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Section name with number: Biological science Plant growth promoting capability of Rhizobacteria from *Sorghum bicolor*

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Abstract

Plant growth- promoting rhizobacteria (PGPR) are useful free living bacteria isolated from the rhizosphere, which when applied to seeds or crops, enhanced the plant growth by different mechanisms. The use of PGPR is increases regularly by farmers. Rhizosphere of many plants has microbial diversity depending on the variety of plants and effect of root exudates which promotes growth and also the crop yield or quality. During the present study such 30 plant growth promoting rhizobacteria were isolated from rhizospheric soil of Sorghum bicolor cultivated at Jalgaon North Maharashtra Region of India. The rhizospheric soil from fields having alkaline pH were collected at first stages of growth and were tested for various PGPR traits. Most efficient bacterial isolates were screened on the basis of their positive activity for, IAA production, ACC-deaminase activity, P-solubilization, Zn-solubilization and N2fixation are directly promote plant growth. Compared to bulk soil, rhizosheric bacteria are more potential to promote plant growth. Out of five different traits screened bacteria were having more than 50 % traits supporting plant growth out of 30 isolates. Such PGPR species can be further explored as bioinoculants.

Keywords: Rhizobacteria, IAA, ACC-deaminase, PGPR.

Introduction

India is agriculture dependant country. All over India variety of crops were grown depending on the area, native soil texture and weather. Sorghum (*Sorghum bicolor (*L) Moench) is one of the usual grown crop, it is vital life sustaining crop in many parts of the world, ranking fifth after wheat (*Tritium* species), rice (*Oryza* spp) maize (*Zea mays*) and barley (*Hordeum vulgare*)[1]. There is an ranking in grain producing country among them India tanks second after USA. In India it is the third important crop after rice, wheat. Sorghum is primarily used for the food in the different area of India Importance states producing sorghum crop are Maharashtra, Karnataka, Andhra pradesh and Madhya Pradesh. Sorghum grain is used as staple food in several forms baked, cooked or fermented. Nutritionally *Sorghum* is superior to all other cereals and therefore it requires to improve the crop yield. Uncertainty of monsoon, scarcity of water for the agriculture land therefore crops which required less water is grown by farmer. *Sorghum* requires less water than others, so is likely to be grown and produce better yields in hotter and drier areas. Plant rhizospheric region is a dynamic and versatile environment of acute plant microbe interactions for supplying essential macro and micro nutrients by developing nutrient pool. Multiple types of biological interactions between microorganisms and plants take place in the soil. Numerous microorganisms such as algae, bacteria, protozoa and fungi coexist in the rhizospheric region, but bacteria are the super abundant among them. Plants only prefer those bacteria contributing virtually to their fittingness by releasing sugars, amino acids, organic acids, vitamins, enzymes and organic or inorganic ions through roots exudates producing a very scoop environment where diversity is low.

Plant Growth Promoting Rhizobacteria: PGPR are free living bacteria, present in the rhizosphere, which when applied to plants, enhanced the growth of the plant or reduce the damage from soil born plant disease. Biofertilizers or PGPRs are use in the replacement of synthetic and chemical fertilizer, it shows better effect, economically also sound, and ecofriendly. Plant growth promoting rhizobacteria are beneficial to plant growth promotion activity by different mechanisms in various ways.

Phosphate solubilization: Phosphorus is important element for the growth of plant,this requirement is fulfil by taking phosphate ions from soil[2]. But phosphate ions are very active state so reacted with different ions such as Mg^{2+}, Ca^{2+}, Al^{3+} and Fe^{3+} and form precipitates. Therefore phosphorus is present in soil is extremely insoluble and unavailable to plants in these forms. As a result, the amount available to plants is usually a small proportion. It has been reported that many bacteria in rhizospher is having a ability to solubilise the phosphate by producing acid and other metabolites. Such type of bacteria known as, phosphate solubilizing bacteria (PSB)[3].

Nitrogen fixation for plant use: Nitrogen is also an essential elements for all types of life. Regrettably no plant species is capable for fixing atmospheric N_2. Thus for this procedure requireds microorganisms, they fix atmospheric N_2 and

convert it in to ammonia.Plants depend on biological nitrogen fixation (BNF), out of which symbiotic nitogen fixation contributes 80% and the remaining comes from free living Nitrogen fixation[4].

Production of Plant Hormones: Phytohormones are important regulatory substance of plant. They required for plant growth and development is also regulated by phytohormone producing PGPR's. Phytohormones such as auxins and cytokine production by PGPR's have been reported by many researchers[5]. Auxins is an important phytohormone, which promotes root cell division, root initiation, and cell enlargement. Indole-3-acetic acid (IAA) produced by PGPR's are reported to increase root growth and length, modifying the plant morphological functions to uptake more nutrients from the soil.

Lowering Plant Ethylene Levels: Agricultur crop is affected by different factors, so yield is in stress conditions. The PGPR's producing enzyme ACC deaminase activity are present in various soils and assures improvement of plant growth and development under stress conditions such as heavy metal stress, phytopathogens, flooding, drought and high salt concentration. Ethylene is a significant phytohormone, but excess secretion of ethylene will lead to root curling and shortening, even it can result in plant death under extreme conditions. ACC deaminase activity of PGPR helps plant to fight under stress condition by hydrolyzing ACC, the precursor of ethylene, to alpha-ketobutyrate and ammonia, and encourages plant growth under stress environment[6].

The present study shows that, screening of such rhizospheric oorganisms which give positive reaction of different PGPR activity which having capabilities to enhance the plant growth.

Material and Methods

Sampling: Sampling form the sorghum cultivated fields situated in jalgoun districts of North Maharashtra region will be carried out in present study. Four different types of composite soil samples viz, bulk soil, Rhizosphere soil and Ectorhizosphere and Endo Rhizosphere of roots from sorghum field with alkaline pH were collected by appropriate standard technique[7]. PGPR were isolated and screened for the plant growth promoting activity was carried out using different selective media e.g. Yeast Extract Mannitol Agar, Ashbey's medium, Kings B Medium, Nutrient Agar Medium, etc. Following capabilities of the bacteria were tested supporting directly to increase growth and yield.

IAA production: The production of indole acetic acid (IAA) by effect of -tryptophan on IAA production was assayed by using Salkowski method [8]. The isolates were grown in nutrient broth medium, supplemented with tryptophan (2 mg ml-1), at 28˚C for 48 hr and centrifuge 10,000 rpm for 15 min. Take two ml of supernatant was mixed with 4 ml of Salkowski reagent which consisted of 4.5 g of $FeCl_3$ per litre in 10.8 M H_2SO_4 and incubate at room temperature for 30 min. development of a pink color indicates IAA production[6].

ACC Deaminase activity: The rhizobacteria having ACC deaminase producing ability, detected by adding sole source of nitrogen as ACC (1-aminocyclopropane-1-carboxylate) into minimal medium .Minimal medium ***(g/l)*** DL-malic acid, 5; KOH, 4; K_2HPO_4, 0.5; $MgSO_4 \cdot 7H_2O$, 0.2; $CaCl_2$, 0.02; NaCl, 0.1;$FeSO_4 \cdot 7H_2O$, 0.5; (mg/l): $NaMoO_4 \cdot 2H_2O$, 2; $MnSO_4 \cdot H_2O$, 10; 0.5% alcoholic solution (or dissolved in 0.2 *N* KOH) of Bromothymol blue, 2 ml; vitamin solution, micronutrient solution and agar, 0.0175 to 0.5%; 1000 ml distilled water, pH 6.8.Growth shows that the organism having ability to produce ACC-Deaminase.

Phosphate solubilization: Phosphate solublizing ability of the isolate was checked on Pikovskaya (PVK) medium incorporated with tricalcium phosphate (TCP) [$Ca_3(PO4)_2$] by Fiske and Subbarow method[9]. Formation of transparent halo zone around the developing colonies indicated phosphate solublizing ability of the bacteria.

Nitrogen Fixation: The Rhizobacteria having nitrogen fixation ability they fix atmospheric nitrogen . Nfb medium use for detection of nitrogen fixing microbes. NFb Medium ***(g/l)*** DL-malic acid, 5; KOH, 4; K_2HPO_4, 0.5; $MgSO_4 \cdot 7H_2O$, 0.2; $CaCl_2$, 0.02; NaCl, 0.1;$FeSO_4 \cdot 7H_2O$, 0.5; (mg/l): $NaMoO_4 \cdot 2H_2O$, 2; $MnSO_4 \cdot H_2O$, 10; 0.5% alcoholic solution (or dissolved in 0.2 *N* KOH) of bromothymol blue, 2 ml; vitamin solution, micronutrient solution and agar, 0.0175 to 0.5%; 1000 ml distilled water, pH 6.8. Growth shows that the organism having ability to fix atmospheric nitrogen.

Zn solubilization: The isolates were inoculated into modified PKV medium, glucose-10.0 g; ammonium sulphate-1.0 g; potassium chloride-0.2 g; dipotassium hydrogen phosphate-0.2 g; magnesium sulphate-0.1 g; yeast-0.2 g; distilled water - 1000 ml, pH 7.0) containing 0.1% insoluble zinc compounds (ZnO, $ZnCO_3$ and ZnS). The test organisms were inoculated on these media and incubated at 28ºC for 48 hours. Formation of transparent halo zone around the developing colonies indicated zinc solublizing ability of the bacteria.

Results and Discussion

Thirty bacterial isolates were isolated from four different soil samples viz bulk soil, rhizospheric soil, ectorhizospher, endorhizosphere from *Sorghum bicolor* grown in alkaline soil from North Maharashtra region. All the isolates were designated as shown in Table 1, in that ectorhizospher having highest number of organism isolated than other sample. The ectorhizospher is more important region for plant, having useful PGPR. All isolates has shown significant PGPR activity. A total thirty bacterial isolates were screened for

phosphate solubilization, N_2 fixation, Zn solubilization,and ACC deaminase production.

Phosphate solubilization: On modified PVK agar, of which twelve isolates showed the development of phosphate solublization around growth (figure-4). Rhizospheric and endorhizospheric region shows highest number of organism positive for phosphate solubilization. (table 6 and 4). Phosphate is important element required for plant growth, solubilization is direct mechanism which supports plant growth (table-2).

Nitrogen fixing organisms are also isolated by using Nfb minimal media, total 24 bacteria were shows growth on minimal media nitrogen fixation property is very important because nitrogen is not taken by plants own mechanism, nitrogen is a one of the basic element of life. 80% organisms having potential to fix atmospheric nitrogen. (figure-5) Ectorhizospheric organisms shows highest number of organisms having ability to fix atmospheric nitrogen as compare with other samples (table-2 and 3).

ACC were incorporated in nitrogen free minimal medium, Growth on medium shows ACC deaminase producing organisms; (figure-5) are also isolated among these 30 isolates 23 organisms are having ability to synthesize ACC deaminase enzyme (table-2). Out of 11 ectorhizospheric isolates 7 isolate positive for ACC deaminase production (table-3) Which is helpful for the plant under stress conditions. Sorghum plant is generally planted in regions were scarcity of water therefore such isolates are very useful for the plant growth promotion.

IAA (Indole-Acetic Acid) production is very important characteristic as concern to plant growth organism grown on nutrient agar containing tryptophan and after reaction with Salkowski reagent many Sorghum rhizospheric bacteria is also give positive reaction by producing pink color. Out of 30 isolates 27 shows IAA production. Ectorhispheric all 11 isolates shows IAA production (table-3) IAA is useful auxin during first stage of plant growth because elongation and growth promotion is important during first stage. It required stimulation by IAA during development (table -2).

Zn is also a required element for growth and development of plant (table-2). PKV modified medium containing insoluble Zn shows growth and zone of solubilization observed around growth (figure-3) Rhizospheric and endorhizospheric both region shows very good result of Zn solubilization (table-6 and 4).Out of 30 isolates 27 isolate shows Zn solubilization. After discussion of all individual traits and samples. It combine shows that among these 30 organisms 6 organism shows all trait positive.

Table-1
Number of soil samples and their codes

Sample No.	Sample Location	No. of isolates	Isolate code
1	Bulk Soil	5	BS/6.12/A/1,BS/6.12/Y/1, BS/6.12/Y/2, BS10/6.12/A/1,BS/6.12/A/2
2	Rhizospheric soil	7	RS/6.12/C/1,RS/6.12/C/2,RS/6.12/C/3 RS/6.12/C/4, RS.01/6.12/C/1,RS.01/6.12/C/2 RS.01/6.12/C/3, RS.01/6.12/C/3
3	Ectorhizospheric region	11	Ecto/6.12/A/1,Ecto/6.12/A/2, Ecto/6.12/A/1 Ecto/6.12/A/2 Ecto/6.12/A/3 Ecto/6.12/C/1 Ecto/6.12/C/2 Ecto/UP/6.12/C/2 Ecto/UP/6.12/A/3 Ecto/6.12/C/3
4	Endorhizospheric region	7	ER/UP/6.12/A/1 ER/UP/6.12/A/2 ER//6.12/A/1 ER/UP/6.12/C/2 ER/UP/6.12/A/3 ER/UP/6.12/N/1 ER/UP/6.12/N/2

Table-2
Samples showing direct PGPR traits

Sr. No.	Sample Type	ACC	IAA	"P"Solubilization	N2 Fixation	ZN solubilization	Total
1	Bulk Soil (5)	5	3	1	5	4	7
2	Rhizospheric soil (8)	6	8	5	6	8	7
3	Ectorhizospheric soil (11)	7	11	3	9	9	9
4	Endorhizospheric soil (7)	5	6	4	4	7	7

Table-3
Direct charecteristics of isolates from EctoRhizosphere soil

Sr. No.	Isolate Code	ACC	"P"Solubilization	N2 fixation	ZN solubilization	IAA Production	Total
1	Ecto/6.12/A/1	-	-	+	+	+	3
2	Ecto/6.12/A/2	+	-	+	+	+	4
3	Ecto/6.12/A/1	-	+	+	+	+	3
4	Ecto/6.12/A/2	+	+	+	+	+	5
5	Ecto/6.12/A/3	+	-	+	-	+	3
25	Ecto/6.12/C/1	+	-	+	+	+	4
26	Ecto/6.12/C/2	+	-	+	+	+	4
11	ECT/UP/6.12/C/2	_	-	+	+	+	3
12	ECT/UP/6.12/A/3	+	+	+	-	+	4
20	Ecto/6.12/C/3	+	-	_	+	+	3
	Total	7	3	9	9	11	38/55

Table-4
Direct charecteristics of isolates from Endo Rhizosphere region

Sr. No.	Isolate Code	ACC	N_2	"P" Solubilization	ZN solubilization	IAA Production	Total
6	ER/UP/6.12/A/1	+	+	-	+	+	3
7	ER/UP/6.12/A/2	+	+	+	+	+	5
8	ER/str/6.12/A/1	+	+	+	+	-	4
11	ER/UP/6.12/C/2	-	-	-	+	+	1
12	ER/UP/6.12/A/3	+	+	-	+	+	3
29	ER/UP/6.12/N/1	+	-	+	+	+	3
30	ER/UP/6.12/N/2	+	-	+	+	+	3
		6	4	4	7	6	27/35

Table-5
Direct characteristics of isolates from Bulk soil

Sr. No.	Isolate Code	ACC	"P"Solubilization	N_2 fixation	ZN solubilization	IAA production	Total
1	BS/6.12/Y/1	+	-	+	+	+	4
2	BS/6.12/Y/2	+	+	+	-	+	4
3	BS10/6.12/A/1	+	-	+	+	+	4
4	BS/6.12/A/1	+	-	+	+	-	3
5	BS/6.12/A/2	+	-	+	+	-	3
	Total	5	1	5	4	3	18/30

Table-6
Direct characteristics of isolates from Rhizospheric soil

Sr. No.	Isolate Code	ACC	"P"Solubilization	N_2 fixation	ZN solubilization	IAA Production	Total
1	RS/6.12/C/1	+	-	-	+	+	3
2	RS/6.12/C/2	+	+	+	+	+	5
3	RS/6.12/C/3	+	-	+	+	+	4
4	RS/6.12/C/4	+	++	+	+	+	5
5	RS.01/6.12/C/1	+	--	+	+	+	5
6	RS.01/6.12/C/2	+	+	+	+	+	5
7	RS.01/6.12/C/3	-	+	+	+	+	4
8	RS.01/6.12/C/3	_	+	_	+	+	3
		6	5	6	8	8	33/40

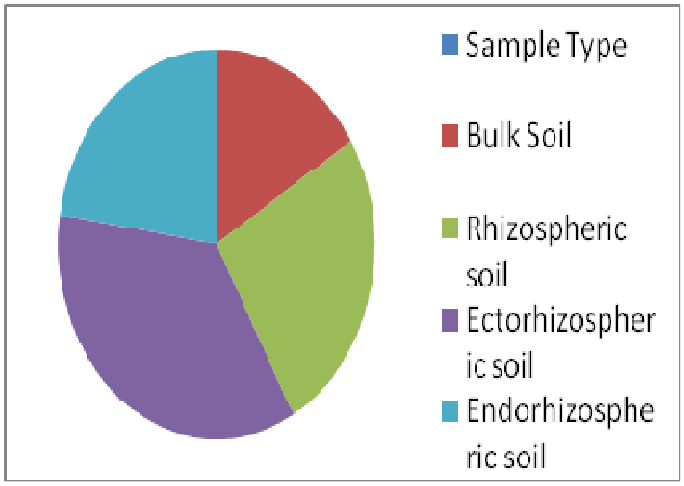


Figure-1
Distribution of soil samples containing isolates.

Figure-4
Phosphate solubilization

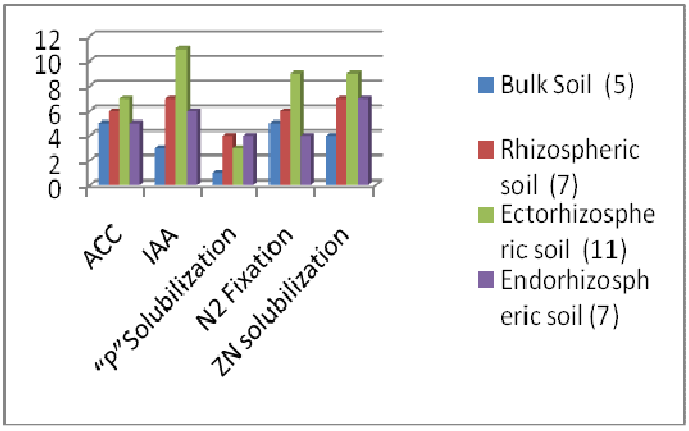
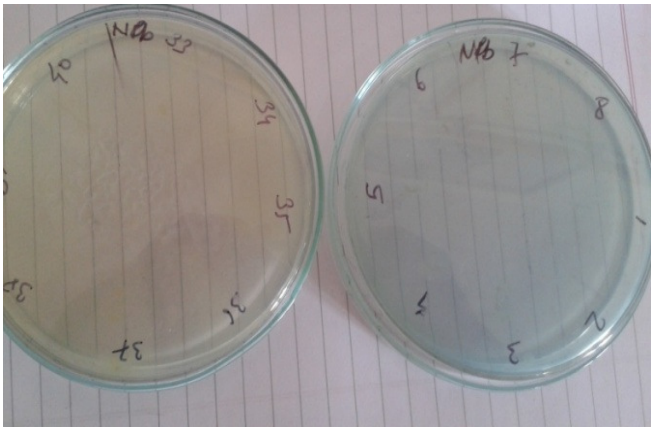


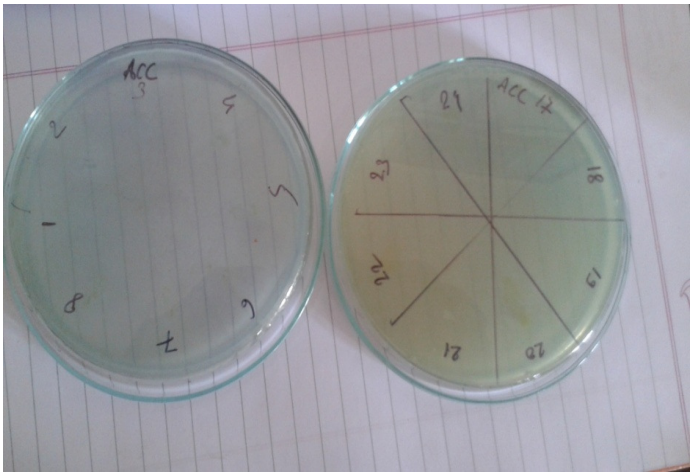
Figure-2
Graphical representation of Direct PGPR traits



Negative **Positive**

Figure-5
Nitrogen fixation

Figure-3
zinc solubilization



Positive **Negative**

Figure-6
Showing Negative and Positive Resut on ACC Minimal Medium Blue color shows positive result

Conclusion

From the results, it is clear that rhizobacteria showed variation in plant growth promoting trait. Many isolates shows multiple traits positive. These organisms may utilize for the formation of consortium, which gives best result for plant growth After selection of such isolates are exploited as biofertilizer. However, further work at field condition for comparison of different isolates on field conditions.

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