Open Access
CODEN: IJBNHY
ISSN: 2278-778X
Original Research Article
International Journal of Bioassays

Molecular characterization of potential salt tolerant bacteria for soybean growth promotion

Anukool Vaishnav[1,2], Sarita Kumari[1,2], Shekhar Jain[1,2], Devendra Kumar Choudhary[2], Kanti Prakash Sharma[1*]

[1]Department of Biological Science, College of Arts, Science & Humanities (CASH), Mody University of Science & Technology, Lakshmangarh, Sikar 332311, Rajasthan, India.
[2]Amity Institute of Microbial Technology (AIMT), Block 'E-3', 4th Floor, Amity University Campus, Sector-125, Noida-201303, Gautam Buddha Nagar, UP, India.

Received: October 16, 2016; **Accepted**: November 23, 2016
Available online: 1st December 2016

Abstract: Salinity is a major limiting factor for soybean crop productivity. To enhance the tolerance of soybean against salt stress, it is essential to understand the diversity of microbiota harboured by soybean rhizosphere. Earlier studies have demonstrated that local adaptation of plants to habitat imposed stresses is driven by their closely associated microbes. The present study aimed to isolation and characterization of salt tolerant rhizobacteria with respect to their functional plant growth promotion ability. A total of 43 bacterial isolates were recovered from soybean rhizospheric soil grown in Bundi district, Rajasthan, India. Out of them, one bacterial isolate strain AU was found to tolerate 10% NaCl stress and significantly enhanced soybean seedlings growth under 100mM NaCl condition. Molecular phylogenetic analysis placed this isolate closely to *Pseudomonas simiae* OLi^T with 99.93% similarity. Molecular characterization of functional genes revealed that AU bacterium possessed genes like *IaaM* (IAA production), *g6pd* (Pi-solubilization) and *sid* (siderophore production). Here, we show that soybean rhizosphere possessed a salt tolerant plant growth promoting bacterium strain AU, which may have impacts on alleviation and tolerance of salt stress in legume plants.

Key words: Genes; Plant growth promoting rhizobacteria; Salinity; Soybean

Introduction

Abiotic stresses are serious environmental factors in arid and semi-arid climatic area that restrict the growth and productivity of plants worldwide. Among these stresses, soil salinity contributes a major proportion in destruction of cultivated land area and reduction of crop productivity. Various number of salts e.g. sodium chloride (NaCl), sodium sulphate (Na_2SO_4), sodium nitrate ($NaNO_3$), magnesium sulphate ($MgSO_4$), magnesium chloride ($MgCl_2$), potassium sulphate (K_2SO_4), calcium carbonate ($CaCO_3$) etc. could be dissolved in saline soil, although NaCl causes most of the salt problems for higher plants in nature. Today, it is big challenge to increase the efficiency and sustainability of global agriculture system. Because this system is regularly marked by scarcity of water resources, environmental pollution and increased salinization of soil and water. These challenges create continued poverty and food insecurity by which people become chronically malnourished.

Many resident micro-flora of stress environment perform all functions of life for survival of their own and associated biological entities. Some genera, like *Bacillus*, *Paenibacillus* and *Pseudomonas* are actively being used to alleviate abiotic stresses (Choudhary *et al.,* 2015). In saline environment, halophilic bacteria and their metabolites have exhibited many potential which are suitable for vast agricultural, industrial and environmental applications. The successful restoration of plant growth under salinity condition after inoculation with plant growth promoting rhizobacteria (PGPR) provides the basis for a suitable alternative to improve crop growth and yield in saline soils. These PGPRs are mostly present in near the roots due to presence of root exudates secreted by plants and used as nutrient source by them. There is clear evidence that a diverse group of root-associated microbes is essential for promoting plant adaptation to salinity (Arora *et al.,* 2013; Shrivastava *et al.,* 2015; Egamberdieva *et al.,* 2015). Beneficial effect of PGPB under salinity has been related to hydraulic conductance, osmolyte accumulation, sequestering toxic Na^+ ions, maintaining higher stomatal conductance and photosynthetic activities (Dodd *et al.,* 2012).

***Corresponding Author**:
Dr. Kanti Prakash Sharma,
Department of Biological Science,
College of Arts, Science & Humanities (CASH),
Mody University of Science & Technology,
Lakshmangarh, Sikar 332311, Rajasthan (India), India.
E-mail: kantipsharma@rediffmail.com

Soybean is a *numero uno* crop with major source of dietary protein contains all the essential amino acids particularly glycine, tryptophan and lysine, vitamins (A & D) and minerals used in place of cow's milk. The oil produced from soybean is highly digestible, contains no cholesterol and used mainly in cooking, margarine and salad dressings. On the other hand, the extreme efforts on alternative sources of energy stimulated soy oil-based lubricant and fuel products that replace non-renewable petroleum products. It finds a cheaper source of high quality proteins and has potential to reduce malnutrition, a dominant problem in poor sections of society in the country. Based on these attributes soybean is the most promising component of the Climate Smart Agriculture Concept (FAO, 2013). Soybean is classified as moderately salt sensitive crop, severely affected by decreasing symbiotic interaction results in reduction of nodule formation and the amount of nitrogen fixed (Prudent *et al.,* 2015). Several abiotic factors are responsible for deprived production of soybean in India. Most of the areas under soybean cultivation are rainfed; hence drought is a major constraint in soybean production. The Northwest areas of India are frequently facing an erratic behavior of monsoon, which affecting planting. Hence, the present study was conducted in an attempt to isolate and characterize salt tolerant plant growth promoting bacteria from soybean cultivated region in Bundi district, Rajasthan, India and evaluate their ability to improve seedling germination of soybean under salt affected conditions.

Material and Methods

Sampling site and sample collection

The rhizobacterial population were explored from the rhizosphere of soybean plants in the month of August from Bundi district in Rajasthan, India. Geographically the sample collection area are located in the Hadoti region lies between 75°59' to 75°73' E & 25°43' to 25°54' N. Five rhizospheric soil samples from healthy soybean plants grown at different provinces were collected by using random sampling method with the help of a steel corer (pre-sterilized with 95% ethanol) in sterile polyethylene bags. Plants were uprooted along with good amount of rhizosphere soil, brought immediately to the laboratory in polyethylene bags and air-dried within 2 hrs.

Isolation of bacteria

Bacterial isolation was conducted under a sterile environment in microbiological safety. All materials used for isolation were pre-sterilized for 15 minutes at 121°C and 15 psi pressure. Ten gram of rhizospheric soil sample was measured and mixed into 90 mL of distilled water for 30 min at 150 rpm. After that, ten-fold series dilutions (10^{-1} to 10^{-6}) of the suspension were prepared and an aliquot of 0.1 mL from 10^{-6} dilution was spread over nutrient agar (NA) medium. Petri plates were incubated at 28°C for three days and bacterial colonies were counted on every day.

Screening of salt tolerant bacterial isolates

Bacterial inoculums was prepared by harvesting bacterial cells from 24 hrs cultures on nutrient agar plates at 28 ± 2 °C in BOD incubator. The inoculum was suspended in sterile physiological saline (0.85%) water to yield 10^8colony forming units (CFU/ml) and used it for further study. Once pure cultures of bacterial isolates were obtained, the intrinsic tolerance of the isolates towards salinity was studied by observing their growth under different concentration of sodium chloride (NaCl- 2, 4, 6, 8, 10 and 12%) in nutrient broth (NB) medium. Bacterial cultures were incubated at 28°C in BOD incubator for 72 hrs. The optical density was measured at 600 nm in every 24 hrs.

Bacterial screening on the basis of pot trials

Bacteria which found to tolerate higher concentration of NaCl were selected for inoculation in soybean seeds. A gnotobiotic pot experiment was conducted in plant growth chamber (28+2°C and 70% humidity) to confirm their plant growth promoting potential. The soybean seeds were surface sterilized (1-min, 70% ethanol soaking followed by 3-min, 0.1% $HgCl_2$ soaking, rinsed repeatedly in sterile distilled water) and treated with bacterial strains (10^8 CFU/mL) for 1 hr. Seeds were sown in pre-sterilized soil amended with 100mM NaCl. Three seeds per pot were maintained with six replicates. Un-inoculated pot served as control. The growth of the seedlings was recorded after 15 days. Seed germination, root/shoot length and number of lateral root were recorded as parameters of plant growth.

Biochemical characterization

After evaluation of pot trial experiment, one bacterial strain AU was selected for further study. The gram staining was done using 24 hrs old culture by Himedia Kit. The stained slide was visualized under compound microscope with oil immersion. The cell morphology and shape were observed under stereomicroscope. Motility was observed by the hanging drop method and in semi-solid media. Biochemical characterization of AU isolate was done as per the procedures outlined by Cappuccino and Sherman (1992).

Molecular characterization and phylogenetic analysis

The bacterial genomic DNA was extracted according to method of Moore *et al.,* (1999) with minor modification. Amplification of 16S rDNA region was done by using universal primers. In addition, three PGP activity genes were amplified by using their specific primers namely tryptophan-

2-monooxygenase for IAA production (*IaaM*), glucose-6 phosphate dehydrogenase for gluconic acid production (*g6pd*) and siderophore (*sid*). These specific primers were designed by IDT oligo-analyzer software and synthesized from Sigma-Eldrich (India). Primer list is provided along with their annealing temperatures (Table 1). PCR reaction condition was as following: pre-denaturation at 95°C for 5 min, denaturation at 95°C for 30 sec, annealing for 30 sec, extension at 72°C for 40 sec followed by 35 cycles and final extension at 72°C for 7 min. Amplified product was analysed in 1% agarose gels, purified and sequenced both strands using respective forward and reverse primers. The nucleotide sequences were di-deoxy cycle sequenced with fluorescent terminators (Big Dye, Applied Biosystems) and run in ABI 3730xl DNA analyzer (Applied Biosystem, USA). Sequence was compared with nucleotides database provided by the National Center for Biotechnology Information using the BLAST (Basic Local Alignment Search Tool) and submitted in GenBank.

Table 1: Details of gene specific primers tested in the present study

Gene	Primer sequences (forward & reverse)	Annealing temperature (C^0)
IaaM	F- 5'-GACTTCCCCAACTCGATGCTG-3' R- 5'-ATCCACATCTTTTGCGAGAACAG-3'	62
g6pd	F- 5'-ACAAACAGGTTCTGATTGCCG-3' R- 5'-TGGGGCTATTTCGACAAGGC-3'	58
sid	F- 5'-CCATTGCATTAGGTCCAGAAATG-3' R- 5'-GCCAATGCCAATGTGGATTAC-3'	60
16s-rDNA	F- 5'-AGAGTTTGATCCTGGCTCAG-3' R- 5'-AAGGAGGTGATCCAGCCGCA-3'	60

Results and Discussion

Bacterial isolation and screening

A total of 43 bacterial colonies with different morphological features were selected for further study. All the bacterial isolates were further sub-cultured and purified on nutrient agar medium. The purified cultures were finally maintained as agar slants stored at 4°C and as 50% glycerol stocks in -80°C for further use. The bacterial isolates differed in their ability to tolerate salinity in form of NaCl. All isolates could tolerate salinity up to 2% NaCl. A decrease in isolate number was observed with increase in NaCl concentration. A number of 19 isolates could tolerate 4 % NaCl, 16 isolates could tolerate 6% NaCl, 5 isolates could tolerate 8% NaCl, 2 isolates could tolerate 10% NaCl and one isolates was able to tolerate 12 % NaCl concentration (Table 2). These results are in agreement with other studies which showed that the number of viable colonies of rhizospheric strains declined with increasing concentration of salt (Naz *et al.,* 2009; Omar *et al.,* 2009). Bacterial cells showed tolerance against higher salt concentration could be due to the synthesis of protective factors and adaptation of current environmental conditions (Finkel and Kolter, 1999).

Table 2: Growth of bacterial isolates under different NaCl concentrations

S. No.	Strain codes	NaCl (%)
1	AT	2 –6
2	AT1	2 – 4
3	AT2	2 – 4
4	AT3	2 – 4
5	AT4	2 – 6
6	AT5	2 – 6
7	AT6	2 – 4
8	AT7	2 – 6
9	AT8	2 – 8
10	AT9	2 – 4
11	AT10	2 – 6
12	AT11	2 – 12
13	AT12	2 – 8
14	AT13	2 – 8
15	AM	2 – 6
16	AM1	2 – 4
17	AM2	2 – 4
18	AM3	2 – 8
19	AM4	2 – 4
20	AM5	2 – 6
21	AM6	2 – 4
22	AM7	2 – 4
23	AM8	2 – 6
24	AM9	2 – 4
25	AM10	2 – 4
26	AM11	2 – 6
27	AM12	2 – 8
28	AM13	2 – 6
29	AM14	2 – 6
30	AM15	2 – 6
31	AM16	2 – 4
32	AU	2 –10
33	AU1	2 – 4
34	AU2	2 – 4
35	AU3	2 – 6
36	AU4	2 – 6
37	AU5	2 – 4
38	AU6	2 – 6
39	AU7	2 – 10
40	AU8	2 – 4
41	AU9	2 – 4
42	AU10	2 – 6
43	AU11	2 – 4

0.8 OD at 600nm was considered for growth measurement

Standardization for bacterial inoculums

On the basis of salt tolerance property, a number of three bacterial isolates AT11, AU and AU7 were selected for pot trial study. All three bacterial strains augmented root/shoot length and lateral roots up to some or greater extent as compared with un-inoculated treatment under 100mM NaCl. The AU bacterial isolate was found more prominent than AT11 and AU7. AU bacterial strain was exhibited with higher seed germination, root/shoot length and lateral root utmost nearly 90%, 12.8/7.5cm and 66 respectively (Table 3). Hence, bacterial strain AU was selected for identification and characterization. There are several reports in which bacteria were isolated from stress affected environments and studied their beneficial effects on plant growth (Ramadoss *et al.,* 2013; Habib *et al.,* 2015). Ramadoss *et al.,*

(2013) reported that *Hallobacillus* sp. SL3 and *Bacillus halodenitrificans* PU62 were able to withstand high salt concentration (20% NaCl) and were able to facilitate wheat seedlings growth promotion in the presence of growth inhibitory levels of salt.

Table 3: Effect of bacterial treatments on plant growth parameters

Bacterial treatment	Germination (%)	Shoot length (cm.)	Root length (cm.)	Lateral root (number)
AT11	70±3.1	6.8±0.7	4±1	30±1.1
AU	90±1.8	12.5±0.2	7.8±0.2	66±1.3
AU7	66±3.6	7±0.6	3.9±0.6	26±0.9
Un-inoculated	55±2.5	4.7±0.8	3.2±0.5	16±1.1

Values represent the means ± SD, n=6

Morphological and biochemical characteristics

The AU isolate was Gram-negative motile rods approximately 1–1.5 µm in size, produced yellow pigment on tryptic soy agar and fluorescent pigment on King's B agar. The isolate was formed round and non-spreading colony. The bacterium was catalase and oxidase positive and able to hydrolysed starch and casein in their respective medium. The isolate reduced nitrate into nitrite and citrate was assimilated (Table 4). In terms of overall cellular morphology and biochemical characteristics, the AU isolate was somewhat resembled member of the family Pseudomonadaceae and tentatively identified as *Pseudomonas fluorescence.* Brown and Lobury (1968) suggested that direct detection of fluorescence around the colonies could be used for the identification of fluorescent pseudomonads.

Table 4: Biochemical characters of AU bacterial isolate

Biochemical characters	AU isolate
Colony morphology	Oval, greenish, flat, erose, smooth, shiny, translucent
Colony size	Medium
Gram reaction	Negative (pink)
Fluorescence	+
Motility	+
Hydrolysis	
Starch	+
Casein	+
Catalase, oxidase & citrate test	+

+ indicates for positive for respective test

Molecular identification and PGP genes characterization

About 1.5 kb fragment of 16S rRNA gene of the AU isolate was sequenced. The sequence obtained was analysed to determine the phylogenetic position of the AU isolate. Sequence database searches (GenBank and EZtaxons) revealed that the AU strain was phylogenetically most closely related to members of the genus *Pseudomonas.* Phylogenetic tree obtained using the neighbour-joining methods revealed that AU strain showed a similarity of 99.93% with *Pseudomonas simiae* OLi^T with '*P. fluorescens* intrageneric cluster' (Fig. 1). Pseudomonads are predominant bacteria in the rhizosphere with versatile functions. They are known to produce plant hormones, siderophores, antibiotics, enzymes like proteases and glucanases and solubilize minerals which have made them the most promising group of PGPR involved in the biocontrol of plant diseases and abiotic stress tolerance (Choudhary, 2012). They are capable of rapid growth and utilize various substrates as nutrients therefore show efficient colonization with a wide variety of crops including cereals, pulses, oilseeds and vegetables (Santoyo *et al.,* 2012). The AU strain 16S rRNA gene sequence (1380bp) was submitted to NCBI with KJ184311 accession number.

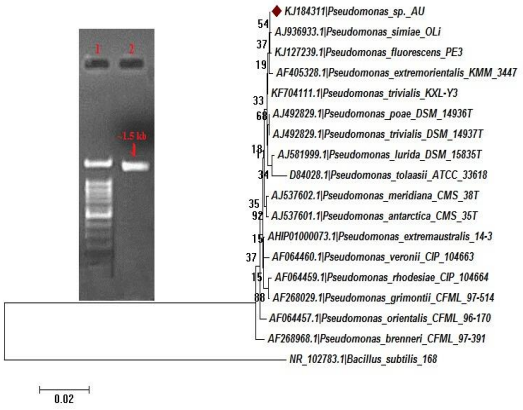

Figure 1: Construction of phylogenetic tree based on 16S rRNA gene sequencing by neighbour joining method

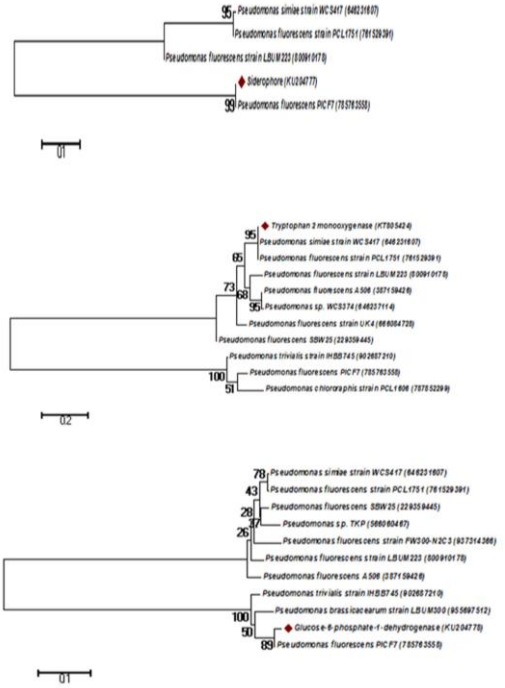

Figure 2: Phylogenetic analysis of siderophore, tryptophan 2 monooxygenases and glucose-6-phosphate-1 dehydrogenase genes isolated form AU bacterial isolate

Amplification with specific gene primers showed sharp and single bands for *IaaM* (330bp), *g6pd* (630bp) and *sid* (360bp). As expected, all three tested PGP genes of AU bacterial isolate showed similar to the *P. fluorescence* intrageneric cluster (Fig. 2). Glucose-6-phosphate dehydrogenase (*g6pd*) is involved in gluconic acid production via 6-phosphogluconate (6-PGA) in bacteria (Ramachandran *et al.,* 2006). Gluconic acid plays an important role in phosphate solubilizing activity (Sashidhar and Podile, 2010). Vyas and Gulati (2009) observed that 19 fluorescent *Pseudomonas* strains were produced gluconic acid during the solubilisation of tricalcium phosphate in liquid culture. Similarly, the enzyme tryptophan-2-monooxygenase (IaaM) converts tryptophan to indole-3-acetamide (IAM) and then IAM is converted to IAA by an IAM hydrolase (IaaH). The *IaaM* gene has been characterized in plant-associated bacteria for confirmation of IAA biosynthesis through IAM pathway (Yin *et al.,* 2014; Kyndt *et al.,* 2015). The production of IAA by PGPR is usually correlated with direct effects on plant growth (Cassán *et al.,* 2014). Siderophores are small peptides synthesized by non-ribosomal peptide synthetases and its biosynthesis genes have been characterized in different bacterial strains (Adams *et al.,* 1994; Najimi *et al.,* 2008). Siderophores chelate iron compounds and making available to plant roots and unavailable to the phytopathogens and protecting plant health (Rajkumar *et al.,* 2010). All gene sequences were submitted in NCBI and GenBank accession numbers are as follows: *sid* (KU204777); *g6pd* (KU204778) and *IaaM* (KT805424).

In this study, we have shown that AU bacterium, isolated from soybean rhizosphere is able to withstand high salt concentrations and can facilitate plant growth promotion in the presence of growth inhibitory levels of salt. The plant growth promotion properties were also confirmed by molecular characterization of PGP genes. AU bacterium was found to possessed genes related to IAA, gluconic acid and siderophore production activity. Hence, these findings permit us to conclude that the AU bacterium can be used as bioinoculants for saline environments.

Acknowledgment

Authors would like to acknowledge Prof. Ajit Varma, Amity University, Noida, and Dr. Yogesh Shouche, National centre of cell science (NCCS), Pune for providing molecular facilities in their lab and scientific suggestions.

References

1. Adams C, Dowling DN, O'Sullivan DJ, O'Gara F. "Isolation of a gene (pbsC) required for siderophore biosynthesis in fluorescent *Pseudomonas* sp. strain M114". *Molecular Genetics and Genomics* 243 (1994): 515524.
2. Arora NK, Tewari S, Singh S, Lal N, Maheshwari DK. PGPR for protection of plant health under saline conditions. D.K. Maheshwari (ed.), *Bacteria in Agrobiology: Stress Management*. (2013): 239-258. Springer-Verlag Berlin Heidelberg
3. Brown VI, Lowbury EJL. Use of an improved cetrimide agar medium and other culture methods for *Pseudomonas aeruginosa. Journal of Clinical Pathology* 18 (1968): 752-756.
4. Cappuccino JC, Sherman N. *Microbiology: A Laboratory Manual.* 3 Edn, Benjamin/cummings Pub. Co. New York. (1992): 125–179.
5. Cassán F, Vanderleyden J, Spaepen S. Physiological and agronomical aspects of phytohormone production by model plant-growth-promoting rhizobacteria (PGPR) belonging to the genus *Azospirillum. Journal of Plant Growth Regulation* 33 (2014): 440-459.
6. Choudhary DK. Microbial rescue to plant under habitat-imposed abiotic and biotic stresses. *Applied Microbiology and Biotechnology* 96 (2012): 1137-1155.
7. Choudhary DK, Kasotia A, Jain S, Vaishnav A, Kumari S, Sharma KP, Varma A. Bacterial-mediated tolerance and resistance to plants under abiotic and biotic stresses. *Journal of Plant Growth Regulation* (2015): 1-25.
8. Dodd IC, Pe´ rez-Alfocea F. Microbial amelioration of crop salinity stress. *Journal of Experimental Botany* 63 (2012): 3415–3428.
9. Egamberdieva D, Shurigin V, Gopalakrishnan S, Sharma R. Microbial strategies for the improvement of legume production in hostile environments. In: Azooz MM and Ahmad P (Eds.). *Legumes under Environmental Stress: Yield, Improvement and Adaptations*, (2015): 133.
10. Finkel SE, Kolter R. Evolution of microbial diversity during prolonged starvation. *Proceeding of National Academy of Sciences* USA 96 (1999): 4023–4027.
11. Habib SH, Saud HM, Ismail MR, Kausar H. Biomolecular characterization of stress tolerant plant growth promoting rhizobacteria (PGPR) for growth enhancement of rice. *International Journal of Agriculture and Biology* 18 (2016): 184-191.
12. Kyndt T, Quispe D, Zhai H, Jarret R, Ghislain M, Liu Q, Gheysen G, Kreuze JF. The genome of cultivated sweet potato contains *Agrobacterium* T-DNAs with expressed genes: An example of a naturally transgenic food crop. *Proceeding of National Academy of Sciences* USA 112 (2015): 5844–5849.
13. Moore E, Arnscheidt A, Krüger A, Strömpl C, Mau M. Simplified protocols for the preparation of genomic DNA from bacterial cultures. *Molecular Microbial Ecology Manual* 1 (1999): 1-15.

14. Najimi M, Lemos ML, Osorio CR. Identification of siderophore biosynthesis genes essential for growth of *Aeromonas salmonicida* under iron limitation conditions. *Applied Environmental Microbiolgy* (2008) 2341–2348.

15. Naz I, Bano A, Hassan T-U. Isolation of phytohormones producing plant growth promoting rhizobacteria from weeds growing in Khewra salt range, Pakistan and their implication in providing salt tolerance to *Glycine max* L. *African Journal of Biotechnology* 8 (2009): 5762-5766.

16. Omar MNA, Osman MEH, Kasim WA, Abd El-Daim. Improvement of salt tolerance mechanisms of barely cultivated under salt stress using *Azospirillum brasilense*. In: Ashraf M, Ozturk M, Athar HR (eds). *Salinity and water stress.* (2009): 133-147 Springer, Netherlands

17. Prudent M, Vernoud V, Girodet S, Salon C. How nitrogen fixation is modulated in response to different water availability levels and during recovery: A structural and functional study at the whole plant level. *Plant and Soil*:(2015): 1-12.

18. Rajkumar M, Ae N, Prasad MN, Freitas H. Potential of siderophore-producing bacteria for improving heavy metal phytoextraction. *Trends in Biotechnology* 28 (2010): 142-9.

19. Ramachandran S, Fontanille P, Pandey A, Larroche C. Gluconic acid: properties, applications and microbial production. *Food Technology and Biotechnology* 44 (2006): 185-95.

20. Ramadoss D, Lakkineni VK, Bose P, Ali S, Annapurna K. Mitigation of salt stress in wheat seedlings by halotolerant bacteria isolated from saline habitats. *Springer Plus* 2 (2013):1.

21. Santoyo G, Orozco-Mosqueda MDC, Govindappa M. Mechanisms of biocontrol and plant growth-promoting activity in soil bacterial species of *Bacillus* and *Pseudomonas*: a review. *Biocontrol Science and Technology* 22 (2012): 855-872.

22. Sashidhar B, Podile AR. Mineral phosphate solubilization by rhizosphere bacteria and scope for manipulation of the direct oxidation pathway involving glucose dehydrogenase. *Journal of Applied Microbiology* 109 (2010): 1–12.

23. Shrivastava P, Kumar R. Soil salinity: A serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. *Saudi Journal of Biological Sciences* 22 (2015): 123–131.

24. Vyas P, Gulati A. Organic acid production *in vitro* and plant growth promotion in maize under controlled environment by phosphate-solubilizing fluorescent *Pseudomonas*. *BMC Microbiology* 9 (2009): 174.

25. Yin C, Park J-J, Gang DR, Hulbert SH. Characterization of a tryptophan 2-monooxygenase gene from *Puccinia graminis* f. sp. *tritici* involved in auxin biosynthesis and rust pathogenicity. *Molecular Plant-Microbe Interactions* 27 (2014): 227–235.

Cite this article as:
Anukool Vaishnav, Sarita Kumari, Shekhar Jain, Devendra Kumar Choudhary, Kanti Prakash Sharma. Molecular characterization of potential salt tolerant bacteria for soybean growth promotion. *International Journal of Bioassays* 5.12 (2016): 5118-5123.
DOI: http://dx.doi.org/10.21746/ijbio.2016.12.001

Source of support: Nil
Conflict of interest: None Declared