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CHARACTERIZING RHIZOSPHERIC PLANT GROWTH PROMOTING BACTERIA FOR THEIR EFFECTS ON OAT (*AVENA SATIVA*)

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ABSTRACT

Plant growth promoting rhizobacteria (PGPR) are group of useful microorganism that colonize plant rhizosphere and enhance growth by their various direct and indirect effects on plants. In our present study, 30 bacterial strains were isolated from rhizospheric soil of wheat, rice and black gram crop. Plant growth promoting traits selected for screening bacterial isolates were IAA production, p-solubilization, ammonia production, HCN production and extra cellular enzyme production like catalase and amylase. In addition, growth of PGPR isolates was optimized under different temperature and pH. Out of the 30 isolates, 4 isolates *viz* SG-13, SG-14, SG-16 and SG-18 were found to be promising for the plant growth promoting attributes studied. The isolates were then tested on *Avena sativa* (Haritika RO-19) under pot conditions for their effects on seed germination and vigour index and were found to promote growth and seed germination. They also increased water stress tolerance in oat plants by enhancing root proliferation and improving relative water content (RWC) in leaf and significantly increasing root shoot ratio as compared to uninoculated control. These bacterial isolates can be potential biofertilizers in crop management under water stress field conditions.

KEYWORDS: ***Avena sativa, plant growth promoting rhizobacteria, seed germination, vigour index***

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INTRODUCTION

Plant growth promoting rhizobacteria (PGPR) are group of bacteria that actively colonize roots and increase plant growth and yield. The rhizosphere has important functions in plant nutrition, health and yield. Different types of substances which include carbohydrates (sugars and oligosaccharides), organic acids, vitamins, nucleotides, flavonoids, enzymes, hormones and volatile compounds[1] diffuse from plant roots into the rhizosphere, which stimulate microbial growth and activity. PGPR through its interactions with rhizospheric microorganism contributes significantly to improvement of soil fertility and ecological sustainability. Direct effect of PGPR on plant growth, include increased uptake of nitrogen, synthesis of phytohormones, solubilization of minerals such as phosphorus and production of siderophores that chelate iron and make it available to the plant root.[2] Indirect benefits of PGPR include production of antibacterial compounds that are effective against certain plant pathogens and pests[3] and eliciting induced systemic resistance against a number of plant diseases.[4] Under stress conditions too, PGPR have shown positive effects on many parameters like germination rate, tolerance to drought, plant weight and yield improvement.[5-6] Different stress ameliorating mechanisms of rhizobacteria include production of chaperons and sugars,[7] synthesis of drought stress enzyme 1-aminocyclopropane 1-carboxylic deaminase (ACC deaminase),[8] exopolysaccharides production (EPS)[9], production of low molecular weight organic compound like trehalose[10] and enhanced root respiration[11], which influence plant physiology. High soil temperature in North-Western parts of India is a major constraint for biological activities in soil. In these areas and other tropical regions, the temperature in the upper 5–6 cm layer of the soil reaches up to 40–50°C during the summer season exposing inoculants to elevated temperatures for varying lengths of time. Such high temperatures in the surface soils have adverse effects on microbial processes and on symbiosis. Exposure to prolonged high temperature results in partial or complete elimination of rhizobia.[12] The optimum temperature range for most rhizobia is 25-31°C[13] and the upper temperature limits are between 32-47 °C.[14] Therefore screening rhizobacteria for growth promoting traits should also take into account their adaptability to altered temperature and pH in the changing global scenario, as much study on PGPR inoculation has been done with respect to improving growth and productivity of cereals (rice, maize and wheat) under irrigated conditions. Oat (*Avena sativa*) is one of the important crops belonging to family Poaceae widely cultivated in India, China and Europe. Oat cultivation is attracting increasing interest not only because of an increase in market demand but also because it is well adapted to a wide range of soil types and performs better than other small-grain cereals grown on marginal soils.[15] Oats are sensitive to hot and dry weather, and hence, drought becomes the main limiting factor for its cultivation in Mediterranean and similar climatic regions.[15] The average world oat grain production in 1999–2003 amounted to about 25.9 million tone/year from 12.7 million ha. The main producing countries are the Russian Federation (5.8 million tone/year in 1999–2003, from 3.8 million ha), Canada (3.3 million tone/year from 1.4 million ha) and the United States (2.0 million tone/year from 0.9 million ha).[16] Current trends in agriculture are focused on the decline in the use of pesticides and inorganic fertilizers, emphasizing for alternative ways to sustainable agriculture. PGPR therefore, can be a promising sustainable approach to meet the current yield demand, maintaining soil health. Hence, PGPR isolates possessing plant growth promoting abilities and adapted to altered climates can prove to be effective biofertilizers. Seed priming that involves exposing quiescent seeds to a solution or matricum of low water potential improve seed germination and seedling emergence particularly under adverse condition like reduced water availability[17] and salinity.[18] Seed priming with potential strains of PGPR increases seed germination and promote healthy seedling.[19]Therefore, the present study was undertaken to screen efficient PGPR's from rhizospheric soil capable of surviving under altered temperature and pH and to evaluate their effect on germination and vigour index of oat crop by seed priming technique.

MATERIALS AND METHODS

Isolation and preliminary screening of bacterial isolates

Soil sample were collected from rhizospheric region of different crops *viz* wheat, rice and black gram growing at three different district of state Uttar Pradesh *viz* Jounpur, Chunar and Varanasi and from district Reeva of state Madhya Pradesh of India and stored at 4°C for further study. The soil at the collection site was loam with good permeability. The bacteria were isolated from rhizospheric soil sample by serial dilution plate technique on Nutrient Agar (NA) media containing per liter of distilled water: Peptic digest of animal tissue 5 g, Sodium chloride 5 g, Beef extract 1.5 g, Yeast extract 1.5 g, agar 15 g maintained at pH 7.4±0.2. Plate were incubated at 28°C for 24 to 48 h. Colonies were picked from these plates and maintained as pure cultures on NA media with periodic transfer to fresh media and stock for further use. All isolates were morphologically characterized based on colony morphology and Gram staining.

Screening for plant growth promoting activities

A total of 30 isolates obtained from rhizospheric soil of different crops were screened for different plant growth promoting traits.

Phosphate solubilization

Inorganic phosphate solubilizing activity was measured by plates assay as described by Pikovaskaya[20] on Pikovaskaya's Agar Medium (PVK) containing per liter of distilled water, yeast extract 0.5 g, dextrose 10 g, $Ca_3(PO_4)_2$ 5 g, $(NH_4)_2SO_4$ 0.5 g, KCl 0.2 g, $MgSO_4$ 0.1 g, $MnSO_4$ 0.0001 g, $FeSO_4$ 0.0001 g, and agar 15 g. Tricalcium phosphate $Ca_3(PO_4)_2$ was added to the media to measure its phosphate solubilization by the isolates. The halo transparent zone formed around the colony was presumptive conformation of phosphate solubilization and was measured after 3 days of incubation at 28°C.

IAA Production

Auxin production by selected bacterial isolates was determined in terms of IAA equivalents with L-tryptophan. Indole acetic acid (IAA) production was detected as described by Bric et al. (1991).[21] NA broth amended with 5 mM tryptophan inoculated with bacterial culture was incubated at 30°C for 48 h. One milliliter sample of each culture was centrifuged (10,000 x g for 10 min) and the supernatant was collected for further analysis, which involved the addition of 2 ml Salkowski reagent, followed by incubation for 1 h at room temperature under dark conditions. Development of pink colour indicated IAA production.

Ammonia production

Ammonia production as described by Cappuccino and Sherman et al. (1992)[22] was observed in peptone water broth containing 1000 ml of distilled water with peptone 10 g, NaCl 5 g and maintained at pH 7.2±0.2. 10 ml of peptone water broth was taken in each test tube, and inoculated with 1 ml of bacterial suspension (3.9×10^7cfu ml^{-1}) and was incubated for 72 h at 28°C. After incubation, added 0.5 ml of Nessler's reagent in each tube. Development of dark brown color indicated production of ammonia.

HCN production

HCN production by bacterial isolates was determined by colour change of filter paper following Alstrom and Burns et al. (1989).[23] One hundred microliter of bacterial suspension was inoculated on nutrient agar medium containing 4.4 gL^{-1} glycine. Filter paper soaked in a reagent solution (2.5 g picric acid and 0.125 g sodium carbonate, 1000ml distilled water) was placed in the upper lid of petridishes. To prevent volatilization, the plates were sealed with parafilm and incubated at 28°C for four days. The plates without inoculation of bacterium served as control. A change in colour of the filter paper from yellow to light brown, brown or reddish brown was recorded as weak (+), moderate (++) or strong (+++) reactions, respectively.

Extracellular enzyme activities

Catalase activity

Fresh culture (24 h old) of bacteria was placed on a glass slide and one drop of H_2O_2 (30%) was dropped on the colony; appearance of gas bubbles indicated the catalase activity [24] and was recorded as weak (+), moderate (++), and strong (+++) respectively.

Amylase activity

The bacterial isolates were spot inoculated on starch agar medium containing per liter of distilled water, beef extract 3 g, peptone 5 g, soluble starch 20 g and agar 15 g. Plates were incubated at 28°C for 72 h. At the end of incubation period, the plates were flooded with iodine solution, kept for a minute and then poured off. After few minutes blue colour faded rapidly and a hollow zone around the bacterial colonies appeared. The colour less zone surrounding the colonies indicated the production of amylase.[25]

Temperature tolerance

Bacterial growth at different temperature was determined in YEM broth inoculated with 50 µL of isolated culture (10^9 CFU ml^{-1}) and incubated at temperatures 20, 28, 35, 42 and 45°C, respectively. Optical density was measured after 48 h at 420 nm to check bacterial growth.

pH tolerance

Growth of bacteria at different pH was determined in YEM broth maintained at pH 5, 7, 9 and 11 inoculated with 50 µL of isolated culture (10^9 CFU ml^{-1}). Optical Density was measured after 48 h at 420 nm to check bacterial growth.

Seed treatment with PGPR

Out of the 30 bacterial isolates, the isolates with promising plant growth promoting traits were tested on oat in the soil system. Seed of oats (*Avena sativa* L.) variety Haritika RO-19 were surface sterilized with 0.2% $HgCl_2$ for 2 min, then rinsed in sterile distilled water for 10 min. Seed were soaked for 7-8 h in 25 ml of YEMA having prescreened bacterial suspension in their log phase containing 10^9 CFU ml^{-1} and kept at 30°C in rotator shaker (90 rpm). Control seeds were soaked in sterile medium. The seeds were then dried overnight aseptically in laminar air flow and used for green house experiments. The seed were sown in earthen pots containing sterile soil and placed in a temperature control growth chamber. Six plants per pot were maintained at 60 % water holding capacity. The effect of PGPR treatment on seed germination and vigour analysis were carried out by paper towel method.[26] After 7 days of germination, the percent germination was calculated by the following eq 1

$$\text{Germination (\%)} = \frac{\text{No. of germinated seed}}{\text{total no. of seed}} \times 100 \qquad (1)$$

To assess plant vigour, root and shoot length of each individual seedling was measures after 5 days. The vigour index[28] was calculated using the following eq.2

$$\text{Vigour index} = (\text{mean root length} + \text{mean shoot length})\text{X Germination \%} \qquad (2)$$

The treatments were arranged in a completely randomized design with three replications. At the end of the experimental period, the plants were uprooted and the shoot and root length, fresh weight and RWC were measured. Biomass was dried to constant weight in oven at 80°C for recording the dry weight.

RESULTS

Morphological characterization and gram's staining of 30 bacterial isolates showed 10 of them to be gram positive, while 20 bacterial isolates were gram negative in nature (Table 1).

Table 1
Characterization of bacterial isolates for specific plant growth promoting traits

Site of soil sample collection	Bacterial isolate	Gram staining (G^+/G^-)	p-solubilization zone of clearance (cm)	IAA production	Ammonia production	HCN Production	Catalase Production	Amylase Production
Jounpur	SG1	+	-	-	-	+	+	-
Jounpur	SG2	+	3.1	-	-	+	+++	-
Jounpur	SG3	+	4.0	+	-	-	++	+
Jounpur	SG4	-	-	++	-	++	+	-
Jounpur	SG5	-	-	-	-	-	+	-
Jounpur	SG6	-	1.4	++	-	-	+	+
Reeva	SG7	-	3.2	+++	-	-	+++	-
Reeva	SG8	-	2.09	+	++	-	+	-
Reeva	SG9	+	1.18	-	-	-	+	+
Reeva	SG10	-	1.29	-	-	+	+	-
Reeva	SG11	-	1.40	-	+	++	-	-
Reeva	SG12	-	1.18	-	-	-	+	-
Chunar	SG13	-	1.25	+++	-	-	+	-
Chunar	SG14	-	1.45	+++	+++	+++	++	+
Chunar	SG15	+	1.45	+++	-	-	++	-
Chunar	SG16	-	2.6	-	+++	++	+	+
Chunar	SG17	-	1.33	-	+	+	+	+
Chunar	SG18	-	1.5	-	-	-	+	+
Chunar	SG19	+	1.6	++	-	-	+	-
Chunar	SG20	+	1.14	-	-	-	+	+
Varanasi	SG21	-	-	-	-	-	+++	-
Varanasi	SG22	-	3.17	-	-	-	+	-
Varanasi	SG23	-	4.25	-	-	-	+	-
Varanasi	SG24	-	5.66	-	-	-	-	-
Varanasi	SG25	+	1.12	+	-	+	+	+
Varanasi	SG26	-	3.50	-	-	+	++	-
Varanasi	SG27	+	3.75	-	-	-	-	-
Varanasi	SG28	-	1.91	-	-	-	-	-
Varanasi	SG29	+	1.16	+	-	-	+	+
Varanasi	SG30	-	1.66	-	-	-	+++	+

Negative, +weak, ++ moderate, +++ strong activity

Phosphate solubilization

Out of the 30 isolates, 26 showed phosphate solubilizing activity on Pikovaskaya's medium by forming a clean transparent zone around the colony (Table 1). These isolates produced organic acid and phytase that converted insoluble phosphorus to soluble phosphorus. The range of halo zone recorded was 1.12 to 5.66cm. (Fig. 1). Eight isolates showed a halozone of more than 3cm.

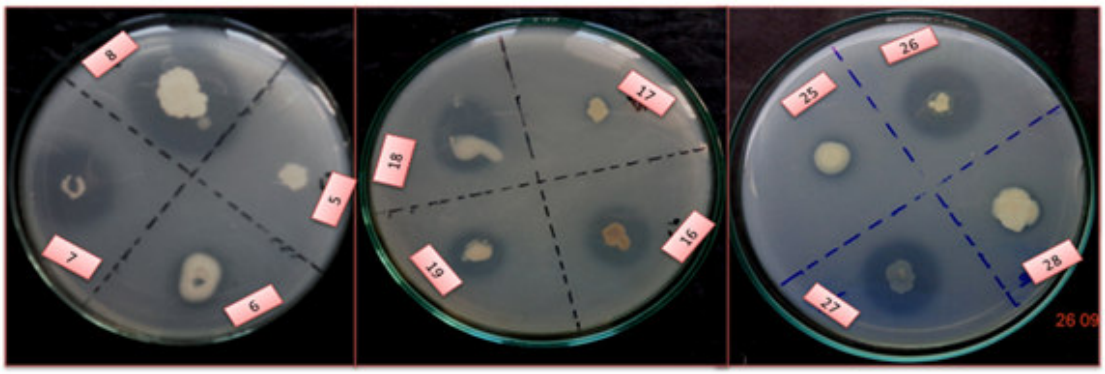

Figure 1
Phosphate solubilization by bacterial isolates

IAA production

Out of 30 bacterial isolates, 11 produced IAA in tryptophan yeast medium (Table 1). Maximum IAA production was observed by isolates SG7, SG12, SG13 and SG14 based on development of pink colour (Figure. 2).

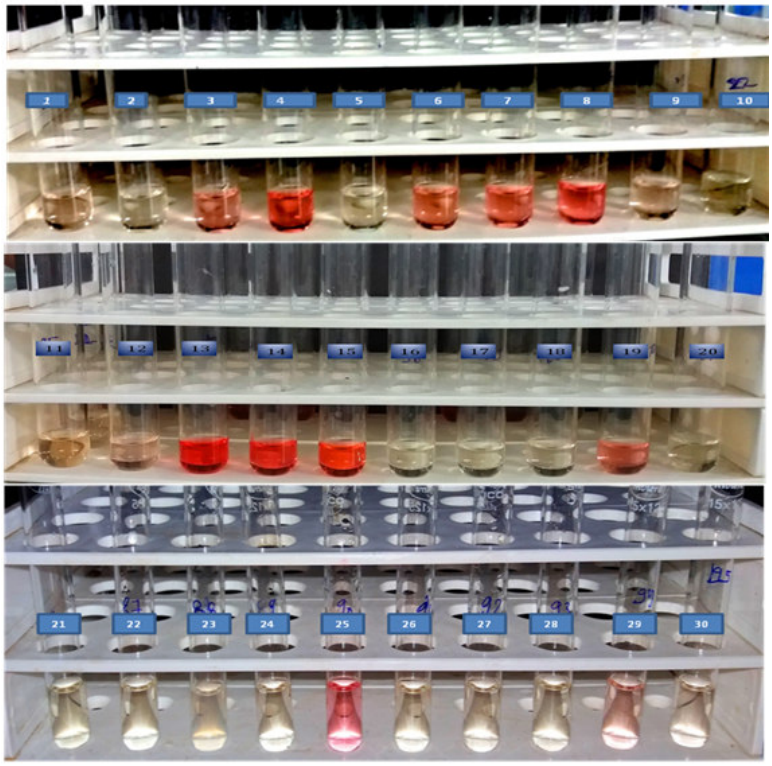

Figure. 2
IAA production by bacterial isolates

Ammonia production
Out of 30 isolates, five were recorded positive for ammonia production. (Table 1). Maximum ammonia production was observed by isolates SG14 and SG16. (Figure. 3)

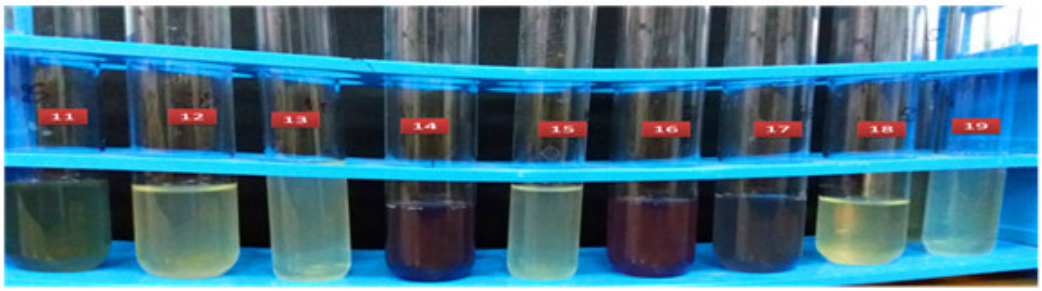

Figure 3
Ammonia production by bacterial isolates

HCN production
The production of HCN in excess may play a critical role in the control of fungal pathogen as a biocontrol agent.[27]
Among the 30 isolates, 10 isolates were recorded positive for HCN production (Table 1). Maximum HCN production was obseved by SG14 (Figure. 4).

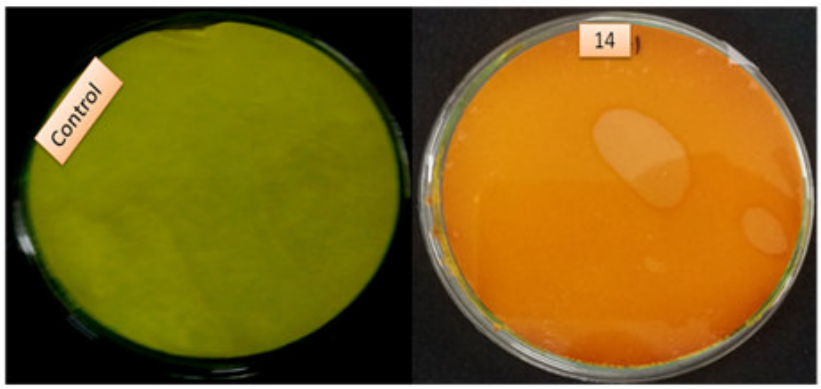

Figure 4
HCN production by bacterial isolate

Catalase production

Out of 30 bacterial isolates, 8 isolates showed good catalase activity (Table 1). Maximum catalase activity was recorded by isolates SG3, SG7, SG21 and SG30 (Figure. 5).

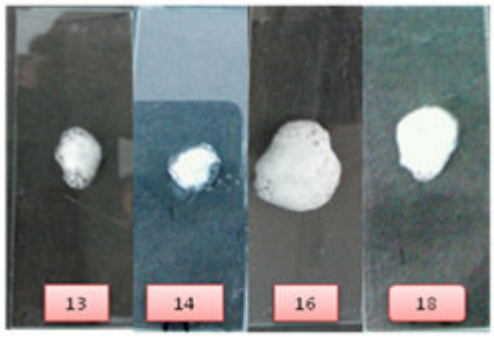

Figure 5
Catalase production by bacteria

Amylase activities

Amylase activity was determined by the appearance of clear zone on starch medium (Table 1). Out of 30, isolates 11 isolates showed Amylase activity and maximum starch utilization was done by SG14 bacterial isolate (Fig. 6).

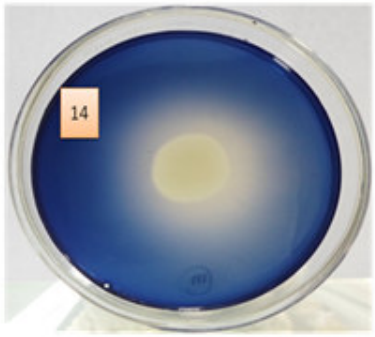

Figure 6
Amylase production by bacterial isolate

Effect of temperature on growth of bacterial isolates

Maximum growth of bacterial isolates was observed at 28°C. There was a gradual and uniform decrease in bacterial growth rate with increase in temperature from 28°C to 45°C. Minimum growth of bacterial isolates was observed at 45°C. Temperature below 28°C and above 45°C did not favour growth in most of the isolates (Figure. 7).

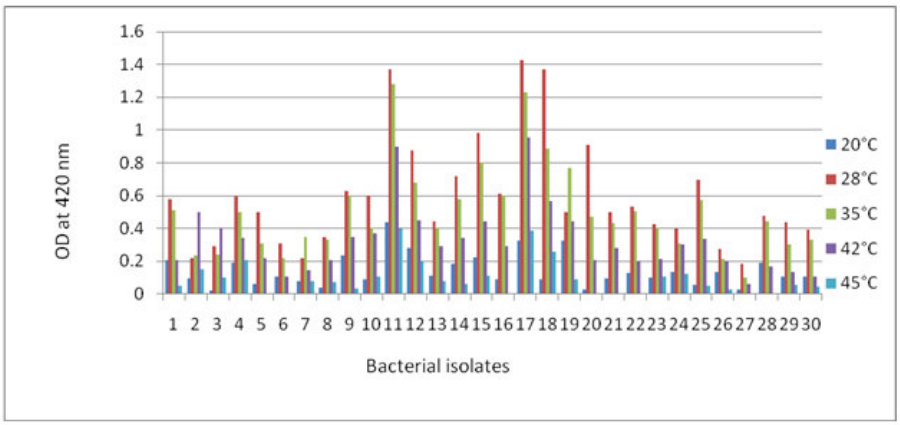

Figure 7
Growth of bacterial isolates at different Temperature

Effect of pH on growth of bacterial isolates

Maximum growth of all the bacterial isolates were obtained at pH7 and minimum growth was observed at pH11(Figure. 8).

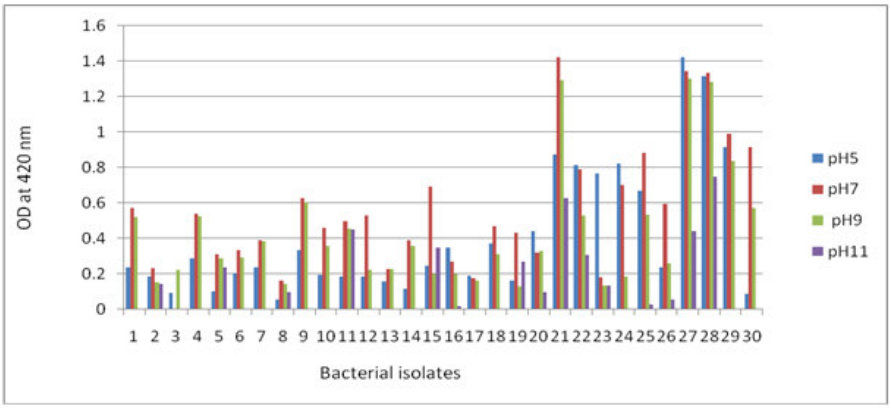

Figure 8
Growth of bacterial isolates at different pH

Effect of bacterial isolates on plant growth parameters

Maximum fresh weight of shoot was observed after treatment of bacterial isolates SG13 and was 256.8% more compared to control. SG18 treatment when compare to control showed 242.6% increase in fresh root weight and was recorded maximum. Maximum increase in dry weight of root (20%) and shoot (290%) was observed by SG16 isolate as compared to control. The bacterial isolate SG18 and SG14 showed maximum value of root length and shoot length with 83% and 84.4% increase respectively as compare to control. As compare to control Relative water content increase was maximum in plants treated with SG18 bacterial isolate and was 18.45% enhanced. Considerable increase in percentage of seed germination was observed after various inoculations. The increase in oat germination percentage ranged from 53-73% after seed priming by different isolates, with maximum increase observed by the bacterial isolate SG14. High vigour index in oat was observed after PGPR inoculation. The bacterial isolate SG14 showed maximum vigour index value of 2701.7 and was 226 % more as compare to control (Fig.9, Table 2).

Figure 9
Effect of PGPR inoculation on vigour index of Avena sativa

Table 2
Effects of bacterial isolates on various growth attributes of Avena sativa under control conditions

S. No	Bacterial isolates	Fresh Root Weight (g)	Fresh Shoot Weight (g)	Mean Root Length (cm)	Mean shoot Length (cm)	Dry root weight	Dry Shoot weight	Leaf area	Germin Ation in (%)	Vigour index	Relative Water content
1	SG13	0.1260± 0.00153	.2073± 0.00328	9.6± 0.1527	12.8667± 0.145	0.02± 0.001	0.0203± 0.0008	3.51± 0.094	80.00± 5.77	1794.0± 106	80.11± 1.25
2	SG14	0.1433± 0.00273	.1867± 0.00433	10.80± 0.2309	18.5± 0.378	0.031± 0.001	0.02± 0.00115	5.203± 0.123	86.66± 8.81	2701.70± 766.8	82.20± 2.20
3	SG16	0.1337± 0.0026	.1867± 0.00491	12.0333± 0.3480	18.5667± 0.260	0.0123± 0.008	0.0273± 0.00203	5.467± 0.144	86.00± 3.33	2648.7± 679.1	73.86± 4.08
4	SG18	0.1730± 0.00265	.2020± 0.00462	12.1333± 0.2603	17.6± 0.288	0.0087± 0.008	0.0207± 0.000176	4.994± 0.085	76.66± 8.81	1886.3± 132	84.22± 0.372
5	Control	0.0507± 0.00769	.0580± 0.01474	6.6± 0.1527	10.0333± 0.120	0.01± 0.001	0.007± 0.00058	2.235± 0.225	50.00± 5.77	827.33± 83.95	71.10± 1.48

Data shows mean ± SD, derived from the independent replicates.

DISCUSSION

Maintaining and restoring soil health to support plant growth is essential for sustainable agriculture. Thus, inoculation of efficient PGPR can enhance soil health and crop productivity even under multi stress environmental condition. Rhizobacteria enhance plant growth by a variety of mechanisms like phytohormone production, solubilization of phosphorus, enhanced stress tolerance, pathogen suppression and enhanced nutrient uptake. The search for best multifarious bacteria non-specific for host plant is the necessity of sustainable agriculture. In the present investigation, 30 bacterial isolates were screened for their growth promoting attributes of which four isolates *viz* SG13, SG14, SG16 and SG18; were promising for more than one growth promoting attribute. The present results showed a high level of phosphate solubilization by the microbes showing more than 1cm zone of clearance during phosphate solubilizing assay, which favored the plant growth directly. Phosphorus being a major nutrient required in plant growth and development and with a limitation of the added phosphorus in form of NPK being fixed in the soil, inoculating bacterial isolates with good phosphate solubilizing capacity and improving phosphorus mobilization can be a helping hand to the plant. Making phosphorous in bio-available form for plants is an important trait for PGPR selection. Rhizobacteria have been reported to produce organic acids such as citrate, lactate, succinate that dissolve the mineral phosphates as a result of anion exchange or chelation of Fe and Al ions associated with phosphate. This leads to an increase in the soluble P in the rhizosphere resulting in more uptake by the plants .[29,30] The isolates showing good phosphorus solubilization were also efficient in improving oat plant growth. Plants are unable to utilize phosphate because 95-99% phosphate present in the soil is in insoluble, immobilized, and precipitated form. Plants absorb phosphate only in two soluble forms, the monobasic (H_2PO_4) and the dibasic (HPO_4^{2-}) ions. Increased phosphorus availability to plants leads to plentiful growth of root as evident in the present study and was in accordance to previous studies.[31] Another important trait of plant growth promotion that was detected in most of the bacterial isolates was the production of growth hormone IAA. Isolates SG13, SG14 produced relatively high concentration of IAA compared to other isolates. Production of high level of IAA by bacterial isolates directly promoted plant growth as they increased the root length.[32, 33] Thus, the test isolates were extremely promising in promoting root growth during early stage of seed germination and plant growth and thereby improving vigour index. The seed germination percentage almost got increased by 2 fold on inoculation of bacterial isolate SG13 and SG14. Augmentation in the root length was up to 129% after inoculation of strain SG14. Increased root length not only support plant growth by exploring a greater volume of soil thereby increasing nutrient availability and absorption, but are also of significance in plants exposed to drought stress as they increase water availability to plants.[34] A relatively higher RWC in leaves of oat was observed after PGPR inoculation. SG18 showing maximum increase in shoot length was also efficient in increasing the RWC maximally compared to other strains and control. Such PGPR can also significantly overcome the stress effects of water deficit in plants. HCN production was detected in 36.6% bacterial isolates. SG14 and SG16 which also showed a number of other plant growth promoting traits were positive for HCN production. HCN production by PGPR mainly has antifungal role and hence inhibit the growth of phytopathogenic fungi.[35] Production of HCN in excess played a critical role in inhibiting the growth of phytopathogenic fungi in wheat seedling.[27] A number of studies suggest that PGPR enhances the growth, seed emergence, crop yield contribute to the protection of plant against certain pathogens and pests.[36] Bacterial strain showing catalase activity must be highly resistant to environmental, mechanical and chemical stress. All the bacterial isolates were positive for catalase activity. Temperature above 25°C has negative effects not only on photosynthetic performance of plants but also on microbial process like nodulation. Water stress conditions are accompanied by an increase in temperature and alteration in soil pH. Therefore the bacterial isolates were also tested for their growth behavior at the same extreme soil temperature and pH as experienced by soil exposed to water stress. While all the isolates showed best growth at 28°C, a uniform decrease in bacterial growth of SG13, SG14, SG16 and SG18 upto 34, 54, 53 and 59% was observed at 42°C. SG14 and SG16 bacterial isolates showed considerable growth even under altered temperature conditions there by showing their wide adaptability to high and low temperature. Variation in pH of growth media also affected bacterial growth. Although an increase or decrease in pH by 2 units affected the growth rate of bacteria, however it did not, stop completely the multiplication of bacteria. Inoculation of PGPR *viz* SG14 and SG16 possessing most of the plant growth promoting traits showed increased percent germination and vigour index of oat crops. Screening bacteria for a number of growth promoting attributes can be a promising approach in enhancing seed germination, plant development and suppression of fungal diseases. In our current study, the enhancement in early stages of plant growth parameters by the bacterial inoculants SG13, SG14, SG16 and SG18 was supported by the greater number of PGPR traits possessed by these isolates and hence they could prove effective in improving oat crop production and maintenance of soil fertility.

CONCLUSION

Identification of efficient PGPR and target plants to get optimal growth under changing climatic conditions is a major challenge. It is inferred from the present study that bacterial isolates are effective in improving seed germination root and shoot length plant growth and development of *Avena sativa*. The bacterial isolates SG13, SG14, SG16 and SG18 were efficient phosphate solubilizer, indole acetic acid producer, ammonia producer and temperature tolerant as well as good in surviving in diverse pH.

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CONFLICT OF INTEREST

Conflict of interest declared none.

REFERENCES

1. Han HS, Lee KD. Plant Growth Promoting Rhizobacteria effect on antioxidant status, photosynthesis, mineral uptake and growth of Lettuce under soil salinity. Res J Agric Biol Sci. 2005;(3):210-15.
2. Kloepper JW, Ryu CM, Zhang S. Induced systemic resistance and promotion of plant growth by *Bacillus* spp. Phytopathology. 2004 Nov; 94(11):1259-66.
3. Herman MAB, Nault BA, Smart CD. Effects of plant growth promoting rhizobacteria on bell pepper production and green peach aphid infestations in New York. Crop Protect. 2008 June 27; (6):996-1002.
4. Jetiyanon K, Kloepper JW. Mixtures of plant growth promoting rhizobacteria for induction of systemic resistance against multiple plant diseases. Biol Contr. 2002 July 24; (3):285-91.
5. Bowen GD, Rovira AD. The rhizosphere and its management to improve plant growth. Adv Agron. 1999; 66(8):1-102.
6. Mirza MS, Ahmad W, Latif F, Haurat J, Bally R, Normand P, Malik KA. Isolation, partial characterization, and the effect of plant growth-promoting bacteria (PGPB) on micro-propagated sugarcane *in vitro*. Plant Soil. 2001 Nov; 237(1):47-54.
7. Berjak P. Unifying perspectives of some mechanisms basic to desiccation tolerance across life forms. Seed Sci Res. 2006 March; 16(1):1-15.
8. Zahir ZA, Munir A, Asghar HN, Arshad M, Shaharoona B. Effectiveness of rhizobacteria containing ACC-deaminase for growth promotion of peas (*Pisum sativum*) under drought conditions. J Microbiol Biotech. 2008 May;18(5):958- 63.
9. Alami Y, Achouak W, Marol C, Heulin T. Rhizosphere soil aggregation and plant growth promotion of sunflowers by an exopolysaccharides-producing *Rhizobium* sp. strain isolated from sunflower roots. Appl Environ Microbiol. 2000 August; 66(8):3393-8.
10. Zahran HH. *Rhizobium*-Legume Symbiosis and Nitrogen Fixation under Severe Conditions and in an Arid Climate. Microbiol Mol Biol Rev. 1999 Dec;63(4):968-89.
11. Volpin H, Phillips DA. Respiratory elicitors from *Rhizobium meliloti* affect intact alfalfa roots. Plant Physiol.1998 july; 116:777-83.
12. Nehra K, Attar SY, Anita RS, Vashishat RK. Characterization of heat resistant mutant strains of *Rhizobium* sp. [*Cajanus*] for growth, survival and symbiotic properties Indian J Microbiol. 2007 Dec;47:329-35.
13. Somasegaran P, Hoben HJ. Handbook for Rhizobia. Methods in Legume-*Rhizobium* Technology. New York: Springer-Verlag; 1994. p. 240-58.
14. Munevar F, Wollum AG. Effects of the addition of phosphorus and inorganic nitrogen, carbon and nitrogen mineralization in adepts from Columbia. Soil Sci Soc Am J. 1977 Dec; 41(3):540-5.
15. Stevens EJ, Armstrong KW, Bezar HJ, Griffin WB, Hampton JG. Fodder oats: an overview. In J. M. Suttie and S. G. Reynolds (eds) Fodder Oats: a world overview. Plant Production and Protection Series.No. 33, FAO, Rome; 2004. p.11-18.
16. FAO. FAOSTATE statical analysis database. Food and agriculture organization of the united nation, Rome, Italy; 2003.
17. Frett JJ, Pill WG. Germination characteristics of osmotically primed and stored impatient seeds. Scientia Horticulturae. 1989 Sept; 40(2):171-9.
18. Wiebe JJ, Muhyaddin T. Improvement of emergence by osmotic seed treatments in soil of high salinity. Acta Horticulturae. 1978; 198:91-100.
19. Prasad JK, Gupta SK, Raghuwanshi R. Screening multifunctional plant growth promoting rhizobacteria strains for enhancing seed germination in wheat (*Triticum aestivum* L.) Int J Agric Res. 2017 March;12(2):64-72.
20. Pikovskaya RI. Mobilization of phosphorous in soil in connection with vital activity of some microbial species: Mikrobiologiya. 1948; 17:362-70.
21. Bric JM, Bostock RM, Silverstone SE. Rapid *in-situ* assay for indole acetic acid production by bacteria immobilized on nitrocellulose membrane. Appl Environ Microbiol. 1991 Feb; 57(2):535-8.
22. Cappuccino JC, Sherman N. Microbiology: A Laboratory Manual, 3 Edn, Benjamin/cummings Pub. Co. New York. 1992:125-79.
23. Alstrom S, Burns RG. Cyanide production by rhizobacteria as a possible mechanism of plant growth inhibition. Biol Fertil Soils. 1989;7:232-8.
24. Fadden MC. Biochemical tests for identification of Medical bacteria. Williams and Wilkins, Baltimore, USA; 1980:51-4.
25. Collins CH, Patricia M, Lyne J, Grange M. Collins and Liyne's Microbiological Methods, 7th Edn, Butterworth-Heinemann, UK;1995. p.117.
26. ISTA. International rule for seed testing. Seed Sci Technol. 1993;21:25-30.
27. Abdul- Baki AA, Anderson JD. Vigour determination in soybean seed by multiple criteria. Crop Sci. 1973 April;13(6):630-3.
28. Flaishman MA, Eyal Z, Zilberstein A, Voisard C, Haas D. Suppression of *Septoria tritici* blotch and leaf rust of wheat by recombinant cyanide producing strains of *Pseudomonas putida*. Mol. Plant Microbe Interact. 1996; 9(7):642- 5.
29. Shahzad SM, Arif MS, Riaz M, Iqbal Z, Ashraf M. PGPR with varied ACC-deaminase activity induced different growth and yield response in

maize (*Zea mays* L.) under fertilized conditions. Eu J Soil Biol. 2013; 57:27-34.

30. Kumar A, Kumar A, Devi S, Patil S, Payal C, Negi S. Isolation, screening and characterization of bacteria from Rhizospheric soils for different plant growth promotion (PGP) activities: An *in vitro* study. Rec Res Sci Tech. 2012; 4:1-5.
31. Kumar A, Maurya BR, Raghuwanshi R. Isolation and characterization of PGPR and their effect on growth, yield and nutrient content in wheat (*Triticum aestivum* L.). Biocatalysis Agri Biotech. 2014 Oct;3(4):121-8.
32. Rana Saharan B, Joshi M, Prasanna R, Kumar K, Nain L. Identification of multI-trait PGPR isolates and evaluating their potential as inoculants for wheat. Ann Microbiol. 2011 Dec; 61(4):893-900.
33. Selvakumar G, Kundu S, Gupta AD, Shouche YS, Gupta HS. Isolation and characterization of non rhizobial plant growth promoting bacteria from nodules of Kudzu (*Pueraria thunbergiana*) and their effect on wheat seedling growth. Curr Microbiol. 2008 Feb; 56(2):134-9.
34. Sinha S, Raghuwanshi R. Synergistic effect of arbuscular mycorrhizal helper bacteria on physiological mechanism to tolerate drought in *Eclipta prostrate* (L.). Pure Applied Microbiol. 2016 June; 10(2):1117-29.
35. Blumer C, Hass D. Mechanism, regulation and ecological role of bacterial cyanide biosynthesis, Arch of Microbiol. 2000 March; 173(3):170-7.
36. Geetha K, Chaitanya K, Bhadraiah B. Isolation and Characterization of PGPR Isolates From Rhizosphere Soils of Greengram In Warangal District of Telangana. Int J Pharm Bio Sci. 2014 Oct; 5(4):153-63.

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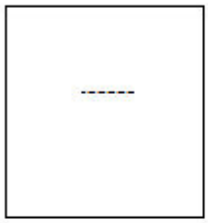

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