INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
22–0273/2022/28–4–246–256
DOI: 10.17957/IJAB/15.1976
http://www.fspublishers.org

Full Length Article

Evaluation of Plant Growth-Promoting Halotolerant Potassium Solubilizing Rhizobacteria Isolated from Paddy Crop under Salinity Stress

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Received 28 June 2022; Accepted 23 September 2022; Published 16 October 2022

Abstract

Salinity is an important developing factor that reduces cultivated area because of the salt deposit, which causes a reduction in crop yield. Soil salinity causes imbalance nutrition in plants and thus reduces plant growth. The current study focused on *Acinetobacter pittii, Rhizobium pusense, Cupriavids oxalaticus,* and *Ochrobactrum ciceri* for phosphorus solubilization, Indole acetic acid production, siderophores production, ammonia production, ACC deaminase activity, and exopolysaccharides production in different media amended with 3, 5 and 7% NaCl and compared with control having no NaCl. The NaCl stress adversely affected the plant growth-promoting properties of potassium solubilizing rhizobacteria; however, all strains were capable of all plant growth-promoting properties under a maximum of 7% NaCl stress. The results show that phosphorus solubilization, ammonia production, ACC deaminase activity and exopolysaccharides production were maximum in *A. pittii* (30.18 µg/mL, 16.17 µg/mL, 2.142 µmol α-ketobutyrate/mg/h and 250.58 µg/mL, respectively) whereas least phosphorus solubilization and ACC deaminase activity were in *R. pusense* (22.41 µg/mL and 1.250 µmol α-ketobutyrate/mg/h, respectively) and ammonia and exopolysaccharides production were least in *C. oxalaticus* (14.52 and 135 µg/mL, respectively). Indole acetic acid and siderophores production were maximum in *O. ciceri* (60.55 and 51.11 µg/mL, respectively) whereas the least indole acetic acid was in *C. oxalaticus* (1.67 µg/mL) and least siderophores were in *R. pusense* (31.24 µg/mL). These results demonstrated that *A. pittii* and *O. ciceri* could exhibit better plant growth-promoting properties under high saline conditions. In crux, application of halotolerant rhizobacteria seemed a viable option to improve plant growth by increasing plant nutrient availability under saline conditions.

Keywords: Salinity; Potassium; Rhizobacteria; Plant nutrients; Halotolerant

Introduction

Various environmental factors, such as temperature, salinity, pathogens and drought, seriously influence agricultural productivity by reducing the growth and development of crops. It is estimated that area of salt affected soils has increased from 45 million to 62 million hectares since the 1990s making salinity the main factor in plant growth and productivity (Chele *et al.* 2021). Salinity is more apparent in the coastal agri-ecosystems because of the continuous entrance of seawater, mishandling of coastal irrigation land, and weak farming practices. Excessive salts in soil modify cellular metabolism initiating many physiological, morphological, biochemical, and molecular variations in plants. Excessive salts in soil adversely affect plant growth and development, causing osmotic stress. Salinity directly affects water accessibility, accumulation of toxic ions like Na^+ and Cl^- in the cells, nutrient disparity, and oxidative stress (Munns and Tester 2008). Excessive accumulation of Na^+ in plant cells may create metabolic disorders in functions where high K^+ or Ca^{+2} and low Na^+ are essential for perfect functioning (Tester and Davenport 2003).

The application of plant growth-promoting rhizobacteria (PGPR) is one of the most encouraging techniques used in the plant to in plants to reduce the harmful effects of salinity and improve growth (Shameer and Prasad 2018). Microorganisms in the soil substantially lessen plant salt stress, subsequently increasing crop production (Etesami and Beattie 2018). Furthermore, rhizobacteria having various PGP properties can improve the growth and development of plants. For example, *Bacillus* spp. capable to produce various phytohormones could increase plant resistance against salinity stress and improve plant growth (Rajendran *et al.* 2008). Moreover, it has been confirmed that applying PGPR enhanced plant growth under salinity stress conditions (Han and Lee 2005).

To cite this paper: Ashfaq M, HM Hassan, AHA Ghazali (2022). Evaluation of plant growth-promoting halotolerant potassium solubilizing rhizobacteria isolated from paddy crop under salinity stress. *Intl J Agric Biol* 28:446–456

A significant portion of the total P in the soil exists in fixed forms like $Ca_3(PO_4)_2$, $AlPO_4$, $FePO_4$ and organic phosphorus, which are unavailable for plants. The availability of P becomes reduced in saline soil, and saline ions inhibit the amount of P taken up by the plant (Rojas-Tapias *et al.* 2012). Phosphate-solubilizing rhizobacteria can solubilize the insoluble P by manufacturing various organic acids (Chen and Liu 2019). Thus, phosphorus becomes available to the plants, and there is a sustainable decrease in the pH of rhizosphere soil. Phosphorus solubilizing rhizobacteria can be a valuable bio inoculant for plants to reduce the effects of salinity and recover the quantitative and qualitative characteristics associated with plant and soil efficiency.

Indole acetic acid (IAA) is one of the most important plant hormones that have direct effects on increasing plant growth. The PGPR helps plants overcome the harmful effects of abiotic stresses by producing IAA, which directly improves plant growth, even in other growth-inhibiting chemicals (Bianco and Defez 2009). The amino acid tryptophan produced as root exudates are converted into IAA by PGPR in rhizoplane and is taken up by the root cells and stimulate auxin signal transduction pathway and different auxin responding factors (Shameer and Prasad 2018). The harmful effects of salinity may be minimized and plant growth can be improved by applying IAA-producing rhizobacteria.

Siderophores are low molecular weight organic compounds generated by rhizobacteria under low iron environments (Ahmad *et al.* 2016). The chief role of such compounds is to chelate iron and make it accessible for microbial and plant cells. Siderophore-producing rhizobacteria also play an essential role against phytopathogens. The Fe present in the soil is firmly bound with the siderophores produced by rhizobacteria and it becomes unavailable to plant pathogens, consequently hindering phytopathogen growth (Beneduzi *et al.* 2012; Ahmed and Holmström 2014).

The production of ammonia (NH_3) is a critical plant growth-promoting property of PGPR. The application of ammonia-producing PGPR provided the plant's nitrogen and significantly improved plant growth and biomass accumulation (Marques *et al.* 2010).

Salinity can boost ethylene synthesis *via* raised levels of 1-Amino cyclopropane-1-carboxylic acid (ACC). Any constraint on increased ethylene produced in plants can promote the plant's growth in saline soils. The ACC-deaminase yielded by various PGPR enhances the scarcity of ACC, decreasing the harmful concentration of ethylene in the plants under salt-stressed conditions. ACC produced in plant tissues is immediately cleaved into α-ketobutyrate and ammonia by ACC-deaminase (Hontzeas *et al.* 2004). The injection of ACC-deaminase-producing PGPR can facilitate plant growth in stressed environmental circumstances, including salinity, flooding, drought, heavy metal contamination and phytopathogens.

Exopolysaccharides (EPS) are high-molecular-weight compounds that contain sugar residues and widely vary in structure and role. The impact of EPS producing PGPR on the amalgamation of root clinging soils has been described by Alami *et al.* (2000). EPS producing PGPR can effectively increase the volume of soil macropores and soil aggregation, as a result, water and fertilizer accessibility to the plant increases. EPS-producing PGPR can also fix cations, including Na^+ (Upadhyay *et al.* 2011). Thus, the increased population of EPS-producing rhizobacteria in the rhizosphere is likely to reduce the concentration of Na^+ available for plant absorption and thus lessen the salt stress on plants grown in saline environments.

In the current study, four salt-resistant KSB (*A. pittii*, *R. pusense, C. oxalaticus* and *O. ciceri)* isolated from salt-affected rice fields in the coastal area of Kuala Muda, State Kedah, Malaysia, were evaluated for phosphorus solubilizing ability, IAA production, siderophores, ammonia, ACC deaminase and exopolysaccharides under salinity stress. The objective of study was to find the strain which could show the good plant growth promoting properties under salinity stress.

Materials and Methods

Four potential potassium solubilizing rhizobacteria *A. pittii*, *R. pusense, C. oxalaticus* and *O. ciceri* were subjected to evaluate their capability for other plant growth-promoting properties under NaCl stress. The strains were taken from the microbiological lab at The School of Biological Sciences, Universiti Sains Malaysia isolated and identified by Ashfaq *et al.* (2020).

Phosphorus solubilizations by KSB under NaCl stress

Four potential potassium solubilization rhizobacteria were subjected to evaluate their capability of phosphorus solubilization under NaCl stress. For phosphorus solubilization, National Botanical Research Institute's phosphate (NBRIP) broth has (g/L) glucose (10 g); $Ca_3(PO_4)_2$ (5 g); $MgCl_2$. $6H_2O$ (5 g); $MgSO_4$. $7H_2O$ (0.25 g); KCl (0.2 g) and $(NH_4)_2SO_4$ (0.1 g) was prepared (Scervino *et al.* 2011). The NBRIP broth was amended with 3, 5 and 7% NaCl to provide salinity stress compared with control without NaCl. One hundred mL NBRIP was taken in 250 mL flasks and the broth was sterilized at 121°C for 15 min. One mL of freshly grown bacterial cultures with optical density (OD) 0.5 was inoculated into 250 mL flasks containing 100 mL NBRIP broth. The flasks were put on a rotary shaker (180 rpm) for eight days at 28 ± 2°C. At 4 and 8 days after incubation (DAI) the bacterial culture was drawn from flasks and filtered through Whatman paper no. 1. The filtered culture was centrifuged at 10000 rpm for 10 min and available phosphorus was measured using the molybdenum blue method (Murphy and Riley 1962). After mixing with the Murphy-Riley reagent, the samples were kept for thirty

minutes to develop blue color, and absorbance was recorded at 712 nm using a spectrophotometer (Kowalenko and Babuin 2007). The experiment was conducted in three replicates. The quantity of phosphorus solubilized was determined from the standard curve of phosphorus prepared from the 2 μg/mL solution of KH_2PO_4.

Indole acetic acid production by KSB under NaCl stress

Indole acetic acid production by KSB strains was evaluated according to Gordon and Weber (1951). The nutrient broth having L-tryptophan @ 0.2 mg/mL (Bharucha *et al.* 2013) was amended with 3, 5 and 7% NaCl. After adjusting OD (0.5), bacterial cultures were inoculated into 250 mL flasks containing 100 mL of nutrient broth. The flasks were placed on a rotary shaker (180 rpm) for 5 days at 28 ± 2°C. IAA production by rhizobacteria was recorded using the Salkowski reagent (2% 0.5 *M* $FeCl_3$ in 35% perchloric acid) (Ehmann 1977). At the 3rd and 5th DAI, the samples were drawn to measure IAA quantity. The culture was centrifuged at 10000 rpm for 15 min. One mL of cell-free supernatant was added with two mL of Salkowski reagent and left at room temperature in the dark for twenty minutes (Gordon and Weber 1951) to develop the color. The IAA produced was measured using a spectrophotometer at 535 nm wavelength. The experiment was conducted in three replicates. The standard solution of 100 μg/mL indole acetic acid was prepared in deionized water to calculate the quantity of IAA. This standard solution was diluted to 5, 10, 15, 20, 25 and 30 μg/mL to get working solutions and standard curve was drawn.

Siderophores production by KSB under NaCl stress

Siderophores production was evaluated by Chrome Azurol S (CAS) Shuttle Assay (Schwyn and Neilands 1987). Succinate medium containing (g/L), K_2HPO_4 (6 g); KH_2PO_4 (3 g); $(NH_4)_2SO_4$ (1 g); $MgSO_4.7H_2O$ (0.2 g); and Succinic acid, 4 g amended with 3, 5 and 7% and without NaCl. Isolates were grown in a succinate medium and incubated under shaking conditions (180 rpm) for four days at 28 ± 2°C. After incubation every 48 h, the bacterial culture was taken out from the flasks and centrifuged at 10000 rpm for 10 min. Five hundred microliters of the supernatant were added to 0.5 mL of the CAS solution. After twenty minutes of incubation, OD was recorded at 630 nm by using a spectrophotometer. A reference sample containing 0.5 mL of CAS solution and 0.5 mL of uninoculated succinate medium was also prepared. The experiment was conducted in three replicates. The percentage of siderophores units produced by KSB strains was calculated by using this formula:

$$\%\text{siderophores} = \left(\frac{\text{Ar} - \text{As}}{\text{Ar}}\right) \times 100$$

Where, Ar is Absorbance of reference sample and As is Absorbance of inoculated sample.

Ammonia production by KSB under NaCl stress

The KSB strains were grown in peptone water modified with an additional 3, 5 and 7% NaCl (Desale *et al.* 2014) and without NaCl to determine the ammonia production. Autoclaved flasks containing 100 mL peptone water were inoculated with 1 mL of fresh-grown bacterial cultures and placed in a rotary shaker (180 rpm) for 96 h at 28 ± 2°C (Hansda *et al.* 2017). Two mL of peptone water with bacterial culture was drawn and centrifuged at 10000 rpm for 10 min. One mL of the supernatant was mixed with 1 mL of Nessler's reagent. The optical density was recorded at 450 nm using a spectrophotometer (Goswami *et al.* 2014). The trial was conducted in three replicates. The quantity of ammonia was calculated using the standard curve prepared from the standard solution of ammonium sulfate (Hansda *et al.* 2017).

ACC deaminase activity by KSB under NaCl stress

The bacterial strains were grown in 15 mL tryptic soy broth (TSB) for 24 h. After 24 h, the contents of the tubes were centrifuged at 8000 × g for 10 min at 4°C. The supernatant was removed and the pellets were washed with 5 mL DF salts in a minimal medium without ACC and centrifuged for 10 min. The supernatant was removed and suspended the cell pellets in 5 mL of 0.1 *M* Tris-HCl with a pH of 7.6. Again, centrifuged at 8000×g at 4°C for 10 min and discarded the supernatant. The washing procedure was repeated twice. The pellet was suspended in 7.5 mL Dworkin-Foster (DF) salts minimal medium amended with 3, 5 and 7% concentrations of NaCl in a fresh culture tube and control having no NaCl. As a sole nitrogen source, 45 μL of 0.5 *M* ACC solution was added to each tube. The inoculated culture was incubated on a rotary shaker (180 rpm) at 28 ± 2°C for 24 h. After 24 h of shaking, the tubes have centrifuged the tubes at 8000 × g for 10 min at 4°C. The supernatant was removed and suspended in the pellet in 5 mL of 0.1 *M* Tris-HCl (pH 7.6). Again, the tubes were centrifuged at 8000 × g at 4°C for 10 min and discarded the supernatant. The washing procedure was repeated twice. The bacterial pellets were resuspended in 1 mL of 0.1 *M* Tris-HCl with pH 8.5 and centrifuged at 13000 rpm for 5 min. The supernatant was discarded and suspended cell pellets in 600 μL 0.1 *M* Tris HCl with pH 8.5, then added 30 μL of toluene and vortexed for 30 s. Two hundred μL of colonized cells were transferred into a fresh 1.5 mL centrifuge tube and 20 μL of 0.5 *M* ACC was added to each tube. After a brief vortex, the tubes were incubated at 28°C for 15 min. After incubation, 1 mL of 0.56 *M* HCl was added to the tubes and the tubes were vortexed and centrifuged for 5 min at 13000 rpm at room temperature. One mL of supernatant was transferred to the glass tube and the supernatant was mixed with 800 μL of 0.56 *M* HCl. Furthermore, 300 μL of the 2, 4-dinitrophenylhydrazine was also poured into the tubes. The tubes were vortexed and then

incubated at 28°C for 30 min. After incubation, 2 mL 2 N NaOH was mixed. The absorbance was measured using a spectrophotometer at 540 nm along with the standard solutions. The ACC deaminase activity of KSB strains was calculated from the standard curve of α-ketobutyrate prepared from 10, 20, 30, 40 and 50 *µM* solutions. The experiment was conducted in three replicates.

Exopolysaccharides production by KSB under NaCl stress

The exopolysaccharides production by bacterial strains was evaluated using ATCC No. 14 broth (Mu'minah *et al.* 2015). ATCC No. 14 broth amended with 3, 5 and 7% NaCl and with NaCl was autoclaved at 121°C for 15 min and cooled at room temperature. One mL of freshly grown bacterial cultures was inoculated into 250 mL flasks containing 100 mL of ATCC No. 14 broth. The flasks were put in a rotary shaker (180 rpm) for eight days at 28 ± 2°C. Three mL of bacterial culture was drawn at 4 and 8 DAI. The cell culture was centrifuged at 10000 rpm for 20 min. Three volumes of chilled acetone were mixed with 1 mL of supernatant for the precipitation of exopolysaccharides. The cell-free supernatant and acetone mixture was stored at 4°C overnight. The solution was centrifuged at 8000 rpm for 10 min. The precipitated exopolysaccharides were collected and resuspended in 1 mL of distilled water. Three volumes of acetone were again used to reprecipitate the dissolved exopolysaccharides. The dissolved exopolysaccharides were used to estimate EPS using glucose as a standard by the phenol-sulfuric acid method (Do *et al.* 2009). One mL of aqueous phenol and 5 mL of concentrated H_2SO_4 were added to the test tubes containing 1 mL of exopolysaccharides solution. After vigorous shaking, they were allowed to stand for 20 min. The absorbance was recorded at 490 nm wavelength. The exopolysaccharides were calculated from the standard curve obtained from the standard solution of glucose.

Statistical analysis

The data about the quantitative determination of PGP properties were subjected to analysis of variance (ANOVA), following Duncan's test. The statistical studies were conducted using IBM SPSS Statistics v. 25. The experiments were conducted in three replications.

Results

Phosphorus solubilizations by KSB under NaCl stress

With the increased NaCl stress, the quantity of phosphorus solubilization decreased; however, all four tested strains could solubilize phosphorus under the highest NaCl stress (7%). On the 4th and 8th days after incubation, the highest phosphorus (36.42 and 41.92 µg/mL, respectively) solubilization was noted in 0% NaCl, significantly higher than all NaCl concentrations (Table 1). Statistically, the lowest phosphorus (9.40 and 10.89 µg/mL, respectively) was measured at 7% NaCl concentration followed by 5% NaCl stress (16.14 and 19.17 µg/mL, respectively).

At 4th DAI, the highest phosphorus solubilization was recorded in *A. pittii* (41.68, 21.96 and 11.73 µg/mL, respectively) in the medium having 0, 5 and 7% NaCl whereas the *O. ciceri* solubilized the highest phosphorus in the medium having 3% NaCl (Table 1). The lowest phosphorus (28.22 and 16.97 µg/mL, respectively) solubilization was recorded in *R. pusense* under treatment having no and 3% NaCl. With a 5% concentration of NaCl, the lowest phosphorus (13.35 µg/mL) was recorded in *O. ciceri*. At 8th DAI, *A. pittii* solubilized the highest phosphorus (45.85, 24.40 and 13.10 µg/mL, respectively) in the medium having 0, 5 and 7% NaCl whereas *O. ciceri* solubilized the highest phosphorus (44.71 µg/mL) in the medium having 3% NaCl. The *R. pusense* solubilized the lowest phosphorus (35.08, 28.10 and 9.11 µg/mL, respectively) in the medium with 0, 3 and 7% NaCl whereas *C. oxalaticus* solubilized the lowest phosphorus (16.12 µg/mL) in the medium with 5% NaCl. The P solubilization by different KSB strains varied according to the potential. On average, at 4th and 8th DAI maximum phosphorus (26.57 and 30.18 µg/mL, respectively), solubilization was observed in *A. pittii*, whereas the lowest phosphorus solubilization was recorded in *R. pusense* (16.74 and 22.41 µg/mL, respectively) (Fig. 1).

Indole acetic acid production by KSB under NaCl stress

The salinity stress significantly affected IAA production by bacterial strains. Regardless of KSB strains, at both 3rd and 5th DAI the highest IAA (35.38 and 39.09 µg/mL, respectively) was produced under control treatment without NaCl, whereas the lowest was in 7% NaCl (8.77 and 11.23 µg/mL, respectively) (Table 1).

In the medium having no NaCl, significantly higher production of IAA was recorded at 3rd and 5th DAI by *O. ciceri* (78.78 and 85.60 µg/mL, respectively) followed by *A. pittii* (31.70 and 37.56 µg/mL, respectively) (Table 1). The lowest IAA production was recorded by the *C. oxalaticus* KSB strain. Results with 3% concentrations of NaCl at 3rd and 5th DAI showed that significantly higher IAA (58.44 and 60.09 µg/mL, respectively) production was recorded in *O. ciceri*. In the case of a 5% concentration of NaCl, significantly higher IAA (43.47 and 53.41 µg/mL, respectively) production was recorded with *O. ciceri*, followed by *A. pittii* (1.67 and 2.93 µg/mL, respectively). The highest IAA (33.94 and 43.09 µg/mL, respectively) was produced by *O. ciceri* under 7% NaCl stress at 3rd and 5th DAI, respectively.

The quantity of IAA produced by KSB strains varied according to the efficiency of the strains. On an average basis, *O. ciceri* produced a significantly higher quantity of

Table 1: Phosphorus solubilization and IAA (μg/mL) production by four KSB strains under different NaCl concentrations

	Strain ID	NaCl level							
		0%		3%		5%		7%	
		4th Day	8th Day	4th Day	8th Day	4th Day	8th Day	4th Day	8th Day
(P)	*A. pittii*	41.68 ± 2.4a	45.85 ± 0.70a	30.91 ± 0.69b	37.37 ± 0.9b	21.96 ± 1.61a	24.40 ± 0.7a	11.73 ± 0.11a	13.10 ± 0.4a
	R. pusense	28.22 ± 1.26b	35.08 ± 1.6b	16.97 ± 2.72c	28.10 ± 0.4c	14.17 ± 2.41b	17.36 ± 0.4b	7.62 ± 1.16b	9.11 ± 0.9b
	C. oxalaticus	37.34 ± 1.58a	44.09 ± 0.9a	18.95 ± 0.53c	36.84 ± 2.0b	15.10 ± 0.53b	16.12 ± 1.2b	7.32 ± 1.48b	9.11 ± 1.0b
	O. cicero	38.44 ± 1.34a	42.66 ± 1.0a	37.17 ± 1.09a	44.71 ± 0.9a	13.35 ± 0.87b	18.80 ± 2.1b	10.96 ± 0.48a	12.27 ± 0.1a
	Mean	36.42 ± 1.67a	41.92 ± 1.32a	26.00 ± 2.62b	36.75 ± 1.72b	16.14 ± 1.22c	19.17 ± 1.20c	9.40 ± 0.69d	10.89 ± 0.60d
(IAA)		3rd Day	5th Day	3rd Day	5th Day	3rd Day	5th Day	3rd Day	5th Day
	A. pittii	31.70 ± 0.78b	37.56 ± 0.68b	16.90 ± 01.14b	28.14 ± 0.73b	1.67 ± 0.30b	2.93 ± 0.67b	0.54 ± 0.07b	0.59 ± 0.31b
	R. pusense	26.54 ± 0.66c	28.47 ± 0.58c	5.31 ± 0.56c	6.04 ± 0.73c	0.98 ± 0.03bc	2.13 ± 0.13b	0.61 ± 1.02b	0.92 ± 0.01b
	C. oxalaticus	4.51 ± 0.29d	4.76 ± 0.29d	0.60 ± 0.03d	0.98 ± 0.05d	0.02 ± 0.00c	0.62 ± 0.02c	0.00 ± 0.00b	0.35 ± 0.02b
	O. cicero	78.78 ± 0.41a	85.60 ± 1.55a	58.44 ± 0.48a	60.09 ± 0.54a	43.47 ± 0.68a	53.41 ± 0.40a	33.94 ± 0.48a	43.09 ± 0.19a
	Mean	35.38 ± 8.16a	39.09 ± 8.91a	20.31 ± 6.88b	23.81 ± 7.02b	11.53 ± 5.56c	14.77 ± 6.73c	8.77 ± 4.38d	11.23 ± 5.55d

Mean ± standard error. Values sharing same letters differ non-significantly ($P > 0.05$)

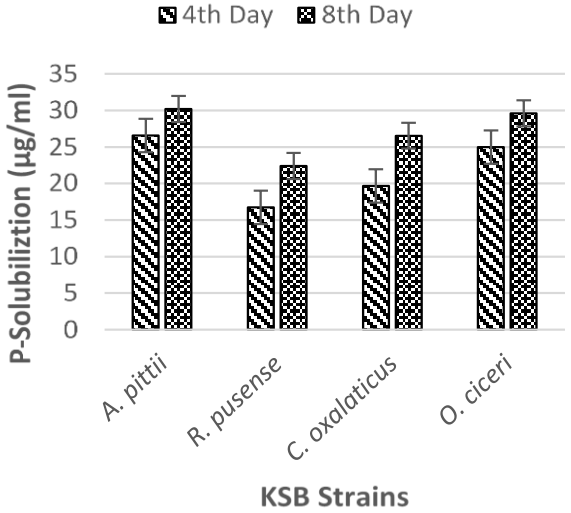

Fig. 1: Average phosphorus (P) solubilization (μg/mL) by 4 strains of KSB under NaCl stress (0, 3, 5 and 7%) on the 4th and 8th day

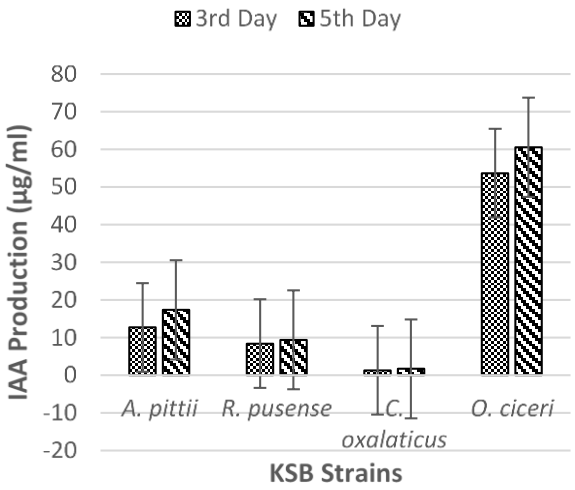

Fig. 2: Average Indole Acetic Acid (IAA) production (μg/mL) by 4 strains of KSB under NaCl stress (0%, 3%, 5%, and 7%) on the 3rd and 5th day

IAA (53.66 and 60.55 μg/mL, respectively), followed by *A. pittii* (12.70 and 17.30 μg/mL, respectively) at 3rd and 5th DAI whereas the lowest IAA was recorded in *C. oxalaticus* (1.28 and 1.67 μg/mL, respectively) (Fig. 2).

Siderophores production by KSB under NaCl stress

The number of siderophores increased with NaCl stress up to 3%; however, further increases in NaCl stress (5 and 7% NaCl) decreased the quantity of siderophore production. Significantly higher siderophores were produced in the medium at 2nd and 4th DAI, having 3% NaCl (48.93 and 71.76%, respectively) followed by 0% NaCl (3.62 and 34.08%, respectively). The lowest siderophores (8.22 and 15.25%, respectively) were recorded in the medium with 7% NaCl stress at the 2nd and 4th DAI.

At 2nd DAI, the highest siderophores (72.69 and 12.35%) were produced by *O. ciceri* in the medium having 0 and 7% NaCl, respectively (Table 2). The highest siderophores (54.32%) were measured in *C. oxalaticus* with 3% NaCl stress and in *A. pittii* (12.38%) with 5% NaCl stress., The lowest siderophores (12.44%) were produced by *C. oxalaticus* in the medium with 0% NaCl and *R. pusense* (42.46, 4.24 and 3.98%, respectively) in the medium with 3, 5 and 7% NaCl stress. At 4th DAI, the highest siderophores (73.43, 79.21, 33.56 and 18.25%) were recorded in *O. ciceri* in the medium with 0, 3, 5 and 7% NaCl stress, respectively. The lowest siderophores (15.52%) were produced by *C. oxalaticus* in the medium without NaCl whereas in the medium having 3 5 and 7% NaCl, the lowest siderophores (60.29, 23.33 and 12.30%, respectively).

The siderophores produced varied according to the efficacy of potassium solubilizing rhizobacteria. On an average basis, at the 2nd DAI, *O. ciceri* produced the highest quantity (37.62%) of siderophores, followed by *A. pittii* (21.25%) (Fig. 3). The lowest siderophores (15.93%) were produced by *R. pusense* (19.97%). At 4th DAI, the highest quantity of siderophores (51.11%) was produced by *O.*

Table 2: Siderophores (%) and ammonia (µg/mL) production by four KSB strains under different NaCl concentrations

	Strain ID	NaCl level							
		0%		3%		5%		7%	
		2nd Day	4th Day	2nd Day	4th Day	2nd Day	4th Day	2nd Day	4th Day
(SID.)	*A. pittii*	17.72 ± 0.89c	18.34 ± 0.71c	44.77 ± 0.67b	72.62 ± 0.75b	12.38 ± 0.50a	31.51 ± 0.30b	10.14 ± 0.63b	16.04 ± 0.15b
	R. pusense	29.22 ± 1.34b	29.03 ± 1.03b	42.46 ± 0.63c	60.29 ± 1.50c	4.24 ± 0.15c	23.33 ± 0.54d	3.98 ± 0.89d	12.30 ± 0.66d
	C. oxalaticus	12.44 ± 0.78d	15.52 ± 0.44c	54.32 ± 0.63a	74.92 ± 1.24b	6.78 ± 0.38b	30.63 ± 0.60b	6.40 ± 0.17c	14.42 ± 0.32c
	O. ciceri	72.69 ± 0.99a	73.43 ± 1.48a	54.18 ± 0.49a	79.21 ± 0.59a	11.25 ± 0.68a	33.56 ± 0.95a	12.35 ± 0.93	18.25 ± 0.46a
	Mean	33.62 ± 7.15b	34.08 ± 7.02b	48.93 ± 1.64a	71.76 ± 0.59a	8.66 ± 1.01c	29.76 ± 1.19c	8.22 ± 1.02c	15.25 ± 0.68c
(AMM)		2nd Day	4th Day	2nd Day	4th Day	2nd Day	4th Day	2nd Day	4th Day
	A. pittii	15.99 ± 0.37b	20.35 ± 0.91a	17.58 ± 0.18a	18.03 ± 0.12a	14.92 ± 0.37a	15.98 ± 0.19b	9.63 ± 0.37ab	10.32 ± 0.11a
	R. pusense	18.17 ± 0.37a	18.36 ± 0.51b	14.37 ± 0.76c	17.57 ± 0.78a	11.02 ± 0.44b	16.63 ± 0.30a	8.22 ± 0.37b	9.60 ± 0.73a
	C. oxalaticus	14.89 ± 0.37b	16.72 ± 0.59c	14.80 ± 0.71ab	16.42 ± 0.81ab	10.51 ± 0.50b	15.15 ± 0.68ab	8.66 ± 0.33b	9.82 ± 0.44a
	O. ciceri	18.00 ± 0.33a	18.63 ± 0.99b	15.21 ± 020b	14.52 ± 0.71b	14.17 ± 0.27a	13.62 ± 1.05b	11.65 ± 0.33a	8.47 ± 0.64a
	Mean	16.76 ± 0.33a	18.51 ± 0.44a	15.49 ± 0.44b	16.63 ± 0.49b	12.65 ± 0.53c	15.34 ± 0.43c	9.54 ± 0.48d	9.55 ± 0.40d

Mean ± standard error. Values sharing same letters differ non-significantly ($P > 0.05$)

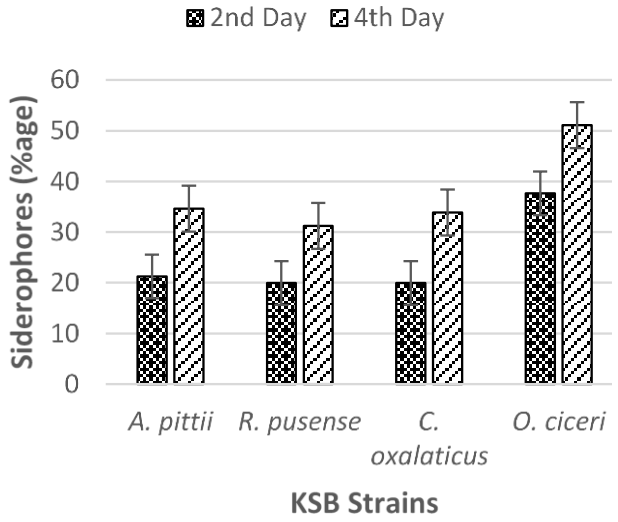

Fig. 3: Average siderophores production (%) by 4 strains of KSB under NaCl stress (0, 3, 5 and 7%) on the 2nd and 4th day

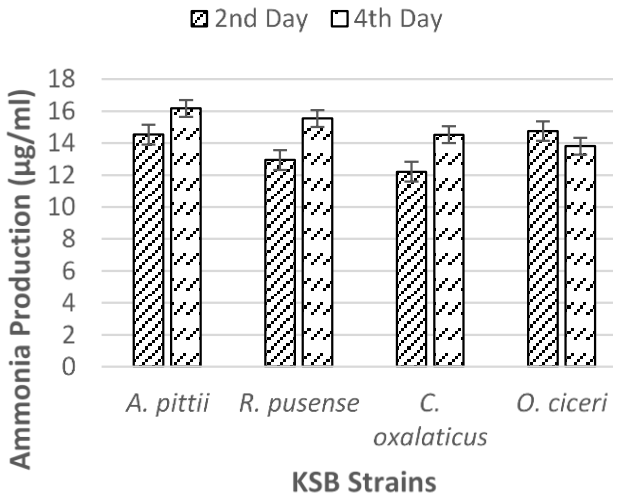

Fig. 4: Average ammonia production (µg/mL) by 4 strains of KSB under NaCl stress (0, 3, 5 and 7%) on the 2nd and 4th day

ciceri, followed by *A. pittii* (34.63%), which is at par with *C. oxalaticus* (33.87%). The lowest siderophores were produced by *R. pusense* (31.28%).

Ammonia production by KSB under NaCl stress

Regardless of the KSB strain, an increase in salinity stress reduced ammonia production. Without NaCl concentration at 2nd and 4th DAI, the highest ammonia (16.76 and 18.51 µg/mL, respectively) was produced followed by 3% NaCl stress (15.14 and 16.63 µg/mL, respectively) (Table 2). Conversely at 2nd and 4th DAI, the lowest ammonia (9.54 and 9.55 µg/mL, respectively) was produced at 7% NaCl, followed by 5% NaCl concentration (12.65 and 15.38 µg/mL, respectively).

At 2nd DAI, *R. pusense* produced the highest ammonia (18.17 µg/mL) in the medium without NaCl. Whereas the lowest ammonia (14.89 µg/mL) was produced by *C. oxalaticus*. The highest ammonia (17.58 and 14.92 µg/mL) was produced by *A. pittii* in the medium amended with 3% and 5% NaCl, respectively. At a 7% NaCl concentration, *O. ciceri* produced the highest ammonia (11.65 µg/mL); however, all other four KSB are at par for ammonia production. At 4th DAI, the highest ammonia (20.35, 18.03 and 10.32 µg/mL) was produced by *A. pittii* in the medium amended with 0, 3 and 7% NaCl stress, respectively. The *R. pusense* produced the highest ammonia (16.63 µg/mL) in the medium amended with 5% NaCl. The lowest ammonia was produced by *C. oxalaticus* (16.72 µg/mL) in the medium without NaCl whereas *O. ciceri* produced the lowest ammonia (14.52, 13.62 and 8.47 µg/mL) in the medium amended with 3, 5 and 7% NaCl, respectively.

The quantity of ammonia under NaCl varied according to the efficacy of rhizobacteria (Fig. 4). On average, under all treatments, at 2nd DAI, the highest ammonia (14.75 µg/mL) was produced by *O. ciceri*, followed by *A. pittii* (14.53 µg/mL). In contrast, the lowest ammonia was recorded in *C. oxalaticus* (12.21 µg/mL). At the 4th DAI, the

Table 3: EPS production (μg/mL) and ACC deaminase activity (μmol α-ketobutyrate/mg/h) by four KSB strains under different NaCl concentrations

(EPS)	Strain ID	NaCl level							
		0%		3%		5%		7%	
		4th Day	8th Day	4th Day	8th Day	4th Day	8th Day	4th Day	8th Day
	A. pittii	130.00 ± 3.21a	256.66 ± 1.85a	138.66 ± 3.90a	266.33 ± 1.85a	199.66 ± 0.33a	285.66 ± 0.33a	116.00 ± 7.50a	193.66 ± 1.20a
	R. pusense	75.66 ± 1.76b	157.33 ± 6.33b	111.00 ± 3.05b	203.00 ± 2.08b	158.33 ± 5.92b	192.33 ± 8.19b	58.66 ± 3.84b	152.66 ± 7.21b
	C. oxalaticus	84.00 ± 2.88b	86.00 ± 5.13d	145.00 ± 1.52a	173.33 ± 3.17c	194.33 ± 5.66a	205.33 ± 3.48b	56.00 ± 3.21b	75.33 ± 4.91c
	O. ciceri	58.66 ± 3.92c	113.00 ± 0.57c	96.33 ± 6.96b	135.33 ± 6.74d	129.33 ± 6.88c	273.66 ± 4.09a	37.00 ± 3.05c	153.00 ± 6.11b
	Mean	87.08 ± 8.06c	153.25 ± 19.63c	122.75 ± 6.89b	194.50 ± 14.54b	170.41 ± 8.90a	239.16 ± 12.48a	66.91 ± 9.13d	143.66 ± 13.11d
(ACC)		0%NaCl		3%NaCl		5%NaCl		7%NaCl	
	A. pittii	2.366 ± 0.14a		2.456 ± 0.11a		2.123 ± 0.42a		1.623 ± 0.25a	
	R. pusense	1.733 ± 0.11b		1.383 ± 0.01c		1.060 ± 0.01b		0.823 ± 0.08b	
	C. oxalaticus	2.103 ± 0.03a		1.760 ± 0.05b		1.070 ± 0.05b		0.803 ± 0.11b	
	O. ciceri	2.400 ± 0.11a		1.886 ± 0.02b		1.376 ± 0.12b		1.050 ± 0.13b	
	Mean	2.151 ± 0.09a		1.871 ± 0.12b		1.407 ± 0.16c		1.074 ± 0.12d	

Mean ± standard error. Values sharing same letters differ non-significantly ($P > 0.05$)

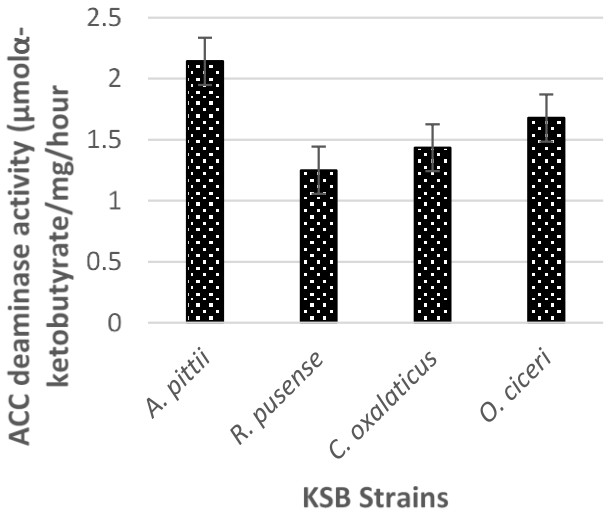

Fig. 5: ACC deaminase activity (μmol α-ketobutyrate/mg/h) by 4 strains of KSB under NaCl stress (0, 3, 5 and 7%)

highest ammonia (16.17 μg/mL) was produced by *Acinetobacter pittii*, followed by *R. pusense* (15.54 μg/mL). The *O. ciceri* produced the lowest ammonia (13.84 μg/mL), followed by *C. oxalaticus* (14.78 μg/mL).

ACC deaminase activity by KSB under NaCl stress

The concentration of NaCl in the DF minimal salt medium significantly affected the ACC deaminase activity of KSB strains (Table 3). The highest ACC deaminase activity (2.151 μmol α-ketobutyrate/mg/h) was noted in the medium having no NaCl followed with 3% NaCl concentration (1.871 μmol α-ketobutyrate/mg/h). The lowest ACC deaminase activity (1.074 μmol/mg/h) was observed under 7% NaCl stress.

Without NaCl stress, the highest ACC deaminase activity (2.400 μmol/mg/h) was observed in *O. ciceri* whereas the lowest in *R. pusense* (1.733 μmol α-ketobutyrate/mg/h). Under 3, 5 and 7% NaCl the highest ACC deaminase activity (2.456 μmol α-ketobutyrate/mg/h, 2.123 μmol α-ketobutyrate/mg/h and 1.623 µmol α-ketobutyrate/mg/h) was recorded in *Acinetobacter pittii.* The lowest ACC deaminase activity (1.383 μmol α-ketobutyrate/mg/h and 1.060 μmol α-ketobutyrate/mg/h) was recorded in *R. pusense* in the medium amended with 3 and 5% NaCl whereas in *C. oxalaticus* (0.803 μmol α-ketobutyrate/mg/h) under 7% NaCl stress.

The efficacy of ACC deaminase activity varied according to their potential (Fig. 5). On an average basis under all treatments, the highest ACC deaminase activity (2.142 μmol α-ketobutyrate/mg/h) was recorded in *A. pittii*, followed by *O. ciceri* (1.678 μmol α-ketobutyrate/mg/h). The lowest ACC deaminase activity (1.250 μmol α-ketobutyrate/mg/h) was measured by *R. pusense*.

Exopolysaccharides production by KSB under NaCl stress

The exopolysaccharides production increased to 5% NaCl concentration; however, further increase in NaCl stress decreased the production of exopolysaccharides. At 4th and 8th DAI, the highest exopolysaccharides (170.41 and 239.16 μg/mL, respectively) were produced at 5% NaCl, followed by 3% NaCl stress (122.75 and 194.54 μg/mL, respectively) (Table 3). The lowest exopolysaccharides (66.91 and 143.66 μg/mL, respectively) were produced in the medium, having 7% NaCl, followed by the medium with 0% NaCl concentration (87.08 and 153.25 μg/mL, respectively).

At 4th DAI, *A. pittii* produced the highest exopolysaccharides (130.00, 199.66 and 116.00 μg/mL) in the medium amended with 0, 5 and 7% NaCl whereas under 3% NaCl stress, *C. oxalaticus* produced the highest exopolysaccharides (145.00 μg/mL). when it was inoculated in the medium having no NaCl. In contrast, *O. ciceri* produced the lowest exopolysaccharides (58.66, 96.33, 129.33 and 37.00 μg/mL, respectively) under 0, 3, 5 and 7% NaCl stress. At 8th DAI, *A. pittii* produced the highest exopolysaccharides (256.66, 266.33, 285.66 and 193.66

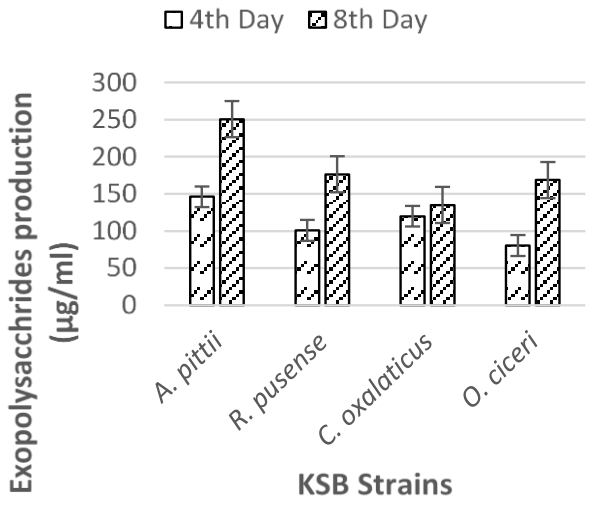

Fig. 6: Exopolysaccharides (μg/mL) production by 4 strains of KSB under NaCl stress (0, 3, 5 and 7%) 4th and 8th day

μg/mL, respectively) in the medium amended with 0, 3, 5 and 7% NaCl. The lowest exopolysaccharides (86.00 and 75.33 μg/mL, respectively) were produced by *C. oxalaticus* under 0 and 7% NaCl stress. Under 3% NaCl stress, *Ochrobactrum ciceri* (135.33 μg/mL) produced the lowest exopolysaccharides, whereas *R. pusense* produced the lowest exopolysaccharides (192.33 μg/mL) in the medium containing 5% NaCl concentration.

The quantity of exopolysaccharides production varied according to the efficacy of rhizobacteria. On an average basis, at 4th and 8th DAI, *A. pittii* produced the highest exopolysaccharides (146.08 and 250.58 μg/mL, respectively) (Fig. 6). In contrast, the lowest exopolysaccharides were recorded in *O. ciceri* (80.33 μg/mL) at 4th DAI and in *C. oxalaticus* (135.00 μg/mL) at 8th DAI.

Discussion

High salinity decreases the available phosphorus and saline ions (Ca^{++}, Na^{+}, Cl^{-} etc.) control phosphorus absorption by plant roots (Beji *et al.* 2017). Adopting salt-resistant phosphorus solubilizing rhizobacteria is a successful technique to increase phosphorus availability and minimize the effects of salinity on plant growth. All the strains were from the saline rhizosphere, and the interaction effects between isolates and NaCl stress were substantial. The NaCl stress in the medium significantly affected the phosphorus solubilization; however, all the strains could solubilize phosphorus up to 7% NaCl stress. There was a significant reduction in P solubilization with increasing NaCl stress; this might be because NaCl stress adversely affects cell growth and propagation, which causes lesser P solubilization. The *A. pittii* had the highest phosphorus solubilization among these strains, followed by *O. ciceri*. Jiang *et al.* (2020) isolated 23 phosphorus solubilizing bacteria, including *Bacillus*, *Acinetobacter*, *Pseudomonas*, *Brevibacillus*, *Gordonia*, *Chryseobacterium*, *Ensifer* and *Paenibacillus*, from saline soils. All PSB in this study could solubilize tricalcium phosphate $Ca_3(PO_4)_2$, ranging from 65 to 496 mgL^{-1}. Likewise, Nautiyal *et al.* (2000) isolated NBRI0603, NBRI2601, NBRI3246, and NBRI4003 phosphorus solubilizing strains and were subjected to growth and phosphorus solubilization in the presence of NaCl (2.5, 5, 7.5 and 10% NaCl). All the strains could solubilize phosphorus up to 10% NaCl stress. Srinivasan *et al.* (2012) isolated 12 PSB from saline soil, which could solubilize mineral phosphorus up to 2 *M* NaCl stress.

Indole acetic acid (IAA) has been considered the most dominant, physiologically active, naturally occurring auxin, produced in larger quantities than any other related compounds (Harikrishnan *et al.* 2014). IAA is one of the essential auxins which enhances early root growth. It promotes lateral and adventitious root formation, enabling the plants to develop more root surface area and absorb more nutrients from the soil (Chaiharn and Lumyong 2011). The salt-tolerant PGPR produces IAA, which is essential for root initiation, cell enlargement, and cell division, which helps plants to manage salt stress (Egamberdieva *et al.* 2019). In the current study, the strains could produce IAA under the salinity stress up to 7% NaCl; however, the production of IAA decreased with the increased NaCl concentration (Dilfuza 2012). The *O. ciceri* and *A. pittii* are high IAA-producing strains under saline conditions. *A. pittii* was also reported by Afzal *et al.* (2015) as high IAA-producing rhizobacteria than other strains.

Most micronutrients, including iron (Fe), are deficient in saline soils and plant growth is highly reduced (Rabhi *et al.* 2007; Yousfi *et al.* 2007). The growth of plants under saline soils is adversely affected by salinity and deficiency of Fe simultaneously. Siderophores are low molecular weight metal-chelating mediators that plants and microorganisms produce in Fe deficient conditions (Crowley *et al.* 1991). In the present study, the highest siderophores were produced at 3% NaCl concentration compared to the siderophores produced in the medium with no stress, which is supported by Sadeghi *et al.* (2012). They also concluded that siderophores production by PGPR isolated from saline soil increased with NaCl stress up to 300 m*M*. The addition of NaCl decreased siderophores production. Argandoña *et al.* (2010) also reported a lesser level of siderophore production was noted at increased salt stress. In the present study, the highest siderophores were produced by *O. ciceri* under NaCl stress. Príncipe *et al.* (2007) also isolated 1 *M* NaCl salt resistant *Ochrobactrum* spp. from saline soils of Argentina capable of siderophores production. The application of siderophores-producing rhizobacteria may prove a promising tool for empowering plants to handle iron deficiency in saline soils (Ferreira *et al.* 2019).

Ammonia production by PGPR influences plant growth directly and indirectly. Ammonia production by rhizobacteria directly supports plant growth by providing

nitrogen. It is an essential macronutrient to synthesize chlorophyll, proteins, enzymes, DNA and RNA (Rodrigues *et al.* 2016). The application of nitrogen increases the salinity resistance of plants as nitrogen plays nutritional and osmotic roles in saline soils (Chen *et al.* 2010). Ammonia production by rhizobacteria may help the plant for nitrogen requirements and minimize the root colonization of host plants by pathogens. In the present study, all the KSB could produce ammonia up to 7% NaCl stress; however, ammonia production decreased with NaCl stress. The highest ammonia was produced by *O. ciceri* and *A. pittii*. Sachdev *et al.* (2010) isolated the *Acinetobacter* rhizobacteria from the wheat field, which exhibited plant growth-promoting properties such as nitrogen fixation, phosphorus solubilization, ammonia production and siderophores production.

Under stress conditions, ethylene production increases due to ion toxicity and osmotic stress (Zhang *et al.* 2010; Tavakkoli *et al.* 2011). Increased ethylene production causes harmful effects on root growth (Belimov *et al.* 2009), decreasing overall plant growth due to water and nutrient restrictions. Previous studies showed that ACC deaminase-producing PGPR could reduce the destructive effects of ethylene on root growth by cleaving its direct precursor ACC into ammonia and α-ketobutyrate (Glick *et al.* 1998; Mayak *et al.* 2004). Ammonia and α-ketobutyrate are used as sources of nitrogen and carbon by rhizobacteria. The α-ketobutyrate is a precursor of various amino acids, such as leucine, used in protein biosynthesis (Glick 2014). In this study, all four KSB strains could utilize ACC as a nitrogen source under 3, 5 and 7% NaCl stress conditions and control. The findings are supported by the results of Bal *et al.* (2013), Zhou *et al.* (2017) and Nascimento *et al.* (2018) and *A. pittii* had the highest ACC utilization rate, followed by *O. ciceri* and *C. oxalaticus*, whereas the lowest ACC degradation was observed in *R. pusense*. In increased salinity stress, the strains showed an expected decrease in ACC degradation, which causes ethylene accumulation in the soil. The constant high ACC deaminase activity of *A. pittii* shows its efficiency for plant growth promotion for a wide range of adverse conditions that would result in the production of ethylene (Gulati *et al.* 2009; Ahmad *et al.* 2016). The results of current study are in accordance with the findings of previous experiments (Nadeem *et al.* 2010; Ahmad *et al.* 2011) regarding ACC-deaminase activity under salinity stress.

Exopolysaccharide (EPS) is a composite mixture of macromolecular electrolytes excreted as mucus on the external surface of bacterial cells. EPS provides a physical fence around plant roots and enhances plant growth under salinity stress (Vaishnav *et al.* 2016). EPS also bind to cations, including Na^+ in saline soils (Geddie and Sutherland 1993), thus alleviating the salt stress effect. It increases soil aggregation for nutrients and water uptake, thus resulting in better plant growth under saline environments (Ashraf *et al.* 2004). The number of EPS produced increased with NaCl stress up to 5%; however, it decreased with a further increase in NaCl stress. The results are supported with findings of Qurashi and Sabri (2012). They also reported that EPS production by rhizobacteria increased with the increase in NaCl stress from 0 to 1 *M* NaCl and decreased with further increase in NaCl stress from 1.5 to 2.5 *M* NaCl. Sandhya and Ali (2015) also reported that EPS production by PGPR improved with the increase in NaCl stress up to 1.4 *M* concentration. An abundant EPS is produced in hostile environments (Bomfeti *et al.* 2011; Tewari and Arora 2014). The highest quantity of EPS was produced by *A. pittii* under saline environments. Bechtaoui *et al.* (2019) also reported the *Acinetobacter* spp. as potential EPS-producing rhizobacteria.

Conclusion

The results of this study revealed that the KSB strains isolated from saline conditions own significant plant growth-promoting properties that are assumed to play an essential role in saline soils. These results demonstrated that *A. pittii* and *O. ciceri* could exhibit excellent plant growth-promoting properties under high saline conditions and the findings will meaningfully contribute to the pool of knowledge. The inoculation of the above strains to plants in salinity stress would considerably enhance plant growth.

Acknowledgements

The authors would like to acknowledge the School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia, for providing research facilities and a research environment.

Author Contributions

MA planned Research, HMH and AHAG supervised research.

Conflicts of Interest

The authors declare that they have no conflict of interests

Data Availability

Data presented in this study will be available on a fair request to the corresponding author

Ethics Approval

Not applicable in this paper.

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