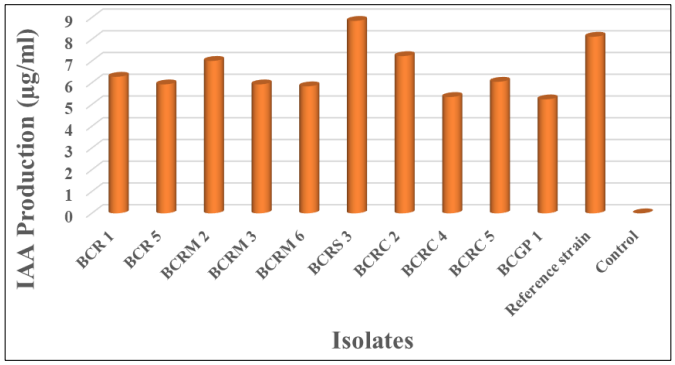

Fig 1: Indole Acetic Acid production by efficient *Bacillus* isolates

Phosphorous solubilization by *Bacillus* isolates

Screening of *Bacillus* isolates for the P solubilization. The variability in the solubilization of P by 10 isolates was varied from 15.36 mm to 11.11 mm as shown in the Table 2. BCRS 3 shows highest solubilizing zone of 15.36 mm which is followed by the isolate BCRC 2 with 14.82 mm of solubilization zone. Lowest P solubilization zone was formed by BCGP 1 with 11.11 mm (Table 2) (Fig 2). Calvo P *et al.* (2010) [7], isolated 43 *Bacillus* isolates, of the forty-three isolates 25 were able to solubilize tricalcium phosphate with halo size varying from 1 to 9 mm.

The phosphate solubilization efficiency is directly correlated with the clear halo zone that the *Bacillus* isolates form. In this study, the colonies on pikovskaya agar medium containing tricalcium phosphate as a phosphate source develop a distinct halo zone surrounding the isolate (Ashish T *et al*., 2016) [3].

Table 2: Phosphorous Solubilization zone produced by different *Bacillus* spp. isolates from cereal rhizosphere

S. No.	*Bacillus* isolates	Diameter (mm)
1.	BCR 1	13.0
2.	BCR 5	11.46
3.	BCRM 2	14.60
4.	BCRM 3	12.54
5.	BCRM 6	11.74
6.	BCRS 3	15.36
7.	BCRC 2	14.82
8.	BCRC 4	11.83
9.	BCRC 5	13.44
10.	BCGP 1	11.11
11.	Reference strain	14.80
12.	Control	0.00
	S.Em±	0.12
	CD at 1%	0.49

Siderophore production by *Bacillus* isolates

All the 10 isolates showed positive results for the production of siderophore. The variability in the production of siderophore by 10 isolates was varied from 80.01% to 52.92% as shown in the Table 3. BCRS 3 shows highest siderophore production of 80.01% followed by BCRC 2 with 75.60%. Lowest siderophore production was seen in the isolate BCGP 1 with 52.92% (Table 3) (Fig 3). Sharma SK, *et al*. (2013) [13]. Who characterised *Bacillus amyloliquefaciens* strain Sks Bnj 1 was positive for siderophore on CAS agar medium by yellow to orange halo formation on blue background.

The presence of iron Chelators may contribute to the production of high siderophores. Due to the pathogen's starvation from available iron in the environment, this iron chelation is crucial for the isolates.

Table 3: Siderophore production by efficient *Bacillus* isolates from cereal rhizosphere

S. No.	*Bacillus* isolates	Siderophore production (%)
1.	BCR 1	65.22
2.	BCR 5	53.48
3.	BCRM 2	71.91
4.	BCRM 3	63.49
5.	BCRM6	57.32
6.	BCRS 3	80.01
7.	BCRC 2	75.60
8.	BCRC 4	56.99
9.	BCRC 5	68.03
10.	BCGP 1	52.92
	Reference strain	72.3
	Control	0.231
	S.Em±	1.04
	CD at 1%	2.96

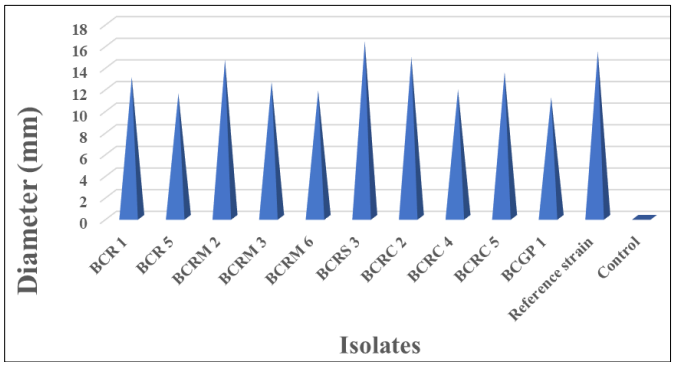

Fig 2: Phosphorous Solubilization zone produced by efficient *Bacillus* isolates

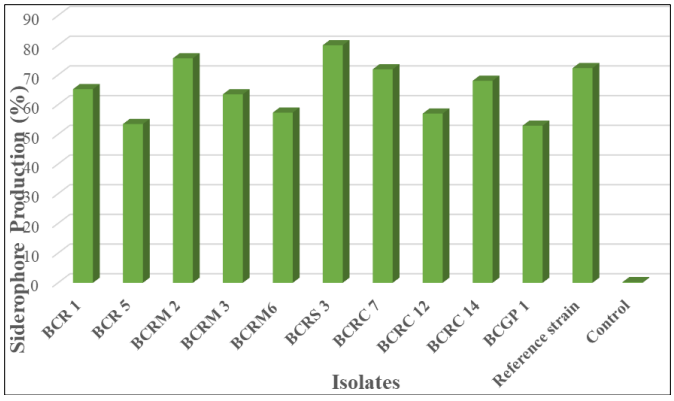

Fig 3: Siderophore Production by efficient *Bacillus* isolate

Conclusion

Because of their many advantageous properties, *Bacillus* species contribute both directly and indirectly to increased soil fertility and plant growth. Direct methods include the supply of nutrients through processes like biological nitrogen fixation and phosphate solubilization and the synthesis of chemicals like IAA that promote plant growth. indirect methods by using biocontrol activity to produce HCN and siderophores. These are affordable and environmentally friendly. Therefore, the use of effective *Bacillus* isolates with a variety of advantageous activities aids in raising agricultural profitability and enhancing the standard of living for small and marginal farmers.

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