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Full Length Research Paper

Effect of salinity on free living - diazotroph and total bacterial populations of two saline soils

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Salinization of soil is a serious problem and is increasing steadily in many parts of the world. Recently, soil biotechnology application can improve the potential of saline soils land use in agriculture. The aim of this research was determining the salinity effect on free-living diazotroph and total bacterial populations in two saline soils. Soil samples with salinity of 35 and 70 dS/m selected. The heterotrophic and free-living diazotrophic bacterial populations were counted on nutrient agar and free-nitrogen medium. The salinity effect on population of each soil was determined on same media supplemented with 0.44 and 1.02 M NaCl (equal with EC 35 and 70 dS/m of each soil). The results showed that, the free-living diazotrophic and total heterotrophic bacterial populations in soil sample 1 was significantly ($P < 0.05$) more than soil sample 2. Also the heterotrophic and diazotrophic bacterial population in each soil sample were significantly less in the presence of salt. Soil salinity is a stress factor relating to microbial selection process and can reduce bacterial diversity and control microbial abundance, composition and functions. Use of soil halotolerant or halophyl bacterial strains which can either fix atmospheric nitrogen will be environmentally begin approach for nutrient management and ecosystem function for saline soils.

Key words: Salinity, bacterial population, diazotrophic, heterotrophic.

INTRODUCTION

Nearly 40% of world's surface has salinity problems (Jadhav et al., 2010). Salinization of soil is a serious problem and is increasing gradually in many parts of the world, particularly in arid and semiarid areas. At present, out of 1.5 billion hectares of cultivated land around the world, about 77 million hectares is affected by excess salt content (Evelin et al., 2009). The importance of soil salinity for agricultural yield is enormous as it affects the establishment, growth and development of plants leading to huge losses in productivity (Mathur et al., 2007). Recently, soil biotechnology application can improve the potential of saline soils land use in agriculture.

Knowledge about microbiological processes and factors on which they depend makes possible the use of many microorganisms in agriculture. Soil is an ecosystem colonized by various groups of microorganisms, which are its living constituent. Active microbiological processes in soil enhance the rate of synthesis and mineralization of organic matter which then leads to better plant nutrition. Some of the microorganisms, particularly valuable bacteria and fungi can develop plant performance under stress condition and, therefore, improve yield (Evelin et al., 2009). At high salinity level, it was found that treatments supplied by biofertilization with yeast decreased the adverse effect of salinity. Halophilic microorganisms are already in use for some biotechnological processes, such as commercial production of β-carotene, polymers, enzymes, compatible solutes (Jadhav et al., 2010).

Application of yeast produced the highest spikes

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number irrespective of salinity level and cultivar type (Gomaa et al., 2008). Na^+-dependent strains also have been isolated recently from soils in Louisiana and also reported new Na^+-dependent isolates were obtained from Canadian soils (Page and Shivprasad, 1991). Maria Mercedes Palomino (2009) reported that *Bacillus subtilis* is a Gram-positive sporulating bacterium able to adapt to wide variations in osmotic and saline strength. Investigated the mechanism of osmotic stress adaptation (osmoregulation) in *Agrobacterium tumefaciens* biotypes I and III are (salt-tolerant) tolerant to 2% NaCl (Smith et al., 1990). A novel Corynebacterium species that originated from saline soil in the west of China was characterized (Chen et al., 2004). In a trial to isolate endophytic bacteria from the saline soil ; from root, shoot and leaf of rice plant cultivated in highly saline soil, two species belonging to the family of Enterobacteriaceae have been isolated (Tanawy, 2009).

Free living prokaryotes with the ability to fix atmospheric di-nitrogen are ubiquitous in the soil. The capacity for nitrogen fixation is widespread among bacteria .The estimated contribution of free-living N_2 fixing prokaryotes to the N_2 input of soil ranges from 0 to 60 kg ha^{-1} $year^{-1}$ (Mirzakhani et al., 2009). A novel species, Swaminathania salitolerans was isolated from the rhizosphere, roots and stems of salt-tolerant, mangrove-associated wild rice using nitrogen-free, semi-solid LGI medium at pH 5.5 (Loganathan and Nair, 2004). The aim of this research was determining the salinity effect on free living-diazotroph and total bacterial populations of two saline soils and investigating salt tolerance of nitrogen-fixing isolates.

MATERIALS AND METHODS

Soils study

Two saline soil samples were collected from the *Aeluropus littoralis* rhizospher and transferred to the laboratory. *A. littoralis* is a C4 perennial halophyte monocotyledonous plant belonging to the same family as wheat. Growing as weed in dry salty areas or marshes, it is salt-secreting, rhizomatous and is used as forage.

The chemical and physical properties of the saline soils were determined. The total C content was identified by elemental analysis, whiles the total nitrogen content, N_2, was determined by the Kjeldahl method (Bremner and Mulvaney, 1982). The Olsen method was used to determine the available Phosphorus (P) content (Olsen and Sommers, 1982). The extractable calcium, magnesium were measured by titration method. The potassium and sodium content were measured by flame photometry. The soil's pH and electrical conductivity (EC) also were measured.

Heterotrophic bacterial populations

Heterotrophic bacterial populations of two saline soils were determined. 1 g of each soil were suspended in phosphate buffer then serial dilution was carried out. So the amount of 100 µl were cultured on nutrient agar (without salt) and nutrient agar supplemented with 0.51 M NaCl (equal with 35 dS/m) for soil sample 1 and 1.02 M NaCl) equal with 70 dS/m) for soil sample 2. After 72 h incubation in 32°C the forming colony were counted by colony counter instrument.

Free-living diazotrophic bacterial populations

Free-living diazotrophic bacterial populations of two saline soils were determined. The samples preparation were done as mentioned above and cultured on free-nitrogen media (without salt) that involved (g/L): 10 g Sucrose, 10 g Glucose, 0.2 g MgSO4.7H2o, 0.15 g KH2PO4, 0.005 g FeCl3, 0.1 g CaSO4.2H2o, 0.05 g K2HPO4, 0.02 g CaCl2, 0.003 g Na2MoO4, 7.2 ± 0.2 pH (Atlas, 1946) and free-nitrogen media supplemented with 0.51 M NaCl (equal with 35 dS/m) for soil sample 1 and 1.02 M NaCl) equal with 70 dS/m) for soil sample 2. After 72 h incubation in 32°C, the forming colonies were counted by colony counter instrument.

Free-living diazotrophic bacterial identification

After the purification of free living diazotrophic isolates from saline soils, their identification was done by the help of gram staining and biochemical tests such as Motility, Indol, Glucose, Manitol, Lactose, Catalase.

Salt tolerance of free-living diazotrophic bacterial isolates

The salt tolerances of diazotrophic bacterial isolates were determined on nutrient agar and on free nitrogen media supplemented with different concentrations of NaCl (0.14, 0.29, 0.58, 0.88, 1.16, 1.47, 1.76, 2.06, 2.35, 2.65, 2.94 and 3.25 M).

RESULTS AND DISCUSSION

The chemical and physical properties of two saline soils are shown in Table 1. The average numbers of heterotrophic and free living diazotrophic bacteria in present and absence of salt are given in Figures 1 and 2 respectively. The result analyzed SPSS statistical with Duncan comparison. There was a significant difference ($P < 0.05$) between the bacterial populations of two soils in each experimental condition. In the next step, the fact that the nitrogen-fixing bacteria are important for ecology and agriculture in hence we identified free-living diazotrophic bacterial and so the salt tolerances of bacterial were determined in the nutrient agar and free-nitrogen mediums for comparison of the effect presence and absence N on the resistants to salt bacteria so different concentrations of NaCl added to the mediums. One of the isolates showed the maximum inhibitory concentration (MIC). This isolate could tolerate the concentration of 2.94 M NaCl in the nutrient agar medium, and according to biochemical tests, it is a member of the genus bacillus. The other isolates that could tolerate the NaCl concentration of 2.65 and 0.88 M belong to the genera of Agrobacterium, Enterobacter and Corynebacterium, respectively, but genus bacillus could tolerate the concentration of 1.76 M NaCl in the free nitrogen medium. The other isolates that could tolerate the NaCl concentration 1.16 and 0.58, M were belonging to the genera of Agrobacterium, Enterobacter and

Table 1. The chemical and physical properties of soils.

Type soils	Ca^{+2} meq/l	Mg^{+2} meq/l	K^{+} meq/l	Na^{+} meq/l	N meq/l	P mg/kg	O.M%	O.C%	Ec (dS/m)	pH
Soil 1	48	36	7	250	0.045	4.5	0.77	0.45	35	6.98
Soil 2	110	68	23	415	0.038	3.4	0.53	0.31	70	8.13

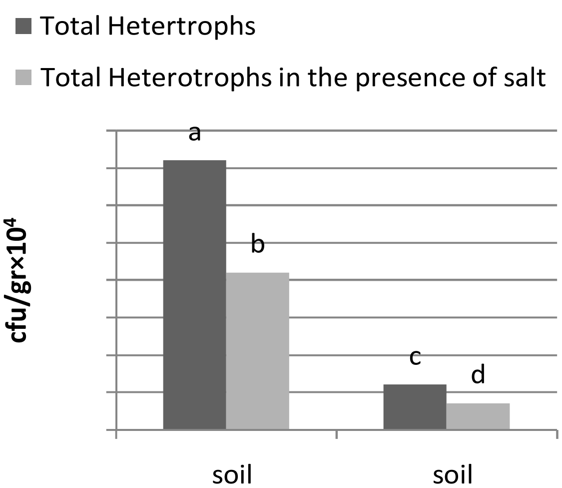

Figure 1. Heterotrophic bacterial populations and salinity effect on them.

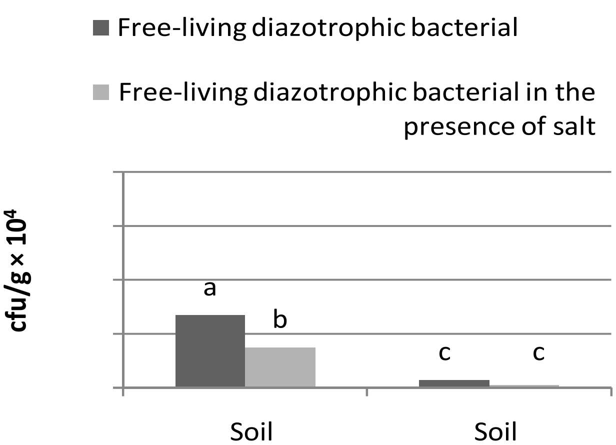

Figure 2. Free-living diazotrophic bacterial populations and salinity effect on them.

Corynebacterium, respectively. There was a significant difference ($P < 0.05$) between the bacterial isolates MIC with nutrient agar and MIC with free nitrogen media. The results are in Figure 3.

The total heterotrophic bacterial populations in soil sample 1 was significantly ($P < 0.05$) more than soil sample 2. Also the results showed that the heterotrophic bacterial population in each soil sample was significantly less in the presence of salt (Figure 1). Soil salinity is a stress factor relating to microbial selection process and can reduce bacterial diversity and control microbial abundance, composition and functions (Ibekwe et al., 2010). Soluble ion concentrations (especially Na^{+}) greater than about 0.15 M in soil leads to hyper osmotic conditions which forces water to diffuse out of a microbial cell. The cells will then shrink or plasmolysis. In addition, the high Na^{+} concentration also causes the water associated with such solutes to become unavailable to microorganisms. Basically, the effect of Na^{+} on the growth of different species of microorganisms will differ due to growing water activity of each microorganism. Microorganisms under hypertonic environments either die or remain dormant except halotolerant and halophilic microorganisms that can combat this problem (Chookietwattana, 2003). The salt requirement and tolerance properties of the bacteria are highly variable and may vary according to the growth temperature and the nature of the nutrients available (Quillaguamán et al., 2004). Several classifications have been proposed; one that is very well accepted considers the optimum growth of the micro-organisms at different salt concentrations.

Kushner and Kamekura defined several categories of micro-organisms on the basis of their optimal growth: (1) non-halophiles are those that grow best in media containing less than 0.2 M NaCl (1% salt). (2) slight halophiles grow best in media with 0.2 to 0.5 M NaCl (1-3% salt). (3) moderate halophiles grow best with 0.5 to 2.5 M NaCl (3-15% salt). (4) extreme halophiles show optimal growth in media containing 2.5 to 5.2 M NaCl (15-32% salt) (Ventosa, 2006). Hence the results of salt resistant diazotrophic bacteria on the nutrient agar showed that Corynebacterium and Enterobacter genera were moderately halophiles and Bacillus and Agrobacterium genera were extreme halophiles. Moderately and extremely halophilic bacteria are the most important group in hypersaline habitats, and receive much attention from microbiologists. Only moderately and extremely halophilic bacteria survive and play a major ecological role. The slightly halophilic bacteria constitute a low proportion of the total microbial population (Rodriguez-Valera, 1988) in such habitats. Most extreme halophiles are archaeobacteria while the moderate and slight halophiles are members of archaeobacteria and eubacteria. Ibekwe et al. (2010) reported that salinity and pH caused severe decrease in the rhizosphere

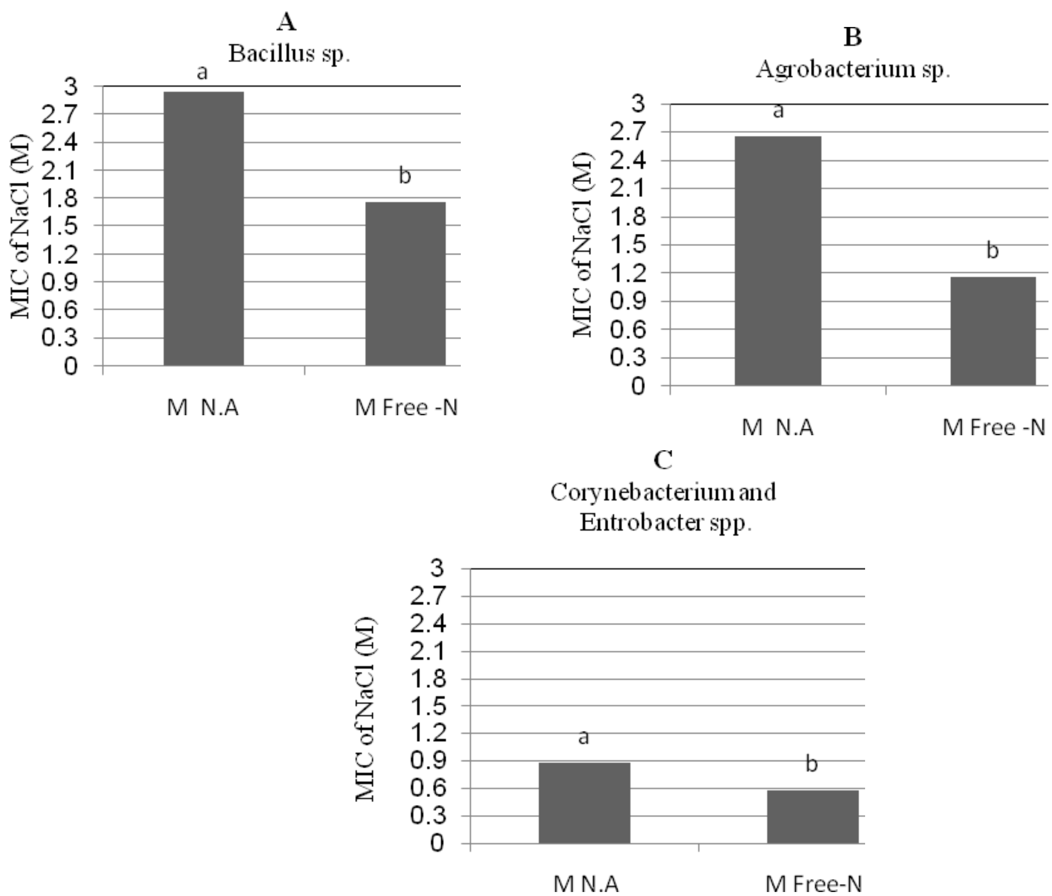

Figure 3. Salt resistance of isolated bacteria in heterotrophic (N.A media) and diazotrophic condition (free N_2 media).

bacterial population (Ibekwe et al., 2010). The effect of NaCl added to the rhizosphere can also change the population of fluorescent pseudomonads. The most abundant population was found at 0.2% which was 3.9 x 10^4 cfu/g soil and with increasing salinity decrease bacterial populations (Khalif et al., 2002). The salinity negatively affects biological activity by high osmotic strength (low water potential) which can be attributed to the toxic effect on microbial growth, except tolerant halophilic bacteria (Yildirim et al., 2008). Generally, high soil salinity can interfere with the growth and activity of soil microbes hence it indirectly affects the nutrient availability to plants. Therefore, the study of interaction between soil microorganisms and plant is needed.

The results showed that the free-living diazotrophic bacterial population in the absence of salt was higher than in saline media but this difference only was significant ($P < 0.05$) in soil 1. The free-living diazotrophic bacterial population of soil 1 was significantly more than soil 2 in experimental condition (Figure 2). Montserrat et al. (2005) reported that saline stress inhibits nitrogen fixation quite significantly, inhibiting both the synthesis and activity of nitrogenase. If nitrogenase did not enhance the activity, then the bacteria can not be alive because there is no synthesis of amino acid and protein. Nitrogenase (acetylene reduction) activity in *Anabaena* sp. strain L-31 is significantly enhanced by the addition of sucrose, but is inhibited upon addition of sodium chloride (Fernandes and Apte, 2000). Butale et al. (2010) reported salinity in soil is developed due to accumulation of excessive salts and predominant nitrogen fixing bacteria in salt deposited soils find it difficult to fix nitrogen due to high pH and salinity.

The failure of nitrogen fixing activity of some nitrogen-fixing organisms in high salinity clearly inhibits the induction of lupines. In such soils, microorganisms tolerating high concentration of salt and yet capable of fixing nitrogen are of importance in increasing its fertility (Jadha et al., 2010). Kouas et al. (2010) reported about N_2 fixing capacity, salt stress that caused 77% decrease in the nitrogen quantity at 200 mM NaCl as compared to the control. Activity in Azospirillum was reported to be more sensitive to salt stress than cellular growth on combined nitrogen. The effect of NaCl stress on acetylene reducing activity is more pronounced on nitrogenase biosynthesis than on nitrogenase activity. This observation is in good agreement with the effect of salinity on the activity of *nif*H and *nif*A promoters expressed through β-galactosidase. Increased NaCl concentrations inhibit the synthesis of dinitrogenase reductase and reduces the relative amounts of the inactive form (Tripathi et al., 2002).

The unfavorable effects of salinity on soil fertility are numerous. Its effects on uptake of nutrients, absorption of moisture and soil structure are well known. So we can use different halotolerant or halophile bacteria for production of biofertilizer for optimum use of saline land potential in agriculture. The excessive use of chemical fertilizers have generated several environmental problems, some of these problems can be stopped by use of biofertilizers, which are natural, beneficial and ecologically friendly. The biofertilizers provide nutrients to the plants and maintain soil structure (Mirzakhani et al., 2009). Use of soil microorganisms which can either fix atmospheric nitrogen, solubilize phosphate, synthesis of growth promoting substances or by enhancing the decomposition of plant residues to release vital nutrients and increase humic content of soils, will be environmentally begin approach for nutrient management and ecosystem function (El-Ghany et al., 2010).

REFERENCES

Atlas RM (1946). Hand book of microbiological media. Gerry jaffe markeing (ed). Publisher. Robert stern.

Bremner JM, Mulvaney CS)1982). Nitrogen-total. In: A L Page, R H Miller, D R Keeney(Eds). Methods of soil analysis, Part 2-Chemical and microbiological properties. 2nd ed., Agronomy, 9: 595-522.

Butale SV, Raut AA, Sawant TB (2010). Application of moderately haloalkaliphilic nonsymbiotic diazotrophs of Lonar lake to saline soils. J. Int. Microbiol. Res., 2(2): 01-04.

Chen HH, Li WJ,Tang SK, Kroppenstedt RM, Stackebrandt E, Xu LH, Jiang Cl (2004). Corynebacterium halotolerans sp. nov., isolated from saline soil in the west of China. J. Syst. Evol. Microbiol., 54: 779-782.

Chookietwattana K (2003). Diversity of halophilic bacteria in saline soil at nong bo reservoir,mahasarakham province,Thailand. PhD dissertation, Suranaree University of Technology.

El-Ghany A, Bouthaina F, Arafa Rhawhia AM, El-Rahmany Tomader A, El-shazly, Mona Morsy (2010). Effect of Some Soil Microorganisms on Soil Properties and Wheat Production under North Sinai Conditions. J. Appl. Sci. Res., 4(5): 559-579.

Evelin H, Kapoor R, Giri B (2009). Arbuscular mycorrhizal fungi in alleviation of salt stress. J. Ann. Bot., 104(7): 1263-1280.

Fernandes TA, Apte SK (2000). Differential regulation of nitrogenase activity by ionic and osmotic stresses and permeable sugars in the Cyanobacterium Anabaena sp. strain L-31. J. Plant. Sci., 150: 181-189.

Gomaa AM, El-Mesiry T, Rady M (2008). The Potential Impact of Biofertilization, Antioxidant and Micronutrients Application in Reducing Salinity Stress on Two Wheat Cultivars. J. Aust. Basic. appl. Sci., 2: 575-582.

Ibekwe Am, Poss JA, Grattan SR, Grieve CM, Suarez D (2010). Bacterial diversity in cucumber (Cucumis sativus) rhizosphere in response to salinity, soil pH, and boron. J. Soil. Biol. Biochem., 42: 567-575.

Jadhav GG, Salunkhe DS, Nerkar DP, Bhadekar RK (2010). Isolation and characterization of salt-tolerant nitrogen-fixing microorganisms from food. J. Eur. Asia. Bio. Sci., 4: 33-40.

Khalif AA, Abdorhim H, Bayoumi Hamuda AFM, Kecskés M (2002). Detection and enumeration of enzymatic activities and fluorescent pseudomonads population in the rhizosphere of white clover due to salinity. 5th International multidisciplinary conference, pp. 251-256.

Kouas S, Slatni T, Salah IB, Abdelly C (2010). Eco-physiological responses and symbiotic nitrogen fixation capacity of salt-exposed Hedysarum carnosum plants. J. Afr. Biotechnol., 9(44): 7462-7469.

Loganathan P, Nair S (2004). Swaminathania salitolerans gen. nov., sp. nov., a salt-tolerant, nitrogen-fixing and phosphate-solubilizing bacterium from wild rice (Porteresia coarctata Tateoka). Int. J. Syst. Evol. Microbiol., 54: 1185-1190.

Mathur N, Singh J, Bohra S, Vyas A (2007). Arbuscular mycorrhizal status of medicinal halophytes in saline areas of Indian Thar Desert. J. Soil. Sci., 2: 119–127.

Mirzakhani M, Ardakani MR, AeeneBand A, Rejali F, Shirani AH (2009). Response of Spring Safflower to Co-Inoculation with Azotobacter chroococum and Glomus intraradices Under Different Levels of Nitrogen and Phosphorus. J. Am. Agric. Biol. Sci., 4: 255-261.

Montserrat A, Rafael FC, Liamas I, Inmaculada L, Fernando M, Juan MC, Quesada E, Emilia Q, Ana DM (2005). The moderately halophilic bacterium Halomonas maura is a free-living diazotroph. FEMS. Microbiol. Lett., 244: 69-74

Olsen SR, Sommers LE (1982). Phosphorus. P. 403-430. In A.L. Page(ed.) Methods of soil analysis, Agron. No. 9, Part 2: Chemical and microbiological properties, 2nd ed., Am. Soc Agron., Madison, WI, USA.

Page WJ, Shivprasad S (1991). Azotobacter salinestris sp. nov. , a Sodium-Dependent, Microaerophilic, and Aeroadaptive Nitrogen-Fixing Bacterium. J. Int. Syst. Ba. Cteriol., 41(3): 369-376.

Palomino MM, Sanchez-Rivas C, Ruzal SM (2009). High salt stress in Bacillus subtilis: involvement of PBP4* as a peptidoglycan hydrolase. J. Res. Microbiol., 160: 117-124.

Quillaguamán J, Hatti-Kaul R, Mattiasson B, Alvarez MT, Delgado O (2004). Halomonas boliviensis sp. nov., an alkalitolerant, moderate halophile isolated from soil around a Bolivian hypersaline lake. J. Int. Syst. Evol. Microbiol., 54: 721-725.

Rodriguez-Valera F (1988). Characteristics and microbial ecology of hypersaline environments. In: Rodriguez-Valera F (ed). Press . Boca Raton. Halophilic Bacteria., 1: 45-51.

Smith LT, Smith GM, Madkour MA (1990). Osmoregulation in Agrobacterium tumefaciens: Accumulation of a Novel Disaccharide Is Controlled by Osmotic Strength and Glycine Betaine. J. Bacteriol., 172(12): 6849-6855.

Tanawy EA (2009). Accquainting with salt tolerant endophytic bacteria isolated from rice plant grown in highly saline soil in egypt. J. Int. Academy. Res., 1(2): 72-79.

Tripathi AK, Nagarajan T, Verma SC, Rudulie DL (2002). Inhibition of Biosynthesis and Activity of Nitrogenase in Azospirillum brasilense Sp7 Under Salinity Stress. J. Curr. Microbio., 44: 363-367.

Ventosa A (2006). Unusual micro-organisms from unusual habitats: hypersaline environments. In Logan NA, Lappin-scott HM, Oyston PCF (eds). SGM symposium. 66: Prokaryotic diversity – mechanisms and significance.

Yildirim E, Turan M, Donmez MF (2008). Mitigation of salt stress in radish (Raphanus Sativus l.) by plant growth promoting rhizobacteria. J. Roum. Biotechnol. Lett., 13(5): 3933-3943.