INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
18F–076/2018/20–8–1915–1920
DOI: 10.17957/IJAB/15.0755
http://www.fspublishers.org

Full Length Article

Molecular Characterization of Salt-Tolerant Bacteria Isolated from Greenhouse Vegetable Cultivation Soils

Jian Zhang[1,2*], Pengcheng Wang[1,2], Hongmei Tian[1,2], Yan Wang[1,2] and Haikun Jiang[1,2,3]
[1]*Institute of Horticulture, Anhui Academy of Agricultural Sciences, Hefei 230031 Anhui Province, China*
[2]*Key Laboratory of Genetic Improvement and Ecophysiology of Horticultural Crops, Hefei 230031 Anhui Province, China*
[3]*School of Horticulture, Anhui Agricultural University, Hefei 230036 Anhui Province, China*
[*]For correspondence: microbiol@126.com

Abstract

The aim of this study was to isolate salt-tolerant bacteria (STB) and identify their molecular properties from greenhouse vegetable cultivation soils in Shou county, China. The STB comprised 21–31% of the total viable cell count. A total of 12 strains were isolated from greenhouse saline soil and were classified as STB. All the isolates were Gram positive. Most of the isolates displayed plant growth-promoting trait, such as indole production. The majority of STB was positive for oxidase. Although the number of STB was only a third of the total viable cells, BOX-PCR and ERIC-PCR DNA fingerprinting showed different cluster groups, suggesting that the diversity of salt resistance bacterial isolates colonizing greenhouse soils differed. All the 12 sequenced isolates belonged to the cluster of *Bacillus* sp., which means that this genus is dominant in greenhouse soils. Only *Bacillus* sp. exhibited a strong ability to adapt in greenhouse saline soils. It was presumed that accumulation of salt ions and decreased pH value in the greenhouse soils could increase the osmotic pressure, which directly affected the number of bacteria with salt resistance. However, the obtained salt tolerant isolates should be further investigated for their application to sustainable agriculture.

Keywords: 16S rDNA; Bacteria; Greenhouse condition; Saline soil; Salt ions

Introduction

Soil salinization is increasing steadily in many areas of the world and has become one of the major disturbing factors that limit crop production, thereby threatening food security (Allakhverdiev *et al.*, 2000; Rengasamy, 2006). Excessive accumulation of salt ions negatively affects not only the physical and chemical properties of soil but also microbial communities and vegetative growth (Tejada *et al.*, 2006). Currently, greenhouse vegetable cultivation accounts for 11.6% of the national agricultural acreage in China and continues to increase rapidly (Maomao *et al.*, 2014). Now, salinity has become one of the limiting factors of crop cultivation in the greenhouse, because greenhouses have a semi-closed environment accompanied by high humidity, temperature, and the shortage of light. Most of all, they intercept rainfall and lead to a high concentration of all kinds of ions (Impron *et al.*, 2008). Consequently, great degeneration has been detected not only in soil quality but also in the microbiological community.

Given the wide distribution of saline soils, exploring the effect of salinization on the soil microbial community would be helpful (Bacilio *et al.*, 2004; Dodd and Pérez-Alfocea, 2012). The soil microbial community plays a vital role not only in the interconversion, degradation, and stabilization of soil organic matter but also in the cycling of nutrients vital for plant growth (Rath *et al.*, 2016). Plant growth-promoting bacteria, which are important components in the soil, can be used as alternative treatment to alleviate salt stress; they can be inoculated in crop seeds and plant seedlings or root. Salt tolerant bacteria can survive under saline conditions. Their beneficial traits, such as producing indole acetic acid, can help plants relieve the effect of salt stress (Mayak *et al.*, 2004). The application of rhizobacteria is one of the promising ways to reduce salt stress effect on plants. Salt-tolerant bacteria (STB) can modulate or ameliorate the saline damage to plant growth (Tiwari *et al.*, 2011). For instance, some *Bacillius* strains have reportedly tolerated (w/v) 8% NaCl and have shown PGP traits (Upadhyay *et al.*, 2009). Some bacteria, such as those with ACC deaminase activity, have been used to reduce the negative effects of salinity (Siddikee *et al.*, 2011). Hence, obtaining bacteria from saline soils would give potential candidates that could relieve saline stress in crop plants. Microbes that can endure in extreme environmental conditions are potentially suitable for utilization in various agricultural practices (Egamberdieva and Kucharova, 2009; Filippidou *et al.*, 2016).

To cite this paper: Zhang, J., P. Wang, H. Tian, Y. Wang and H. Jiang, 2018. Molecular characterization of salt-tolerant bacteria isolated from greenhouse vegetable cultivation soils. *Int. J. Agric. Biol*., 20: 1915–1920

However, to date, information on the analysis of STB from greenhouse soil is lacking. Therefore, the present work mainly aimed to isolate and characterize native STB from greenhouse soil. DNA fingerprinting techniques, including BOX-PCR and ERIC-PCR (Enterobacterial Repetitive Intergenic Consensus PCR), have been performed to identify the genetic relatedness of the obtained isolates. All the bacteria were identified on the basis of 16S ribosomal DNA partial sequences.

Materials and Methods

Materials

Soil samples were collected from the experimental basements in Shou County (116.27° E and 32.40° N), Huainan City, China, from three greenhouse agricultural sites. Collected samples were preserved at 4°C in labeled pre-sterilized bags until analysis and isolation.

Primary Screening of Salt Resistant Bacteria

The serial dilution was changed from 10^{-3} to 10^{-5} by transferring 1 mL of saline soil solution. For the selective screening of STB, approximately 0.1 mL volume of the resulting soil solution was obtained and then mixed with the Luria Bertani (LB) agar plates contained peptone 10.0 g/L, yeast extract, 5.0 g/L, sodium chloride 10.0 g/L, and agar 15.0 g/L. The medium without salt was used as the control. The pH of the medium was adjusted to 7.0 before autoclaving at 121°C for 25 min. After 72 h of incubation, the plates were observed for any kind of development on the culture medium. After preliminary screening of soil samples containing salt tolerant isolates, streak plate technique was used during isolation. Control plates with LB media and without salt were also prepared. Colonies differing in morphological characteristics were selected, purified, and preserved on different plates for further studies.

Phenotypic and Biochemical Characterization of Bacterial Isolates

The bacterial isolates were characterized based on cultural, morphological and biochemical characteristics, according to Bergey's Manual of systemic Bacteriology (Holt *et al.*, 1994). All isolates were initially characterized by Gram staining under light microscopy. The direct observation of isolated colonies on the LB agar medium was proceeded by color, shape, and texture.

DNA Fingerprinting

BOX-PCR: Bacterial DNA was extracted from the obtained isolates using the bacterial DNA Kit (Tiangen, China) according to the manufacturer's procedures. DNA amplifications were performed with 50 mL volumes containing 2 m*M* primer AR1 (5'-CTACG GCAAGGCGACGCTGACG-3') and 20 ng of genomic DNA. The reaction mixture was subjected to initial denaturation at 95°C for 5 min in an ABI cycler. This step was followed by 30 cycles at 90°C for 1 min, at 52°C for 1 min, at 72°C for 2 min, and a final extension at 72°C for 5 min. Horizontal electrophoresis was used to analyze PCR products in 2.0% agarose gels at 80 V for 2 h (McGee *et al.*, 2001).

ERIC-PCR

The primers used had the following sequences: 5'-ATGTAAGCTCCTGGGGATTCAC-3' and 5'-AAGTAAGTGACTGGGGGT-3'. Initially, DNA template was denatured for 5 min at 95°C. PCR was carried out for 35 cycles at 94°C for 30 s, at 52°C for 1 min, and at 72°C for 1 min, with a final elongation step at 72°C for 7 min. Horizontal electrophoresis was used to analyze PCR products in 2.0% agarose gels at 80 V for 2 h. The profiles were photographed under UV light (Bouchiba *et al.*, 2017). Comparative analysis of electrophoretic BOX-PCR and ERIC-PCR patterns was performed by using NTSYS software by Unweighted Pair Group Method with Arithmetic Averages (UPGMA).

Sequencing of 16S rDNA Gene

Genomic DNA was obtained by the method described as a section of DNA fingerprinting. Characterization of each selected strain was identified by partial sequencing of the 16S ribosomal DNA gene. The primers 27f and 1495r were used for amplification (Weisburg *et al.*, 1991). The 50 μL PCR mixtures constituted the following: 0.5 μM of each primer; and 1 μL of template DNA. The PCR was performed as follows: a hot start at 94°C for 5 min; followed by 30 cycles at 94°C for 1 min, at 54°C for 40 s, and at 72°C for 3 min; with a final extension at 72°C for 7 min. The 16S rDNA partial genes were sequenced by Sangon Biotech Co., Ltd. (Shanghai). The obtained sequences were subjected to BLAST analysis with the NCBI database to determine the closely related bacteria (http://blast.ncbi.nlm.nih.gov/Blast.cgi) (Yu *et al.*, 2011). Similarity sequences with high scores were selected from the NCBI database. Based on the sequences, software MEGA 6.0 was applied to construct a phylogenetic tree using Neighbor-Joining method (Tamura *et al.*, 2013).

Statistical Analysis

Data were captured in Microsoft Excel Software, version 2007 which was used to calculate means and standard deviations. Fisher's protected LSD test at the $p \leq 0.05$ level was performed by using the SPSS software package (version 19.0).

Results

Total viable cell count (130 CFU) on LB solid medium without NaCl was much higher than the number (33 CFU) in LB medium containing (*w/v*) 10% salt (Table 1). The number of STB was 21%–31% of the total viable cell count. After further identification, twelve isolates were obtained from greenhouse saline soil by LB medium and were purified. The colonies were whitish, white and yellowish. Out of 12 isolates, four strains were isolated from cabbage soil, one strain was separated from soil planted with tomato and celery, and seven strains were derived from cucumber soil. All the isolates were Gram positive, motile rods, and could form endospores (Fig. 1). The majority of bacteria were positive for oxidase (Table 2).

In this work, twelve isolates were selected to perform the Rep-PCR. The diversity of 12 isolates was identified by BOX-PCR and ERIC-PCR DNA fingerprinting. Cluster analysis grouped isolates by BOX-PCR, as well as by ERIC-PCR similarity. The dendrogram is shown in Fig. 2. For BOX-PCR, 12 isolates were grouped into 2 clusters, which contained isolates from either the same or different sites. For ERIC-PCR, 12 isolates were grouped into 3 clusters. These techniques are not only considered as useful tools to detect microbial ecology but also highly discriminating methods that enable differentiation of genetic diversity at the intra-species level. After analysis of all the representative isolates, they were further retained for 16S rDNA sequencing.

All strains displaying salt tolerance were identified based on a 16S rDNA partial sequence. All acquired sequences were deposited in the NCBI GenBank, and the accession numbers are listed in Table 3. All 12 sequenced isolates belonged to the cluster of *Bacillus* spp., which means that this genus is dominant in greenhouse soils. Based on the Neighbor-Joining method, one phylogenetic tree was constructed from 16S rRNA gene sequences (Fig. 3). Therefore, the bacterial strains that exhibited salt resistance activity still presented a small spectrum of microbial diversity in greenhouse soil.

Table 1: Total viable cell count in solid medium

LB medium (CFU)	LB containing (w/v) 10% NaCl (CFU)	Percent (%)
120	26	21.67
160	50	31.25
110	24	21.82
Average **130**	**33**	**24.91**

Table 2: Morphological and biochemical characteristics of salt-tolerant isolates

Bacterial isolates	Colony color	Gram nature	Cell shape	Indole	Oxidase
SX1	White	+	Rod	-	+
SX 2	Whitish	+	Rod	+	-
SX 3	Yellowish	+	Rod	-	+
SX 4	White	+	Rod	-	+
SX 5	Yellowish	+	Rod	-	+
SX 6	Whitish	+	Rod	+	+
SX 7	White	+	Rod	+	-
SX 8	Whitish	+	Rod	+	+
SX 9	Whitish	+	Short Rod	+	+
SX 10	Yellowish	+	Rod	+	+
SX 11	Yellowish	+	Rod	+	+
SX 12	White	+	Rod	+	-

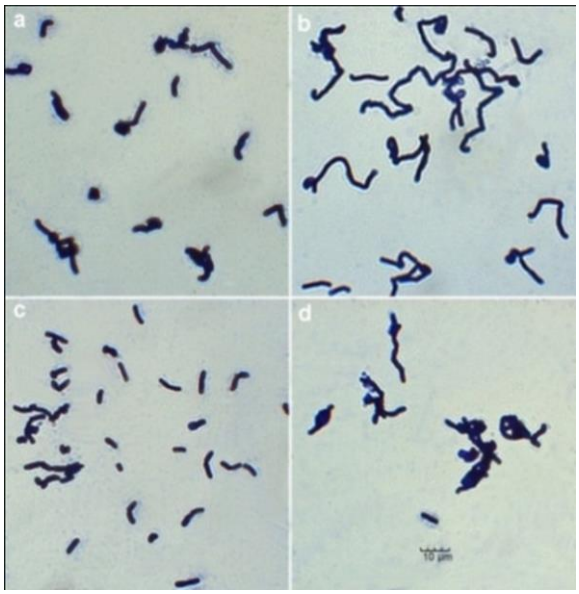

Fig. 1: Single-cell form of isolates under optical microscope view. (a) SX1, (b) SX2, (c) SX9, and (d) SX10. Bar represents 10 μm

Discussion

Only a third of the total viable bacteria could survive at 10% salt stress. Twelve isolates were obtained from the greenhouse soils, and they grew well on the LB agar containing (*w/v*) 10% salt; this number was higher than that previously reported (Upadhyay *et al.*, 2009). All isolates were Gram positive. Although the number of STB was only a third of the total viable cells, BOX-PCR and ERIC-PCR DNA finger printing showed different cluster groups, thereby suggesting that the diversity of salt resistance bacterial strains colonizing greenhouse soils are still different. Those techniques are useful tools to make a distinction between genetic diversities, especially at the intra-species level (Rai *et al.*, 2016). Moreover, those techniques are efficient not only for identifying STB in the soil but also for characterizing the different isolates and exploring their distribution during vegetable production. Most of the isolates displayed plant growth-promoting trait and produced indole, thereby indicating that they have a double function for enhancing plant growth.

Table 3: Analysis of salt-tolerance isolates according to the 16S rDNA partial sequence

Isolate	Organisms identified	Accession number*	Closest type strain in NCBI data base	16S rDNA identity (%)
SX1	*Bacillus* spp.	MF431747	*Bacillus megaterium* KF933665	99.0
SX 2	*Bacillus* spp.	MF431748	*Bacillus megaterium* JQ229806	99.0
SX 3	*Bacillus* spp.	MF431749	*Bacillus* sp. KC119104.1	99.0
SX 4	*Bacillus* spp.	MF431750	*Bacillus megaterium* KU647242	99.0
SX 5	*Bacillus* spp.	MF431751	*Bacillus megaterium* KT588644	99.0
SX 6	*Bacillus* spp.	MF431752	*Bacillus megaterium* EU979528	99.0
SX 7	*Bacillus* spp.	MF431753	*Bacillus megaterium* KU647208	99.0
SX 8	*Bacillus* spp.	MF431754	*Bacillus megaterium* KM659224	99.0
SX 9	*Bacillus* spp.	MF431755	*Bacillus megaterium* KR063189	99.0
SX 10	*Bacillus* spp.	MF431756	*Bacillus megaterium* KU647259	99.0
SX 11	*Bacillus* spp.	MF431757	*Bacillus megaterium* KX350034	99.0
SX 12	*Bacillus* spp.	MF431758	*Bacillus megaterium* KT588644	99.0

*The accession number of the isolates deposited in the Genbank (NCBI)

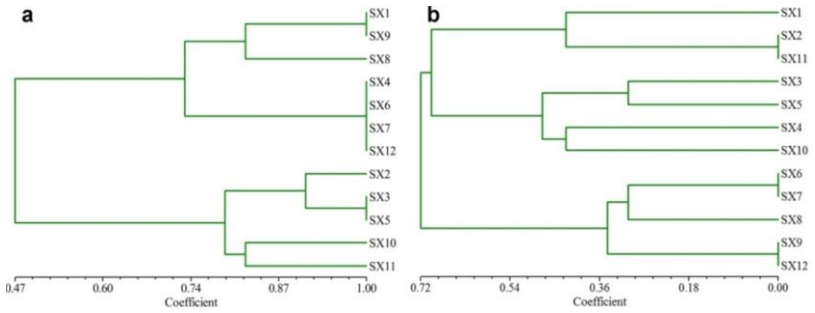

Fig. 2: Dendrograms constructed by unweighted pair group method with arithmetic mean (UPGMA) showing dissimilarity among BOX-PCR (a) and ERIC-PCR (b) of the obtained isolates from greenhouse soils

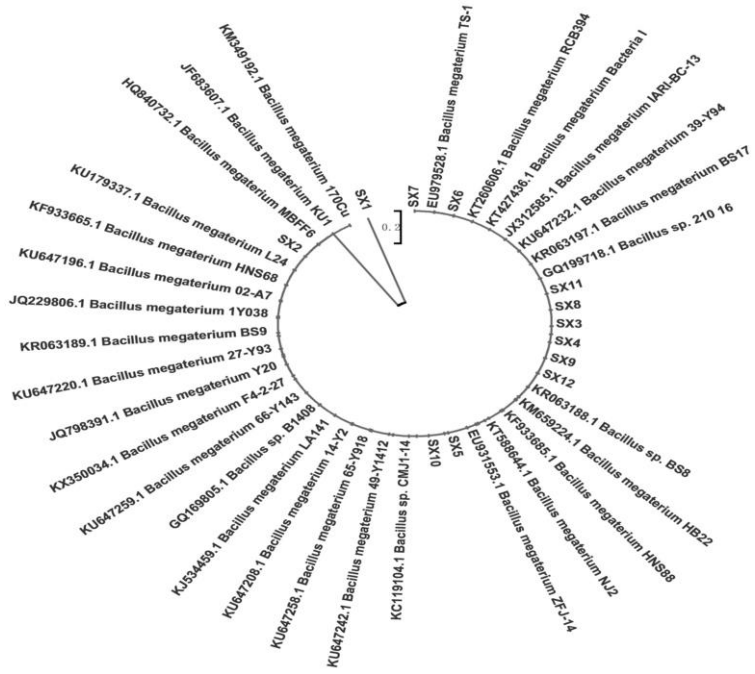

Fig. 3: Neighbor-joining phylogenetic tree based on 16S rRNA gene sequence shows the position of isolated strains with the species of each genus. Bootstrap percentage values as obtained from 2,000 resamplings of the data set are given at the nodes of the tree. Bar represents 0.2 substitutions per nucleotide position

According to the 16S rRNA partial gene sequence, all the bacteria belonged to *Bacillus* spp., which is typically Gram positive. Generally, *Bacillus* spp. is dominant in root-adhering soil, especially under extreme environment conditions (Laguerre *et al.*, 1994), and the present results were consistent with previous findings (Johri, 2011). Only *Bacillus* spp. exhibited a strong ability to adapt to greenhouse saline soils. Given the current research results, the soil pH was significantly reduced under greenhouse conditions by intensive application of chemical fertilizers (Shi *et al.*, 2009). Moreover, soil samples taken from greenhouse soils had high concentrations of nutrient elements, such as nitrogen, phosphorus, potassium, and salt ions, e.g., Mg^{2+}, Ca^{2+}, and Na^{+}; and this observation was found not only in our study (data not shown) but also in the data of other research groups (Lao *et al.*, 2003). Sodium chloride is the most soluble and abundant salt released (Robinson *et al.*, 2017). Salinization in the greenhouse is caused by the overuse of fertilizer to achieve high crop yield (Qadir *et al.*, 1998). In this case, fertilizers cannot be removed from the soil by rainfall because greenhouses have a semi-closed environment. Salt ions can be transferred from the subsoil to the top soil under the driving force of water evaporation (Cho *et al.*, 2009). As a result, a high concentration of ions exists in surface soil under greenhouse conditions.

Accumulation of ions and decreased pH value could significantly increase the osmotic pressure in the soil, which directly increases the number of bacteria with salt resistance. *Bacillus* spp. has relatively thick and dense cell walls (Beveridge, 1978; Dajkovic *et al.*, 2017), thereby allowing it to survive better in saline soils. The present findings suggest that bacterial communities display a significantly change in greenhouse soils. Notably, the majority of soil bacteria could not tolerate salt. However, approximately 21%–31% of soil bacteria adapt to saline soil, even though the soil pH significantly decreased and a large amount of salt ions were accumulated in greenhouse soil.

Conclusion

Number of salt-tolerant bacteria was only a third of total viable cells in the detected sample soils. Twelve isolates were obtained in this study and all the isolates were Gram positive. Based on the 16S rRNA gene sequence, all bacteria were *Bacillus* sp. Accumulation of salt ions and decreased pH value could significantly increase the osmotic pressure in the greenhouse saline soils, which directly increased the number of bacteria with salt resistance. The present study is the first to analyze STB in greenhouse soils. Results contribute to knowledge on their resistance to salinity stress. The obtained isolates should be further investigated for their application to sustainable agriculture.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (Grant No.31701968), and the Funds (Nos.17B0307, 18C0309, and 17D0306) from Anhui Academy of Agricultural Sciences. We gratefully acknowledge anonymous referees who provided valuable and constructive suggestions to improve this manuscript. Our thanks are also due to professors from Anhui Agricultural University for their helpful discussions.

References

Allakhverdiev, S.I., A. Sakamoto, Y. Nishiyama, M. Inaba and N. Murata, 2000. Ionic and osmotic effects of NaCl-induced inactivation of photosystems I and II in *Synechococcus* spp. *Plant Physiol.*, 123: 1047–1056

Bacilio, M., H. Rodriguez, M. Moreno, J.P. Hernandez and Y. Bashan, 2004. Mitigation of salt stress in wheat seedlings by a gfp-tagged *Azospirillum lipoferum*. *Biol. Fertil. Soils*, 40: 188–193

Beveridge, T.J., 1978. The response of cell walls of *Bacillus subtilis* to metals and to electron-microscopic stains. *Can. J. Microbiol.*, 24: 89–104

Bouchiba, Z., Z.F. Boukhatem, Z. Ighilhariz, N. Derkaoui, B. Kerdouh, H. Abdelmoumen, Y. Abbas, M.M.E. Idrissi and A. Bekki, 2017. Diversity of nodular bacteria of *Scorpiurus muricatus* in western Algeria and their impact on plant growth. *Can. J. Microbiol.*, 63: 450–463

Cho, J.M., K.J. Kim, K.Y. Chung, S. Hyun and K. Baek, 2009. Restoration of saline soil in cultivated land using electrokinetic process. *Sep. Sci. Technol.*, 44: 2371–2384

Dajkovic, A., B. Tesson, S. Chauhan, P. Courtin, R. Keary, P. Flores, C. Marlière, S.R. Filipe, M.P. Chapot-Chartier and R. Carballido-Lopez, 2017. Hydrolysis of peptidoglycan is modulated by amidation of meso-diaminopimelic acid and Mg^{2+} in *Bacillus subtilis*. *Mol. Microbiol.*, 104: 972–988

Dodd, I.C. and F. Pérez-Alfocea, 2012. Microbial amelioration of crop salinity stress. *J. Exp. Bot.*, 63: 3415–3428

Egamberdieva, D. and Z. Kucharova, 2009. Selection for root colonising bacteria stimulating wheat growth in saline soils. *Biol. Fertil. Soil.*, 45: 563–571

Filippidou, S., T. Wunderlin, T. Junier, N. Jeanneret, C. Dorador, V. Molina, D.R. Johnson and P. Junier, 2016. A combination of extreme environmental conditions favor the prevalence of endospore-forming firmicutes. *Front. Microbiol.*, 7: 1707

Holt, J.G., N.R. Kreig, P.H.A. Sneath, J.T. Staley and S.T. Williams, 1994. *Bergey's Manual of Systematic Bacteriology*, 9th edition, Vol. 1, pp: 1935–2045. Baltimore, MD: Williams & Wilkins

Impron, I., S. Hemming and G.P.A. Bot, 2008. Effects of cover properties, ventilation rate, and crop leaf area on tropical greenhouse climate. *Biosyst. Eng.*, 99: 553–564

Johri, B., 2011. Bacterial diversity in a bagasse-based compost prepared for the cultivation of edible mushrooms *Agaricus bisporus*. *J. Agric. Technol.*, 7: 1303–1311

Laguerre, G., M.R. Allard, F. Revoy and N. Amarger, 1994. Rapid identification of *rhizobia* by restriction fragment length polymorphism analysis of PCR-amplified 16S rRNA genes. *Appl. Environ. Microbiol.*, 60: 56–63

Lao, M.T., S. Jiménez, E. Eymar, E.J. Fernández and R. Jiménez, 2003. Determination of spatial variability of nutrient composition of soil solutions in greenhouses by using suction cups. *Commun. Soil Sci. Plant Anal.*, 34: 865–879

Maomao, H., S. Xiaohou and Z. Yaming, 2014. Effects of different regulatory methods on improvement of greenhouse saline soils, tomato quality, and yield. *Sci. World J.*, 2014: 1–11

Mayak, S., T. Tirosh and B.R. Glick, 2004. Plant growth-promoting bacteria confer resistance in tomato plants to salt stress. *Plant Physiol. Biochem.*, 42: 565–572

McGee, L., L. McDougal, J. Zhou, B.G. Spratt, F.C. Tenover, R. George, R. Hakenbeck, W. Hryniewicz, J.C. Lefévre, A. Tomasz and K.P. Klugman, 2001. Nomenclature of major antimicrobial-resistant clones of *Streptococcus pneumoniae* defined by the pneumococcal molecular epidemiology network. *J. Clin. Microbiol.*, 39: 2565–2571

Qadir, M., R.H. Qureshi and N. Ahmad, 1998. Horizontal flushing: a promising ameliorative technology for hard saline-sodic and sodic soils. *Soil Till. Res.*, 45: 119–131

Rai, S., P.L. Kashyap, S. Kumar, A.K. Srivastava and P.W. Ramteke, 2016. Identification, characterization and phylogenetic analysis of antifungal *Trichoderma* from tomato rhizosphere. *SpringerPlus*, 5: 1939

Rath, K.M., A. Maheshwari, P. Bengtson and J. Rousk, 2016. Comparative toxicities of salts on microbial processes in soil. *Appl. Environ. Microbiol.*, 82: 2012–2020

Rengasamy, P., 2006. World salinization with emphasis on Australia. *J. Exp. Bot.*, 57: 1017–1023

Robinson, H.K., E.A. Hasenmueller and L.G. Chambers, 2017. Soil as a reservoir for road salt retention leading to its gradual release to groundwater. *Appl. Geochem.*, 83: 72–85

Shi, W.M., J. Yao and F. Yan, 2009. Vegetable cultivation under greenhouse conditions leads to rapid accumulation of nutrients, acidification and salinity of soils and groundwater contamination in South-Eastern China. *Nutr. Cycl. Agroecosyst.*, 83: 73–84

Siddikee, M.A., B.R. Glick, P.S. Chauhan, W.J. Yim and T. Sa, 2011. Enhancement of growth and salt tolerance of red pepper seedlings (*Capsicum annuum* L.) by regulating stress ethylene synthesis with halotolerant bacteria containing 1-aminocyclopropane-1-carboxylic acid deaminase activity. *Plant Physiol. Biochem.*, 49: 427–434

Tamura, K., G. Stecher, D. Peterson, A. Filipski and S. Kumar, 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol.*, 30: 2725–2729

Tejada, M., C. Garcia, J.L. Gonzalez and M.T. Hernandez, 2006. Use of organic amendment as a strategy for saline soil remediation: Influence on the physical, chemical and biological properties of soil. *Soil Biol. Biochem.*, 38: 1413–1421

Tiwari, S., P. Singh, R. Tiwari, K.K. Meena, M. Yandigeri, D.P. Singh and D.K. Arora, 2011. Salt-tolerant rhizobacteria-mediated induced tolerance in wheat (*Triticum aestivum*) and chemical diversity in rhizosphere enhance plant growth. *Biol. Fertil. Soil.*, 47: 907

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

Weisburg, W.G., S.M. Barns, D.A. Pelletier and D.J. Lane, 1991. 16S ribosomal DNA amplification for phylogenetic study. *J. Bacteriol.*, 173: 697–703

Yu, X., X. Liu, T. Zhu, G. Liu and C. Mao, 2011. Isolation and characterization of phosphate-solubilizing bacteria from walnut and their effect on growth and phosphorus mobilization. *Biol. Fertil. Soil.*, 47: 437–446

(Received 20 April 2018; Accepted 03 May 2018)