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Functional Attributes of Psychrotolerant Rhizobacteria from Wheat (*Triticum aestivum* L.) Rhizoshpere

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A B S T R A C T

Cold-tolerant PGPRs are known to improve plant growth and nutrient uptake by an array of mechanisms, under stress and nutrient imbalance conditions. On the basis of growth potential we selected 43 PGPRs from pool of 85 rhizobacterial isolates obtained from wheat rhizospheric soil collected from different wheat growing fields. Phosphate and zinc solubilization efficiency along with phytohormones and siderophore producing ability at 10 and 20°C was also assessed. Rhizobacterial isolates A-43, A-13, A-12, A-36, A-25, A-22 and A-34 was potent phosphate and zinc solubilizers. IAA production was estimated in the range from 2.12 to 43.24 µg/ml and 2.40 to 68.36 µg/ml in the presence of tryptophan at 10 and 20°C respectively. Maximum GA production was recorded with A-43, A-13, A-12 and A-25 at 10 and 20°C. Similarly highest siderophore production was shown by bacterial culture A-6 (250.4 µg/ml and 90.5 µg/ml) and A-43 (225.9 µg/ml and 80.6 µg/ml) at both temperatures. Also, isolates A-43, A-36, A-13 and A-12 were resistant to many antibiotics like ampicillin (25 µg/disc), tetracycline (10 µg/disc), streptomycin (10 µg/disc) etc. These four potent rhizobacterial isolates improved germination, root and shoot length and fresh weight of wheat seedlings as compared to uninoculated control under axenic conditions.

Introduction

Wheat (*Triticum aestivum* L.) is a major cereal crop that sustains humanity and it is the staple food of more than 40% of human population. India is the second largest producer of wheat (94.90 million tons) next only to China (125.60 million tons) and covers the largest area under its cultivation (29.80 m ha), which is about 13.53 % of the world wheat area (217 m ha) (FAOSTAT 2014). The most favorable climatic condition for wheat cultivation is cold temperature during the vegetative growth period followed by warm temperature for the grain to mature and ripen.

The plant growth promoting rhizobacteria (PGPR) facilitates plant growth and development with a spectrum of direct and indirect mechanisms under stress and non-stress conditions (Nadeem *et al.,* 2014). The rhizobacteria affect plant growth directly by increasing nutrient cycling, biological nitrogen fixation (Ahmad *et al.,* 2008), siderophore production, solubilization of

phosphorus and zinc, synthesis of phytohormones or indirectly by synthesis of biocontrol compounds to inhibit phytopathogens (Lucy *et al.*, 2004).

Nutrient deficiency in addition to various abiotic (cold temperature) and biotic stress is one of the important yield limiting factor in wheat. To overcome micronutrient deficiency, improvement of Zn bioavailability in cultivated soils may improve crop growth and also enhance Zn contents in the staple food grains which would possibly diminish major health risks. Also, Biofortification is a current approach aimed at increasing the bioavailability of micronutrients such as Zn and Fe in the staple crops of specific region (Stein, 2010).

In order to improve plant microbe interaction under low temperature conditions, it is essential to exploit cold adapted strains of rhizospheric bacteria that can influence plant growth and development. Psychrotolerant PGPRs are widespread in the agro-ecosystem and play a range of roles including plant-growth-promotion, micronutrient solubilisation and alleviation of cold stress in plants.

Bioinoculants for the improvement of micronutrient deficiency is promising due to its ecological, economic, and eco-friendly nature. So, keeping this in view present study deals with the characterization of low temperature tolerant rhizobacterial isolates for their multiple plant growth promoting traits at low temperature from the rhizosphere of wheat crop.

Material and Methods

Isolation of rhizobacteria

All the isolates of *Pseudomonas, Bacillus* and *Serratia* were isolated from the 27 wheat rhizoshperic soil samples by standard microbiological techniques collected from different locations of Punjab, Himachal Pradesh, Haryana and U.P., India. Spread plating was done on Nutrient agar (NA) for *Bacillus* and *Serratia* and on King's B for *Pseudomonas* (King *et al.*, 1954). The isolates were grown at 10°C and 20°C in respective media and growth in terms of optical density was recorded at 600 nm. Isolates showing maximum growth both at 10°C and 20°C were selected for further analysis. All the functionality traits were assessed at 10°C and 20°C.

In vitro screening of bacterial isolates for their plant growth promoting activities

Zn solubilisation

Qualitative assay for Zn solubilisation: All the bacterial isolates were first screened by plate assay for their efficiency to solubilize zinc on Tris-minimal medium supplemented separately with zinc oxide (ZnO) [1.244 /L] = 15.23mM and zinc carbonate ($ZnCO_3$) [1.9882 g/L] = 5.0mM at a concentration equivalent to 0.1% Zn (Fasim *et al.*, 2002). Zinc solubilization efficiency (SE) was calculated as described by Ramesh *et al.*, (2014).

$$SE = \frac{\text{Diameter of solubiliza tion halo zone}}{\text{Diameter of colony}} \times 100$$

Effect of Zinc compounds on pH of the medium: Bacterial isolates showing zones of clearance with ZnO/ ($ZnCO_3$) on solid medium were inoculated in minimal medium, supplemented with the same compounds (1mg/ml, each) and glucose (10mg/ml).

Inoculated flasks were incubated at 10 and 20^0C for 3 to 5 days and samples were drawn at regular interval to record pH of culture broth (Joshi *et al.*, 2013).

Phosphate-solubilization

All the isolates were screened on Pikovskaya's agar plates and NBRI-BPB medium described by Mehta and Nautiyal (2001) for phosphate solubilising. The solubilization index was calculated by Edi-Premono *et al.,* (1996)

$$\text{Phosphate Solubilization Index} = \frac{\text{Total diameter (colony + halo zone)}}{\text{Diameter of colony}}$$

Quantitatively P-solubilization was recorded at intervals of 3 days up to 15 days of incubation (Jackson, 1973).

IAA and gibberellic acid production

Characterization of isolates for the production of IAA and gibberellic acid was determined as per the method given by Gordon and Weber (1951) and Borrow *et al.,* (1955) respectively.

Production of siderophore

Siderophore production of isolates was done both qualitatively and quantitatively as per the method described by Schwyn and Neilands (1987) and Arnow (1937) respectively.

Intrinsic antibiotic resistance spectra

An antibiotic resistance spectrum of the isolates was studied by using different antibiotics.

Filter paper discs (Hi-Media) containing a standard concentration of antibiotics viz. ampicillin (25µg/disc), chloramphenicol (25µg/disc), tetracycline (30µg/disc), streptomycin (25µg/disc), streptomycin (10µg/disc), carbenicillin (100µg/disc), kanamycin (30µg/disc) and tetracycline (30µg/disc) were used.

Germination of wheat seeds under axenic conditions

Germination tests were carried out to determine the effect of potent rhizobacterial isolates on germination and seedling growth of wheat variety (WH1105) under axenic conditions as per the method of described Khalid *et al.,* (2004).

Results and Discussion

Isolation and characterization

A total 85 rhizobacterial isolates were isolated from wheat rhizospheric soil from 27 different wheat growing fields. Isolates 40 from Kings B medium showed the characteristic yellowish-green pigmentation and 41 isolates from NA showed transparent to creamish, yellow to orange in color and 4 isolates from NA showed an entire margin and produced red color pigmentation. On the basis of cultural morphological and biochemical tests, these were tentatively assigned to genera *Bacillus*, *Pseudomonas*, and *Serratia.*

The growth in terms of optical density recorded at 10°C and 20°C showed that rhizobacterial isolates exhibited growth potential at both 10ºC and 20°C when incubated for 24 hours. Isolates A-1, A-40, A-43, A-13, A-12, A-36 and A-25 showed relatively good growth at 10°C however, it was lower than that at 20°C (Fig. 1 and 2).

Zinc solubilization

Forty three of the rhizobacterial isolates produced distinct halo zone on Tris-minimal medium supplemented with 0.1% ZnO and $ZnCO_3$ indicating zinc solubilization at 28°C. They also showed clear halo zone on modified PVK medium supplemented with ZnO (Plate 1), formation of halo zone

initiated after 2 days, reaching a maximum upto 15 days. Out of these, 38 rhizobacterial isolates showed zinc solubilization at 20°C and 14 isolates at 10°C.

In plate assay, all the selected isolates could effectively solubilize the insoluble Zn compounds at 10 and 20°C. At 10°C, size of the solubilization zone ranged from 0.5 to 1.45cm in ZnO incorporated medium (Plate 2). The formation of halo by the microorganisms is reported to be due to the movement of acidity corresponding with the solubilization of the metal compound (Saravanan *et al.,* 2003). The S.E. ranged from 100 to 290 (Table 1). Maximum solubilization was exhibited by four isolates A-12 (S.E. = 290) followed by A-43 (S.E. = 280), A-36 (S.E. = 260) and A-13 (S.E. = 240) in ZnO amended media. Whereas at 20°C size of the halo zone ranged from 0.31 to 2.9cm and S.E. = 42.86 to 966.67. Maximum halo zone diameter was shown by A-43, 13 (2.9), A-36, 34 (2.7), A-22 (2.6) and A-12 (2.3) respectively, (Plate 3) and maximum solubilisation shown by isolate A-43 (S.E. = 966.67) followed by A-36 (S.E. = 675), A-13 (S.E. = 580) and A-12 (S.E. = 575). On average, four rhizobacterial isolate A-43, A-13, A-36, and A-12 showed zinc solubilization at both temperatures. However, their solubilization potential was more at 20°C.

The zone of solubilization was comparatively higher in ZnO amended medium as compared to $ZnCO_3$. At 10°C, the size of the solubilization zone ranged from 0.5 to 1.45cm in zinc carbonate supplemented medium. The range of S.E. (90 to 280) was found to be less as compared to zinc oxide. Maximum solubilisation was shown by isolates A-12 (S.E. = 280) which was same as in zinc oxide followed by A-43 (S.E. = 260), A-36 (S.E. = 260) (Table 1). However, isolate A-13 showed higher solubilization of zinc carbonate as compared to zinc oxide. Whereas, at 20°C size of the halo zone ranged from 0.5 to 2.8 cm, S.E. = 35.71 to 800. Maximum solubilisation showed by isolate A-43 (S.E. = 800) and A-36 (S.E. = 625) which is somewhat similar to zinc oxide. However, S.E. = 733.33 by isolate A-13 and S.E. = 583.33 by A-12 was found to be higher in the zinc carbonate medium. On average, rhizobacterial isolates A-43, A-36 and A-13 were found to be potent zinc solubilizers in zinc oxide as compared to zinc carbonate amended media at 20°C. The result showed varied solubilization potential among the bacterial isolates in term of zinc compounds. This might be related to differences in the locations from which they were isolated.

Eight of the culture filtrates showed a drop in pH after 10 days growth. A significant pH drop from pH 7.0 by some rhizobacteria in the uninoculated medium was observed. The pH drop ranged from 7 to 6.06 at 10°C and 7 to 3.73 at 20°C. Among ten isolates, A-43 showed a drop in pH from 7.0 to 3.73 followed by A-25 (pH 4.36), A-13 (pH 5) and A-36 (pH 5.16), in zinc oxide supplemented cultures at 20°C (Fig. 3). Similar findings were also reported by Desai *et al.,* (2012), they inferred that higher availability of Zn is directly proportional to the acidic pH of the medium, which was positively correlated with the production of organic acids.

Surprisingly, isolates A-36 and A-12 showed an increase in pH from 7 to 7.52 at 10°C. In some potent strains, pH did not fall drastically thereby suggesting that in those strains other mechanisms may be active and this aspect is being accentuated. Goteti *et al.,* (2013) in their studies also found no significant correlation between pH and solubilization of nutrients.

The overall result showed that maximum solubilization occurred in the presence of zinc

oxide supplemented media at 20°C as compared to zinc carbonate and A-43, A-36, A-13, A-12, A-22, and A-34 were promising isolates in terms of Zn solubilization.

Phosphate solubilization

Screening of 85 isolates showed that 43 were phosphate solubilizers as evidenced by orangish yellow halos on NBRI-BPB media at 28°C. Screening of P-solubilizing ability at low temperature revealed of the 43 bacterial isolates from wheat rhizosphere 35 solubilized phosphate at 10°C and 38 at 20°C.

On the basis of solubilization index, ten isolates were further selected for quantitative estimation. At 10°C, P-solubilization on Pikovskaya's agar medium started on the 4th day and increased upto 15th day. Maximum solubilization index was shown by isolate A-43 (4.5) followed by A-25, 12 (4.3) and A-13 (4.1). Whereas, at 20°C solubilization started a day earlier and increased upto 15th day. Maximum solubilization index was found in isolate A-15 (6.6) followed by A-43(6.4), A-13 (6.2) and A-12 (6.1) (Table 2 and 3).

All the isolates efficiently released the bound phosphate from calcium in the PVK broth at 10°C and 20°C. The relative efficiency of 10 isolates in solubilizing TCP was evaluated *in vitro* as a function of time. It was seen that increasing amount of P was released by most of the isolates till the 9th day at 10°C and 6th day at 20°C thereafter it started decreasing with incubation. This may be due to utilization of released P by rhizobacteria. The phosphate solubilization ranged from 0.4 to 11.4 mg/100 ml at 10°C and 3.2 to 23.7 mg/100 ml at 20°C. Maximum solubilization at 10°C was by isolate A-25 (13.1 mg/100 ml) after 9 days of incubation followed by A-13, A-12, A-36, A-34 and A-29 (10.5 to 7.8 mg/100 ml) (Table 2). Rhizobacterial isolate A-43 was potent solubilizer and showed efficient solubilization 11.4 mg/100 ml after 6 days. Surprisingly, isolate A-3 started solubilization of phosphorus on 3rd day itself (3.7 mg/100 ml) and showed a decline thereafter. Isolate A-29 (6.6 mg/100 ml) found to be maximum solubilizer after 12 days and two isolates A-6 (7.4 mg/100 ml), A-15 (6.1 mg/100 ml) after 15 days of incubation.

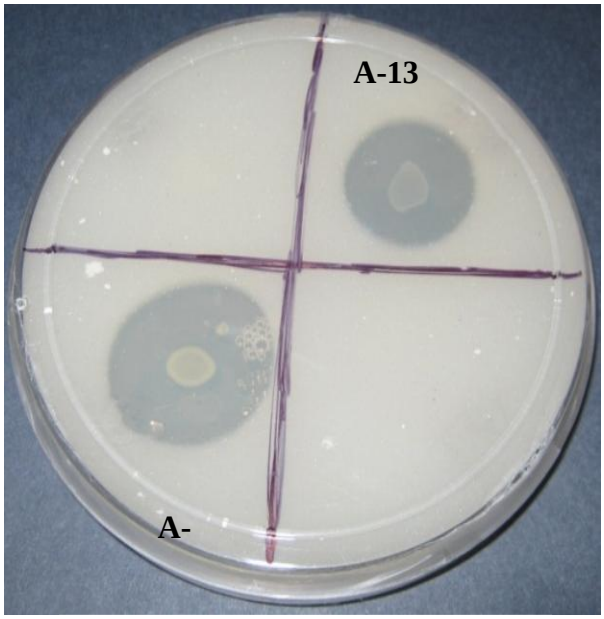
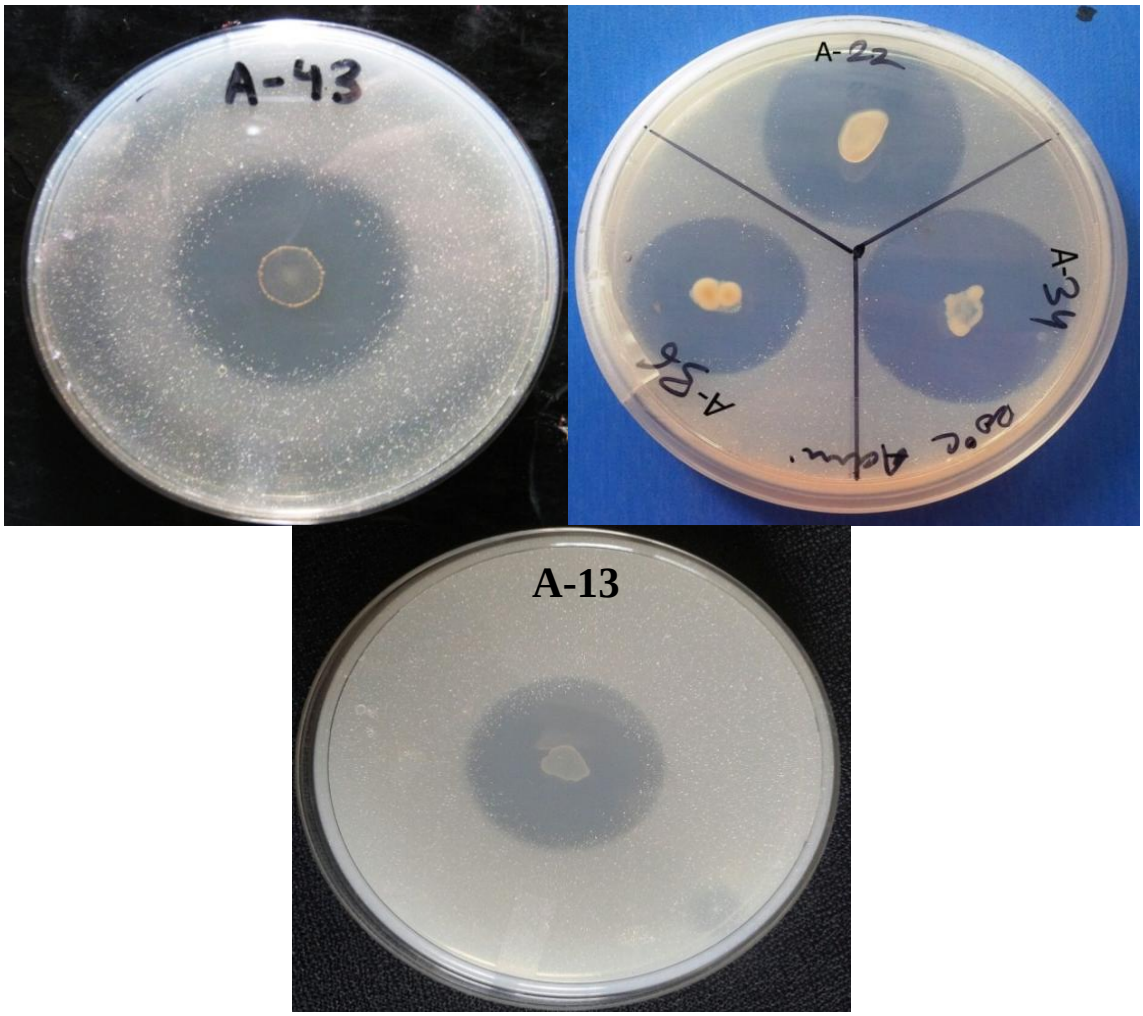


Plate.1 Zinc solubilization on modified PKV medium at 28°C

Plate.2 Zinc solubilization on Tris-minimal medium (0.1% $ZnCO_3$) at 10°C

Plate.3 Zinc solubilization on Tris-minimal medium (0.1% ZnO) at 20°C



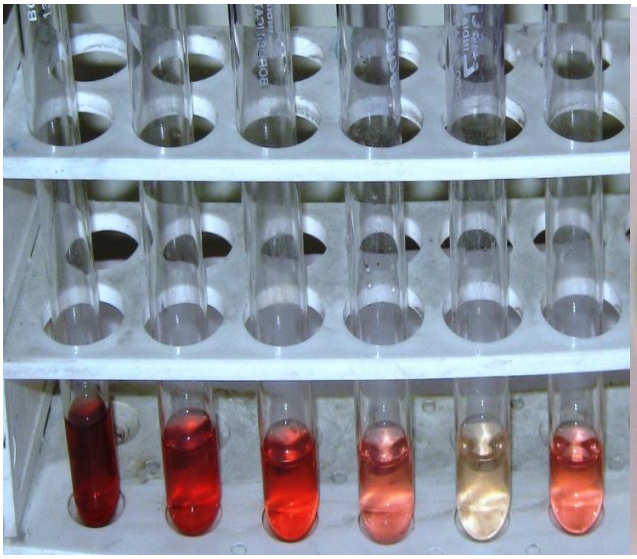


Plate.4 IAA production at 10 and 20°C

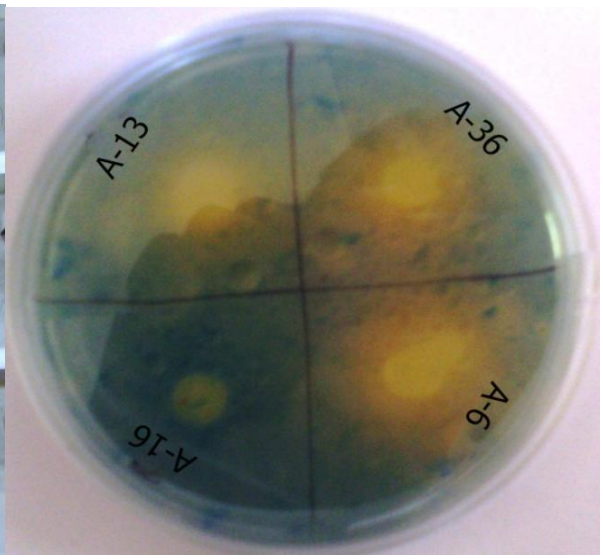


Plate.5 Siderophore production by rhizobacterial isolates 20°C

Plate.6 Effect of potent rhizobacterial isolates on germination and seedling growth of wheat under axenic conditions

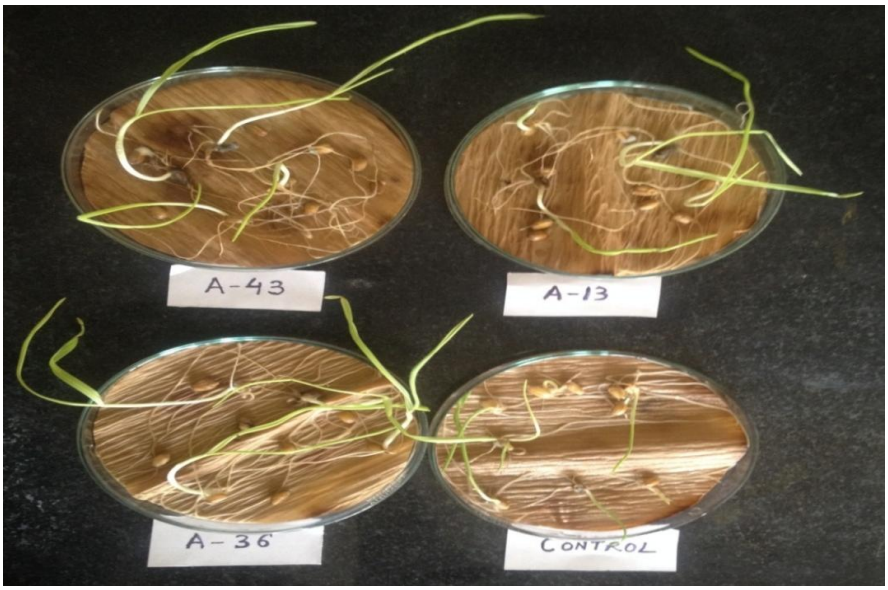


Fig.1 Growth profile of rhizobacterial isolates at 10 °C

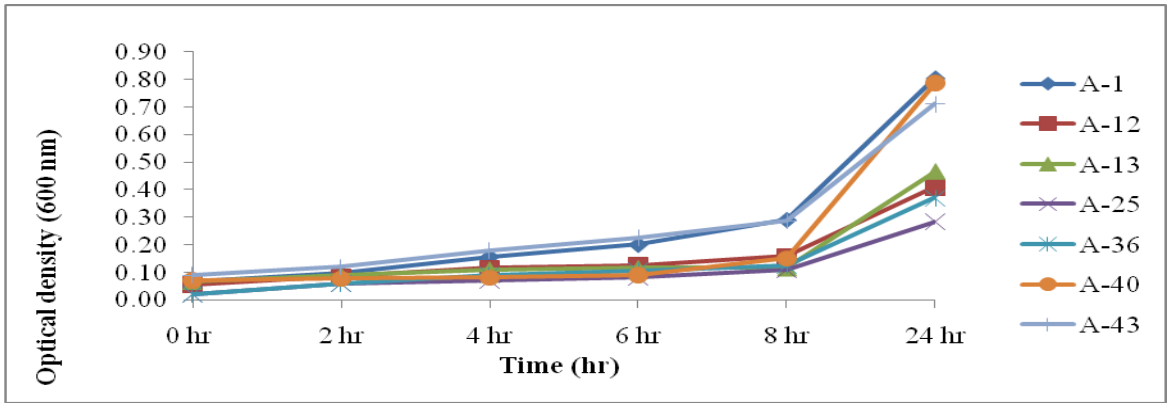


Fig.2 Growth profile of rhizobacterial isolates at 20ºC

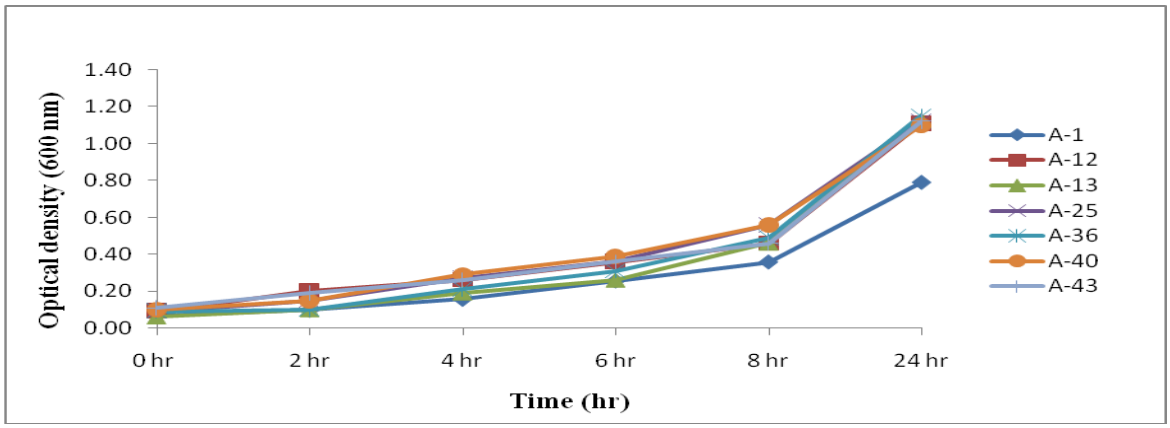


Fig.3 Relative changes in pH in zinc oxide supplemented medium by different zinc solubilizers

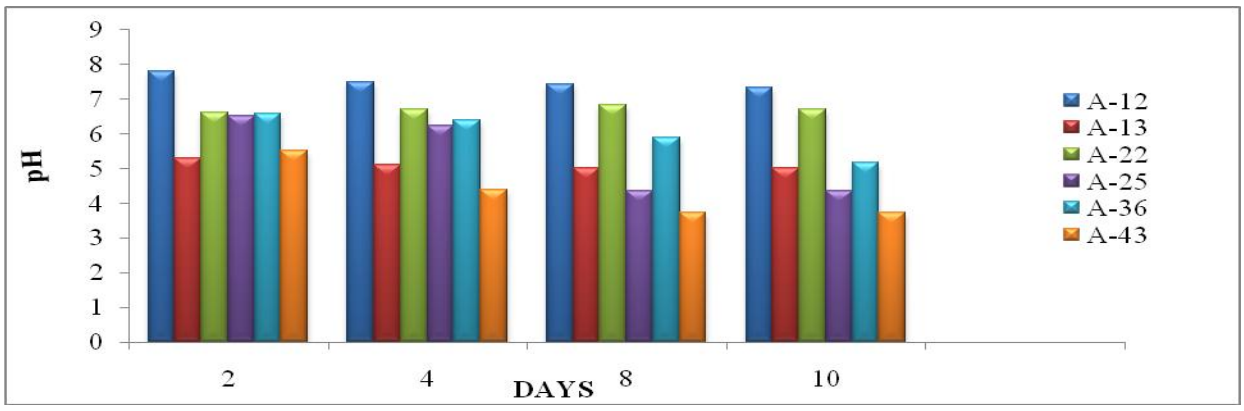


Table.1 Zinc solubilisation by different rhizobacteria at 10 and 20°C

Rhizobacterial Isolates	Solubilization Efficiency			
	At 20°C		At 10°C	
	ZnO	$ZnCO_3$	ZnO	$ZnCO_3$
A-1	55.56	116.67	-	-
A-2	112.50	45.45	250.00	175.00
A-3	287.50	380.00	-	-
A-4	63.27	150.00	-	-
A-5	125.00	111.11	-	-
A-6	300.00	425.00	200.00	233.33
A-7	85.71	112.50	-	-
A-8	114.29	88.89	-	-
A-9	91.67	110.00	160.00	166.67
A-11	380.00	487.50	100.00	100.00
A-12	575.00	583.33	290.00	280.00
A-13	580.00	733.33	240.00	250.00
A-14	150.00	50.00	-	-
A-15	380.00	366.67	-	-
A-16	212.50	300.00	225.00	120.00
A-17	150.00	105.13	-	-
A-19	437.50	500.00	-	-
A-21	350.00	35.71	-	-
A-22	520.00	416.67	100.00	200.00
A-23	316.67	81.82	-	-
A-24	500.00	666.67	-	-
A-25	485.37	240.00	233.33	90.00
A-27	100.00	77.78	-	-
A-28	124.49	78.95	-	-
A-29	390.00	566.67	116.67	120.00
A-31	73.68	60.00	-	-
A-32	66.67	387.50	-	-
A-33	73.33	60.00	-	-
A-34	450.00	81.82	-	-
A-35	66.67	78.95	-	-
A-36	675.00	625.00	260.00	260.00
A-37	350.00	500.00	116.67	160.00
A-38	103.39	122.22	-	-
A-39	142.86	83.33	225.00	225.00
A-40	42.86	115.38	-	-
A-41	300.00	300.00	-	-
A-42	400.00	525.00	-	-
A-43	966.67	800.00	280.00	260.00

- means absent.

Table.2 Phosphate solubilization as a function of time by rhizobacterial isolates at 10°C

Rhizobacterial Isolates	P-solubilization (mg/100ml) Incubation period (days)					P-solubilization Index(cm)
	3rd	6th	9th	12th	15th	
A-3	3.7	1.9	1.7	0.6	0.4	2.2
A-6	0.4	1.7	4.2	2.9	7.4	2.1
A-12	4.8	4.6	8.1	6.3	5.2	4.3
A-13	8.1	9.6	10.5	7.5	4.1	4.1
A-15	0.4	1.1	2.1	4.3	6.1	2.2
A-22	0.5	1.4	4.2	1.0	0.4	2.0
A-25	5.0	6.3	13.1	10.3	6.9	4.3
A-29	1.0	0.6	2.5	6.6	6.3	2.3
A-34	5.1	6.3	7.8	7.2	7.5	3.9
A-36	3.3	7.3	7.9	6.3	3.1	2.5
A-43	9.6	11.4	7.7	7.6	7.5	4.5

Table.3 Phosphate solubilization as a function of time by rhizobacterial isolates at 20°C

Rhizobacterial Isolates	P-solubilization (mg/100ml) Incubation period (days)					P-solubilization Index(cm)
	3rd	6th	9th	12th	15th	
A-3	3.0	8.3	5.3	3.1	2.7	2.7
A-6	3.1	7.0	4.3	3.2	2.9	3.7
A-12	10.4	17.1	16.2	12.2	9.2	6.1
A-13	8.8	21.1	11.3	10.8	8.3	6.2
A-15	7.2	10.9	15.3	10.1	9.8	4.4
A-22	5.3	11.8	7.7	7.6	7.4	5.8
A-25	13.4	23.7	18.3	15.4	11.1	6.6
A-29	4.3	11.4	6.2	5.3	3.7	5.4
A-34	4.8	5.4	14.2	6.6	2.5	5.5
A-36	9.1	19.9	12.5	9.3	8.7	5.9
A-43	5.7	18.9	15.8	12.0	11.1	6.4

Table.4 Relative production of IAA equivalents by rhizobacteria

Rhizobacterial Isolates	IAA equivalents (µg/ml)			
	At 10°C		At 20 °C	
	L-TRP (-)	L-TRP (+)	L-TRP (-)	L-TRP (+)
A-1	2.31	4.50	2.31	10.06
A-2	0.60	2.37	1.04	3.82
A-3	3.29	11.26	5.16	5.75
A-4	4.05	3.29	2.85	4.90
A-5	0.76	3.61	9.18	17.23
A-6	1.84	4.68	1.30	12.57
A-7	2.84	3.68	2.91	15.16
A-8	1.48	2.16	2.19	9.92
A-9	2.05	3.80	4.20	21.5
A-10	0.98	3.70	2.11	7.73
A-11	3.37	5.59	1.26	5.43
A-12	4.51	20.21	6.77	23.61
A-13	2.08	18.83	6.54	26.52
A-14	3.79	6.25	7.58	21.68
A-15	2.72	9.25	2.44	18.64
A-16	6.23	10.25	4.13	23.25
A-17	1.91	6.84	2.81	5.41
A-18	2.10	4.14	1.33	18.25
A-19	3.52	6.92	3.55	21.36
A-20	2.04	2.48	4.62	4.38
A-21	3.55	4.20	5.38	6.98
A-22	7.04	18.30	2.68	23.63
A-23	0.78	4.21	2.94	4.53
A-24	0.77	3.37	3.10	4.32
A-25	3.20	3.38	1.80	4.44
A-26	1.37	3.99	4.79	6.96
A-27	2.58	8.22	9.31	14.15
A-28	1.18	2.43	2.50	7.49
A-29	3.05	5.10	1.65	2.77
A-30	1.42	3.87	2.36	3.87
A-31	2.13	7.02	6.77	21.91
A-32	0.74	3.38	3.38	9.12
A-33	1.18	3.91	2.78	4.25
A-34	2.60	3.69	9.03	9.55
A-35	2.08	2.77	5.40	9.39
A-36	5.21	19.25	2.96	30.77
A-37	1.21	2.12	1.10	2.40
A-38	12.25	35.66	26.92	39.81
A-39	9.12	5.85	3.38	9.63
A-40	6.24	43.24	13.53	68.36
A-41	0.87	3.35	2.09	4.79
A-42	3.70	8.12	4.98	11.85
A-43	7.04	21.62	8.26	24.83

Table.5 Siderophore Production by rhizobacterial isolates

Rhizobacterial Isolates	Siderophore Production		
		Catechol type (µg/ml)	
	Dia (cm)	10ºC	20ºC
A1	1.8	51.2	96.8
A-2	2.9	55.8	98.3
A-6	3.4	90.5	250.4
A-11	1.8	29.0	86.2
A-12	3.1	71.9	105.2
A-13	2.1	76.3	110.6
A-16	1.3	31.2	69.6
A-29	2.9	30.9	74.0
A-36	3.0	64.1	117.9
A-43	2.5	80.6	225.9

Table.6 Effect of potent rhizobacterial isolates on germination and seedling growth of wheat under axenic conditions

Rhizobacterial isolates	PGP traits				
	% Germination	Root Length (cm)	Shoot Length (cm)	Root Fresh Weight (mg)	Shoot Fresh Weight (mg)
A-43	85	16.0	9.5	0.073	0.380
A-36	80	13.3	8.7	0.081	0.416
A-13	85	13.0	9.1	0.057	0.337
Control	80	11.6	6.2	0.042	0.197

Values represent mean of two replicates with 10 seeds per replication

However at 20°C, isolate A-25 (23.7 mg/100 ml) exhibited maximum P-solubilization followed by A-13, A-36, A-43, A-12, A-22, A-29, A-3 and A-6 (21.1 to 7.0 mg/100 ml) after 6 days of incubation (Table 3). Two of the isolates (A-15 and A-34) however, exhibited maximum phosphate solubilization after 9 days of incubation (14.2 to 15.3 mg/100 ml). Thus, a degree of variability in the relative P-solubilization potential of rhizobacterial isolates was observed and found to be temperature dependent.

Indole acetic acid production

A total of 43 selected isolates of *Pseudomonas* (twenty), *Bacillus* (nineteen) and *Serratia* (four) were tested for the quantitative estimation of IAA in the presence and absence of tryptophan. IAA ranged from 2.12 to 43.24 µg/ml after 5 days of incubation at 10°C whereas at 20°C IAA production ranged from 2.40 to 68.36 µg/ml after 5 days of incubation (Plate 4). IAA production by potent rhizobacterial isolates was more at 20°C than at 10°C. Surprisingly, four isolates A-3, A-17, A-11, and A-29 showed higher IAA production at 10°C as compared to 20°C (Table 4).

Production of siderophore

In our study, we found that 27 of the 43 isolates exhibited formation of orange-halos

on CAS agar, reflecting the transfer of ferric ions from the medium to the siderophores. The isolate A-6 being the highest producer (3.4 cm), followed by A-12 (3.1cm) and A-36 (3.0cm) (Plate 5). Among 27 rhizobacterial isolates, siderophore producing isolates were further tested for catecholate type of siderophores. Out of the 27 isolates producing orange halo on CAS plates, 10 tested positive for production of catechol-type siderophore (29.0 to 90.5 µg/ml) at 10°C and (69.6 to 250.4 µg/ml) at 20°C (Table 5). Maximum siderophore production was shown by A-6 (250.4 µg/ml and 90.5 µg/ml) and A-43 (225.9 µg/ml and 80.6 µg/ml) at both temperatures. Isolates A-36, A-13, A-12 and A-2 (117.9, 110.6, 105.2 and 98.3 µg/ml) were maximum producers at 20°C, whereas at 10°C, isolate A-13, A-12, A-36 and A-43 (76.3, 71.9, 64.1 and 80.6 µg/ml) were maximum producers.

Gibberellic acid production

On the basis of zinc and phosphate solubilising ability, 18 rhizobacterial isolates were estimated for gibberellic acid production (10 and 20°C). All the selected isolates showed gibberellic acid production (Table 6), however, relative variation in GA production ranged from 11.2 to 60.7 µg/ml at 10°C and 18.8 to 113.4 µg/ml at 20°C. The maximum gibberellic acid produced was recorded in the case of A-43 (60.7 µg/ml) followed by A-13, A-36 and A-12 (50.5, 43.4 and 43.3 µg/ml) at 10°C. Whereas, at 20°C isolate A-13, A-43, A-12, A-25 and A-10 (113.4,104.8, 97.6, 86.0 and 80.4 µg/ml) were maximum GA producers.

Intrinsic Antibiotic Resistance (IAR) spectra of rhizobacterial isolates

The four rhizobacterial isolates were tested for their reactivity to antibiotics with the understanding that isolates having resistance against large number of antibiotics may compete well in soil and may have broader role in disease resistance.

Rhizobacterial isolates A-43, A-36, A-13 and A-12 were found to be resistant to ampicillin and Tetracycline. Isolate A-13 and A-12 were found to be resistant against streptomycin whereas A-36, A-13 and A-12 were found to be resistant against carbenicillin. However, the isolates A-43 and A-12 were found to resistant against Kanamycin.

Effect of potent rhizobacterial isolates on germination and seedling growth of wheat under axenic conditions

Germination tests were carried out to determine the effect of inoculation with zinc solubilizing rhizobacteria on seed germination. It revealed that under *in vitro* conditions, seed treatment with PGPR strains improved seedling germination, vigor, emergence and seedling stand as compared to uninoculated control. Maximum germination was recorded with A-43 and A-13 (85%) followed by A-36 (80%) which was at par to control (Plate 6).

The increase may be attributed to the IAA producing potential of these two isolates. The improvement in seed germination by PGPR was also reported by Shaukat *et al.*, (2006), they observed that some PGPR increased seed emergence, in some cases achieving increases up to 100% greater than controls. Isolate A-43 recorded increased root length upto 16cm, followed by A-36, A-13 as compared to uninoculated control (11.6 cm).

Maximum root fresh weights was also recorded with A-36 (0.081mg/seedling) and A-43 (0.073 mg/seedling) as compared to uninoculated control (0.042 mg/seedling) (Table 7). This could be due to the plant growth promoting traits acting in synergism.

The maximum increase 53.2% in shoot length of wheat seedlings was observed in response to inoculation with rhizobacterial isolate A-43, followed by A-13 and A-36 (46.8 and 40.3%). However, maximum shoot fresh weight was found by isolate A-36 (0.41 mg/seedling) followed by A-43, A-13 (0.38 and 0.33 mg/seedling), respectively as compared to control (0.19 mg/seedling). In confirmation to our observations, Ryu *et al.*, (2003) also observed that PGPR treatment increased germination rate and root/ shoot growth, while Dal-Bello *et al.*, (2002) observed that seed bacterization proved a successful method for enhancing biological control of plant disease.

Application of plant growth promoting rhizobacteria, can be a useful component to overcome micronutrient deficiencies and promote plant growth. This study has revealed the multiple abilities of plant growth promotion by low-temperature tolerant rhizobacteria isolates (A-43, A-13, A-12, and A-36). Axenic germination assay revealed the enhancement in seedling emergence, root and shoot length, and weight by A-43, A-36, and A-13. On the basis of their multiple PGP traits (zinc and phosphate solubilization, IAA equivalent, Gibberellic and Siderophore production) and their potential to enhance growth parameter under axenic conditions, it would be prudent to the evaluate these as biofertilizers seeds or soil drench to test for improvement of nutritional conditions and ultimately growth and yield of wheat crops under field conditions.

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