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# Alleviation of salinity stress in rice plant by encapsulated salt tolerant plant growth promoting bacteria *Pantoea agglomerans* strain KL and its root colonization ability

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## ABSTRACT

The present investigation indicates the effects of 0 to 8% NaCl stress on plant growth promoting traits such as ACC (1-aminocyclopropane-1-carboxylate) deaminase, phosphate solubilization, IAA (indole acetic acid), ammonia and exopolysaccharide production of *Pantoea agglomerans* strain KL isolated from salt-stressed soil of Kolhapur, Maharashtra, India. We have studied the effect of encapsulated inoculum (EI) and free inoculum (FI) of *P. agglomerans* strain KL on the alleviation of salinity stress (100 mM NaCl) and promotion of rice plant growth in the pot experiment. The present study showed significant improvement in plant growth supplemented with EI in terms of increased length, biomass, photosynthetic pigment and decreased level of proline, malondialdehyde. Furthermore, EI supplemented plant exhibited decreased sodium and increased calcium and potassium uptake. Root colonization study revealed the survival of encapsulated organism which was less after 10 days. However, a significant number of colony forming unit were noted after 20 and 30 days. In addition, the scanning electron microscopic analysis of salt-stressed plant root showed tremendous root colonization by EI. Hence, the present study demonstrates the potency of *P. agglomerans* strain KL in the expression of plant growth promoting traits and amelioration of salt stress by EI.

## ARTICLE HISTORY

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## KEYWORDS

Plant growth promoting traits; *Pantoea agglomerans* strain KL; Encapsulated inoculum; salinity stress; root colonization

## Introduction

The productivity of numerous plants is affected severely by various stress factors that result in yield loss. Salt stress is a serious agricultural problem that causes reduction in plant growth (Cicek and Cakirlar 2002), mainly rice (*Oryza sativa* L.) production in arid and semiarid regions of irrigated areas (Koyro 2006). Rice is a major staple food for around 70% of the population worldwide (Hasegawa et al. 2000) providing essential daily calories for a billion numbers of people (Singh et al. 2012). Presence of salt stressor increases the uptake of plant Na<sup>+</sup> and reduces Ca<sup>2+</sup> and K<sup>+</sup> uptake which is the reason for imbalance in the nutritional status of a plant (Neel et al. 2002).

Ethylene is a gaseous hormone playing an important role in plant growth (Bleecker and Kende 2000). Its concentration increases during stress that inhibits the overall plant growth. ACC is the immediate precursor of ethylene; it is typically excluded from plant roots (Penrose and Glick 2001; Penrose et al. 2001). ACC deaminase is an enzyme that catabolize ACC to produce  $\alpha$ -ketobutyrate

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and ammonia, produced by plant growth promoting bacteria (PGPB) that colonize in the root of plants or may be free-living (Lugtenberg and Kamilova 2009). Such PGPB eliminates the inhibitory effects of stress ethylene and facilitates long root formation in plant growing in presence of salinity (Penrose and Glick 2003). In addition, PGPB promotes the plant growth directly through phosphate solubilization, nitrogen fixation and production of IAA, siderophore and indirectly by controlling the growth of pathogens (Tank and Saraf 2003; Lucy et al. 2004). Due to such a beneficial profile, we can use PGPB as biofertilizer for saline soil. However, the successful and safe way to inoculate PGPB in the soil is the encapsulation method by using biodegradable matrices (Vassilev et al. 2001), the best alternative for free cell use as an inoculum. The inoculation of a plant by free cells might result in difficulty in survival and root colonization due to its susceptibility to environmental and stress factors (Wu et al. 2012). This can be overcome by the use of encapsulated inoculum. Encapsulation provides suitable microenvironment which reduces the cell loss (Rekha et al. 2007), releases the cells slowly in target soil and maintain the ability of PGPB to promote plant growth. Furthermore, it maintains sufficient numbers of live cells, which assures long-term effectiveness (Wu et al. 2014).

In this study, we have isolated potent isolate named AB-7 from salt-affected soil of Kolhapur, Maharashtra, India and investigated for expression of plant growth promoting traits (PGPT) in presence and absence of salt stressor (0–8% NaCl). In addition, we have prepared encapsulated AB-7 isolate alginate beads. The beads were analyzed by SEM (Scanning electron microscope). Furthermore, we have evaluated the efficacy of EI and FI to ameliorate salinity stress (100 mM NaCl) on rice plant. Analysis of plant was done after 30 days for different parameters. In addition, the root colonization potential of encapsulated and free AB-7 isolate was studied.

## **Materials and methods**

### ***Isolation of micro-organisms***

The salt-affected soil of Ganeshwadi village of Kolhapur district was collected and analyzed for EC (Electrical conductivity), pH, organic carbon, total nitrogen and available phosphate content. All the analytical techniques were performed according to APHA (1998). Micro-organisms were isolated from the soil sample on the Nutrient agar plates by using serial dilution technique and used further.

### ***Plant growth promoting traits of isolates in salt stress***

All the PGPT of isolates were studied in the presence of salt stress (0, 1, 2, 3, 4, 5, 6, 7 and 8% NaCl).

### ***ACC deaminase activity***

Quantitative ACC deaminase activity was assayed by using the method of Honma and Shimomura (1978) with few modifications. The 1 ml fresh cultures of isolates were inoculated aseptically in tryptic soya broth supplemented separately with salt stress. The ACC deaminase activity was determined from the amount of  $\alpha$ -ketobutyrate, by comparing absorbance of a sample with a standard curve of  $\alpha$ -ketobutyrate (0.1–1  $\mu$ mol) at 540 nm.

### ***Phosphate solubilization***

The isolates with ACC deaminase activity were used further. One-milliliter fresh culture of the isolate was inoculated in 100 ml of Pikovskaya's liquid medium (Pikovskaya 1948) containing different salt stressor, separately in 250 ml Erlenmeyer flask. Flasks were incubated on a rotary shaker (120 rpm) and after every 24 h amount of soluble phosphate was determined by Fiske and

Subbarow method (1925). The concentration of soluble phosphate was determined from a standard graph of  $\text{KH}_2\text{PO}_4$ .

### ***IAA production***

Sterile Nutrient broth (100 ml) with 0.2% tryptophan was added separately with the salt stressor, inoculated with 1 ml fresh culture of isolate and incubated under shaking conditions (120 rpm) at 30°C. After 24 h of incubation, the IAA production by the isolates was quantified by the method of Gordon and Weber (1951). The concentration of IAA was determined from a standard curve of IAA (10–100  $\mu\text{g ml}^{-1}$ ).

### ***Ammonia production***

The isolates were inoculated separately into 10 ml sterile peptone water having different salt stress and incubated for 48 h at 30°C. After incubation, Nessler's reagent (0.6 ml) was added to the tube. The uninoculated medium was used as blank and ammonia production was indicated by the development of brown to yellow color (Marques et al. 2010).

### ***Exopolysaccharide production***

The exopolysaccharide production ability of the isolates was qualitatively determined by the protocol of Nicolaus et al. (1999) in different salinity stress.

### ***Biochemical properties and identification of isolate***

Gram staining and Indole, Methyl Red, Voges Proskauer, Citrate utilization (IMViC) tests were performed following the standard protocol (Prescott et al. 2002). The ability of a selected isolate to utilize different carbohydrates was tested using a carbohydrate utilization test kit (KB 009, Himedia). A potent organism was identified using 16S rDNA sequencing. DNA was isolated from potent organism and 16S rDNA gene was amplified by Polymerase chain reaction using forward (5'AGHGTBTGHTCMTGNCTCAS 3') and reverse (5'TRCGGYTMCTTGTWHCGACTH 3') primers. One hundred  $\mu\text{l}$  of PCR reaction mixture contains DNA (20 ng), 400 ng each primer, PCR buffer (10x), 2.5 mM of each dNTPs and Taq polymerase (3U). PCR condition includes, initial denaturation (at 95°C for 5 min), followed by 35 cycles of 94°C (for 30 sec), 50°C (for 30 sec), 72°C (for 1 min and 30 sec) and the final extension step was carried at 72°C (for 7 min). Purified PCR product was then sequenced and analysis was done using BLAST tool (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). The other species sequences were used for phylogenetic analysis. The Neighbour-Joining method was used to infer evolutionary history (Saitou and Nei 1987). The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The Maximum Composite Likelihood method was used to calculate evolutionary distances (Tamura et al. 2004) and was in the units of the number of base substitutions per site. There were a total of 1397 positions in the final dataset. An evolutionary analysis was conducted in MEGA6 (Tamura et al. 2013).

### ***Encapsulation of isolate***

The potent isolate having the ability to express PGPT in salt stressor was inoculated in sterile Nutrient broth and incubated under shaking condition at 30°C (200 rpm) for 24 h. Bacterial pellet was collected after centrifugation at  $5590 \times g$  for 15 min and mixed with 1.5% sodium alginate solution. The prepared mixture having an organism and sodium alginate was splashed in 200 ml cold calcium chloride solution using an injection needle to form immobilized isolate beads. After 2 h, the solution of calcium chloride was drained. The regular encapsulated beads that formed

were washed with sterile distilled water, placed in petri plate and dried at room temperature. The encapsulation of isolate was analyzed by using SEM.

### ***Effect of potent encapsulated isolate on plant growth***

The encapsulated beads containing potent salt tolerant plant growth promoting bacteria (SPGPB) were used further to assess its ability to ameliorate salt stress in rice plants. The experiment was carried out in the month of September (Average temperature within 30–35°C and more than 30% humidity). The soil used for pot study was sterilized by autoclaving and transferred to pots having a size of 15 × 15 cm. Rice seeds were surface sterilized by treating with 70% ethanol for 1 min followed by several times washing with sterilized distilled water. The surface sterilized seeds were planted in each pot (5 seeds per pot). The experimental design contains different groups which includes, non-salt-stressed soil (NS), 100 mM salt-stressed soil (SS), 100 mM salt stressed soil with free cells of AB-7 inoculum ( $10^8$  cells ml<sup>-1</sup>) (SS+FI) and 100 mM salt stressed soil with encapsulated beads of AB-7 inoculum (SS+EI). In EI the beads were added in soil near seeds. For imposing salt stress, the pots were watered with 100 mM NaCl solution after every 48 h. The experiment was repeated for three times and after 30 days of sowing all the plantlets were uprooted and analyzed for length, weight, chlorophyll (Arnon 1949), proline (Bates et al. 1973) and Na<sup>+</sup>, Ca<sup>2+</sup> and K<sup>+</sup> contents (Ryan et al. 2007).

### ***Lipid peroxidation***

Peroxidation of lipid was assayed by measuring the amount of malondialdehyde (MDA) produced by the method of Heath and Packer (1968). The plant material was ground in 1% trichloroacetic acid and centrifuged at  $6708 \times g$  for 10 min. After incubation, 1 ml of supernatant was added with 4 ml of thiobarbituric acid (0.5%). The reaction mixture was heated at 95°C for 30 min, cooled and again centrifuged at  $1677 \times g$  for 5 min. The absorbance of the resulting supernatant was measured at 532 nm and compared with a standard curve of MDA (0.1–10 nmol).

### ***Rhizospheric competence***

To find out rhizospheric competence of isolate in salt-stress conditions the root-adhering soil was used as a sample. The plants were uprooted; soil suspension was prepared, serial dilution of soil suspension was prepared by using sterile saline (0.75% NaCl) and spread on sterile Nutrient agar plates and incubated at 30°C for 48 h. Finally, root colonization potential of the isolate was determined from a number of bacterial colonies forming unit g<sup>-1</sup> root biomass (cfu g<sup>-1</sup>).

### ***SEM analysis***

For the rhizospheric competence analysis, the root sample of salt-stressed rice plants inoculated with alginate beads was taken, washed thoroughly for the removal of root-adhering soil. Root sample was then fixed with glutaraldehyde followed by immersion in 50–100% acetone. Root sample dried in an oven at 50°C for 48 h. The dried sample was coated with gold and analyzed by SEM.

### ***Statistical analysis***

Results were developed as an average of three determinations. Analysis of variance was carried out of all data at  $p < 0.05$  using Microsoft Office data analysis tool pack.  $p \leq 0.05$  difference was considered as significant.

## Results

### Isolation of micro-organisms

The salt-affected soil of Ganeshwadi was whitish in color with EC of 4.5 dS m<sup>-1</sup>, alkaline pH (8.1), 0.41% organic carbon, 0.73% total nitrogen and 15 mg kg<sup>-1</sup> of available phosphate content. Twenty-five bacterial isolates were isolated from a soil sample, maintained on Nutrient agar slants and used for further study.

### Plant growth promoting traits of isolates in salt stress

#### ACC deaminase activity

Among 25 isolates, 1 isolate named AB-7 was found to have maximum ACC deaminase activity. The quantitative assay showed that 3% salt stress significantly induced the ACC deaminase activity of AB-7 and 6% completely inhibits enzyme activity. ACC deaminase activity of AB-7 was 258.5 ± 0.95 μmol mg<sup>-1</sup> protein h<sup>-1</sup> in 3% salt stress while 220.1 ± 1 μmol mg<sup>-1</sup> protein h<sup>-1</sup> in non-salt-stress condition (Table 1).

#### Phosphate solubilization

The salt stress of 1, 2 and 3% slightly increased while 4% leads to a significant increase in phosphate solubilization over the control. AB-7 isolate showed phosphate solubilization of 2304 ± 2 μg ml<sup>-1</sup> in 4% salinity while only 835 ± 3.6 μg ml<sup>-1</sup> was detected under non-stressed conditions after 144 h of incubation (Figure 1).

#### IAA production

Isolate AB-7 produced increasing IAA with an increase in salinity stress. At 8% salt stress 53.60 ± 1.2 μg ml<sup>-1</sup> of IAA was produced after 96 h of incubation while 21.50 ± 0.82 μg ml<sup>-1</sup> of IAA was produced under a non-saline condition under same incubation period (Figure 2).

#### Ammonia and exopolysaccharide production

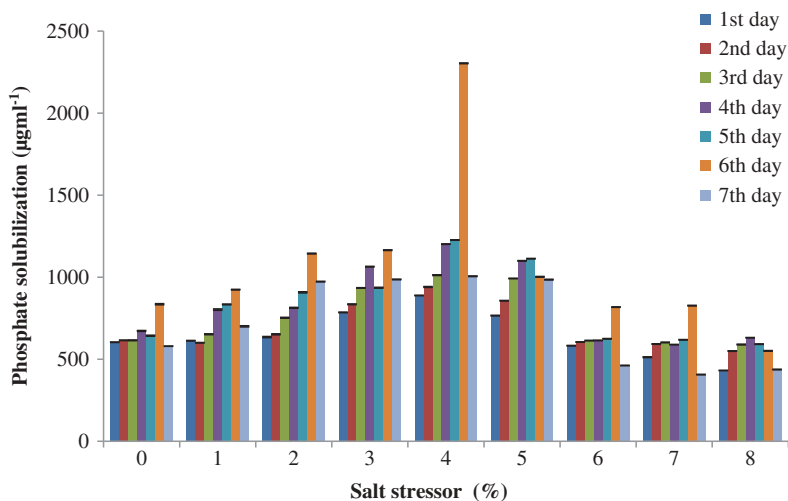
Ammonia production by AB-7 isolate was reduced at 5% salt stress and completely inhibited at 6%. While exopolysaccharide production ability of isolate was observed in non-stress and up to 8% of salinity stress (Table 1).

**Table 1.** Plant growth promoting traits of *Pantoea agglomerans* strain KL in the presence and absence of salinity stress.

Salt stressor (%)	Plant growth promoting traits				
	ACC deaminase activity (μmol α-ketobutyrate mg <sup>-1</sup> protein h <sup>-1</sup> )	Phosphate Solubilization (μg ml <sup>-1</sup> )	IAA Production (μg ml <sup>-1</sup> )	Ammonia Production	Exopolysaccharide production
0	220.1 ± 1.00	835 ± 3.60	21.50 ± 0.82	++	++
1	222.83 ± 0.55	924.2 ± 2.0	22.84 ± 0.97	++	++
2	224.86 ± 0.25	1144 ± 2.08	24.73 ± 0.96	++	++
3	258.5 ± 0.95	1165 ± 2.0	25.34 ± 0.69	++	++
4	217.23 ± 0.80	2304 ± 2.08	25.72 ± 1.01	++	++
5	201.43 ± 1.26	1113.2 ± 1.48	26.34 ± 1.03	+	++
6	-	817.6 ± 1.64	31.33 ± 1.01	-	++
7	-	826.8 ± 1.78	33.51 ± 1.03	-	++
8	-	631.3 ± 1.25	53.60 ± 1.22	-	++

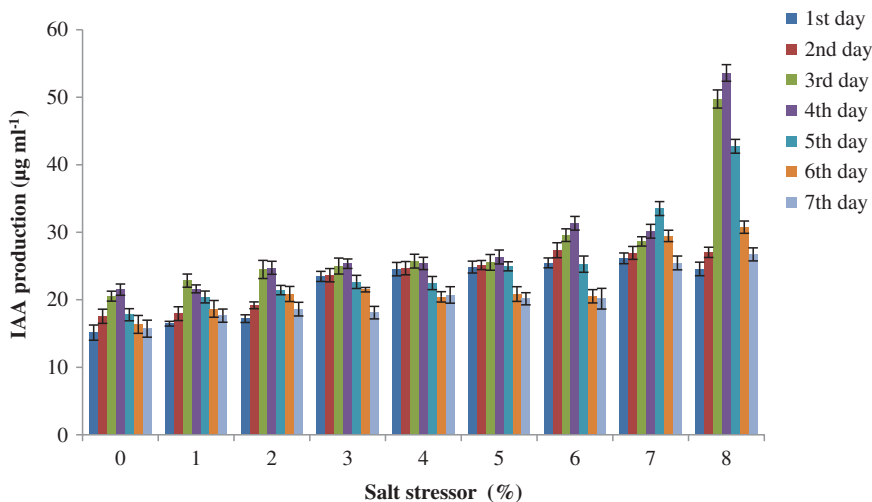
The present table indicates the highest activity of strain KL in presence of 0–8% NaCl stress.

Data are presented as the average of three replicates and  $p < 0.05$  according to Graph Pad software. All the values are expressed in mean ± S.D. format ( $n=3$ ). ++, Very good; +, Good; —, Negative  
ACC, 1-aminocyclopropane-1-carboxylate; IAA, indole acetic acid



**Figure 1.** Phosphate solubilization by *Pantoea agglomerans* strain KL in Pikovskaya's medium in presence and absence of salt stressor at 30°C after every 24 h.

(Data are presented as the average of three replicates and  $p < 0.05$  according to Graph Pad software). All the values are expressed in mean  $\pm$  S.D. format ( $n=3$ )



**Figure 2.** IAA production by *Pantoea agglomerans* strain KL in Nutrient broth containing 0.2% tryptophan in presence and absence of salt stressor at 30°C after every 24 h.

(Data are presented as the average of three replicates and  $p < 0.05$  according to Graph Pad software). All the values are expressed in mean  $\pm$  S.D. format ( $n=3$ )

### Identification of isolate

The biochemical properties of potent isolate are summarized in supplementary Table 1. The isolate AB-7 was identified by 16S rDNA gene sequence analysis as *Pantoea agglomerans* strain KL. The partial 16S rDNA gene sequence was deposited in Gene Bank database under the accession number of KX879607. The phylogenetic tree of *Pantoea agglomerans* strain KL is given in supplementary Figure 1.

## Encapsulation of isolate

The encapsulated beads of *P. agglomerans* strain KL were spherical in shape. The encapsulated beads have a diameter of 3 mm and weight of 25 mg. The uniform distribution of isolate on the surface and in the interior of beads was observed by SEM analysis of sodium alginate beads.

## Plant analysis

Results of plant analysis showed a reduction in the growth of rice plants in 100 mM salt stressor under non-bacterized as well as bacterized conditions as compared to plant growing in non-salt-stress condition. However, bacterization of salt-stressed plants with AB-7 in free and encapsulated form decreased reduction in plant growth. In presence of salt stressor of 100 mM the shoot and root length, fresh and dry weight of rice plant was found to be  $16.46 \pm 0.25$  cm,  $6.5 \pm 0.2$  cm,  $0.55 \pm 0.02$  g and  $0.062 \pm 0.002$  g, respectively. The supplementation of salt-stressed plant with EI and FI improved plant growth. However, EI was more effective in promoting plant growth over FI (Table 2). EI added plant showed shoot and root length of  $21.46 \pm 0.15$  and  $8.33 \pm 0.25$  cm and fresh plus dry weight of  $0.70 \pm 0.01$  and  $0.085 \pm 0.00$  g after 30 days (Table 2).

After 30 days of growth, the chlorophyll content was reduced in salt-affected plant ( $4.23 \pm 0.06$  mg ml<sup>-1</sup> g<sup>-1</sup> FW) over non-salt affected plant ( $5.21 \pm 0.10$  mg ml<sup>-1</sup> g<sup>-1</sup> FW). However, bacterization of salt-stressed rice plants with EI improved chlorophyll content (Table 2).

In the case of proline content, the significant increase was observed in presence of salinity ( $4.51 \pm 0.02$  μmol g<sup>-1</sup> FW) over non-salinity non-inoculated rice plant ( $3.47 \pm 0.11$  μmol g<sup>-1</sup> FW). Reduction in proline content was observed in plants supplemented with EI followed by FI after 30 days of growth (Figure 3).

Figure 4 shows an increase in Na<sup>+</sup> and a decrease in Ca<sup>2+</sup> and K<sup>+</sup> content in non-bacterized plants in 100 mM salt stressor. The bacterization of salt-affected plant with EI notably decreased the Na<sup>+</sup> and increased Ca<sup>2+</sup> and K<sup>+</sup> content after 30 days of growth.

## Lipid peroxidation

In the present study, increase in MDA contents were observed at 100 mM salt stressor level as compared to non-stressed rice plant. The non-salinity affected rice plant had MDA contents of  $122.3 \pm 3.21$  and  $154 \pm 3.60$  mg g<sup>-1</sup> DW in shoot and root, respectively. The non-bacterized salinity stressed rice plant showed an increase in MDA content in plants shoot ( $382.33 \pm 2.51$  mg g<sup>-1</sup> DW) and root ( $424 \pm 3.60$  mg g<sup>-1</sup> DW) after 30 days of growth. However, EI bacterized salt-stressed plant shoot and root exhibited a considerably reduced level of MDA over salinity stressed control plant (Figure 5).

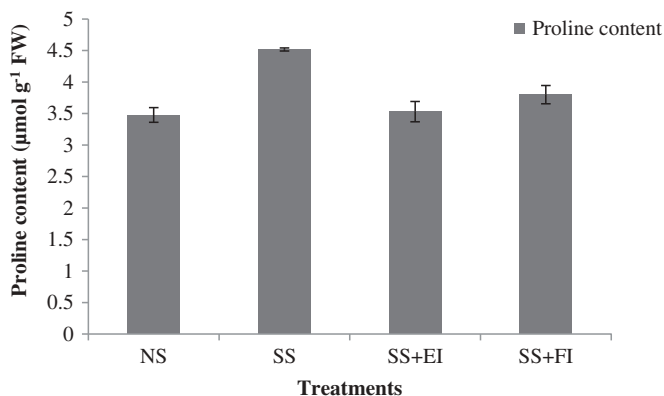
**Table 2.** Effect of encapsulated and free *Pantoea agglomerans* strain KL on rice plant growth in presence of 100 mM salt stressor after 30 days in pots.

Treatment	Growth parameters				Chlorophyll content (mg ml <sup>-1</sup> g <sup>-1</sup> FW)
	Shoot length (cm)	Root length (cm)	Fresh weight (g)	Dry weight (g)	
NS	$23.26 \pm 0.15$	$9.26 \pm 0.20$	$0.73 \pm 0.01$	$0.34 \pm 0.0$	$5.21 \pm 0.10$
SS	$16.46 \pm 0.25$	$6.5 \pm 0.2$	$0.55 \pm 0.02$	$0.06 \pm 0.0$	$4.23 \pm 0.06$
SS+EI	$21.46 \pm 0.15$	$8.33 \pm 0.25$	$0.70 \pm 0.01$	$0.08 \pm 0.0$	$4.48 \pm 0.02$
SS+FI	$19.33 \pm 0.25$	$8.16 \pm 0.15$	$0.61 \pm 0.01$	$0.07 \pm 0.0$	$4.11 \pm 0.02$

Data are presented as the average of three replicates and  $p < 0.05$  according to Graph Pad software. All the values are expressed in mean  $\pm$  S.D. format ( $n = 3$ ).

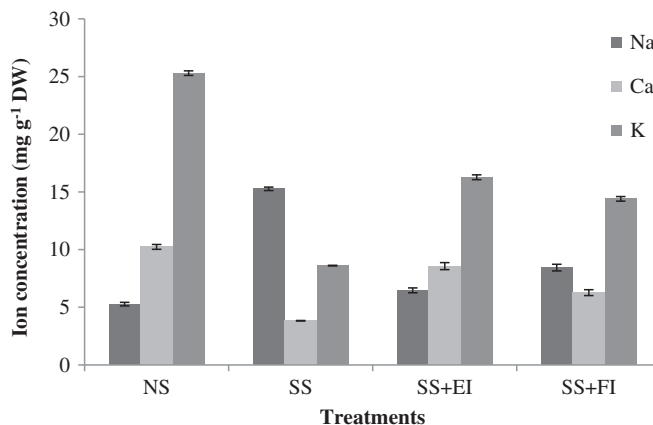
NS, non-salt stressed soil; SS, 100 mM salt stressed soil; SS+EI, 100 mM salt stressed soil with encapsulated beads of AB-7 inoculum; SS+FI, 100 mM salt stressed soil with free cells of AB-7 inoculum ( $10^8$  cells ml<sup>-1</sup>).





**Figure 3.** Effect of encapsulated and free *Pantoea agglomerans* strain KL on proline content of rice plants in presence of 100 mM salt stressor after 30 days in pots.

(Data are presented as the average of three replicates and  $p < 0.05$  according to Graph Pad software). All the values are expressed in mean  $\pm$  S.D. format ( $n=3$ ) (Abbreviations: NS, Non-salt stressed soil; SS, 100 mM salt stressed soil; SS+EI, 100 mM salt stressed soil with an encapsulated inoculum of AB-7; SS+FI, 100 mM salt stressed soil with a free inoculum of AB-7)



**Figure 4.** Effect of *Pantoea agglomerans* strains KL on Na<sup>+</sup>, Ca<sup>2+</sup> and K<sup>+</sup> content of rice plants in presence of 100 mM salt stressor after 30 days in pots.

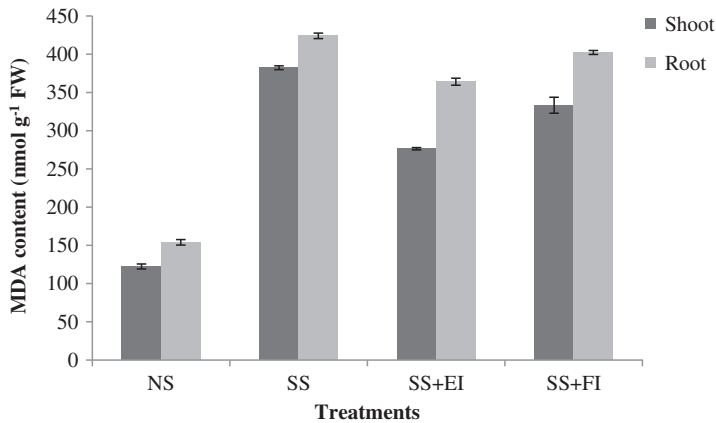
(Data are presented as the average of three replicates and  $p < 0.05$  according to Graph Pad software). All the values are expressed in mean  $\pm$  S.D. format ( $n=3$ ) (Abbreviations: NS, Non-salt stressed soil; SS, 100 mM salt stressed soil; SS+EI, 100 mM salt stressed soil with an encapsulated inoculum of AB-7; SS+FI, 100 mM salt stressed soil with a free inoculum of AB-7)

### Rhizospheric competence and SEM analysis

The root colonization potential of AB-7 under salt-stress condition is given in Table 3. The EI showed maximum of  $8 \times 10^7$  cfu g<sup>-1</sup> root biomass under salt-stress conditions. The FI had a reduction in cfu with a time period. While EI initially showed a low number of cfu g<sup>-1</sup> root, increased bacterial number was observed after 30 days (Figure 6).

### Discussion

A salt stressor is a biotic stress that degrades the soil properties, makes the soil unfit for the growth of isolates and also inhibitory to the growth and development of plants. The most common strategy to solve the salt stressor problem is the use of ACC deaminase producing PGPB. These PGPB lower down the level of stress hormone ethylene due to ACC deaminase enzyme that



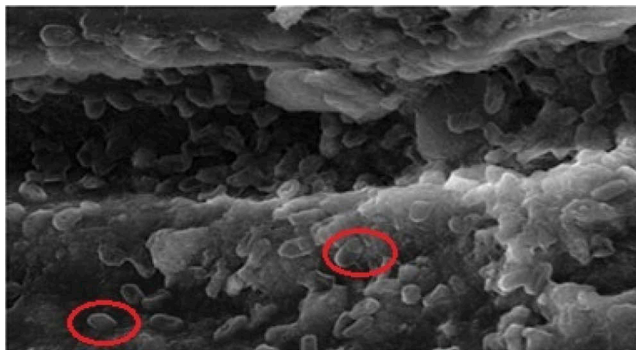
**Figure 5.** Effect of *Pantoea agglomerans* strains KL on MDA content of rice plants in presence of 100 mM salt stressor after 30 days in pots.

(Data are presented as the average of three replicates and  $p < 0.05$  according to Graph Pad software). All the values are expressed in mean  $\pm$  S.D. format ( $n = 3$ ) (Abbreviations: NS, Non-salt stressed soil; SS, 100 mM salt stressed soil; SS+EI, 100 mM salt stressed soil with an encapsulated inoculum of AB-7; SS+FI, 100 mM salt stressed soil with a free inoculum of AB-7)

**Table 3.** Rhizospheric competence (cfu g<sup>-1</sup> root biomass) of *Pantoea agglomerans* strain KL.

Treatment	10 days	20 days	30 days
FI	$7 \times 10^7$	$5 \times 10^6$	$4.2 \times 10^4$
EI	$6.4 \times 10^5$	$7.1 \times 10^6$	$8 \times 10^7$

EI, encapsulated inoculum; FI, free inoculum



**Figure 6.** Root colonization potential of encapsulated *Pantoea agglomerans* strain KL in presence of 100 mM salt stressor after 30 days in pots.

converts ACC into a ketobutyrate and ammonia (Mayak et al. 2004). Such PGPB efficiently alleviate salinity and significantly promotes plant growth. This report highlights the effect of 0–8% NaCl stress on PGPT of *P. agglomerans* strain KL (Table 1). However, ACC deaminase activity, IAA production (Zhang et al. 2011) and phosphate solubilization (Son et al. 2006) in *P. agglomerans* were earlier reported. This study also reports the role of encapsulated inoculum of *P. agglomerans* strain KL on mitigation of salinity stress (100 mM) in rice plant growth.

The pot study results showed significant improvement in plant growth supplemented with EI over FI. The promotion of plant growth by free bacterial inoculation is limited due to constraints in keeping  $10^6$ – $10^7$  cfu ml<sup>-1</sup> in the soil which is the threshold level of PGPB used as an inoculum (Bashan 1986b). The salt-stressed plants bacterized with EI improved shoot length to 130.36%, root

length to 128.20%, fresh weight to 127.10% and dry weight to 136.39% compared to salt-stressed non-inoculated rice plants (Table 2). Similarly, He et al. (2017) reported an increase in fresh and dry weight, length and germination percentage of cotton plants under salinity stress (0.5%) using encapsulated *Pseudomonas putida* Rs-198. The increase in plant growth by EI may be due to the entrapment of adequate number of PGPB in sodium alginate beads (Zohar-Perez et al. 2002) which facilitates controlled release of bacteria from beads to the surrounding soil. The encapsulated beads also ensure a continuous supply of inoculum over a longer time period (Bashan 1986a), resulted in improved plant growth over free inoculum. The ACC deaminase activity in addition to phosphate solubilization and phytohormones production by *P. agglomerans* strain KL could play a prime role in plant growth under salinity (Ramadoss et al. 2013).

The supplementation of EI to salt-stressed rice plant increased the chlorophyll content to 114.56% compared to salinity affected plant, specifies improved photosynthesis in salt-stressed conditions (Table 2). This increase in photosynthetic pigments may be because of the increased plant biomass due to supplementation of EI over FI. According to Guo et al. (2005) the increased plant weight is results of salt tolerance indicating the bacterized stressed plant have limited damage mainly to photosynthetic apparatus.

In the present work, salt-stressed rice plant exhibits an increased  $\text{Na}^+$  and decreased  $\text{Ca}^{2+}$  and  $\text{K}^+$  contents as compared to plant growing under non-salt conditions. The bacterization of rice plants by EI reduced the  $\text{Na}^+$  and increased  $\text{Ca}^{2+}$  and  $\text{K}^+$  uptake (Figure 4). This may be due to the exopolysaccharide production that restricts  $\text{Na}^+$  influx and enables the plant to survive in salt stressor (Kohler et al. 2006).

Exposure of rice plant to salt stressor produced a high level of proline which acts as osmoregulant and plays an important role in protein and cell membrane stabilization (Claussen 2005). The EI decreased proline content in presence of salinity by 21.84% (Figure 3). The ability of strain KL in the reduction of proline under stress conditions denotes that the rice plants are less affected by the salt stressor. In this study, Figure 5 shows a significant increased level of MDA indicating salt stressor-induced increase ROS and damage to plant membrane. The EI application in salt stressor mitigates the salinity-induced lipid peroxidation by reducing MDA content by 27.72 and 14.15% in shoot and root indicating enhanced tolerance to the salt stressor (Figure 5). Similarly, Wu et al. (2014) reported reduced MDA contents in salinity stressed cotton plants provided with encapsulated *Klebsiella oxytoca* Rs-5. Figures 3 and 5 shows significantly decreased level of proline and MDA in EI supplemented plant than the FI indicating effectivity of encapsulated *P. agglomerans* strain KL in mitigating salinity stress.

The *P. agglomerans* strain KL showed  $220.1 \pm 1 \mu\text{mol mg}^{-1} \text{protein h}^{-1}$  of ACC deaminase activity while *Pantoea agglomerans* strain JP3-3 showed  $370 \pm 15 \mu\text{mol mg}^{-1} \text{protein h}^{-1}$  of ACC deaminase activity (Zhang et al. 2011). In the case of the salinity effect, 3% of salt stress significantly increased ACC deaminase activity. Similarly, *Pseudomonas fluorescens* showed slightly increased ACC deaminase activity at 5% NaCl stress while *Bacillus megaterium* and *Variovorax paradoxus* showed reduced ACC deaminase activity with increased salinity stress (2, 5% NaCl) over control (Nadeem et al. 2016).

Salinity causes precipitation of phosphorous in soil hence it is necessary to provide soluble phosphate for the better plant growth by inoculated isolates in stress condition (Gyaneshwar et al. 2002). The studied strain KL showed phosphate solubilization in the presence of a salt stressor. Figure 1 shows 2.7-fold increased phosphate solubilization at 4% of salt stress. In contrast, Son et al. (2006) reported a decrease in phosphate solubilization with an increase in NaCl stress, at 5% NaCl stress only  $584 \mu\text{g ml}^{-1}$  of soluble phosphate was produced by *Pantoea agglomerans* strain R-42 while in the non-salinity condition in optimized medium,  $900 \mu\text{g ml}^{-1}$  of soluble phosphate was produced.

Under non-stress condition studied, isolated produced  $21.50 \pm 0.82 \mu\text{g ml}^{-1}$  IAA and 2.5-fold enhanced IAA production was observed in presence of 8% of salt stress over non-stressed condition, while only  $11.9 \mu\text{g ml}^{-1}$  of IAA was produced by *Pantoea agglomerans* strain JP3-3 (Zhang et al. 2011). The produced IAA induces the physiological response of plants (Upadhyaya et al. 2011) and protects

the plants exposed to the salt stressor by enhancing cellular defense system (Bianco and Defez 2009). In addition, inoculation of salinity affected wheat plants by the halotolerant bacteria improved root length of 71.7% over the non-inoculated control plant (Ramadoss et al. 2013).

The strain KL also showed ammonia and exopolysaccharide production in addition to phosphate solubilization, IAA production and ACC deaminase activity (Table 1). As per Ali et al. (2014) inoculation of such organism with multifarious PGPT is helpful to ameliorate stress and to improve plant growth.

The SPGPB, *P. agglomerans* strain KL expressed PGPT even in presence of a salt stressor. These results demonstrated that salt stressor did not hinder the plant growth promoting beneficial traits of the isolate. In this study, the KL strain displayed significant variation in salt tolerance profile in relation with PGPT (Table 1). According to Yaish et al. (2015) the basic composition of the growth medium affects the expression of plant growth promoting attributes at different salt-stress levels. These results corroborate with Bhise et al. (2017) where the difference in ACC deaminase, phosphate solubilization, IAA, siderophore, ammonia, HCN and exopolysaccharide production was noticed in *Enterobacter cloacae* strain KBPD in presence of 0, 2, 4, 6 and 8% of NaCl stress.

The presence of interested organism added as inoculum in threshold level is necessary to achieve plant colonization and improved plant growth through PGPT of an isolate (Pii et al. 2015). In root colonization studies the initial degree of colonization of EI was less after 10 days over FI however after 20 and 30 days EI showed excellent root colonization which again confirmed by SEM analysis of root sample (Table 3 and Figure 6). Similarly, colonization potential of encapsulated *Pseudomonas putida* Rs-198 was less at 7–21 days and was considerably high after 35 days over free cells inoculum (He et al. 2016). The initial high cfu of FI was due to the sufficient distribution, reproduction and persistence of free inoculum (Russo et al. 2005). The encapsulation of isolate in sodium alginate increased the survival rate of the organism (Weir et al. 1995) and resulted in long-term performance which leads to improved plant growth.

It is evident from this report that the plant response varies among FI and EI. When the isolate is encapsulated it is protected from environmental and mechanical stress (Bashan 1998), hence significant improvement in salt-stressed plant growth is observed. The SEM analysis of plant root is clear evidence of successfully rhizospheric competence of EI. Because of encapsulation, bacteria could deliver in a controlled manner and the rate of survival is also increased in inoculated soil (Wu et al. 2011). These threshold levels of inoculum now help the plant to withstand salinity stress by reducing stress ethylene accumulation through ACC deaminase activity (Mayak et al. 2004).

The isolation and identification of potent isolate to be used as an inoculant has been increased in the last decades (Saharan and Nehra 2011). There is essential need to have a novel isolate to be used as an ideal bioinoculant especially to salinity stressed soil, which is capable of improving plant growth. Currently, research is going on the isolation of PGPB and selection of potent isolate as an inoculant to salinity stressed soil based on PGPT of the isolate. However, it is essential to find out the salt tolerance, plant growth promoting properties and rhizospheric competence of isolate when we are studying the effects of inoculum on the growth of a plant in salinity.

## Conclusion

The present study confirms the capability of *Pantoea agglomerans* strain KL in the expression of plant growth promoting traits even in salinity stress. The comparison of free and encapsulated inoculum revealed the prominent role of encapsulated *P. agglomerans* strain KL in amelioration of salinity stress with powerful root colonization ability. Therefore, the applications of plants with encapsulated *Pantoea agglomerans* strain KL inoculum represent an environmentally friendly approach that could be beneficial to combat salt stressor.

## Disclosure statement

No potential conflict of interest was reported by the authors.

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