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ISOLATION AND EVALUATION OF HALOTOLERANT RHIZOBACTERIA FROM *XANTHIUM STRUMARIUM* L. AS PLANT GROWTH PROMOTING RHIZOBACTERIA

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Abstract

Rhizosphere inhabiting bacteria are indirectly or directly modulate plant growth and physiological attributes by secretion of unique metabolites. This study was conducted to isolate and characterize rhizobacteria from a halophytic plant *Xanthium strumarium* L. to evaluate for their ability to promote growth of maize plant (*Zea mays* L.). For this purpose four different rhizobacteria were isolated from *X. strumarium* growing in Mardan district. All isolates were characterized for the production of phytohormones (Indole acetic acid (IAA) and Cytokinins), nitrogen fixation, phosphate solubilization and salt tolerance ability. All the isolates were biochemically characterized and identified as *Staphylococcus* sp. (Xs1), *Arthrobacter* Sp. (Xs2), *Enterobacter* sp. (Xs3) and *Pseudomonas* sp. (Xs4). All isolates showed IAA and cytokinins production and one isolate showed nitrogen fixing ability but none showed phosphate solubilization. Of the isolated strains Xs1 and Xs2 showed growth in LB agar containing up to 5.5% NaCl concentration. Seed germination experiments revealed increase in root and shoot lengths, fresh and dry weights of maize seedlings in treated seedlings as compared to control. In pot experiment, growth parameters such as root length, shoot length, fresh and dry weight after 15 days of growth showed enhancement as compared to control. *Staphylococcus* sp. (Xs1) was found most effective to increase growth of maize seedlings.

Key words: Biochemical screening, Phytohormone, Salt tolerance, *Zea mays.*

Introduction

Rhizosphere is considered as a region of diversified biological activities and associated plant growth promoting rhizobacteria (PGPR) which progressively affects plant growth and productivity (Liangpeng *et al*., 2007). PGPR induce growth promotion by stimulating uptake of phosphorous, nitrogen and production of phytohormones, whereas hindering growth of plant pathogen and increase resistance is their indirect effect on plant growth (Ahemad & Kibret, 2014; Abbasi *et al*., 2014). Insoluble phosphates like dicalcium phosphate, rock phosphate, tricalcium phosphate and hydroxyl apatite are mainly solubilized by PGPR to convert them as readily available forms (Rodríguez *et al*., 2007). Some other abilities of PGPR include biological nitrogen fixation and phytohormones production. These hormones comprises auxins, gibberellins, cytokinins abscisic acid, salicylic acid, ethylene and jasmonic acid. These plant regulators interact in a complex way to produce their physiological effects (Kochar *et al*., 2013). The utilization of PGPR as biofertilizer is sustainable and an efficient alternative to chemical fertilizers (Akbari *et al*., 2007) which can improve soil fertility by effective plant-soil-microbe continuum (Dastager *et al*., 2011). For improving plant growth and productivity PGPR have been applied to crop plants in different forms (Abbasi *et al*., 2013; Minorsky, 2008).

Soil salinity is one of the major abiotic stress causes osmotic stress, ionic imbalances, specific ion toxicity, nutritional disorders and oxidative damages due to hyper-accumulation of sodium and chloride ions in cytosol thus affect plant growth and development (Abdel-Hamid, 2014). Salinity affects both plant productivity and biodiversity (Fernandez *et al*., 2010). Halophytes have the ability to develop on salted soils (Munns & Tester, 2008). The rhizosphere of halophytes contains high amount of salts and salt ions such as HCO_3, Cl^-, Mg, Na and K (Liangpeng *et al*., 2007). Halophytes can complete their life cycles in NaCl rich environment which is harmful to other plants species and damage 99% of their population. These plants have well developed structural and functional adaptations that enable them to survive in higher salt stress regimes (Flowers & Colmer, 2008). Halophytes adapt various mechanisms against the injurious effects of elevated salinity in soils. These include adjustment of osmotic balance, ion homeostasis, production of defensive metabolites and proteins and stimulation of enzymatic antioxidants systems (Hussain *et al*., 2008). Halophytes have adapted certain features to tolerate high levels of salts due to which they become unable to nurture without presence of salts. These adaptations explain their natural distribution (Grigore *et al*., 2012). Halophytes grow well in hyper-saline environments (Jayaprakashvel *et al*., 2015). The rhizosphere of halophytes may have microorganisms with distinct characteristics (Kebbouche-Gana *et al*., 2009). The interaction between halophytic roots and beneficial microbial association helps the plant to perform normally under stress conditions (Evelin *et al*., 2009). These microbes with diverse metabolic processes have great potential to be used as PGPR for growth improvement

and productivity of economically important crops. They have immense importance in agriculture due to enhancing plant nutrient exchange and decreasing the dependence on chemical fertilizers (Ullah & Bano, 2015). The main objective of this study was to isolate, identify and characterize PGPR indigenous to rhizosphere of *Xanthium strumarium* (a halophyte) and to evaluate their plant growth promoting ability in Maize (*Zea* mays).

Materials and Methods

Isolation of rhizosphere inhabiting bacteria: Soil sample was collected aseptically in polythene bags from rhizosphere of *Xanthium strumarium* L. at depth of 6-15 cm, from district Mardan (Karpagam & Nagalakshmi, 2014). Properties of soil samples like pH and EC were determined. For isolation of rhizobacteria one gram of soil sample was mixed in 100 mL of sterilized distilled water and stirred for 20 minutes (Pandya *et al*., 2014). From soil suspension, serial dilutions 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} were prepared (Sharma *et al*., 2011). From each serial dilution about 0.1 mL was spread on Luria Bertani (LB) agar and Petri plates were incubated in dark at 28-30^{o}C. Appeared colonies were picked separately and were streaked on LB agar plates. The purified bacterial colonies were preserved in 20% glycerol and LB broth in eppendorf tubes at -80^{o}C (Dastager *et al*., 2009).

Biochemical characterization of rhizobacteria: All isolated bacterial colonies were grown on nutrient agar or nutrient broth for 24 h. Then isolates were characterized biochemically by Gram staining, Catalase, Urease and Oxidase reaction, further by Citrate utilization, Mackonkey agar, H_2S production, Indole production, Methyl-Red-Voges-Proskauer (MR-VP) and Gelatin agar test using standard methods (Aneja, 2003).

Test for nitrogen-fixing ability of isolates: The visible finding of nitrogen fixation of the rhizobacteria were detected by using Nitrogen free semisolid malate medium. After the incubation for one week at 37°C, the medium changed color from green to blue green which indicated that the bacterial isolates had nitrogen fixing ability (Gothwal *et al*., 2008).

Phosphate solubilization activity of rhizobacteria: Similarly, the observation of phosphate solubilization for these strains was tested by using Pikoviskaya medium. After one week incubation at 37°C, the appearance of clear halo zone was indicated that isolates had the ability to solubilized phosphate (Sharma *et al*., 2011).

Determination of salt tolerance of the isolates: Salt range of the isolated strains were determined by culturing the isolates with different salt concentrations e.g. 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5,5.5 and 6 % in LB agar medium/broth at 30°C for 48 hours (Kumar *et al*., 2014).

Indole acetic acid (IAA) production in rhizobacteria: For the determination of IAA isolates were cultivated on LB broth containing L- tryptophan (0.1g/L) for 24 hours at 28°C at 150 rpm on a shaking incubator in dark. Broth with bacterial cells was centrifuged at 10,000 *g* for 15 min. The supernatants were carefully separated and transferred to sterile test tubes. 1mL of each supernatant was mixed with 1.5 mL of Salkowski's reagent (50 mL 35% $HClO_4$; 1 mL 0.5 $FeCl_3$) and incubated at room temperature for 30 minutes. Development of pink color by the isolates indicated IAA production (Mohite, 2013). The optical density (OD) of the mixture was recorded at 540 nm on Perkin Elmer Lambda 25 UV/VIS Spectrophotometer.

Cytokinin cotyledon bioassay: For the bioassay of cytokinin, cucumber seeds were purchased from Tarnab farm. First of all seeds were germinated on filter paper in an open plastic tray, autoclaved distilled water was supplied to these seeds. At room temperature seeds were incubated in dark for 7 days (Fletcher *et al*., 1982). From cucumber seedlings cotyledons were removed after 7 days that were grown in dark. In sterilized petri plates the cotyledons were placed containing the specific bacterial broth. Commercially available synthetic cytokinin 6 bezylaminopurine (BAP) in the bioassay was used as positive control; the concentration of BAP in positive control was 25ppm while a negative control was sterile distilled water (Shore & Sathisha, 2010).

The incubation of cotyledons of cucumber adjacent to positive and negative control was done in luminous tube light for 3.5 hours at 22°C. Cotyledons were collected after incubation, and with the help of motor and pestle, crushed with 80% acetone. Than the extracts of chlorophyll were assembled and were centrifuged at 4000 rpm for 10 minutes. The resulting supernatant was used to measured total amount of chlorophyll using spectrometer at 663 and 645 nm (Sadasivam & Manickam, 2010).

Germination assay: The bacterial isolates were tested for their growth promotion/reduction ability. Maize (*Zea* mays) seed were selected for germination experiment. Seeds were inoculated in LB broth (Yadav *et al*., 2010). Five seeds were sterilized in 70% ethanol and were kept in each autoclaved petri dish having filter paper. Hoagland solution and specific bacterial isolate were applied on the filter paper. After 7 days of germination, lengths of seedling and root, fresh weights of shoot and root and shoot dry weights were recorded for observing the growth promotion/reduction in maize seedlings (Kapoor *et al*., 2011).

Sand culture experiment: Nutrient broth inoculated with specific bacterial isolates was incubated for 24 hours at 35°C. Maize seeds were soaked in the nutrient broth for 1 hour. Sterilized sand (100 g) was placed in pots along with the bacterial soaked maize seeds having three replicates of each. Five seeds were placed in each pot having 20 mL of Hoagland solution. After 14 days of growth, observation for root length, shoot length, root fresh weight, shoot fresh weight, root dry biomass, shoot dry biomass, leaves fresh biomass, leaves dry biomass and total chlorophyll were recorded (Aziz *et al*., 2015).

Data analysis

Data of the recorded parameters were subjected to one way analysis of variance, Duncan's New Multiple range test were performed to determine the significant difference (p<0.05) between the means of different treatments.

Results

Biochemical and physiological characterization: Four isolates, isolated from rhizosphere of *Xanthium strumarium* were biochemically characterized. The results of Gram's reaction showed that out of four isolates two were cocci (one Gram positive and other negative) and identified as *Staphylococcus* sp. (Xs1) and *Arthrobacter* Sp. (Xs2), respectively. While other two were Gram negative rods and identified as *Enterobacter* sp. (Xs3) and *Pseudomonas* sp. (Xs4) (Table 1). All isolates were catalase and citrate positive and none of the isolate showed positive reaction to oxidase, VP, MSA, Indole and Macconcky agar. Three of the isolates (Xs1, Xs2 and Xs3) showed positive reactions to MR except Xs4. Isolate Xs4 showed nitrogen fixation and none of isolates showed phosphate solubilization (Table 1).

Salt tolerance: All the isolated strains showed growth in LB agar containing 4.5% of NaCl. Isolates Xs1 and Xs2 showed growth at 5.5% of NaCl, whereas isolates Xs3 and Xs4 did not show any growth even at 5% salt concentration. None of the isolates showed growth at 6% NaCl concentration. Results of salt stress are given in (Table 2).

IAA production: All of the isolates showed significant (p<0.01) increase in IAA production. The concentration of IAA produced by different isolates ranged from 16.52 - 25.20µg/ml. The highest quantity of IAA was 25.20µg/ml produced by isolate Xs1. Most significant amount of IAA produced by Xs1 (25.20µg/ml) (Fig. 1a).

Cytokinin production: All of the isolates showed significant (p<0.01) increase in cytokinins production. The concentration of cytokinin produced by different isolates ranged from 258.03 - 263.74 µg/mL. The highest quantity of cytokinin was 263.74 µg/mL produced by isolate Xs4 (Fig. 1b).

Table 1. Biochemical and physiological characteristics of isolates *Xanthium strumarium*.

Strain	Xs1 (*Staphylococcus* sp.)	Xs2 (*Arthrobacter* sp.)	Xs3 (*Enterobacter* sp.)	Xs4 (*Pseudomonas* sp.)
Shape	C	C	R	R
G stain	+	-	-	-
Catalase	+	+	+	+
Oxidase	-	-	-	-
Urease	-	-	-	+
Macconkey	-	-	-	-
MR	+	+	+	-
VP	-	-	-	-
Citrate	+	+	+	+
MSA	-	-	-	-
Indole	-	-	-	-
H2S	-	+	-	-
Gelatin	+	-	+	-
TSIA	Nd	Nd	Nd	Nd
N_2 fix	-	-	-	+
PO_4	-	-	-	-

Key: (+) Present, (-) Absent, (C) Cocci, (R) Rod

Table 2. Salt range of bacterial isolates from *Xanthium strumarium.*

NaCl concentrations (%)	Strains			
	Xs1 (*Staphylococcus* sp.)	Xs2 (*Arthrobacter* sp.)	Xs3 (*Enterobacter* sp.)	Xs4 (*Pseudomonas* sp.)
1.5	+	+	+	+
2	+	+	+	+
2.5	+	+	+	+
3	+	+	+	+
3.5	+	+	+	+
4	+	+	+	+
4.5	+	+	+	+
5	+	+	-	-
5.5	+	+	-	-
6	-	-	-	-

Key: (+) Present, (-) Absent

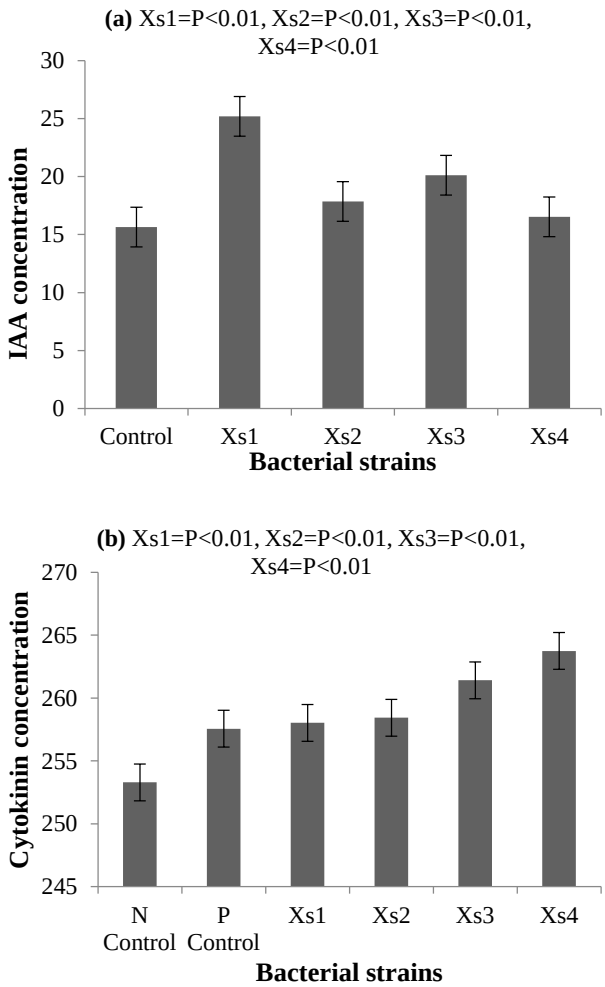

Fig. 1. Production of IAA (a) and cytokinins (b) by rhizobacterial isolates. Error bars are SE from three replicates per same treatment. *Staphylococcus* sp. (Xs1), *Arthrobacter* Sp. (Xs2), *Enterobacter* sp. (Xs3) and *Pseudomonas* sp. (Xs4)

Germination Bioassay

a. Germination percentage: In bioassay maximum germination percentage was obtained with isolate Xs4 (100%) as compared to control (60%). Isolate Xs1 did not show any increase or decrease in germination percentage. Isolates Xs2 and Xs4 showed non-significant increase in germination percentage whereas isolate Xs3 showed significant (p<0.01) decrease in germination percentage (Fig. 2a).

b. Shoot length: The results showed maximum shoot length (up to 65.133 cm) in Xs2 treated seedlings as compared to controls (24.76 cm). Isolate Xs1 (p<0.05), Xs2 (p<0.01) and Xs4 (p<0.01) showed significant increase in shoots lengths. Isolate Xs3 revealed non-significant decrease in shoot length (Fig. 2b).

c. Root length: Results indicated that isolates Xs1 (p<0.05), Xs2 (0.01) and Xs4 (p<0.01) significantly increased the root length in maize. Whereas, isolate Xs3 showed non-significant increase in root length (Fig. 2c). Maximum root length (51.3 cm) was recorded in Xs2 treated seedlings as compared to control (18.36 cm).

d. Fresh weight: Results showed that isolates Xs1 (p<0.01), Xs2 (p<0.01), Xs3 (p<0.05) and Xs4 (p<0.01) showed significant increase in the fresh weight of maize (Fig. 2d). Maximum fresh weight was recorded in the presence of isolate Xs2 (2.066 g) compared to control (0.236 g).

e. Dry weight: Data indicated that isolate Xs1 showed non-significant increase in the dry weights, whereas isolates Xs2 (p<0.01), Xs3 (p<0.01) and Xs4 (p<0.01) significantly increased the dry weights of maize (Fig. 2e). Maximum dry weight was recorded in the presence of isolate Xs4 (0.11687 g) compared to control (0.02733 g).

Seedling growth in sand culture

a. Germination percentage: Maximum germination percentage was obtained in the presence of isolate Xs1 (80%) as compared to control (60%). Isolate Xs1 showed non-significant increase in germination. Isolates Xs3 and Xs4 showed non-significant decrease in germination percentage whereas Xs2 showed significant (p<0.05) decrease in germination percentage (Fig. 3a).

b. Chlorophyll content: Maximum chlorophyll content was noted in the presence of isolate Xs4 (30.23 SPAD units) related to control (24.9 SPAD units). Significant decrease in chlorophyll content was noted in the presence of isolates Xs2 (p<0.05) and Xs3 showed non-significant decrease. Non-significant increase in content of chlorophyll was recorded in the presence of isolates Xs1 and Xs4 (Fig. 3b).

c. Shoot length: The results showed that highest shoot length (69.05 cm) was noted in the presence of isolates Xs1 as compared to control (26.34 cm). Isolate Xs1 showed significant (p<0.01) increase in shoot length. Non-significant decrease in shoot length was noted in the presence of isolate Xs2. Isolates Xs3 and Xs4 showed non-significant increase in shoot length (Fig. 3c).

d. Root length: Maximum root length (86.37 cm) was noted in the presence of isolate Xs1 (p<0.01) as compared to control (26.81 cm). Isolates Xs2 Xs3 and Xs4 showed non-significant increase in root length (Fig. 3d).

e. Fresh weight: Data revealed maximum fresh in plants treated with isolate Xs1 (4.97 g) as compared to control (1.969 g). Isolate Xs1 showed significant (p<0.01) increase in fresh weight. Isolates Xs2 Xs3 and Xs4 showed non-significant increase in fresh weight (Fig. 3e).

f. Dry weight: Results indicated that non-significant increase in the dry was recorded in the presence of all the isolates but isolate Xs1 showed significant (p<0.01) increase in dry weight. Maximum dry weight was recorded in the presence of isolate Xs1 (0.526 g) compared to control (0.0556 g) (Fig. 3f).

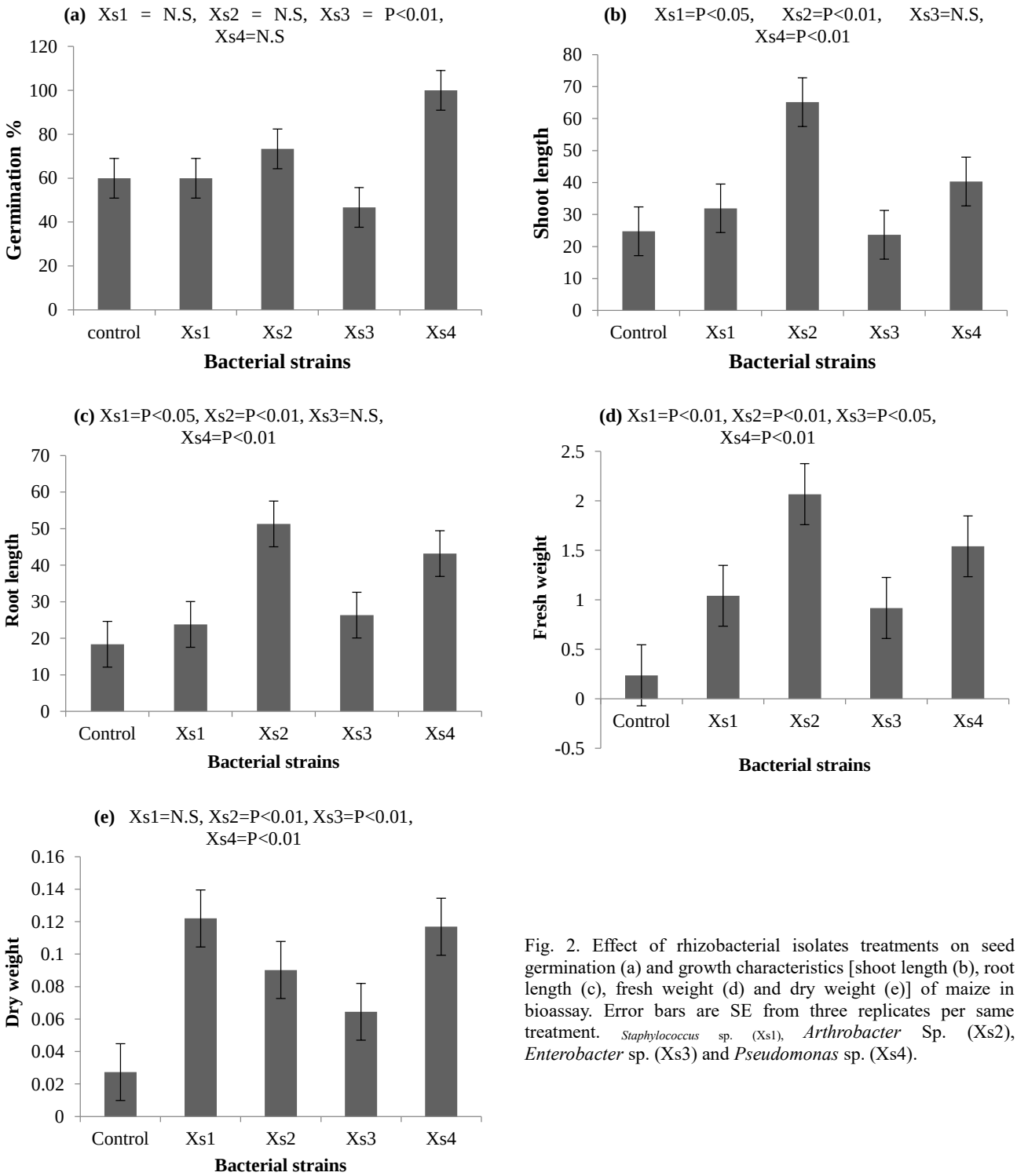

Fig. 2. Effect of rhizobacterial isolates treatments on seed germination (a) and growth characteristics [shoot length (b), root length (c), fresh weight (d) and dry weight (e)] of maize in bioassay. Error bars are SE from three replicates per same treatment. *Staphylococcus* sp. (Xs1), *Arthrobacter* Sp. (Xs2), *Enterobacter* sp. (Xs3) and *Pseudomonas* sp. (Xs4).

Discussion

Rhizosphere of *Xanthium strumarium* showed the presence of four bacterial isolates of which only one isolate showed H_2S production and one exhibited gelatin liquefaction while one isolate was urease positive. Comparable results were attained by Donate-Correa *et al*., (2005). Greater number of Gram negative strains were isolated from the rhizosphere of *Chamaecytisus proliferus* as compared to gram positive strains (Donate-Correa *et al*., 2005). Based on the findngs of this experiment where we found cocci and rod shaped bacterial species it was studied that Parray *et al*., (2013) observed greater abundance of gram negative rods were obtained from rhizosphere of saffron. We performed different biochemical tests of the isolates and identified the isolates as *Staphylococcus* sp. (Xs1), *Arthrobacter* Sp. (Xs2), *Enterobacter* sp. (Xs3) and *Pseudomonas* sp. (Xs4). *Staphylococcus* sp. previously isolated from the mangrove rhizosphere, the habitat with higher saline concentration (Holguin *et al*., 1992) as well as from the rhizosphere of cultivated crop *Arachis hypogaea*, the habitat with normal saline concentration, however, strains were characterized as halotolarant (Sarkar *et al*.,

2012). *Arthrobacter* species were reported from saline infested zone of wheat and tomato rhizosphere with stress tolerant activity (Upadhyay *et al*., 2009; Banerjee *et al*., 2010). Similarly, *Enterobacter* (George *et al*., 2013; Shoebitz *et al.*, 2009) and *Pseudomonas* strains (Lugtenberg & Dekkers, 1999; Ozawa *et al*., 2007) were isolated from the rhizosphere of diverse plant species with their intrinsic ability to tolerate abiotic stresses. Nitrogen fixation ability of the isolates is a vital process for plant growth promotion. The transmission of nitrogen among diazotrophic nitrogen fixers and the roots of various crops have been verified (Tajini *et al*., 2012). Several studies have characterized the genus *Pseudomonas* as diazotrophicus microbe that have been isolated from the rhizosphere of cultivated plants including rice (Mirza *et al*., 2006), sugarcane (Ashraf *et al*., 2011) and legumes (Ahmad *et al*., 2008). The use of nitrogen fixers are recommended to decrease the need of chemical fertilizers to reduce the cost of plant production (Saharan & Nehra, 2011).

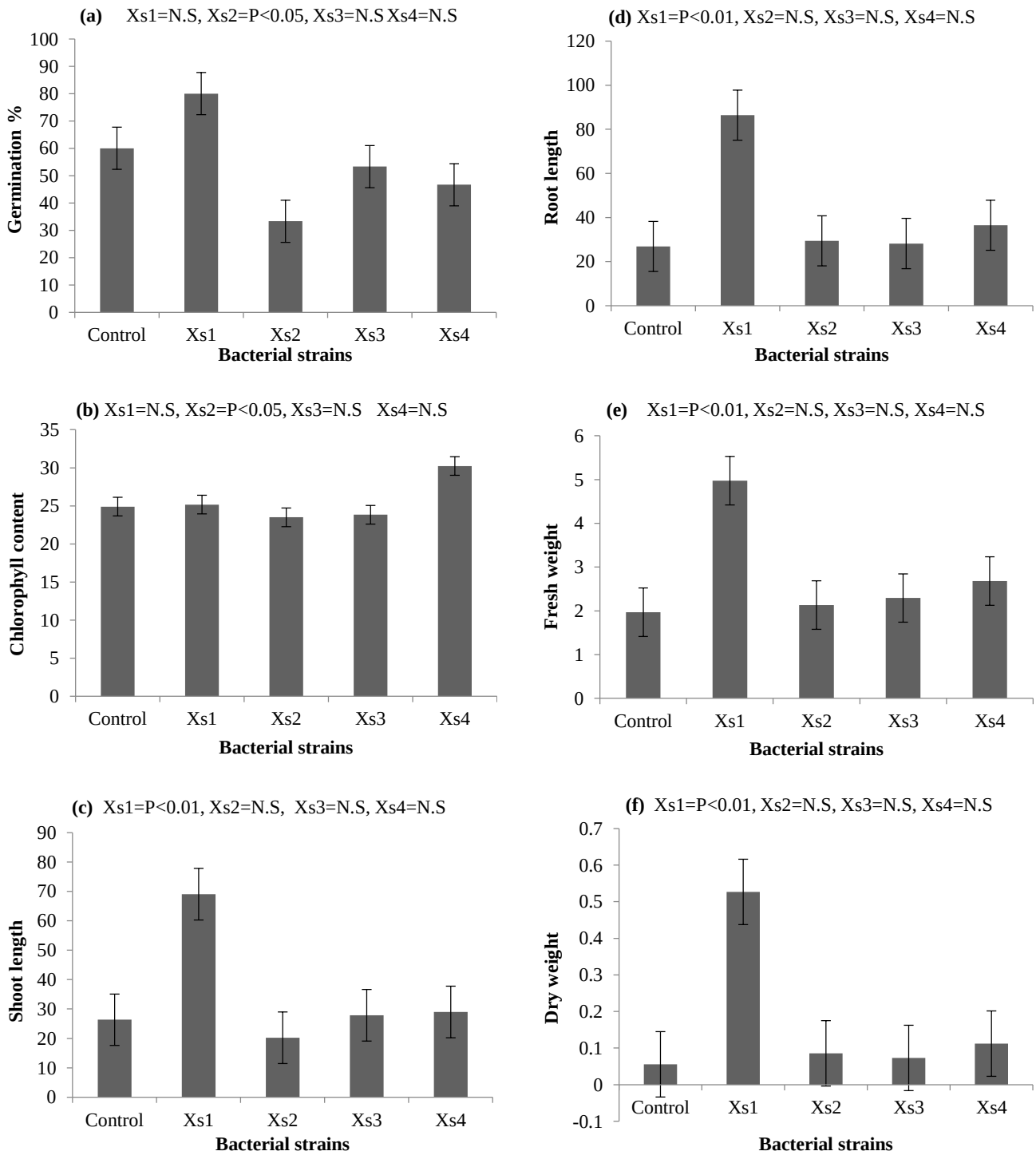

Fig. 3. Effect of rhizobacterial isolates treatments on seed germination (a) and growth characteristics [chlorophyll content (b), shoot length (c), root length (d), fresh weight (e) and dry weight (f)] of maize seedlings grown in sand culture. Error bars are SE from three replicates per same treatment. *Staphylococcus* sp. (Xs1), *Arthrobacter* Sp. (Xs2), *Enterobacter* sp. (Xs3) and *Pseudomonas* sp. (Xs4).

Present studies showed that Xs1 and Xs2 were found to be more salt tolerant strains compared to Xs3 and Xs4. Damodaran *et al*., (2013) observed sixteen strains of rhizobacteria isolated from sodic rhizospheres, of which he reported only five strains that tolerated high salt stress (7.5% NaCl). Another interesting report suggested greater diversity of rhizobacteria in the soil consisting high salinity compared to the soil with lower level of salinity (Yang *et al*., 2016). According to earlier reports bacterial strains isolated from soil of high salinity are expected to tolerate salt stress (Upadhyay *et al*., 2009). Possession of PGP traits by such bacteria on the other hand, would be perfect for practice in supportable agriculture (Egamberdiyeva & Islam, 2008). Some of bacterial isolates in our study displayed different plant growth promoting characters like synthesis of auxins, cytokinin as well as nitrogen fixation. The bacterial isolates could display multiple growth stimulating properties, which may enhance plant growth by different direct or indirect mechanisms (Joseph *et al*., 2012).

Synthesis of phytohormones like IAA in rhizobacteria is one of the most frequently described mechanisms (Patten & Glick, 2002) and consider as the most common and important trait of PGPR (Zahid, 2015). Results showed that Xs1 found most effective in term of IAA synthesis compared to other isolates. Production of auxins is recognized and corporate character of root allied bacteria (Ahmad *et al*., 2005). Cytokinins are main group of plant hormones that produce by PGPR, which is consider as a direct plant growth promoting mechanism (Castro *et al*., 2008). Highest synthesis of Cytokinins was studied in the isolate Xs4, followed by Xs3. Cytokinins are responsible for controlling apical dominance and controls growth of root and shoot, senescence of leaf and development of chloroplast (Oldroyd, 2007). Previously, it was reported that several soil inhabiting bacteria have intrinsic ability to synthesize cytokinins or gibberellins or both crucial plant regulators (Kang *et al*., 2009).

Inoculation of Maize seeds with bacterial isolates considerably enhanced seed germination and different growth parameters. Significant increase in length of shoot and root, fresh and dry mass of maize was detected in seedlings treated with different isolates. Similar results were also reported by Anbumalar & Ashokumar (2015). Seedling length of crops like cotton, Jatropha, okra and sorghum was significantly increased due to PGPR isolate M5 as compared to un-inoculated control (Anbumalar & Ashokumar, 2015). However, improved root length, plant height, fresh and dry weight of both root and shoot was observed with test isolates compared to control (Bhatt & Vyas, 2014). Lwin *et al*., (2012) isolated four best IAA producers (R1, R3, R5, R8) from 18 isolates for further studies on maize where he observed that the rate of seed germination was not affected significantly by treatment with isolates. In pot experiment, rhizobacterial isolate (R1) improved fresh and dry weight of shoot and height while treatment R3 significantly improved fresh and dry weight of root, root length, and number of adventitious roots (Lwin *et al*., 2012). All bacterial isolate increased rate of germination, root length, root number and root mass of the germinated seeds of *Triticum aestivum* and in pot experiment comparative to control (Aziz *et al*., 2015). Inoculation of *Cynara scolymus* with plant growth promoting bacteria significantly increased different growth parameters like rate of seed germination, length and weight of seedlings (Jahanian *et al*., 2012; Jarak *etal*., 2012).

Conclusion

The study concluded that rhizospheric bacteria of *Xanthium strumarium* had multiple plant growth promoting characters. Although all isolated species had great capacity to act as PGPR but *Staphylococcus* sp. (Xs1) performed much better performance as compare to others. So, this study supports the use of PGPR as biofertilizers which effectively improved plant growth and productivity.

References

Abbasi, M.W., N. Ahmed, M.J. Zaki, S.S. Shuakat and D. Khan. 2014. Potential of *Bacillus* species against *Meloidogyne javanica* parasitizing eggplant (*Solanum melongena* L.) and induced biochemical changes. *Plant & Soil*, (1-2): 159-173.

Abbasi, M.W., M.J. Zaki and M. Anis. 2013. Application of *Bacillus* species cultured on different low cost organic substrates for the control of root-knot nematode infection on okra (*Abelmoschus esculentus* Moench). *Pak. J. Bot.*, 45(3): 1079-1084.

Abdel-Hamid, A.M.E. 2014. Physiological and molecular markers for salt tolerance in four barley cultivars. *Europ. Sci. J.*, 10(3): 252-272.

Ahemad, M. and M. Kibret. 2014. Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. *J. King Saud Uni. Sci.*, 26: 1-20.

Ahmad, F., I. Ahmad and M. Khan. 2008. Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. *Microbiol. Res.*, 163: 173-181.

Ahmad, F., I. Ahmad and M.S. Khan. 2005. Indole acetic acid production by the indigenous isolates of *Azotobacter* and fluorescent *Pseudomonas* in the presence and absence of tryptophan. *Turk. J. Biol.*, 29: 29-34.

Akbari, G.A., S.M. Arab, H. Alikhani, I. Allakdadi and M. Arzanesh. 2007. Isolation and selection of indigenous *Azospirillum* spp. and the IAA of superior strains effects on wheat roots. *Wor. J. Agri. Sci*., 3: 523-529.

Anbumalar, S. and P. Ashokumar. 2015. Assessment of the effect of halo-tolerant bacterium on plant growth promotion. *Int. J. Pharm. Chem. & Biol. Sci.*, 5(1): 287-293.

Aneja, K.R. 2003. *Experiments in microbiology, plant pathology and biotechnology*, New Age International (p) limited publishers, pp. 607.

Ashraf, M.A., M. Rasool and M.S. Mirza. 2011. Nitrogen fixation and indole acetic acid production potential of bacteria isolated from rhizosphere of sugarcane (*Saccharum officinarum* L.). *Adv Biol. Res.*, 5: 348-355.

Aziz, K., M. Nawaz, J. Nazir, A. Anjum, T. Yaqub, M. Ahmad, M. Rehman, G. Aziz and M. Khan. 2015. Isolation, characterization and effect of auxin producing bacteria on growth of *Triticum aestivum*. *JAPS, J. Ani. & Plant Sci.*, 25: 1003-1007.

Banerjee, S., R. Palit, C. Sengupta and D. Standing. 2010. Stress induced phosphate solubilization by *Arthrobacter* sp. and *Bacillus* sp. isolated from tomato rhizosphere. *Aust. J. Crop Sci.*, 4(6): 378-383.

Bhatt, P.V. and B.R.M. Vyas. 2014. Screening and characterization of plant growth and health promoting rhizobacteria. *Int. J. Curr. Microbiol. App. Sci.,* 3: 139-155.

Cakmakci, R., M.F. Donmez and U. Erdogan. 2007. The effect of plant growth promoting rhizobacteria on barley seedling growth, nutrient uptake, some soil properties, and bacterial counts. *Turk. J. Agri. & Forest.,* 31: 189-199.

Carrillo, A.E., C.Y. Li and Y. Bashan. 2002. Increased acidification in the rhizosphere of cactus seedlings induced by *Azospirillum brasilense*. *Naturwissenschaften,* 89: 428-432.

Castro, R., E. Valecia-Cantero. and J. Bucio. 2008. Ortíz- and López-. Plant growth promotion by *Bacillus megaterium* involves cytokinin signaling. *Plant Signaling Behavior,* pp., 3: 263-265.

Damodaran, T., V. Sah, R. Rai, D. Sharma, V. Mishra, S. Jha and R. Kannan. 2013. Isolation of salt tolerant endophytic and rhizospheric bacteria by natural selection and screening for promising plant growth-promoting rhizobacteria (PGPR) and growth vigour in tomato under sodic environment. *Afr. J. Microbiol. Res.,* 7: 5082-5089.

Dastager, S., C. Deepa, S. Puneet, C. Nautiyal and A. Pandey. 2009. Isolation and characterization of plant growth-promoting strain Pantoea NII-186 from Western Ghat Forest soil, India. *Letters in Appl. Microbiol.,* 49: 20-25.

Dastager, S.G., C. Deepa and A. Pandey. 2011. Potential plant growth-promoting activity of *Serratia nematodiphila* NII-0928 on black pepper (*Piper nigrum* L.). *World J. Microbiol. & Biotechnol.,* 27: 259-265.

Donate-Correa J., M. Leon-Barrios and R. Perez-Galdona. 2005. Screening for plant growth-promoting rhizobacteria in *Chamaecytisus proliferus* (tagasaste), a forage tree-shrub legume endemic to the Canary Islands. *Plant & Soil,* 266: 261-272.

Egamberdiyeva, D. and K.R. Islam. 2008. Salt-tolerant rhizobacteria: Plant growth promoting traits and physiological characterization within ecologically stressed environments. *Plant-Bacteria Interactions: Strategies & Tech. to Promote Plant Growth,* 257-281.

Evelin, H., R. Kapoor and B. Giri. 2009. Arbuscular mycorrhizal fungi in alleviation of salt stress: a review. *Ann. Bot.,* 104: 1263-1280.

Fernandez, O., L. Bethencourt, A. Quero, R.S. Sangwan and C. Clement. 2010. Trehalose and plant stress responses: friend or foe? *Trends Plant Sci.,* 15: 409-417.

Fletcher, R., V. Kallidumbil and P. Steele. 1982. An improved bioassay for cytokinins using cucumber cotyledons. *Plant Physiol.,* 69: 675-677.

Flowers, T.J. and T.D. Colmer. 2008. Salinity tolerance in halophytes. *New Phytol.,* 179: 945-963.

George, P., A. Gupta, M. Gopal, L. Thomas and G.V. Thomas. 2013. Multifarious beneficial traits and plant growth promoting potential of *Serratia marcescens* KiSII and *Enterobacter* sp. RNF 267 isolated from the rhizosphere of coconut palms (*Cocos nucifera* L.). *World J. Microbiol. & Biotechnol.,* 29(1): 109-117.

Gothwal, R., V. Nigam, M. Mohan, D. Sasmal and P. Ghosh. 2008. Screening of nitrogen fixers from rhizospheric bacterial isolates associated with important desert plants. *Appl. Ecol. Eviron. Res.,* 6: 101-109.

Grigore, M.N., M. Villanueva, M. Boscaiu and O. Vicente. 2012. Do halophytes really require salts for their growth and development? An experimental approach. *Notulae Scientia Biologicae,* 4(2): 23-29.

Holguin, G., M.A. Guzman and Y. Bashan. 1992. Two new nitrogen-fixing bacteria from the rhizosphere of mangrove trees: Their isolation, identification and *In vitro* interaction with rhizosphere *Staphylococcus* sp. *FEMS Microbiol. Lett.,* 101(3): 207-216.

Hussain, T.M., M. Hazara, Z. Sultan, B.K. Saleh and G.R. Gopal. 2008. Recent advances in salt stress biology a review. *Biotechnol. & Mol. Biol. Rev.,* 3: 8-13.

Jahanian, A., M.R. Chaichi, K. Rezaei, K. Rezayazdi and K. Khavazi. 2012. The effect of plant growth promoting rhizobacteria (PGPR) on germination and primary growth of artichoke (*Cynara scolymus*). *Int. J. Agri. Crop Sci.,* 4: 923-929.

Jarak, M., N. Mrkovacki, D. Bjelic, D. Joscaron, T. Hajnal-Jafari and D. Stamenov. 2012. Effects of plant growth promoting rhizobacteria on maize in greenhouse and field trial. *Afr. J. Microbiol. Res.,* 6: 5683-5690.

Jayaprakashvel, M., A. Rajan and A.J. Hussain. 2015. Bioprospecting of halotolerant bacteria from the rhizosphere of a halophyte for the production of industrial enzymes. *Int. J. Pure App. Biosci,* 3: 193-201.

Joseph, B., R. Ranjan Patra and R. Lawrence. 2012. Characterization of plant growth promoting rhizobacteria associated with chickpea (*Cicer arietinum* L.). *Int. J. Plant Prod.,* 1: 141-152.

Kang, S.M., G.J. Joo, M. Hamayun, C.I. Na, D.H. Shin, H.Y. Kim, J.K. Hong and I.J. Lee. 2009. Gibberellin production and phosphate solubilization by newly isolated strain of *Acinetobacter calcoaceticus* and its effect on plant growth. *Biotechnol. Lett.,* 31: 277-281.

Kapoor, N., A. Arya, M.A. Siddiqui, H. Kumar and A. Amir. 2011. Physiological and biochemical changes during seed deterioration in aged seeds of rice (*Oryza sativa* L.). *Amer. J. Plant Physiol.,* 6: 28-35.

Karpagam, T. and P. Nagalakshmi. 2014. Isolation and characterization of phosphate solubilizing microbes from agricultural soil. *Int. J. Curr. Microbiol. & App. Sci.,* 3: 601-614.

Kebbouche-Gana, S., M.L. Gana, S. Khemili, F. Fazouane-Naimi, N.A. Bouanane, M. Penninckx and H. Hacene. 2009. Isolation and characterization of halophilic *Archaea* able to produce biosurfactants. *J. Indust. Microbiol. Biotechnol.,* 36: 727-738.

Kochar, M., A. Vaishnavi, A. Upadhyay and S. Srivastava. 2013. Bacterial biosynthesis of indole-3-acetic acid: signal messenger service. *Mol. Microbial Ecol. of the Rhizosphere,* 309-326.

Kumar, A., B.R. Maurya and R. Raghuwanshi. 2014. Isolation and characterization of PGPR and their effect on growth, yield and nutrient content in wheat (*Triticum aestivum* L. *Biocatalysis & Agri. Biotechnol.,* 3: 121-128.

Liangpeng, Y., M. Jian and L. Yan. 2007. Soil salt and nutrient concentration in the rhizosphere of desert halophytes. *Acta Ecol. Sinica,* 27: 3565-3571.

Lugtenberg, B.J. and L.C. Dekkers. 1999. What makes *Pseudomonas* bacteria rhizosphere competent? *Environ. Microbiol.,* 1(1): 9-13.

Lwin, K.M., M.M. Myint, T. Tar and W.Z.M. Aung. 2012. Isolation of plant hormone (indole-3-acetic acid-IAA) producing rhizobacteria and study on their effects on maize seedling. *Engg. J.,* 16: 137-144.

Minorsky, P.V. 2008. On the inside. *Plant Physiol.,* 146: 1455-1456.

Mirza, M.S., S. Mehnaz, P. Normand, C. Prigent-Combaret, Y. Loccoz, R. Bally and K.A. Malik. 2006. Molecular characterization and PCR detection of a nitrogenfixing *Pseudomonas* strain promoting rice growth. *Biol. & Fertility of Soils,* 43:163-170.

Mohite, B. 2013. Isolation and characterization of indole acetic acid (IAA) producing bacteria from rhizospheric soil and its effect on plant growth. *J. Soil Sci. & Plant Nutr.,* 13: 638-649.

Munns, R. and M. Tester. 2008. Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.,* 59, pp. 651-681.

Oldroyd, G.E. 2007. Nodules and hormones. *Science*, 315: 52-53.

Ortíz-Castro R., E. Valencia-Cantero and J. Lopez-Bucio. 2008. Plant growth promotion by *Bacillus megaterium* involves cytokinin signaling. *Plant Signal. Behav.,* 3: 263-265.

Ozawa, T., J. Wu and S. Fujii. 2007. Effect of inoculation with a strain of *Pseudomonas pseudoalcaligenes* isolated from the endorhizosphere of *Salicornia europea* on salt tolerance of the glasswort. *Soil Sci. & Plant Nutr.*, 53(1): 12-16.

Pandya, N.D. and P.V.J. Desai. 2014. Screening and characterization of GA3 producing *Pseudomonas monteilii* and its impact on plant growth promotion. *Int. Microbiol. App. Sci.*, 3: 110-115.

Parray, J.A., A.N. Kamili, Z.A. Reshi, R. Hamid and R.A. Qadri. 2013. Screening of beneficial properties of rhizobacteria isolated from Saffron (*Crocus sativus* L.) rhizosphere. *Afr. J. Microbiol. Res.*, 7: 2905-2910.

Patten, C.L. and B.R. Glick. 2002. Regulation of indoleacetic acid production in *Pseudomonas putida* by tryptophan and the stationary-phase sigma factor RpoS. *Can. J. Microbiol.*, 48: 635-642.

Rodriguez, H., R. Fraga, T. Gonzalez and Y. Bashan. 2007. Genetics of phosphate solubilization and its potential applications for improving plant growth-promoting bacteria. *First international meeting on microbial phosphate solubilization*. *Springer*, 15-21.

Sadasivam, S. and A. Manickam. 1996. *Biochemical Methods*. New Age International Publishers. *New Delhi*, pp. 251.

Saharan, B.S. and V. Nehra. 2011. Plant growth promoting rhizobacteria: a critical review. *Life Sci. Med. Res.*, 2011: 1-30.

Sarkar, P., S. Yamasaki, S. Basak, A. Bera and P.K. Bag. 2012. Purification and characterization of a new alkali-thermostable lipase from *Staphylococcus aureus* isolated from *Arachis hypogaea* rhizosphere. *Process Biochem.*, 47(5): 858-866.

Sharma, S., M. Kaur and D. Prashad. 2014. Isolation of fluorescent *Pseudomonas* strain from temperate zone of Himachal Pradesh and their evaluation as plant growth promoting rhizobacteria (PGPR). *The Bioscan*, 9: 323-328.

Sharma, S., V. Kumar and R.B. Tripathi. 2011. Isolation of phosphate solubilizing microorganism (PSMs) from soil. *J. Microbiol. & Biotechnol. Res*., 1: 90-95.

Shoebitz, M., C.M. Ribaudo, M.A. Pardo, M.L. Cantore, L. Ciampi and J.A. Curá. 2009. Plant growth promoting properties of a strain of *Enterobacter ludwigii* isolated from *Lolium perenne* rhizosphere. *Soil Biol. & Biochem.*, 41(9): 1768-1774.

Shore, S.J. and G. Sathisha. 2010. Screening of endophytic colonizing bacteria for cytokinin-like compounds: crude cell-free broth of endophytic colonizing bacteria is unsuitable in cucumber cotyledon bioassay. *World J. Agri. Sci.*, 6: 345-352.

Tajini, F., M. Trabelsi and J.J. Drevon. 2012. Combined inoculation with *Glomus intraradices* and *Rhizobium tropici* CIAT899 increases phosphorus use efficiency for symbiotic nitrogen fixation in common bean (*Phaseolus vulgaris* L.). *Saudi J. Biol. Sci.*, 19: 157-163.

Ullah, S. and A. Bano. 2015. Isolation of plant-growth-promoting rhizobacteria from rhizospheric soil of halophytes and their impact on maize (*Zea mays* L.) under induced soil salinity. *Can. J. Microbiol.*, 61(4): 307-313.

Upadhyay, S.K., D.P. Singh and R. Saikia. 2009. Genetic diversity of plant growth promoting rhizobacteria isolated from rhizospheric soil of wheat under saline condition. *Curr. Microbiol.*, 59: 489-496.

Yadav, J., J.P. Verma and K.N. Tiwari. 2010. Effect of plant growth promoting rhizobacteria on seed germination and plant growth chickpea (*Cicer arietinum* L.) under *In vitro* conditions. *In:* Biological Forum. Citeseer, 15-18.

Yang, H., J. Hu., X. Long, Z. Liu and Z. Rengel. 2016. Salinity altered root distribution and increased diversity of bacterial communities in the rhizosphere soil of Jerusalem artichoke. *Sci. Reports*, 6: 1-10.

Zahid, M. 2015. Isolation and identification of indigenous plant growth promoting rhizobacteria from Himalayan region of Kashmir and their effect on improving growth and nutrient contents of maize (*Zea mays* L.). *Front. in Microbiol.*, https://doi.org/10.3389/fmicb.2015.00207.

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