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Full Research Article

ACC-deaminase and EPS Production by Salt Tolerant Rhizobacteria Augment Growth in Chickpea under Salinity Stress

Poonam Kumari[1*] and Veena Khanna[2]

[1]Dept. of Microbiology, [2]Dept. of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, Punjab (141 004), India

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Correspondence to

E-mail: poonam15sep@gmail.com

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Abstract

Application of salt tolerant rhizobacteria containing 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase and exopolysachharide activity in salt-affected soil can play an important role in alleviating soil salinity stress during plant growth. Out of 66 rhizobacterial strains screened for ACC-deaminase activity, 29 isolates exhibited growth in DF minimal salt medium supplemented with ACC. In liquid medium, ACC-deaminase positive isolates showed different efficacy to utilize ACC as a sole source of N and their growth ranged from 0.205-0.774 (OD at 600 nm) as compared to negative control (0.025-0.285). Among these, 10 potent isolates tolerant to 0.6M NaCl concentration were evaluated for ACC-deaminase activity and EPS production at different salinity levels (0.2, 0.4, 0.8, 1.0 and 1.2 NaCl). The three most efficient salt-tolerant ACC deaminase and EPS containing isolates, B20b, B20d and B-I were investigated alone and in combination with chickpea nodulating, *Mesorhizobium ciceris* for their effects on the germination and growth of chickpea under various salinity levels (0, 30, 60, 90 and 120 mM NaCl). The results showed that salinity stress significantly reduced plant growth but inoculation with PGPR enhanced plant growth especially at salinity levels ranging from 30-90 mM NaCl, thus reducing the inhibitory effect of salinity. Combined application of PGPR and *Rhizobium* was more effective under saline conditions, and the combination B-20d+R was the most efficient for improving seedling growth. Biochemical analysis showed higher accumulation of total sugars and protein in response to salinity stress as a protective mechanism to maintain plant turgor required for growth.

1. Introduction

Crop production environments and their associated factors often impose varying levels of abiotic stresses on crops and thereby prevent the full realization of the genetic potential of the plant in terms of yield and quality (Selvakumar et al., 2012). Among various abiotic stresses, salinity is the major environmental stress that adversely affects plant growth and development. High soil salinity stimulates biosynthesis of ethylene by using 1-aminocyclopropane-1-carboxylic acid (ACC) as a precursor, and hence called salt stress-induced ethylene (Chookietwattana and Maneewan, 2012). Numerous reports have documented that ethylene helps in inducing multifarious physiological changes in plants at molecular level but at higher levels is usually deleterious, as it induces defoliation, changes cellular processes leading to growth inhibition, premature senescence, restricted nodulation, all of which reduce crop yield (Lie et al., 2005). Since saline-induced stress in plants is at least partially the result of the plant's production of stress ethylene (Glick, 2014), lowering ethylene levels in plant might afford some protection against this stress and intensify agricultural production.

In this context, the concept of plant growth promoting bacteria (PGPB) containing ACC-deaminase for promotion of plant growth under environmental stress conditions has gained importance (Berg, 2009). The relation between ethylene and rhizobacterial mediated stress alleviation is provided by the enzyme ACC-deaminase which metabolizes ACC in the root of developing plants, thereby reducing the level of ethylene in stressed plant (Selvakumar et al., 2012). However, the ability of inoculated bacteria to survive and colonize the saline rhizosphere remains to be a challenge for successful application since high salinity could influence the survival, growth and activity of microorganisms (Chookietwattana and Maneewan, 2012). The salt-tolerant ACC deaminase-

containing bacteria could thus be advantageous over others to thrive in a new saline environment in the sufficient numbers to deliver beneficial effects on plants.

Besides this, some plant growth-promoting strains can produce bacterial exopolysaccharide (EPS). Production of exopolysaccharides (EPS) in many salt tolerant bacteria is a strategy for growth, adhering to solid surfaces, and to survive adverse conditions. Certainly, they possess a protective nature: the EPSs, forming a layer surrounding a cell, provide an effective protection against high salinity (Poli et al., 2010). Bacterial exopolysaccharide (EPS) can also bind cations including Na^+ thereby alleviate salinity stress by decreasing the content of Na^+ available for plant uptake. There are reports of EPS-producing plant growth-promoting rhizobacteria to significantly enhance the volume of soil macropores and the rhizosphere soil aggregation, resulting in increased water and fertilizer availability to inoculated plants (Upadhyay et al., 2011). Salt-tolerant ACC-deaminase containing plant growth-promoting rhizobacteria (PGPR) can alleviate soil salinity stress during plant growth by reducing ethylene synthesis while bacterial exopolysaccharide (EPS) will ensure its better survival in field and can also help to mitigate salinity stress by reducing the concentration of Na^+ in soil. Considering this, the present research work was framed to screen and select the efficient salt tolerant ACC deaminase and EPS producing rhizobacteria and to study its effectiveness for growth promotion in chickpea (*Cicer arietnum* L.) under in vitro saline conditions.

2. Materials and Methods

The present experiment was conducted in Punjab Agricultural University, Ludhiana, India during the year 2013 and 2014.

2.1. Isolation of rhizobacteria

Rhizosheric soil samples closely adhering to chickpea roots were collected from different sites and serially diluted 10-fold from 10^{-2} to 10^{-6}. Rhizosphere suspension was plated using Nutrient agar and Kings B as isolation media to enrich the bacterial population. Isolates having different colony morphology were picked and maintained on respective slants at 4 °C.

2.2. Screening for ACC-deaminase producing rhizobacteria

This screening procedure comprised two consecutive steps. For primary screening, bacterial isolates were grown in Luria broth medium and cell pellet collected by centrifugation, washed and resuspended in sterile water. The ACC deaminase of bacterial strains was qualitatively investigated by spot inoculating on DF salt minimal medium (Dworkin and Foster, 1958) supplemented with 3.0 mM ACC as a sole nitrogen source.

In the secondary screening step, bacterial isolates showing growth on DF plates were inoculated in 100 ml DF salt minimal medium alone, DF+ACC and DF+$(NH_4)_2SO_4$ and growth was measured at 600 nm after 72 h of incubation.

2.3. Screening for salt tolerant rhizobacteria

Isolates were screened for their salt tolerance potential by inoculating freshly grown cultures in respective broth supplemented with 0.6 M NaCl and growth was measured at 600 nm after 24 h of incubation.

2.4. Screening for salt-tolerant bacterium containing ACC deaminase

Isolates showing maximum O.D. in both the screening steps (salt tolerance and ACC-deaminase) were selected for further study. Salt-tolerant bacterial strains were investigated for their abilities to utilize ACC as a sole nitrogen source by culturing them in 100 mL of DF salt minimal medium supplemented with 3.0 m M ACC as a sole nitrogen source and NaCl over a range of 0.2, 0.4, 0.8, 1.0 and 1.2 (1% inoculum) for 72 h at 30 °C with shaking at 200 rpm. Bacterial growth was measured at 600 nm.

2.5. Screening for salt tolerant EPS producing bacteria

Rhizobacterial isolates were evaluated for EPS production by the method of De Vuyst et al. (1998). 24 h old bacterial culture ($O.D._{600}$ 0.3) were inoculated in 100 ml of medium suggested by Verhoef et al. (2003) supplemented with varying NaCl concentration (0, 0.2, 0.4, 0.8, 1.2 M), incubated at 160 rpm shaker for 48 h at 37 °C, centrifuged at optimized conditions (1000 rpm for 15 min at 4 °C) and supernatant was precipitated using 3 volumes of pre-chilled acetone. Weight of freshly precipitated EPS was taken and quantified in terms of total carbohydrate using glucose as standard.

2.6. Seed bacterization, growth and biochemical analysis

The rhizobacterial isolates were evaluated alone and in combination with *Mesorhizobium ciceris* under salinity stress condition to study their effect on plant growth. Chickpea (*Cicer arietinum*) seeds (var GPF-2) were surface sterilized by immersion in 0.1% $HgCl_2$ for 1 min, followed by 2 min in 70% ethanol, then washed three times with sterile distilled water. The disinfected seeds were immersed in the bacterial inoculum (10^8 CFU mL^{-1}) or mixture of bacteria and *Mesorhizobium ciceris* (1:1 ratio) for one hour and were used.

Germination assays were performed according to International Seed Testing Association. Three replicates of 6 seeds were germinated in sterilized Petri dishes (9.0 cm diameter) containing two sheets of filter papers moistened initially with 4 mL of sterile distilled water supplemented with salinity at 0, 30, 60, 90, and 120 mM.100 µM $CoCl_2$ was used as positive control as it is ethylene inhibitor. The Petri dishes were then

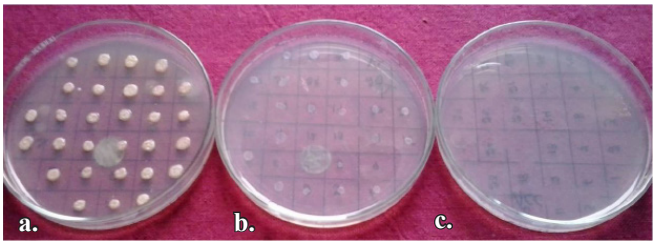
placed under artificial light which provided light intensity at 2,000 lux for 16 h daily and at a temperature of 22 °C. The germination percentage was calculated. Two weeks after germination, different parameters of growth were measured. Total soluble protein (mg g^{-1} fresh weight) in roots was determined following Lowry method (Lowry et al., 1951). Total soluble sugar per gram of fresh weight of plant was measured by the phenol-sulphuric acid method (Dubois et al., 1956) using glucose as a standard.

3. Results and Discussion

The global climate change has witnessed severe consequences especially on agricultural land resulting in great loss in food productivity. Salt stress is one of the major environmental factors limiting growth of plants in different ways depending on plant species. In response to salinity stress, ethylene is produced to a level which has deleterious effect on root growth and nodulation of legumes (Naz et al., 2009). Some PGPR are capable of lowering ethylene levels through the enzyme ACC deaminase. Exopolysaccharide production by these bacteria may also contribute to stress mitigation besides enhancing their survival and competence under salinity condition. Therefore, selection, screening and application of stress tolerant PGPRs having ACC-deaminase and EPS production ability for improved agricultural system would significantly help the farming community by overcoming such drastic climate changes.

3.1. Screening for salt tolerant ACC deaminase and EPS producing rhizobacteria

In the present study, a total of 66 bacterial isolates were isolated and conceded through a series of experiments to screen the most efficient salt tolerant ACC deaminase and EPS producing PGPR. Plate assay carried out for screening the ACC-deaminase containing rhizobacteria showed that 29 isolates (16 from Nutrient agar and 13 from Kings B medium) were able to grow on DF medium supplemented with 3 µM ACC as a sole source of nitrogen compared to DF plate where no growth was observed signifying the secretion of ACC-deaminase (Figure 1). A number of rhizobacteria belonging to genera *Bacillus, Achromobacter, Burkholderia, Enterobacter,* and *Pseudomonas* have been reported to exhibit variable ACC deaminase activity (Chookietwattana and Maneewan, 2012). In the secondary screening step, the ability of 29 rhizobacterial isolates to utilize ACC as a sole source of N with different degrees of efficacy was assessed on the basis of bacterial growth in liquid medium in terms of optical density (OD at 600 nm) which varied in the range of 0.205-0.774 as compared to negative control where no ACC was added to DF minimal media and bacterial growth ranged from 0.025-0.285 (Table 1 and 2). Maximum growth was observed with isolate B20b (OD_{600} 0.744) followed by B20d (OD_{600} 0.639). Isolate Ps14c exhibited about 18-fold increase in growth in DF medium supplemented with ACC compared to DF medium containing no N-source (Table 2). In contrast to this, isolate B20b which recorded maximum optical density at 600 nm in liquid DF medium with ACC showed only about 3-fold increase in growth compared to only DF salts medium. Similar observations have been reported by different workers. Govindasamy et al. (2009) observed that from the initial 236 bacterial isolates screened from the wheat rhizosphere, 40 isolates showed growth on DF minimal medium containing ACC. Their OD_{600} values were also higher in liquid DF medium with ACC, when compared to DF medium without any N source.



a. DF medium+Ammonia; b. DF medium+3mM ACC; c. DF medium

Figure 1: Growth of rhizobacteria on DF minimal salts medium

The salt-tolerant properties of rhizobacteria authenticate their abilities to survive and show the beneficial effects on promoting plant growth in salt-affected soils over the other ACC deaminase containing bacteria which lack salt-tolerant property. Out of 29 ACC-deaminase producing rhizobacterial isolates, 20 were found to possess osmoadaptation property and showed growth at salinity level of 0.6 M, however, considerable variation in growth rate was observed (Table 1 and 2). Maximum growth was recorded with isolate B20b (OD_{600} 0.854) followed by B20d and B-I (OD_{600} 0.777 and 0.756 respectively). Isolate Ps-14c which surpassed others in ACC-deaminse production was quite sensitive to salinity (OD_{600} 0.105). Salt tolerance is an important facet of saprophytic ability and competitiveness among rhizobacterial isolates which help them to perform well in the rhizosphere (Davey and Toole, 2000). Johri et al. (1999) isolated and characterized salinity tolerant phosphate solubilizing bacteria that could survive at 5% NaCl concentration. It has been explained that novel proteins synthesized by salt tolerant *Pseudomonas fluorescens* strain MSP-393 nullified detrimental effects of high osmolarity (Paul et al., 2008).

The subsequent work was carried out to fish-out rhizobacterial isolates and indentify the potential strains which could grow in DF salt minimal medium supplemented with ACC as a sole nitrogen source under various levels of salinity (0.2, 0.4, 0.8, 1.0 and 1.2 M). The rationale of this study was based on

Table 1: Relative efficacy of ACC-utilization and salinity tolerance by rhizobacterial isolates isolated from nutrient agar medium

Isolates	OD at 600 nm			0.6 M NaCl
	Without ACC	With 3 µM ACC	With NH_4	
B5a	0.113±0.004	0.330±0.017	1.790±0.109	0.512±0.012
B5b	0.106±0.003	0.402±0.007	1.897±0.070	0.441±0.025
B7a	0.131±0.009	0.445±0.025	1.809±0.050	0.005±0.000
B8a	0.117±0.009	0.427±0.018	1.802±0.079	0.010±0.001
B9b	0.197±0.009	0.408±0.004	1.845±0.025	0.447±0.052
B9c	0.134±0.019	0.317±0.009	1.604±0.081	0.321±0.039
B9d	0.112±0.007	0.393±0.130	1.436±0.146	0.021±0.003
B11a	0.182±0.012	0.336±0.021	1.817±0.127	0.015±0.000
B17b	0.150±0.014	0.545±0.140	1.578±0.011	0.654±0.031
B18a	0.140±0.011	0.368±0.140	1.470±0.013	0.019±0.001
B20b	0.285±0.011	0.744±0.025	1.683±0.013	0.777±0.044
B20d	0.260±0.005	0.639±0.022	1.775±0.106	0.854±0.031
B21c	0.234±0.019	0.612±0.069	1.661±0.062	0.710±0.065
B22a	0.278±0.010	0.616±0.063	1.470±0.095	0.657±0.032
B28c	0.179±0.011	0.617±0.046	1.861±0.144	0.677±0.044
B-I	0.225±0.014	0.627±0.015	1.391±0.065	0.756±0.023

Table 2: Relative efficacy of ACC-utilization and salinity tolerance by rhizobacterial isolates isolated from Kings B medium

Isolates	OD at 600 nm			0.6 M NaCl
	Without ACC	With 3 µM ACC	With NH_4	
Ps 4b	0.079±0.005	0.333±0.019	1.824±0.013	0.007±0.000
Ps7a	0.095±0.002	0.241±0.023	1.999±0.057	0.225±0.019
Ps13b	0.132±0.007	0.283±0.013	1.811±0.120	0.578±0.028
Ps14c	0.025±0.002	0.445±0.025	2.025±0.136	0.105±0.012
Ps14d	0.129±0.005	0.345±0.025	1.801±0.058	0.321±0.057
Ps15a	0.081±0.001	0.352±0.030	1.830±0.075	0.029±0.002
Ps16a	0.060±0.005	0.208±0.004	1.800±0.086	0.033±0.005
Ps19d	0.128±0.005	0.442±0.024	1.787±0.050	0.345±0.019
Ps20b	0.109±0.005	0.329±0.016	1.799±0.066	0.339±0.029
Ps24d	0.100±0.005	0.351±0.029	1.801±0.034	0.040±0.009
Ps28c	0.077±0.004	0.269±0.011	1.974±0.132	0.417±0.062
Ps29c	0.098±0.005	0.205±0.011	1.888±0.109	0.350±0.039
P-I	0.185±0.003	0.283±0.019	1.723±0.127	0.229±0.027

the hypothesis that inoculation with salt-tolerant bacterium containing ACC deaminase could lower the biosynthesis of salt stress-induced ethylene within plant which then eliminates the inhibitory effects of ethylene and subsequently enhance plant growth (Hontzeas et al., 2006). The 5 potent ACC-deaminase producing rhizobacterial isolates which exhibited optimum growth at 0.6 M salinity level (B20b, B20d, B22a, B28c and B-I) were selected to study the effect of salinity on their growth in DF medium supplemented with ACC. It was observed that growth of all the isolates decreased as concentration of NaCl increased. Four bacterial strains (B-I, B-20d, B20b and B-28c) were able to grow and utilize ACC as a sole nitrogen source up to 1.2 M NaCl concentration (Table 3). However, optimum growth was observed at the salinity ranging from 0.2-0.8 M after which it declined dramatically. Isolate B20d demonstrated the highest O.D. at the salinity ranging from 0.2-0.8 M. Isolates B20b and B28c, however, showed a striking reduction in growth at 0.8 M NaCl salinity. Similar findings have been

reported where salt tolerant and ACC deaminase containing *Bacillus* strains showed maximum OD in the range of 0.2-0.8

Table 3: Effect of different salinity levels on growth of potent ACC-deaminase producing rhizobacteria

Isolate	O.D. at 600 nm				
	0.2 M	0.4 M	0.8 M	1.0 M	1.2 M
B-I	0.515±0.008	0.368±0.023	0.301±0.009	0.047±0.002	0.014±0.001
B28c	0.266±0.024	0.109±0.025	0.087±0.001	0.059±0.002	0.006±0.001
B20d	0.792±0.042	0.537±0.021	0.491±0.023	0.097±0.001	0.039±0.002
B20b	0.637±0.021	0.461±0.024	0.125±0.014	0.083±0.002	0.010±0.001
B22a	0.329±0.016	0.116±0.009	0.060 ±0.005	0.012±0.001	0.000±0.000

M NaCl concentration grown in DF medium supplemented with ACC (Chookietwattana and Maneewan, 2012).

The potential salt tolerant ACC deaminase containing isolates were also checked for EPS production under various levels of salinity (0.2, 0.4, 0.8, 1.0 and 1.2 M). Surprisingly, the salt resistant isolates (B20d, B20b and B-I) which contained highest ACC deaminase over a range of salinity levels also produced maximum EPS (Figure 2). Among various identified EPS-producing bacterial genera, the *Bacillus* sp. are most abundant. Enhanced EPS production was observed with rise in salinity to a certain level which later on declined with further increase in NaCl concentration. However, results showed two different pattern of EPS secretion under varying salt concentration. Isolate B20d and B-I exhibited maximum exopolysachharide at 0.4 M which later declined dramatically. In contrast to this, isolate B20b showed its maximum potential to produce EPS at 1.0 M NaCl concentration. At no salt concentration all the isolates exhibited reduced EPS production. Higher EPS values at higher salt concentration shows protective mechanism employed by bacterial isolates to surpass stress condition. It has been reported that increased production of exopolysaccharide against higher salt stress also favors biofilm formation and protects this mini assembly by retaining a water layer around the cells. Plants are benefitted against stress in this protective environment as EPS can bind cations including Na+ thereby decreasing their availability to inoculated plants. EPS-producing plant growth-promoting rhizobacteria increase the volume of soil macropores and the rhizosphere soil aggregation, resulting in increased water and fertilizer availability to plants (Quresi and Sabri, 2012). The binding properties of their exopolysaccharide would cause soil particles to cement and strengthening aggregates formation (Lucy et al., 2004) and favor plant growth under salt stress.

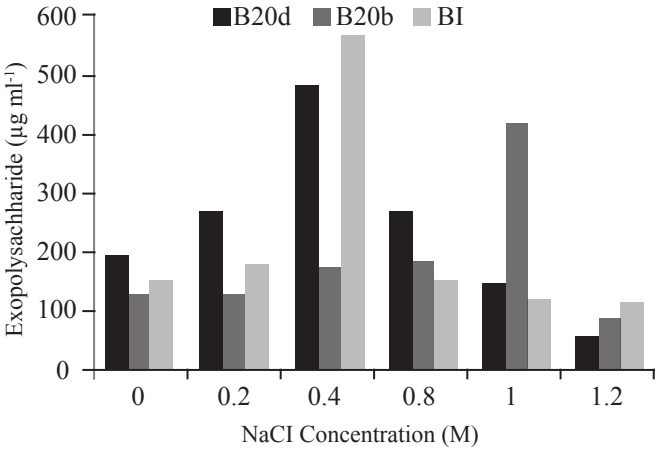


Figure 2: EPS secretion by rhizobacterial isolates under different salt concentration

3.2. Effect of Bacterial inoculation on plant growth

Salinity is one of the most common environmental stress factors that adversely affect nodulation, growth and yield of leguminous crops worldwide. Chickpea, although frequently used over the world due to its higher nutritional value, is severely affected by salt stress. Plant productivity in saline soils is considerably reduced due to nutrient imbalance (Parida and Das, 2005), osmotic stress and more importantly by induction of stress hormone i.e. ethylene. To improve plant growth under stress conditions, and for sustainable crop production, it is necessary to improve salt stress tolerance in crops. Salt tolerant rhizobacteria having EPS and ACC deaminase activity can significantly improve growth and productivity of the plants under salt stress.

The germination assay carried out for studying the effect of selected salt tolerant rhizobacteria showed that in non-inoculated plants, there was a little effect of NaCl stress up to 30 µM. However, reduction in all growth parameters of non-inoculated plants, including germination (83.3%), seedling length, fresh weight and dry weight parameters was observed at higher NaCl concentration. In all inoculated plants, growth parameters, significantly improved towards higher salinity. All the three *Bacillus* isolates, B20d, B-I and B20b improved the root fresh weight (by 71.3%, 26.14% and 28.16% respectively) (Table 4) and root length (by 36.8%, 21.67 and 26.6 % respectively) (Table 5) in germinating seedlings over uninoculated control at 60 µM NaCl concentration. Inoculation of B20d in combination with *Rhizobium* (salt tolerant)

improved the root fresh weight by 63.7% and 74.5% at no salinity stress and 60 mM NaCl concentration respectively (Table 4). In general, it was concluded that bacterial inoculation stimulated the plant growth with and without salt stress. Ahmad et al. (2011) reported that salinity stress significantly reduced plant growth but inoculation with PGPR containing ACC deaminase enhanced plant growth, thus reducing the inhibitory effect of salinity. However, their combined application was more effective under saline conditions, and the combination Mk20×M6 was the most efficient for improving seedling growth and nodulation. Increase in growth parameters at higher salinity may be attributed to ACC-deaminase activity contained by rhizobacteria as it may inhibit the negative impacts of stress hormone ethylene by decreasing its accumulation in germinating seeding. The correlation between ethylene production and seed germination or seedling growth has been well documented (Zapata et al., 2004; Glick, 2005). Nonetheless, the stress protection offered by salt tolerant ACC-

Table 4: Effect of rhizobacteria on growth parameters of plants under salinity stress

Treatments	Weight (mg plant^{-1})							
	0		30 µM		60 µM		90 µM	
	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
B20d	67.50	44.43	60.83	23.16	56.83	-	25.66	-
B20d+R	81.16	62.50	77.16	27.08	58.33	10.00	40.16	-
B-I	56.20	61.66	52.50	31.66	41.83	-	31.66	-
B-I+R	67.89	74.83	61.33	37.51	51.33	-	49.00	-
B-20b	52.00	39.65	46.83	25.15	42.5	-	27.83	-
B20b+R	60.50	85.00	51.70	38.55	49.83	16.4	30.16	-
100 µM $CoCl_2$	76.33	55.50	79.60	36.66	67.66	21.8	50.83	-
Control	49.83	32.83	41.00	18.50	33.16	-	20.16	-
CD (p=0.05)	1.91	1.06	1.23	0.96	1.15	-	1.39	-

Table 5: Effect of rhizobacteria on growth parameters of plants under salinity stress

Treatments	Length (cm plant^{-1})							
	0		30 µM		60 µM		90 µM	
	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
B20d	4.85	1.50	3.76	1.13	3.60	-	2.01	-
B20d+R	7.95	2.45	5.30	1.37	4.38	0.50	4.78	-
B-I	4.06	1.37	3.76	1.03	3.20	-	2.20	-
B-I+R	5.55	2.09	4.72	1.75	3.46	-	2.28	-
B-20b	4.06	1.96	3.61	1.37	3.30	-	2.50	-
B20b+R	5.90	2.21	4.15	1.90	3.33	1.30	2.66	-
0.1 µM $CoCl_2$	5.48	2.00	5.13	1.70	3.75	1.57	2.68	-
Control	3.96	1.10	3.03	0.70	2.63	-	1.94	-
CD (p=0.05)	0.82	NS	0.35	0.47	0.49	-	0.50	-

deaminase containing isolates was comparable to that offered by $CoCl_2$ which is a chemical inhibitor of ethylene.

Inoculation with bacterial isolates helped in development of shoot at 60 µM salt concentration compared to uninoculated control seedlings where no development of shoots was observed at this salinity level (Table 4 and 5). Our results are consistent with the hypothesis that all the three *Bacillus* strains have a tendency to form a biofilm due to EPS secretion at elevated stress so their presence on seedlings maintains sufficient moisture around seeds. This helps them to survive at their germination stage at higher levels of the NaCl stress. It has also been reported that excess of sodium ions in the soil decreases the fresh weight and dry weight values at higher salinity (Nemati et al., 2011).

Biochemical analysis of roots showed maximum accumulation of total soluble sugars and protein contents at 60 µM stress level in bacterized seedlings (Table 6 and 7). *Bacillus* isolate B20d in combination with *Mesorhizobium* recorded maximum

total sugar (131.56 mg g^{-1}) and protein (22.01 mg g^{-1}) contents in roots at 60 µM salinity level. Under saline conditions, osmotic pressure in the rhizosphere solution exceeds that in root cells, influencing water and nutrient uptake. Almost all micro-and macronutrient contents decrease in the roots and shoots with increasing NaCl concentration in the growth medium. Total soluble sugars and soluble protein contents are better indicators of osmotic adjustments in plants in response to stress (Khatkar and Kuhad, 2000). Both these parameters have also been reported to increase with increasing salt stress in non-inoculated plants. Accumulation of total soluble sugars and soluble protein contents at higher level is associated with the maintenance of the plant turgor required for growth under salt stress.

Table 6: Total sugar content in roots of germinated plants

Treatments	Total sugars (mg g fw^{-1})			
	0	30 mM	60 mM	90 mM
B20d	36.86	54.39	101.06	58.70
B20d+R	60.12	78.12	131.56	63.13
B-I	44.25	52.33	101.66	59.3
B-I+R	61.22	62.75	108.9	61.78
B-20b	57.41	58.21	106.17	63.13
B20b+R	62.08	70.12	121.06	78.21
0.1 µM $CoCl_2$	59.53	64.51	113.04	88.0
Control	34.61	58.70	87.72	21.38
CD (p=0.05)	0.97	1.05	1.53	1.14

Table 7: Total protein content in roots of germinated plants

Treatments	Total protein (mg g fw^{-1})			
	0	30 mM	60 mM	90 mM
B20d	11.74	12.87	16.51	10.1
B20d+R	12.2	13.85	22.01	15.44
B-I	12.1	13.03	15.39	10.2
B-I+R	12.56	13.38	17.08	10.87
B-20b	11.33	11.54	16.1	10.51
B20b+R	12.31	13.23	17.69	11.95
0.1 µM $CoCl_2$	14.15	16.77	18.26	12.56
Control	11.13	11.54	12.41	9.08
CD (p=0.05)	0.49	0.67	0.51	0.56

4. Conlusion

Salt tolerant plant growth promoting rhizobacteria producing ACC deaminase and EPS could serve as a suitable bioinoculant for crops growth under saline soils. This study paves the way for the ideal selection of potential bioagents having abiotic stress tolerance and enlists them as candidate strains for further characterization and application.

5. References

Ahmad, M., Zahir, A., Zahir, H., Asghar, N., Asghar, M., 2011. Inducing salt tolerance in mung bean through coinoculation with rhizobia and plant-growth promoting rhizobacteria containing 1-aminocyclopropane-1-carboxylate deaminase. Canadian Journal of Microbiology 5, 578-589.

Berg, G., 2009. Plant microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. Applied Microbiology and Biotechnology 84, 11-18.

Chookietwattana, K., Maneewan, K., 2012. Selection of efficient salt-tolerant bacteria containing ACC deaminase for promotion of tomato growth under salinity stress. Soil and Environment 31, 30-36.

Davey, M.E., O'toole, G.A., 2000. Microbial biofilms: From ecology to molecular genetics. Microbiology and Molecular Biology Reviews 64, 847-867.

De Vuyst, L., Vanderveken, F., Van de Ven, S., Degeest, B., 1998. Production by and isolation of exopolysaccharides from Streptococcus thermophilus grown in milk medium and evidence for their growth associated biosynthesis. Journal of Applied Microbiology 84, 1059-1068.

Dubois, M., Gilles, K.A., Hamilton, J.K., Reberts, P.A., Smiths, F., 1956. Colorimetric method for determination of sugars and related substances. Analytical Chemistry 3, 350-356.

Dworkin, M., Foster, J., 1958. Experiments with some microorganisms which utilize ethane and hydrogen. Journal of Bacteriology 75, 592-601.

Glick, B.R., 2005. Modulation of plant ethylene levels by the bacterial enzyme ACC deaminase. FEMS Microbiology Letter 251, 1-7.

Glick, B.R., 2014. Bacteria with ACC deaminase can promote plant growth and help to feed the world. Microbiological Research 169, 30-39.

Hontzeas, N., Hontzeas, C.E., Glick, B.R., 2006. Reaction mechanisms of the bacterial enzyme 1 aminocyclopropane-1-carboxylate deaminase. Biotechnology Advances 24, 420-426.

Govindasamy, V., Senthilkumar, M., Mageshwaran, V., Annapurna, K., 2009. Detection and characterization of ACC in plant growth promoting rhizobacteria. Journal of Plant Biochemistry and Biotechnology 18, 71-76.

Johri, J.K., Surange, S., Nautiyal, C.S., 1999. Occurrence of salt, pH, and temperature-tolerant, phosphate-solubilizing bacteria in alkaline soils. Current Microbiology 39, 89-93.

Khatkar, D., Kuhad, M.S., 2000. Short-term salinity induced

changes in two wheat cultivars at different growth stages. Biologia Plantarum 43, 629-632.

Lie, Q., Saleh-lakha, S., Glick, B.R., 2005. The effect of native and ACC deaminase-containing *Azospirillum brasilense* Cdl843 on the rooting of carnation cuttings. Canadian Journal of Microbiology 51, 511-514.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry 193, 265-275.

Lucy, M., Reed, E., Glick, B.R., 2004. Applications of free living plant growth-promoting rhizobacteria. Antonie Van Leeuwenhoek 86, 1-25.

Naz, I., Bano, A., Ul-hassan, T., 2009. Morphological, biochemical and molecular characterization of rhizobia from halophytes of Khewra Salt Range and Attock. Pakistan Journal of Botany 41, 3159-3168.

Nemati, I., Moradi, F., Gholizadeh, S., Esmaeili, M.A., Bihamta, M.R., 2011. The effect of salinity stress on ions and soluble sugars distribution in leaves, leaf sheaths and roots of rice (*Oryza sativa* L.) seedlings. Plant Soil and Environment 57, 26-33.

Parida, S.K., Das, A.B., 2005. Salt tolerance and salinity effects on plants. Ecotoxicology and Environmental Safety 60, 324-349.

Paul, D., Nair, S., 2008. Stress adaptations in a Plant Growth Promoting Rhizobacterium (PGPR) with increasing salinity in the coastal agricultural soils. Journal of Basic Microbiology 5, 378-384.

Poli, A., Anzelmo, G., Nicolaus, B., 2010. Bacterial Exopolysaccharides from Extreme Marine Habitats: Production, Characterization and Biological Activities. Marine Drugs 8, 1779-1802.

Qurashi, A.W., Sabri, A.N., 2012. Bacterial exopolysaccharide and biofilm formation stimulate chickpea growth and soil aggregation under salt stress. Brazilian Journal of Microbiology 1183-1191.

Selvakumar, G., Panneerselvam, P., Ganesamurthy, A.N., 2012. Bacterial mediated alleviation of abiotic stresses in plants. In: Maheswari D.K. (Eds.), Bacteria in Agrobiology: Stress management. IIHR, Banglore, Karnataka, India, 205-224.

Upadyay, S.K., Maurya, S.K., Singh, D.P., 2012. Salinity tolerance in free living plant growth promoting rhizobacteria. Indian Journal of Scientific Research 3, 73-78.

Verhoef, R., Waard, P.D., Schols, H.A., Siika-aho, M., Voragen, A.G.J., 2003. *Methylobacterium* sp. isolated from a Finnish paper machine produces highly pyruvated galactan exopolysaccharide. Carbohydrate Research 338, 1851-1859.

Zapata, P.J., Serrano, M., Pretel, M., Amorós, A., Botella, M.A. 2004. Polyamines and ethylene changes during germination of different plant species under salinity. Plant Science 167, 781-788.