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SALT-TOLERANT PGPR STRAIN *PLANOCOCCUS RIFIETOENSIS* PROMOTES THE GROWTH AND YIELD OF WHEAT (*TRITICUM AESTIVUM* L.) CULTIVATED IN SALINE SOIL

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Abstract

Rhizobacteria improve plant growth employing a variety of growth promoting mechanisms including nutrient up-take, root growth, proliferation and biocontrol activities. Present study characterizes a salt-tolerant, IAA producing, phosphate solubilizing bacterial strain SAL-15 containing ACC-deaminase activity and evaluates its potential for enhancing growth and yield of wheat (*Triticum aestivum* L. var. TJ-83) under salinity stress *In vitro* and *In vivo*. The bacterium was identified as *Planococcus rifietoensis* based on 16S *rRNA* sequence analysis. It was catalase/oxidase positive, Gram-positive, rod-shaped, orange colored alkaliphilic bacterium, able to grow up to 65 g/L NaCl salinity in the medium. The bacterium produced 264.2 µg/mL IAA in the tryptophan-supplemented medium, released 16.7 µg/mL phosphorus from inorganic-tricalcium phosphate in the Pikoviskaya's medium and utilized ACC as nitrogen source at 100 as well as 300 mM NaCl concentration in respective media. Salinity severely reduced various growth and yield parameters of wheat (up to 60%) both in pot and field experiments. However, SAL-15 inoculation enhanced growth and yield by alleviating the toxic effects of salinity. Inoculation of SAL-15 resulted in 37% increase in overall plant growth under salt stress, 63% in the presence of inorganic tri-calcium phosphate and >60% in the presence of ACC. Based on the results, we conclude that bacterial isolate SAL-15 can be used as potent bacterial inoculum for yield improvement of wheat under salinity stress.

Introduction

Salinity is a severe problem for temperate and tropical agriculture system affecting 20% of global agriculture land (Mayak *et al.,* 2004). The harmful effects of presence of salts in soil result in increased level of ethylene in root, ionic imbalance and hyper-osmotic condition in plants (Niu *et al.,* 1995; Zhu *et al.,* 1997; Mayak *et al.,* 2004). Pakistan is situated in arid and semi-arid region where high evapo-transpiration results in accumulation and deposition of salt contents on the soil surface. Precipitation, water logging, poor drainage and clearing of trees are the major factors contributing to soil salinity (Measham, 2009). Physical removal of salts from the surface of soil or chemical treatment of soil is not only expensive but can't be applied to vast areas for soil reclamation purposes. The solution lies with using phytoremediation (*i.e*., using the halotolerant plants) or bioremediation (using the salt tolerant bacteria) for reclamation of salt affected soils on large scale.

Wheat (*Triticum aestivum* L.) is the main staple food of Pakistan as well as half of the world. Although the salt shows negligible effects on seed germination and seedling growth but salt sensitivity of wheat is well documented on plant dry weight and biomass as the major energy of the plant is utilized to maintain the osmotic balance under salt stress (Jamal *et al*., 2011; Saqib *et al*., 2012). Plant growth promoting rhizobacteria (PGPR)-induced plants salt stress tolerance has been well studied and is considered to be the cost-effective solution to the problem. PGPR isolated from saline soils improve the plant growth at high salt (Mayak *et al.,* 2004; Yildirim & Taylor, 2005: Barassi *et al.,* 2006). These PGPR tolerate wide range of salt stress and enable plants to withstand salinity by hydraulic conductance, osmotic accumulation, sequestering toxic Na+ ions, maintaining the higher osmotic conductance and photosynthetic activities (Dodd & Alfocea, 2012). The bacteria obtained from saline environment (Quesada *et al.,* 1984; Moral *et al,* 1988) include *Flavobacterium*, *Azospirillum*, *Alcaligenes*, *Acinetobacterium, Pseudomonas*, (Rodriguez *et al.,* 1985; Reinhold *et al*., 1987; Moral *et al.,* 1988; Ilyas *et al*., 2012), *Sporosarcina, Planococcus* (Ventosa *et al*., 1983), *Bacillus* (Upadhyay *et al*., 2009) *Thalassobacillus*, *Halomonas*, *Brevibacterium*, *Oceanobacillus*, *Terribacillus*, *Enterobacter*, *Halobacillus*, *Staphylococcus* and *Virgibacillus* (Roohi *et al*., 2012).

Ethylene is the plant growth regulating hormone produced in response to water logging (Grichko & Glick, 2001), salinity and/or drought (Kausar & Shahzad, 2006; Nadeem *et al.,* 2007; Zahir *et al.,* 2007). PGPR from stressed environment exhibit 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity (Glick *et al.,* 1998; Arshad *et al.,* 2007) which reduces the level of ACC and endogenous ethylene (Glick *et al.,* 1998; Yuhashi *et al.,* 2000) mitigating the deleterious effects of stress on over all plant growth (Ligero *et al.,* 1991; Hirsch & Fang, 1994). The plants inoculated with PGPR having ACC-deaminase are relatively more tolerant to environmental stress (Naveed *et al.,* 2008).

Main objective of this research was to focus on the evaluation of the bacterial strain SAL-15 to stimulate salinity tolerance and promotion of wheat growth and identification of this bacterium using phenotypical and16S *rRNA* sequence analysis. This study will help to device a basis to find out and use of PGPR to improve the plants tolerance in stress environment especially salinity and promote their growth particularly in wheat which is a major staple crop throughout the world.

Materials and Methods

Strain isolation, growth and salt tolerance: The bacterial strain SAL-15 was isolated from rhizosphere soil of wheat growing at high saline and alkaline environment (Table 1) of Biosaline Research Station, Pakka Anna, Faisalabad using dilution plating technique (Somasegaran & Hoben, 1994) and purified by sub-culturing at 28±2°C for 24h. The soil pH was 8.8; EC 9.46 mS/cm and sandy clay loam. Salt tolerance was tested in LB-broth supplemented with 600 mM NaCl. Halophilic nature was tested on halophilic media supplemented with 65 g/L NaCl salt while alkaliphilic nature was tested on solid alkaliphilic medium as described by Akhtar *et al.,* (2008). The appearance of growth on plates and or liquid medium was considered as salt or pH tolerance ability of bacterium.

Characterization and identification of strain SAL-15: Colony morphology, size, color, shape, gum production and growth pattern were recorded after 24 h growth on LB agar plates at 28±2°C. Cell size and motility was observed under light microscope. Acid/alkali production was tested on LB-agar plates containing pH indicator bromothymol blue (0.025% w/v). Gram's reaction was checked as described earlier (Vincent, 1970). Amino-peptidase and cytochrome oxidase tests were performed by using commercially available strips (Merck, Germany) while catalase production was checked by adding a drop of H_2O_2 on bacterial colony on glass slide. Resistance to antibiotics ampillicin (10 µg), gentamycin (10 µg), streptomycin (10 µg) and neomycin (30 µg) was determined on solid antibiotic sulphonamide sensitivity test agar (Merck, Germany) plates using commercially available discs (Bioanalyse® Turkey). The utilization of different carbon sources and enzymatic reactions were performed using the QTS-24 kit (DESTO, Karachi) following the manufacturer's protocol.

Total Genomic DNA of strain SAL-15 was isolated by the alkaline lysis method (Maniatis *et al.,* 1982) and used to amplify the 16S rRNA gene with primers P1 (F) and P6 (R) as described by Tan *et al.,* (1997). Polymerase chain reaction was carried out in thermal cycler (Eppendorf, Germany) as described by Imran *et al*., (2010). Amplified PCR product was purified using QIAquick PCR purification kit (Qiagen, USA), ligated in TA cloning vector pTZ57R/T (Fermentas) and cloned in *E. coli* strain DH5α as described by Maniatis *et al.,* (1982). Cloned PCR product was sequenced commercially from Macrogen (Korea). The gene sequence was compared with others in the GenBank database using the NCBI BLASTn. Multiple sequence alignments were performed by ClustalX and phylogeny was determined by neighbor-joining method.

Table 1. Chemical properties of water and soil samples from Biosaline Research Station, Pakka Anna, Faisalabad.

Character	EC (mS/cm)	pH	Macronutrients (meq/L)						SAR	RSC	Available nutrients (kg/ha)		
			CO_3	HCO_3	Cl^-	Ca^+	Na^+	K^+	(mmol L^{-1})		N	P	K
Soil	7.63	8.25	4	17	48	3	82.93	0.3	40.5	6.4	237	195	325
Water	6.12	8.78	6.12	11.75	23	4	75.13	ND	37.6	11.8	ND	ND	ND

ND= Not-determined

Assays for plant growth promoting abilities

Acetylene reduction assay: Nitrogenase activity was determined through acetylene reduction assay (Hardy *et al.,* 1968). One hundred (100) µL of bacterial culture (at early logarithmic period) was inoculated into 28 mL McCartney vials containing 8 mL semi solid CCM and incubated at 28±2°C for 72-96 h. Two mL air was replaced with 2 mL acetylene gas and kept at 28±2ºC overnight. Reduction of acetylene to ethylene was checked on a gas chromatograph (Thermoquest trace) equipped with a hydrogen flame ionization detector by injecting 20 µL gas sample from vial. The nitrogenase activity was calculated in nmol/ vial/ 24 h as described by Somasegaran & Hoben (1994) by measuring acetylene and ethylene (Somasegaran & Hoben, 1994).

Production of indole-3-acetic acid: Production of indole-3-acetic acid (IAA) was tested by colorimetric method (Gordon, 1951) and quantified by growing bacterium for 7 days in LB-broth supplemented with 100 mg/L tryptophan as precursor of IAA. For estimation of IAA in the presence of salt, LB-tryptophan was supplemented with different concentrations of NaCl (*i.e.*, 100-1100 mM). Seven days grown culture was centrifuged at 10,000 rpm. Supernatant was acidified (up to pH 2.8) with hydrochloric acid and extracted twice with equal volume of ethyl acetate (Tien *et al.,* 1979). The ethyl acetate extracts were air-dried, re-collected in ethanol and analyzed using high-performance liquid chromatograph at a flow rate of 0.5 ml/min on C-18 column. The data was analyzed using Turbochrom software (Perkin Elmer, USA).

Solubilization of tri-calcium phosphates and zinc oxide: Aliquots (10 µL) of overnight grown SAL-15 culture in LB, were spot inoculated onto Pikovskaia's agar (Sigma) containing tri-calcium phosphate as insoluble P source and LGI medium (Cavalcante & Dobereiner, 1988) containing 0.1% zinc oxide as insoluble zinc source. For salt supplementation, 200, 300, 400, 500 and 600 mM NaCl was added in both media individually. The plates were incubated at 28±2°C for 10-14 days and examined daily for the formation of clear zone around the bacterial growth. The appearance of clear zone was considered as positive for phosphate and zinc solubilization activities. Total solubilized phosphate was measured by using Phosphor-molybdate blue color method (Murphy & Riley, 1962). Duplicate 100 mL samples of liquid Pikovskaia's medium supplemented with tri-calcium phosphate, or un-supplemented (control) were inoculated with an overnight grown pre-culture of SAL-15 and grown with constant shaking for 12 days. The available phosphorous was determined in cell-free supernatant by using spectrophotometer (Camspec M350) at 882 nm using standard phosphate Curve (Halder *et al.,* 1990).

Utilization of ACC as sole nitrogen source: The ability of bacterial strain SAL-15 to use ACC as a nitrogen source was tested in 5 mL DF salt minimal medium (Penrose & Glick, 2003) containing 3 µL of 0.5 M ACC. The ACC-deaminase activity was stimulated by constant shaking of the bacterial culture at 160 x g for 24 h at 28±2^{o}C. ACC-deaminase activity was also checked at high salt concentration by growing bacterium in DF-medium supplemented with ACC and different concentrations of NaCl (200, 300, 400, 500 and 600 mM).

Wheat inoculation experiments

Preparation of inoculum and seed coating: Seeds of wheat variety TJ-83 were obtained from Agriculture Research Station, Tandojam, Sindh. SAL-15 was grown overnight in LB broth at 28±2°C with constant shaking. Cells were harvested by centrifugation and re-suspended in normal saline to get an optimum growth (OD 10^8 cells per mL at λ_{600}). Seeds were constantly shaken along-with the bacterial suspension with continuous addition of the sterile carrier material until the seeds become coated with a thin film of bacterial suspension and carrier material. Coated seeds were air-dried before sowing.

Pot trials: Pot experiments were conducted by employing Completely Randomized Design (CRD). Seeds of TJ-83 were surface sterilized with 0.1% $HgCl_2$ for 2 min and washed with sterilized water. Seeds were germinated in dark at 20±2^{o}C on water-agar plates and transplanted to pots after 2 days of germination. SAL-15 was grown overnight in LB-liquid and 1mL culture was directly applied to each of the seedling base 2 days after transplanting. The sand was sterilized by autoclaving thrice before the experiment while natural soil was used without sterilization. The plants were maintained in growth room. Hoagland solution was provided whenever required (Hoagland, 1950).

Trial 1: This experiment was conducted in falcon tubes containing sand salinized thrice with 6 days intervals (@ 300 mM each). First salinization was done before seed sowing while 2nd was done at 6-days old seedling stage and 3rd was done at 12days old seedlings. Hoagland solution and saline water were alternatively provided to plant whenever required. The plants grown without sand salinization were used as positive control.

Trial 2: The experiment was carried out in (9 cm diameter) small pots containing sterilized sand (410 g/pot) containing inorganic tri-calcium phosphate. Three gram tri-calcium phosphate (dissolved in water) was supplied to each pot. The pots without bacterial-inoculation and without phosphorus were used as negative control.

Trial 3: The experiment was conducted in (8 cm diameter) plastic pots containing 31 g natural saline soil from Biosaline Research Station, Pakka Anna. ACC was added @ 3 mM g^{-1} in each pot and plants were inoculated as described earlier. The pots without bacterial-inoculation were used as negative control.

Field trials: Two year consecutive (2008-09; 2009-10) field experiments were designed in a randomized complete block design (RCBD) with three replications in field at Pakka Anna. Soil and water characteristics are mentioned in Table 1. There were total 3 treatments (T1-full N+PK, T2- ½ N+PK, T3-SAL-15+ ½ N+PK) each with 3 replicates in a plot size of 25 m^2. The fertilizer phosphorus and potassium (Engro Chemicals, Pakistan) were added as per recommended rate (*i.e*., 83.98 and 61.75 kg/ha, respectively) during the preparation of field in all the plots. In full N plots, nitrogen was added at recommended rate *i.e*., 177.84 kg/ha while in ½ N plots @ 88.92 kg/ha. Seed was bacterized with SAL-15 inoculum (for T3) and sown @ 74 kg seeds /ha. The crop was irrigated (brackish water) three times during growth.

Measurements and data analysis: Data were recorded from five plants of each replicate, 30 days after planting from pots and 130 days after planting from field. The data were subjected to analysis of variance (ANOVA) with replicates using computer statistical program M-Stat C (Freed & Eisensmith, 1986), and differences among various treatment means were compared by least significant differences test (LSD) at 5% probability level (Steel & Torrie, 1984). Graphs were constructed using Microsoft Excel (2007) and assembled using CorelDraw (R 12).

Results

Characterization and identification of SAL-15: The strain SAL-15 was identified as member of the genus *Planococcus* on the basis of morphological data (Table 2). To further confirm, 16S *rRNA* gene sequence of the strain SAL-15 was analyzed. An amplicon of 1513 bp obtained with primers P1 and P6 was sequenced and homology was searched in NCBI. The BLASTn search indicated that the strain SAL-15 shared 99% homology to the 16S *rRNA* sequence of bacterial strain *Planococcus rifietoensis* 16S *rRNA* isolate Z19-2zhy (AM411996). Such high homology values confirmed that SAL-15 was a *Planococcus rifietoensis* strain as sequence homology above 98% shows the specie similarity (Stackebrandt & Gobel, 1994). Accession number for SAL-15 16S *rRNA* obtained from GenBank was HE573181. The phylogenetic analysis of this strain along with other members of family *Planococcaceae*, showed a clear evolutionary relationship of this strain to the rest of the members of the family (Fig. 1).

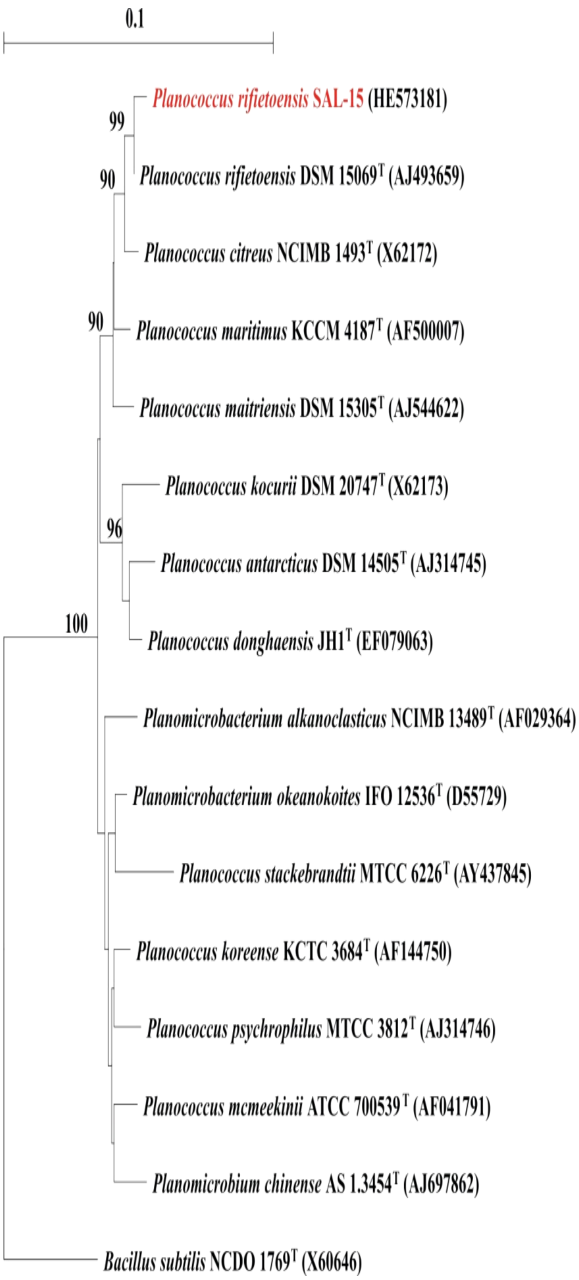

Fig.1. Phylogenetic tree based on 16S rRNA gene sequence of SAL-15 (1513 bp) constructed by Neighbour-joining method. Numbers at branching points are bootstrap values > 70% (1000 re-samplings). The accession numbers of the bacterial strains are mentioned in parentheses.

Assay for characteristics associated with plant growth promotion potential: Indole acetic acid production in tryptophan supplemented medium was observed up to 264.2 µg/mL. SAL-15 was able to produce IAA at 300 and 400 mM salt concentration as determined by development of pink color. P-solubilization data showed that SAL-15 released 16.7 µg/mL phosphorus in Pikoviskaya's medium without additional salt while 16.2 µg/mL in Pikoviskaya's medium supplemented with 300 mM additional salt NaCl. It utilized ACC as sole nitrogen source in normal as well as under salt stress condition (300 mM NaCl). The bacterium did not show nitrogenase enzyme activity (acetylene reduction assay) when grown on NFM and Zn solubilization ability on LG1 medium (Table 2).

Plant growth promotion in pots: The data showed that due to the salinity stress, a decrease of 32% in plant height, 17% in shoot fresh weight, 25% in shoot dry weight, 9% in root length, 24% in root area and 54% in root dry weight was observed in wheat variety TJ-83. SAL-15 mitigated the deleterious effects of salt and showed 63% increase in plant height, 59% in shoot fresh weight, 55.5% in shoot dry weight, 171% in root length, 12% in root area and 80% in root dry weight as compared to un-inoculated. In non-saline control, the inoculation of SAL-15 resulted in increased plant height (up to 57%), plant fresh (47.8%), shoot dry weight (41%), root area (28.5%) and root length (22.6%) as compared to non-saline un-inoculated control plants (Table 3).

The experiment conducted to evaluate the mobilization of inorganic phosphate showed that SAL-15 performed significantly better in increasing the shoot and root growth of the inoculated plant by helping the mobilization of inorganic P present in the plant rhizosphere or root zone. Maximum shoot length (28cm), shoot fresh weight (0.72g), shoot dry weight (0.20 g), root length (24.6 cm), root fresh weight (1.6g) and root area (25.92 cm^2) were observed in the plants inoculated with SAL-15 (Table 3; Fig. 2) and provided with inorganic phosphate which were significantly higher than both un-inoculated control plants (with or without inorganic P).

The data showed that ACC-deaminase containing SAL-15 which is also an IAA producing strain increased root and shoot growth and plant biomass under salt stress in the presence of ACC. Inoculated plants showed 71% increase in plant weight, 94% in root length and 183% in shoot length than un-inoculated control plants (Fig. 3).

Plant growth promotion in fields: The filed experiments conducted at Biosaline Research Station, Pakka Anna showed that on the average SAL-15 inoculation resulted in increased plant growth and yield when used along-with ½ N fertilizer. The increase recorded was; 37-29% in plant height, 5.7-12% in 100 grain weight, 21-36% in biomass, 58-50% in straw weight, 113-38% in grain weight during the years 2008-09 and 2009-10, respectively. The grain yield and biomass of wheat plants inoculated with SAL-15 was significantly better as compared to respective non-inoculated control plants in both the years (Fig. 4). The results showed that in fully-fertilized control plants, biomass was high and grain yield was low while addition of halotolerant PGPR with half fertilization exhibited higher grain yield as compared to biomass.

Table 2. Physio-chemical characteristics of *Planococcus rifietoensis* strain SAL-15 isolated from wheat rhizosphere from Biosaline Research Station, Pakka Anna.

Character studied	SAL-15	Character studied	SAL-15
Colony morphology:		Resistant to antibiotics (10 µg):	
Colour	Orange	Streptomycin	No
Shape	Round	Gentamicin	No
Size	Medium	Ampicillin	No
Appearance	Shinning	Neomycin	Yes
Margins	Entire		
Cell morphology	Rods	Production of IAA:	
Catalase and Oxidase test	Positive	At 100 mM	Yes
Gram staining	Positive	At 300 mM	Yes
P-Solubilization:		Nitrogenase activity:	
At 100 mM	Yes	At 100 mM	No
At 300 mM	Yes	At 300 mM	No
Zn-Solubilization:		ACC-deaminase activity:	
At 100 mM	No	At 100 mM	Yes
At 300 mM	No	At 300 mM	Yes
Production of acid from:		Production of acid from:	
Melibiose	+	Succinate	-
Raffinose	-	Glucose	+
Inositol	+	Mannitol	+
Adonitol	+	Arabinose	+
Maltose	+	Rhamannose	+
		Sorbitol	+
Production of H_2S	-	Fermentation of sodium malonate	-
Lysine decarboxylase	-	Production of ß-galactosidase	-
Arginine dihydrolase	-	Utilization of sodium citrate	UI
Ornithine decarboxylase	-	Urea hydrolysis	-

+ = Shows reaction is positive for the said test, - = shows reaction is negative for the said test
UI= the result of the reaction cannot be identified as positive or negative

Table 3. Response of wheat variety TJ-83 towards inoculation with *Planococcus rifietoensis* strain SAL-15 in salinized sand (Trial 1) and tri-calcium phosphate (Trial 2) in pots.

Treatments	Trial 1					
	Plant height (cm)	**Shoot fresh weight (g)**	**Shoot dry weight (g)**	**Root length (cm)**	**Root area (cm^2)**	**Root dry weight (g)**
Un-inoculated control	12.2 ± 1.4^{b}	0.46 ± 0.16^{b}	0.12 ± 0.00^{b}	4.6 ± 1.3^{c}	4.9 ± 1.5^{b}	0.22 ± 0.07^{a}
SAL-15	19.2 ± 4.1^{a}	0.68 ± 0.07^{ab}	0.17 ± 0.02^{ab}	15.0 ± 1.4^{a}	6.3 ± 0.43^{a}	0.22 ± 0.07^{a}
NaCl control	8.2 ± 1.16^{c}	0.38 ± 0.07^{a}	0.09 ± 0.18^{a}	4.2 ± 1.4^{c}	3.7 ± 0.17^{c}	0.10 ± 0.05^{ab}
SAL-15+NaCl	13.4 ± 1.8^{ab}	0.54 ± 0.101^{a}	0.14 ± 0.03^{a}	11.40 ± 1.0^{ab}	4.2 ± 0.07^{b}	0.18 ± 0.07^{ab}
LSD	**0.0016**	**0.0152**	**0.0152**	**0.0000**	**0.0002**	**0.0708**
	Trial 2					
SAL-15 +P	28 ± 3.6^{a}	0.72 ± 0.18^{a}	0.20 ± 0.06^{a}	24.6 ± 5.8^{a}	25.92 ± 8.6^{a}	1.6 ± 0.55^{a}
P+control	24 ± 3.6^{ab}	0.45 ± 0.35^{a}	0.11 ± 0.08^{a}	14.6 ± 3.9^{b}	19.96 ± 5.9^{b}	1.1 ± 0.97^{ab}
SAL-15 –P	27 ± 1.6^{a}	0.62 ± 0.11^{a}	0.18 ± 0.04^{a}	23.4 ± 6.9^{a}	26.80 ± 3.9^{a}	1.5 ± 0.17^{a}
P zero control	22.2 ± 4.4^{b}	0.40 ± 0.12^{a}	0.10 ± 0.03^{a}	12.8 ± 2.0^{b}	16.18 ± 3.2^{c}	1.1 ± 0.5^{ab}
LSD	**0.6516**	**0.8290**	**0.8290**	**0.0053**	**0.0061**	**0.0003**

values with the same letter within column indicate non-significant difference among treatments with $p \geq 0.05$

Discussion

For growth promotion and induction of resistance in many crops, the effectiveness of PGPR has been well documented (Hafeez *et al.*, 2006; Haq *et al*., 2012). The experimental results reported here show the likelihood of use of PGPR for growth and yield improvement of wheat which is the most important staple crop of world. We have shown that the strain SAL-15 indigenous to highly saline soils of Pakka Anna (Faisalabad) is capable to protect wheat against salt stress. After inoculation with the strain SAL-15, plant height and biomass were significantly improved as compared to the un-inoculated plants both under growth room and field experiments (Table 3; Fig. 2, 3, 4). A significant increase in growth of inoculated treatment promises the practical application of this strain. PGPR have been reported to have potential to promote growth in many crops like barley, sorghum, tomato, (Baldani *et al.,* 1986; cotton (Hafeez *et al*., 2004; Yasmin *et al*., 2013), maize (Naureen *et al*., 2005) and rice (Mehnaz *et al*., 2001).

The strain SAL-15 was identified as *Planococcus rifietoensis* on the basis of 16S *rRNA* sequence analysis. Genus *Planococcus* is known to present in diverse environments, including soil, sediments, seawater, fish and cyanobacterial mats (Hao & Komagata, 1985; Reddy *et al*., 2002; Alam *et al*., 2003; Romano *et al*., 2003; Yoon *et al*., 2003; Mayilraj *et al*., 2005). There have been increasing reports of the presence of this genus in the rhizosphere and plant growth promotion of plants like rose (El Deeba *et al*., 2011) and salicornia rhizosphere (Rueda-Puente *et al*., 2011). *P. halophilus* and *P. rifietoensis* have been reported as halophilic bacteria from this genus. *P. rifietoensis* has been known to contain multiple plant growth promoting traits *e.g*., nitrogen fixation, production of IAA, chitinase, cellulase, lipase and protease (Siddikae *et al*., 2010).

SAL-15 was found to be halophilic and alkaliphilic bacterium having multiple plant growth promoting traits. Our study showed that SAL-15 have an inherent ability to produce IAA as well as solubilization of inorganic phosphate. Moreover, the utilization of ACC as sole nitrogen source makes SAL-15 an attractive supplement for crops grown under stress as ACC deaminase activity help plants to withstand biotic as well as abiotic stress conditions (Mayak *et al*., 2004; Cheng *et al*, 2007; Zahir *et al.*, 2009). It is possible that auxin and ACC-deaminase stimulate root growth in a synchronized manner (Glick *et al*., 2007). Bacteria produce IAA to promote root growth by stimulating cell division or elongation (Patten & Glick, 2002; Glick, *et al*., 1998). In the presence of salt, bacteria showing IAA-activity without ACC-deaminase activity inhibit root growth rather than root elongation showing the importance of and higher synthesis of ACC under stress (Cheng *et al.*, 2007).

In conclusion, this study has demonstrated that halophilic bacterium SAL-15 isolated from alkali-saline soils, is able to survive high salt concentrations (65 g/L NaCl) and pH (> 9), and can improve plant growth at high salt concentration. These results further suggest that the selection and subsequent use of ACC-deaminase containing salt-tolerant bacteria, having a mixture of PGP activities may improve growth of plants in saline conditions. The study hence, recommends the great potential of using strain SAL-15 as bacterial inoculant for production of wheat biofertilizer for saline areas. Moreover, due to the presence of ACC-deaminase activity, the response of SAL-15 can be evaluated in water stress environment.

P + control (un-inoculated) SAL-15 + P (inoculated)

Fig. 2. Effect of inoculation with *Planococcus rifietoensis* strain SAL-15 on wheat growth in the presence of insoluble tri-calcium phosphate.

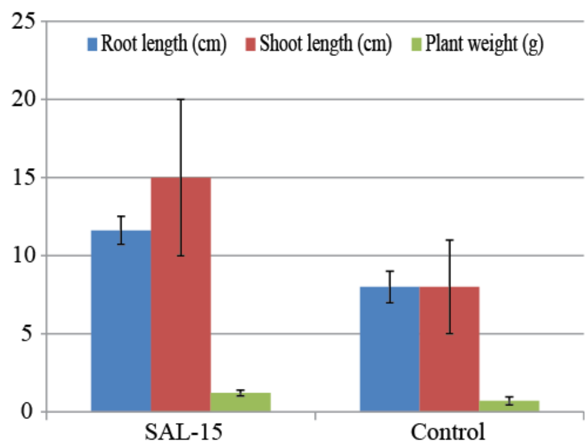

Fig. 3. Effect of inoculation with *Planococcus rifietoensis* strain SAL-15 on growth of wheat in the presence of ACC.

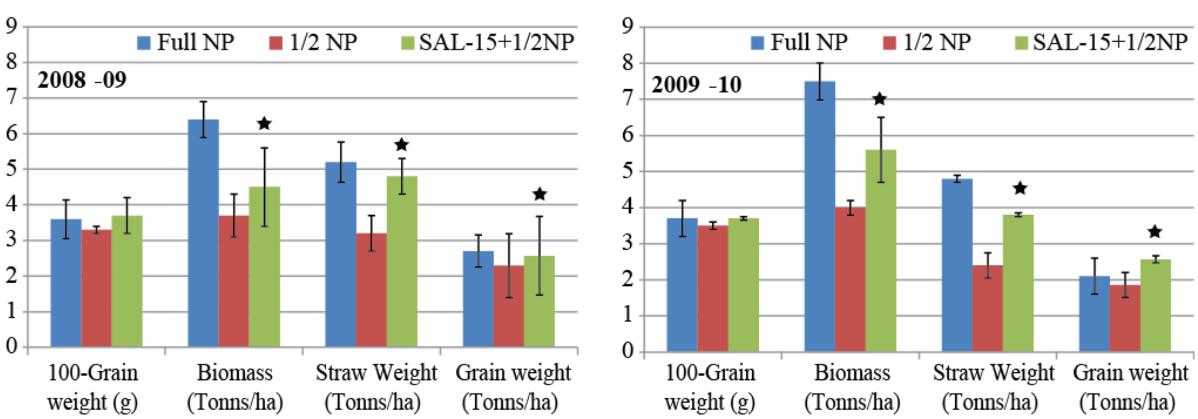

Fig. 4. Response of wheat variety TJ-83 towards bacterial inoculation in field condition at Pakka Anna.
★Represents the values are significant at LSD 0.05. 100 grain weight is represented in grams while biomass, straw weight and grain weight are represented in kg/ha.

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