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ASSESSMENT OF PHYTOHORMONES PRODUCING CAPACITY OF *STENOTROPHOMONAS MALTOPHILIA* SSA AND ITS INTERACTION WITH *ZEA MAYS* L.

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Abstract

In this paper we reported the isolation of *Stenotrophomonas maltophilia* SSA from roots of *Solanum surrattense* Burm. f., a weed growing at arid soil of district Attock, Pakistan. The isolate was characterized for morphological and biochemical attributes and was identified as *Stenotrophomonas maltophilia* SSA on the basis of 16S-rRNA partial sequence analysis. The population size varied from 10^6 -10^7 cfu g^{-1} fresh weight of root. Phytohormones: Indole-3-acetic acid, Gibberellic acid, trans-Zeatin riboside and Abscisic acid were found in culture supernatant as measured by high performance liquid chromatography. The bacterium used as inoculants on *Zea mays* L. seedlings showed significant increase in the growth and proline content of root and shoot both under normal and NaCl stressed conditions.

Introduction

The genus *Stenotrophomonas* phylogenetically resides within the γ-subclass of the Proteobacteria (Moore *et al*., 1997) and plays very important ecological role in the element cycle in nature. It is gram negative *Bacillus* (Gautam *et al*., 2009) and found in a wide variety of environmental and geographical regions including hospitals, extreme environment such as Soda Lake (Denton & Kerr, 1998), rhizosphere of plants (Berg *et al*., 1996) and aquatic habitats (Minkwitz & Berg, 2001).

Stenotrophomonas maltophilia has dual characteristics, on one side it act as nosocomial multidrug-resistant pathogen associated with immuno-suppressed patients (Denton & Kerr, 1998) and on the other side it is non-pathogenic to plants (Wolf *et al.,* 2002) and aids beneficial effects in plant growth and development (Taghavi *et al*., 2009). The production of IAA, nitrogen fixing (Park *et al*., 2005) capability and production of anti-fungal metabolites (Minkwitz & Berg, 2001) by *Stenotrophomonas maltophilia* may reveal its bio-inoculant (Park *et al*., 2005) properties. It also showed tolerance to external osmolarity by the accumulation of certain osmoprotective compounds and can grow well under NaCl stress (Miller & Wood, 1996). Naz *et al*., (2009) reported isolation of Rhizobia from rhizosphere soil of *Solanum surattense* growing in arid zone of Attock. PGPB implies positive effects on root biomass and yield of plants (Shahzad *et al*., 2008).

Weeds harbour PGPR which as bio-inoculant are the best alternative to vegetate agricultural soils (Naz *et al*., 2010) but very few reports are available on this aspect. Keeping this point in mind the present study was aimed to (1) isolate and characterize bacterial strain and to (2) estimate phytohormones viz., GA_3, ABA and trans-Zeatin riboside by this bacterial isolate besides IAA production already documented in literature (3) and to evaluate its potential as bio-inoculant on *Zea mays* L., grown under normal and NaCl stressed (20 dS m^{-1}) conditions.

Materials and Methods

Sampling of the roots: The root samples were collected in five replicates from different localities of district Attock at vegetative phase of commonly growing weed: *Solanum surattense* Burm. f. (Yellow berried night shade). The roots were stored at 4°C for further processing.

Isolation and biochemical characterization of bacterial isolate: Root (1g) was rinsed with tap water, subsequently washed with sterile distilled water and ground in a sterile mortar with 10 ml distilled water. Serial dilutions were prepared and 100 µl from decimal dilutions was used to inoculate Luria Bertani (LB) media. The plates were incubated at 30°C for 24-72 h. Colonies showed similarities with *Stenotrophomonas* were observed under light microscope (Nikon, Japan) and preserved in glycerol for further tests. The viable colonies were counted at 10^6 and 10^7 dilutions using the formula:

$$\text{Viable cell count (CFU/g fresh root weight)} = \frac{\text{Number of colonies}}{\text{Volume of inoculum}} \times \text{dilution factor}$$

Gram staining of the bacterial isolate was done by the method of Vincent (1970) and biochemically characterized on the basis of carbon/nitrogen source utilization pattern using QTS-24 miniaturized identification system (DESTO Laboratories Karachi, Pakistan) following the method of MacFaddin (1980). One day old bacterial culture was used for this purpose; results were noted after 18 h of incubation at 30°C and compared with standard species mentioned in Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994).16S-rRNA gene amplification and sequencing DNA of the bacterial isolate was extracted using the method of Chen & Kuo (1993) and amplified following the method of Weisburg (1991). The polymerase chain reaction (PCR) was performed using two primers rd1 (AAGGAGGTGATCCAGCC) and fd1 (AGAGTTTGATCCTGGCTCAG). The reaction volume contained 1 µl of 50 µg of genomic DNA, 1.5 mM $MgCl_2$, 0.2 mM dNTP mix, 5 µl of 10 X *Taq* buffer, 1 U *Taq* DNA polymerase, 10 pmoles of each primer and

finally the volume was raised to 25 µl by autoclaved pure water. The reactions were carried out in thermocycler (Biometra, Germany). After denaturation at 95°C for 2 min, samples were passed for 30 cycles through the following temperature profile: denaturation at 94°C for 30 sec, annealing at 55 °C for 30 sec, extension at 72°C for 2 min plus one additional cycle for chain elongation at 72°C for 10 min. Amplified PCR products were separated on 1.2% (w/v) agarose gel and visualized under UV trans-illuminator lamp and compared with 1 Kb DNA ladder. The band of almost 1.5Kb was excised from the gels, purified using JET QUICK gel extraction spin kit (GENOMED) and sequenced on the automatic sequencor (CEQ-8800 Genetic analysis system, Beckman Coulter, USA) using CEQ DTCS kit (Beckman Coulter, USA). The obtained sequence was compared with reference species available at National Centre for Biotechnology Information (NCBI) database by employing BLAST algorithm and deposited in NCBI Genbank (www.ncbi.nlm.nih.gov/Genbank/submit.html) with the accession numbers (GQ262775).

Quantification of growth regulating substances in the culture supernatant Luria Bertani (LB) media supplemented with or without tryptophan (10 mg/100 ml) was inoculated with single colony of 24 h old bacterial culture and kept on shaker (ECELLA E24, USA) at 100rpm for 5d, until OD of cultures became equivalent to 1 at 600nm and 10^7 colony forming unit (CFU/g) was obtained on LB agar plates. The bacterial culture was centrifuged at 10,000 rpm for 15 min at 4ºC and supernatant (cell-free liquid culture medium) was used for extraction of phytohormones following the method described by Tien *et al*., (1979). The extracted samples were analyzed on high performance liquid chromatography (Agilent 1100) equipped with variable UV detector and C_{18} column (39 x 300mm) [BondaPack Porasil C-18, 37/50 µm, Waters, Eschborn, BRD). Methanol and water in the ratio of 30:70 v/v were used as mobile phase @ of 1500µl/min. with a run time of 20 min/sample. For identification of hormones, 100µl of sample filtered through a 0.45millipore filter, were injected into column. The growth hormones were identified on the basis of retention time of phytohormone standards (commercially grade, Sigma Chemical USA Company). IAA was eluted at 280nm wavelength while GA_3, t-Zr and ABA were eluted at 254nm respectively. The un-inoculated culture media was treated as blank.

Response of *Zea mays* L. seedlings to *Stenotrophomonas maltophilia* SSA

The seeds of the maize (advance germ plasm line: Islamabad Gold) were surface sterilized with 95% ethanol for 2-3 min and soaked overnight in distilled water prior to soaking in 5d old culture of *Stenotrophomonas maltophilia* (10^6-10^7 cfu g^{-1} fresh weight of root) for 2 h. The seeds soaked in un-inoculated culture media were treated as control. The soaked seeds were sown in pre-sterilized pots (19cm x 17cm) containing sterilized soil and sand (2:1 ratio). The temperature of growth room was maintained at 25 ± 2°C with the photoperiod 16h and the humidity varying from 65-70%. The plants were irrigated with Hogland nutrient solution. Two weeks after inoculation, seedlings were gradually exposed to NaCl by adding 5 dS m^{-1} NaCl per day until the final concentration of 20 dS m^{-1} was reached. Measurements were made 30d after inoculation. Three replicates (five plants / replicate) were used for measurement. Proline contents of shoots and roots were measured following the method of Bates *et al*., (1973).

Statistical analysis: Factorial analysis of variance (ANOVA) and two factor completely randomized design test (CRD) with least significance difference (LSD) was applied using MSTAT C program, version 4.0.

Results

The colonies of *Stenotrophomonas maltophilia* SSA isolated from roots of *Solanum surratense* grown in arid area of Attock appeared round, yellow and with smooth margin on LB medium. The number of viable colonies of isolate ranged from 10^6-10^7 cfu/g fresh weight of root. The isolate was gram -ive, rod shaped, motile in nature and characterized by biochemical tests (QTS-24 miniaturized identification system, DESTO Laboratories Karachi, Pakistan) based on carbon/nitrogen source utilization pattern (Table 1) which revealed the isolate was positive for the tests including sodium citrate, sodium melonate, lysine decarboxylase, ornithine decarboxylase, H_2S production, tryptophan deaminase, indole production, gelatin hydrolysis, acid from glucose, acid from maltose, acid from inositol, acid from sorbitol and acid from raffinose. The isolate was negative for tests viz: ortho nitro phenyl β-D-galactopyranoside, arginine dihydrolase, urea hydrolysis, Voges proskauer, acid from sucrose, acid from mannose, acid from arabinose, acid from rhamnose and acid from adonitol.

Table 1. Biochemical characterization (QTS-24 miniaturized identification system) of bacterial isolate.

Tests	Result	Tests	Result
Ortho nitro phenyl β-D-galactopyranoside	-	Gelatin hydrolysis	+
Sodium citrate	+	Acid from glucose	+
Sodium melonate	+	Acid from maltose	+
Lysine decarboxylase	+	Acid from sucrose	-
Arginine dihydrolase	-	Acid from mannose	-
Ornithine decarboxylase	+	Acid arabinose	-
H_2S production	+	Acid from rhamnose	-
Urea hydrolysis	-	Acid from sorbitol	+
Tryptophan deaminase	+	Acid from inositol	+
Voges proskauer	-	Acid from adonitol	-
Indole	+	Acid from raffinose	+
Organism identified	***Stenotrophomonas* sp.**		

The 16S-rRNA sequence analyses revealed sequence information of 527 nucleotides. The comparison of this sequence depicted 95% similarity (identities 487/512 positions) with the sequence of *Stenotrophomonas maltophilia* R551-3 (Accession No. 011071) already deposited in Gen Bank.

The results (Table 2) revealed that the level of IAA was 5 folds greater in the culture medium supplemented with tryptophan in contrast, the production of GA3, t-Zr and ABA were six, four and five times respectively, greater in without tryptophan when compared to with tryptophan medium. The isolate was used to inoculate seedlings of *Zea mays* L., (advance germplasm line Islamabad Gold) to evaluate its performance under normal and induced salt stress (20 dS/m NaCl) conditions (Table 3). Significant increases were observed in root length, shoot length, root dry weight and shoot dry weight of inoculated *Zea mays* L., plants growing under normal and salt stressed conditions in contrast to un-inoculated (control) plants. The magnitude of stimulation was markedly greater under unstressed condition.

The proline content of root was significantly increased in presence of NaCl both in the un-inoculated and seedlings inoculated with *S. maltophilia* SSA (Table 4). The basal level of proline was also higher in both roots and shoots of inoculated plants, comparatively the roots of inoculated plants accumulated more proline than the shoots.

Table 2. Phytohormones (µg ml^{-1}) produced by the isolate in culture medium supplemented with (0.1 mg ml^{-1}) or without tryptophan.

Phytohormones	- Trp	+ Trp
IAA	50 B	250 A
GA3	26.4 A	4.4 B
t-zr	16 A	4.0 B
ABA	0.4 A	0.08 B

–Trp=without tryptophan, +Trp= with tryptophan. All such values which share a common letter are insignificantly different otherwise, they differ at p≤0.05.

Table 3. Inoculation effects of Bacterial isolate on shoot and root length (cm) and dry weight (g) of 40 days old *Zea mays* L. plants growing under induced salt stress (20 dS m^{-1} NaCl) and unstressed conditions.

Treatments	Shoot length (cm)		Root Length (cm)		Shoot weight (g)		Root weight (g)	
	+NaCl	-NaCl	+NaCl	-NaCl	+NaCl	-NaCl	+NaCl	-NaCl
T1	39.4B	48.2A	20C	30.6A	6.7 B	11.2 A	5 B	8.2 A
T2	28.8C	41.2B	15.4D	25.3B	4.00 C	8.6 B	2.3 C	5.1 B

+NaCl= Seedlings treated with 20 dS m^{-1} NaCl, -NaCl = Seedlings not treated with 20 dSm^{-1} NaCl. T1 represents the inoculation treatment with *Stenotrophomonas maltophilia* SSA while T2 represents un-inoculated plant.

Table 4. Proline contents (mg g^{-1}) of shoots and roots of 40 days old *Zea mays* plants (growing in presence and absence of 20 dS/m NaCl stress) inoculated with *Stenotrophomonas maltophilia* SSA.

Treatments	*Zea mays* shoots		*Zea mays* roots	
	- NaCl	+ NaCl	-NaCl	+ NaCl
T1	12.4 C	16.2 A	14.3 C	19.6 A
T2	10 D	13.9 B	11.4 D	16.3 B

+NaCl= Seedlings treated with 20 dS m^{-1} NaCl, -NaCl = Seedlings not treated with 20 dSm^{-1} NaCl. T1 represents the inoculation treatment with *Stenotrophomonas maltophilia* SSA while T2 represents un-inoculated plant. All such values which share a common letter are insignificantly different otherwise, they differ at p≤0.05.

Discussion

The morphological and biochemical analyses indicated highest (98%) similarity of the isolate with the genus *Stenotrophomonas* when compared with Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994). In addition the phenotypic characteristic of this species correlate well with the molecular analyses based on 16S-rRNA partial sequence analyses. Naz *et al*., (2010) identified three phosphate solubilizing bacteria up to genera level by QTS-24 kit as belonging to genera *Pseudomonas* and these three strains were identified as *Pseudomonas mendocina* Khsr2, *Pseudomonas stutzeri* Khsr3 and *Pseudomonas putida* Khsr4 by 16S-rRNA.

The colony count of *S. maltophilia* SSA appeared to be in the range reported earlier. Tsavkelova *et al*., (2007) quantified 10^6 *Stenotrophomonas* colonies per g root of terrestrial and epiphytic tropical orchids. The population density of bacteria in the rhizosphere and roots depend on nature of root exudates, root morphology, stages of plant growth, physical and as well as chemical properties of soil (Vessey, 2003).

The amount of IAA produced (250 µg/ml) by the isolate in presence of added tryptophan was almost 2x higher than the IAA produced by *Stenotrophomonas maltophilia* PM-1 (112.8 µg/ml) and *Stenotrophomonas maltophilia* PM-26 (139.2 µg/ml) reported by Park *et al*. (2005) and 8X more than *Stenotrophomonas* sp. (31 µg/ml) isolated by Tsavkelova *et al*., (2007). The PGPR strains have been reported to produce IAA either with or without the tryptophan supplement in culture media (Fatima *et al*., 2009; Mehnaz *et al*., 2001). Greater production of IAA obtained during the present study might be due to the presence of tryptophan deaminase enzyme in *Stenotrophomonas maltophilia* that utilized tryptophan as precursor of IAA. Auxin biosynthesis is wide spread among soil and plant associated bacteria (Verma *et al*., 2001) including *Stenotrophomonas maltophilia* (Suckstorff & Berg, 2003).

Literatures are documented regarding the GA_3, ABA and trans-Zeatin riboside production by PGPR (Perrig *et al*., 2007; Boiero *et al*., 2007; Cohen *et al*., 2007) but there is no evidence describing the production of GA_3, ABA and trans-Zeatin by *Stenotrophomonas maltophilia*. This is the first report regarding the GA3, t-zr and ABA

production by *Stenotrophomonas maltophilia* in culture supplemented with or without tryptophan.

The increase in IAA production but decrease in GA_3, t-zr and ABA concentration in culture media suuplemented with tryptophan represents antagonistic interaction of IAA with GA_3, ABA and t-zr as repoted earlier (Naz *et al*., 2010) Present results evaluated that plant growth affected badly by salt stress. Negative effects of salt stress on germination, seedling growth, plant bio mass and some physiological activities of many plant species have been investigated (Hussain *et al*., 2008; Hussain *et al*., 2009; Shah *et al*., 1987; Ashraf *et al*., 1994) but the growth of NaCl stressed plants inoculated with *Stenotrophomonas maltophilia* SSA was better then un-inoculated plants. This is due to *Stenotrophomonas maltophilia* SSA isolated from arid zone of Attock which exhibited the ability to adapt under saline condition and provided tolerance to maize seedlings. Miller & Wood (1996) demonstrated that *Stenotrophomonas* strains have the ability to adapt themselves under salt stress perhaps by the accumulation of osmoprotective compounds. This view was further supported by the finding that proline (an osmoregulant) content was higher in maize plants inoculated with *Stenotrophomonas maltophilia* SSA. Among the mechanism of adaptation by *Stenotrophomonas maltophilia* SSA under saline condition are the ability to produce ABA in culture medium and the ability to augment the production of proline in inoculated plants. PGPR-induce the proline accumulation, membrane integrity and antioxidant enzyme activities but it has not been widely studied for plants which are exposed to salt stress (Kohler *et al*., 2009; Nadeem *et al*., 2007; Paul & Nair, 2008). Microbial inoculation enhanced proline accumulation in the roots thus provides tolerance to plant under salinity stress (Sharifi *et al*., 2007). The link between proline and ABA production has been reported under salinity (Woodward & Bennett, 2004).

Evaluation of this isolate under the field condition and thorough investigation of bioactive metabolites production to unravel its use as a biocontrol agent constitute future research.

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References

Ashraf, M., Z.U. Zafar and M. Tufail. 1994. Intra-specific variation for salt tolerance in a potential oil seed crop, brown mustard.*Brassica juncea* (L.). *J. Agron. Soil Sci*., 38: 449-458.

Bates, L.S., R P, Waldren and I.D. Teare. 1973. Rapid determination of free proline for water stress studies. *Plant. Soil*, 39: 205-207.

Berg, G., P. Marten and G. Ballin. 1996. *Stenotrophomonas maltophilia* in the rhizosphere of oilseed rape-occurrence, characterization and interaction with phytopathogenic fungi. *Microbiol. Res*., 151: 19-27.

Boiero, L., D. Perrig, O. Masciarelli, C. Penna, F. Cassan and V. Luna. 2007. Phytohormones production by three strains of *Bradyrhizobium japonicum* and possible physiological and technological implications. *Appl. Microbiol. Biotechnol*., 74: 874-880.

Chen, W.P. and T.T. Kuo. 1993. A simple and rapid method for the preparation of gram –ive bacterial genomic DNA. *Nucleic acid. Res.,* 21 (9): 2260.

Cohen, A.C., R. Bottini and P.N. Piccoli. 2007. *Azospirillum brasilense* SP 245 produces ABA in chemically-defined culture medium and increase ABA content in *Arabidopsis* plants. *Plant. Growth. Regul*., pp 1-11.

Denton, M and K.G. Kerr. 1998. Microbiological and clinical aspects of infections associated with *Stenotrophomonas maltophilia*. *Clin. Microbiol. Rev* ., 11: 7-80.

Fatima, Z., M. Saleemi, M. Zia, T. Sultan, M. Aslam, Riaz-Ur-Rehman and M.F. Chaudhary. 2009. Antifungal activity of plant growth-promoting rhizobacteria isolates against *Rhizoctonia solani* in wheat. *Afr. J. Biotechnol*., 8(2): 219-225.

Gautam, V., P. Ray, P. Vandamme, S.S. Chatterjee, A. Das, K. Sharma, S. Rana, R.K. Garg, S.K. Madhup, M. Mahajan and M. Sharma. 2009. Identification of lysine positive non-fermenting gram negative bacilli (*Stenotrophomonas maltophilia* and *Burkholderia cepacia* complex). *Indian. J. Med. Microbiol*., 27(2): 128-133.

Holt, J.G., N.R. Kreig, P.H.A Sneath, J.T. Staley and S.T. Williams. 1994. Bergey's Manual of Determinative Bacteriology. Williams and Wilkins, Baltimore, USA, pp. 40-169.

Hussain, K., A. Majeed, K. Nawaz, B.K. Hayat and M.F. Nisar. 2009. Effect of different levels of salinity on growth and ion contents of black seeds (*Nigella sativa* L.). *Curr. Res. J. Biol. Sci*., 1(3): 135-138.

Hussain, K., M. Ashraf and M.Y. Ashraf, 2008. Relationship between growth and ion relation in pearl millet (*Pennisetum glaucum* (L.) R. Br.) at different growth stages under salt stress. *Afr. J. Plant Sci*., 2(3): 23-27.

Kohler, J., J. A. Hernandez, F. Caravaca and A. Roldan. 2009. Induction of antioxidant enzymes is involved in the greater effectiveness of a PGPR versus AM fungi with respect to increasing the tolerance of lettuce to severe salt stress. *Environ. Exp Bot.,* 65(2-3): 245-252.

MacFaddin, J.F. 1980. *Biochemical tests for Identification of Medical bacteria.* Williams and Wilkins, Baltimore, USA, pp. 51-54.

Mehnaz, S., M.S. Mirza, J. Haurat, R. Balley, P. Normand, A. Bano and K.A. Malik. 2001. Isolation and 16S-rRNA sequence analysis of the beneficial bacteria from the rhizosphere of rice. *Can. J. Microbiol*., 47: 110-117.

Miller, K.J. and J.M. Wood. 1996. Osmoadaptation by rhizosphere bacteria. *Annu. Rev. Microbiol*., 50: 101-136.

Minkwitz, A. and G. Berg. 2001. Comparison of antifungal activities and 16S ribosomal DNA sequences of clinical and environmental isolates of *Stenotrophomonas maltophilia*. *J. Clin. Microbiol*., 39: 139-145.

Moore, E., A. Kruger, L. Hauben, S. Seal, R De. Baere, K. De. Wachter, K. Timmis and J. Swings. 1997. 16S-RNA gene sequence analyses and inter and intrageneric relationship of *Xanthomonas* species and *Stenotrophomonas maltophilia*. *FEMS. Microbiol. Lett*., 151: 145-153.

Nadeem, S. M., Z.A. Zahir, M. Naveed and M. Arshad. 2007. Preliminary investigations on inducing salt tolerance in maize through inoculation with rhizobacteria containing

ACC deaminase activity. *Can. J. Microbiol.,* 53: 1141-1149.

Naz I. and A. Bano. 2010. Biochemical, molecular characterization and growth promoting effects of Phosphate solubilizing *Pseudomonas* sp. isolated from weeds grown in salt range of Pakistan. *Plant. Soil*, DOI 10. 1007/s11104-010-0372-8.

Naz I., A. Bano and Tamoor-ul-Hassan. 2009. Morphological, biochemical and molecular characterization of rhizobia from halophytes of Khewra salt range and Attock. *Pak. J. Bot.,* 41(6): 3159-3168.

Park, M., C. Kin, J. Yang, H. Lee, W. Shin, S. Kim and T. Sa. 2005. Isolation and characterization of diazotrophic growth promoting bacteria from rhizosphere of agricultural crops of Korea. *Microbiol. Res*., 160: 127-133.

Paul, D. and S. Nair. 2008. Stress adaptations in a plant growth promoting rhizobacterium (PGPR) with increasing salinity in the coastal agricultural soils. *J. Basic Microbiol.,* 48(5): 378-384.

Perrig, D., M.L. Boiero, O.A. Masciarelli, C. Penna, O.A. Ruiz, F.D. Cassan and M.V. Luna. 2007. Plant growth promoting compounds produced by two strains of *Azospirillum brasilense*, and implications for inoculant formation. *Appl. Microbiol. Biotechnol*., DOI 10.1007/s00253-007-0909-9.

Shah, S.H., J. Gorham, B.P. Forster and R.G. Jones. 1987. Salt tolerance in the Triticeae. *J. Exp. Bot*., 38: 254- 269.

Shahzad, S.M., A. Khalid, M. Arshad, M. Khalid and I. Mehboob. 2008. Integrated use of plant growth promoting bacteria and P-Enriched compost for improving growth, yield and nodulation of chickpea. *Pak. J. Bot*., 40(4): 1735-1441.

Sharifi, M., M. Ghorbanli and H. Ebrahimzadeh. 2007. Improved growth of salinity-stresses soybean after inoculation with salt pre-treated mycorrhizal fungi. *J. Plant. Physiol*., 164: 1144-1151.

Suckstorff, I. and G. Berg. 2003. Evidence for dose dependent effects on plant growth by *Stenotrophomonas* strains from different origins. *J. Appl. Microbiol.,* 95: 656-663.

Taghavi, S., C. Garafola, S. Monchy, L. Newman, A. Hoffman, N. Weyens, T. Barac, J. Vangronsveld, D. Lelie and Der Van. 2009. Genome survey and characterization of endophytic bacteria exhibiting a beneficial effect on growth and development of Poplar trees. *Appl. Environ. Microbiol*., 75 (3): 748-757.

Tien, T.M., M.H. Gaskins and Hubbell. 1979. Plant growth substances produced by *Azospirillum brasilense* and their effect on the growth of pearl millet (*Pennisetum americanum* L). *Appl. Environ. Microbiol*., 37: 1016-1024.

Tsavkelova, E.A., T.A. Cherdyntseva, S.G. Botina and A.I. Netrusov. 2007. Bacteria associated with orchid roots and microbial production of auxin. *Microbiol. Res*., 162: 69-76.

Verma, S.C., J.K. Ladha and A.K. Tripathi. 2001. Evaluation of plant growth promoting and colonizing ability of endophytic diazotrophs from deep water rice. *J. Biotech*., 91: 127-141.

Vessey, J.K. 2003. Plant growth promoting rhizobacteria as biofertilizers. *Plant. Soil*., 255: 571-586.

Vincent, J.M. 1970. A *manual for the practical study of the root nodule bacteria.* Burgess and Son Ltd, Great Britain.

Weisburg, W.G., S.M. Barns, D.A. Pelletier and D.J. Lane. 1991. I6S-ribosomal DNA amplification for phylogenetic study. *J. Biotechnol*., 173: 16-22.

Wolf, A., A. Fritze, M. Hagemann and G. Berg. 2002. *Stenotrophomonas rhizophilia* sp. Nov., a novel plant-associated bacterium with antifungal properties. *Int. J. Sys. Evol. Microbiol*., 52: 1937-1944.

Woodward, A.J. and I.J. Bennett. 2004. The effect of salt stress and abscisic acid on proline production, chlorophyll content and growth pf in vitro propagated shoots of *Eucalyptus camaldulensis. Plant. Cell. Tissue. Organ. Culture*, 82: 189-200.

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