Endophytic colonization of maize (*Zea mays v.*) root plants by PGPRs under salinity stress

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Abstract: Endophytic bacteria are a group of bacteria that actively colonize plant roots and increase plant growth and yield.The current investigation succeeded to isolate 18 entophytic bacterial isolates were isolated from roots of *Zea mays v.* plants grown in saline soil, and evaluate the efficiency of these isolates on the growth parameters of maize plant grown on sterilized saline soil. In vitro, colonization predominantly on root hair zones was studied after 45 days from sowing. All of the obtained isolates were screened for their tolerance to against different concentrations of sodium chloride (0.1, 1, 10,20, 30 and 50 Mm), bacterial growth and nitrogenase activity were determined. Six active tolerant isolates were identified by 16S rDNA sequencing 3 of them identified as *Bacillus polymyxa* and the others identified as *Azospirillum brasilense*. The selected strains were tested in spermsphere model and electron microscopy to investigate the colonization patterns of endophytic bacteria on the roots of *zea mays* plant. Pot experiment, was conducted during summer 2012 at Agricultural Research Center (ARC) Giza, Egypt, to evaluate the efficacy of these endophytes on the growth and productivity under salt stress conditions. Results indicated that the mixture of endophytic bacteria strains significantly increased growth parameters of maize plants. The plants inoculated with these bacteria showed increase in photosynthetic pigments, enzymatic activities. Proline content decreased significantly by inoculation of maize plant with endophytic bacteria when applied individually and or in mixture. The endophytic bacteria significantly improved growth and yield components. The increase in total carbohydrates (62.41mg/g and crude protein (4.81%) respectively as compared to control (uninoculated). Moreover, mixture of endophytic bacterial strains increased 100-grain weight (37.12g),nitrogen, phosphorus and potassium contents in grainsin comparison with control. Therefore, these six strains under study are considered good candidates as plant growth promoters endophytes in maize plants.
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Key words: Endophytic bacteria, Zea mays v., salt stress, colonization patterns,transmission electron microscopy, growth characters, yield.

1. Introduction

Endophytic bacteria can be defined as those bacteria that colonize the internal tissues of various plant showing no external sign of infection or negative effect on their hosts and spend most of their life cycle inside the plant without causing damage or disease symptoms, of the nearly 300 000 plant species that exist on the earth, each individual plant is host to one or more entophytes **(Strobel *et al*., 2004).** Only a few of these plants have ever been completely studied relative to their endophytic biology. Endophytes were divided into facultative and obligate bacteria as proposed by **(Baldani *et al*., 1997).**

Plants can require the presence of associated bacteria for their growth and establishment in different ecosystems. However, little is known about their role and the regulation processes of the plant growth.

In the root system some nitrogen –fixing endophytes bacteria must find their way to penetrate through cracks formed at the emergence of lateral roots or at the zone of elongation and differentiation of root **(Sharma *et al*., 2005).**

Diastrophic endophytes appear to provide more fixed nitrogen as compared to rhizospheric ones because the interior of plant is more suitable for nitrogen fixation in view of low partial oxygen pressure and direct a accessibility of fixed nitrogen to plant. Besides they have plant growth promoting activities such as production of phytohormones, p-solubilization, siderophores production, inhibition of ethylene biosynthesis and resistance of certain pathogens **(Prabhat and Ashok, 2009).** Nitrogen-fixing bacteria have been reported living in the rhizosphere and as endophytes of *Zea mayzs*cultivars like *Bacillus*, *Pseudomonas,Enterobacter* **(Malmqvist *et al*., 1994).**

Endophytes produced exoenzymes necessary to infect and colonize the host **(Lumyong *et al*., 2002)**, the adaptation of host and endophytes may not only be to a particular host,but to endophytic growth on plant organ,in the roots in contrast to the shoots **(Schulz and Boyle 2005)**. Endophytic organisms also supply essential vitamins to plants **(Pirttila *et al*, 2004)**. Moreover, the endophytes have a number of beneficial

effects include osmotic adjustment, stomata regulation, modification of root morphology, enhanced the uptake of minerals and alteration of nitrogen accumulation and metabolism **(Compant *et al.*, 2005)**. Maize plant (*Zea mays L.)* is commonly cultivated in temperate regions where prolonged periods of suboptimal temperature and short-term cold spells often occur in spring during the crucial stages of early vegetative development. Soil salinity is one of the most limiting environmental factors for crop production and it adversely affects the growth and productivity of many crops. The applications of plant growth regulators are found to play an important role in plant responses to stress **Chakrabarti and Mukherjee (2003).**

The tolerance of diazotrophs to osmotic stress becomes of particular interest as it diminished dependence on nitrogen fertilizers and consequently reduces the environmental pollution consequently; the opportunity to find new and beneficial endophytic microorganisms among the diversity of plants in different ecosystems is considerable. Therefore, the aim of this investigation was to isolate some endophytic nitrogen fixers living within the tissues of maize plants grown under salt stress conditions and evaluate their ability to fix atmospheric nitrogen and promote the growth and yield of maize plants under green house condition and finally to be used as inoculants for many agronomic crops.

2. Material and methods

Endophytes isolation

Bacterial endophytes were isolated from the roots of cultivated mazie (Zea mays) grown in saline soil at Sahl El-ussinia Gover, Egypt as described by **Hallmann *et al.* (1997).** Pure cultures of endophytes were maintained on nutrient agar slants **(Difco, 1985)**.

Screeningof endophytic isolates.

Eighteen isolates were sub-cultured from each colony to obtain pure cultures; these isolates were grown on total diazotrophic watanabe medium **(watanabe and Barraquio, 1979)** at 28 C^o for 72 hours. Isolates were divided into two groups according to morphological characterization to *Bacilli*and the other *Azosprilli*. All of the isolates were tested as N_2 fixers through the activity of nitrogenase according to **Somasegaran and Hoben (1994)** and as PGPRs through the indole acetic acid (IAA) production, according to **Gilickmann and Dessause (1995).**

Growth chamber experiment

The two groups of isolates were grown on nutrient broth medium **(Difco, 1985)** with constant shaking on rotary shaker at 150 r p m for 48 hours at (28±2^{o}C). The cells were harvested by centrifugation at 6000 rpm for 15 min and bacterial cells were resuspended in phosphate buffer (0.01 M, pH=7.0). The cell density was adjusted using spectrophotometer to approximately 10^8(CFU) / ml (OD_{595}=0.3) and then used as inocula for treating maize seeds **(Thompson *et al*,.1996).**Seeds of maize (ZeaMays) Giza 321 obtained from Field, Crops Res. Institute. Giza, Egypt, were surface sterilized in 2% calcium hypochlorite solution for 2 hr under agitation, rinsed thoroughly under aseptic conditions in sterile water and soaked in 1:1 (v/v) H_2O_2 for 20 min. Saline soil was sterilized by autoclaved for 4 hours for three days consecutive and taken into sterile polyethylene bags. Sterilized seeds were coated with isolates cells1×10^8 cfu per seed with either of *Bacilli*, or *Azosprilli* group. Where's the uninoculated control was treated with sterilized broth specific medium for each isolates. Coated seeds (5 seeds per bag) were sown 5 mm below the soil surface (1 kilo /bag). After thinning three seedlings in each bag were left. Initially the pots were irrigated with water at 60% WHC. After 30 days of growth inoculated and uninoculated maize plants with intact roots were recovered from the soil and stirred for 1-2 minutes on a vibrating arm shaker for removing attached soil. Growth parameters of the rhizosphere were carried out as described by **Gouzou *et al.* (1993).** Dry weights (g) of root plants were taken after drying the plant material at 70^0C for 72h. Root colonization percentage (%) and the vigor index were calculated by using the formula as described by **Abdul-Baki and Anderson (1973):**

Vigor index = Percent germination× Seedling length (shoot length + root length).

Nitrogenase activities (N_2-ase) in the rhizosphere and Dehydrogenase (DHA) were assayed according to **Somasegaran and Hoben (1994), Skujins and Burns., (1976)** respectively**.**

Selection of salt tolerant PGPR strain:

PGPRs were tested for their tolerance to various concentration of NaCl. Isolates of *bacilli* group were grown on nitrogen deficient medium of **(Hino and Welson., 1958),** while isolates of *Azosprilli* group were grown on nitrogen deficient malate medium according to **Dobereiner *et al.* (1976).** Both selective media were supplemented with various concentrations (0.1,1,10, 20,30 and 50 Mm) of sodium chloride.The tubes were inoculated with 0.2 ml of prepared culture. The tubes were incubated at 28 ± 2^{o}C for 24 hr, for measuring the turbidity by using spectrophotometer at 600 nm according to **Strausberg (1983).** Nitrogenase activity was determined. Uninoculated selective medium for each bacterial group was maintained as negative control.

The most active six tolerant isolates were identified through 16SrDNA.The genomic DNA of PGPRs was amplified by the method as described by **Weisburg (1991).** Sequencing was done using Big Dye terminator cycle sequencing kit v.3.1 (Applied

Bios stems, USA) and the sequencing products were resolved on sequencer (ABI 3730 x l DNA Analyzer (Applied Bios stems, USA) at the GATC biotech in Germany, by Sigma Scientific Service Company. The results were compared by using BLAST algorithm (www. ncbi.nlm.nih.gov/BLAST. The isolates were identified as three of them belonged to *Azosprillum brasilense* (HQ678675, HQ678676, HQ678677) and other three of them as *Bacillus polymyxa* (JQU15993, JQU15994, JQU15995).

Spermsphere model for colonization

Spermsphere model for colonization patterns as described by **Thomas *et al*. (1982)** was used with the selected two strains *Azospirillum brasilense* HQ678675 (A) and *Bacillus Polymyxa* JQU15993(E)**.**. The roots of maize plants were treated with 2, 3,5 Tri-phenyl tetrazolium chloride (2 ml solution of TTC/sample) for 2 hr to detect the colonization of the bacteria into the roots of maize.

Electron microscopy:

Endophytic colonization of maize roots with *Azospirillum brasilense*HQ678675 (A) and *Bacillus Polymyxa* JQU15993(E) were observed by transmission electron microscopy JEOL (JEM-1400 TEM) according to method described by **Bozzola and Russell(1999)**.

Pots experiment

Soil

Saline soil was collected from Sahl EL-Hussinia, station, El-Sharkia Governorate, Egypt.

The physic chemical characteristics of the used soil were illustrated in Table (1).

Table (1): The main physical and chemical properties the of the experimental soil

Course sand (%)	Fine sand (%)	Silt (%)	Clay (%)		Soil Texture	OM (%)		$CaCO_3$ (%)
2.10	38.67	12.32	46.91		clay	0.62		10.36
pH (1:2.5)	EC* (dSm^{-1})	Cations (meq/l)				Anions (meq/l)		
		Ca^{++}	Mg^{++}	Na^{+}	K^{+}	HCO^{-}_3	Cl^{-}	SO^{--}_4
8.34	20.38	11.00	15.05	175	1.3	10.29	172	20.06

Microbial strains used

A -*Azospirillum brasilense* a salt tolerant an active endophytic strain grown on nitrogen deficient malate medium **(Dobereiner *et al*., 1976)** at 28°C for 48hr, cell densities were adjusted to be (10^7cfu/ml).

B- *Bacillus polymyxa*: a salt tolerant an active strain, grown and maintained in nitrogen deficient medium of **Hino and Wilson (1958)**, then incubated for 48hr at 28°C. The pure culture was enriched on nutrient broth medium **(Difco, 1985)** for 48hr at 28°C to reach the maximum growth (10^7cfu/ml).

Experimental design

A pot experiment was conducted during summer season of 2012 at Agricultural Research Center (ARC), Giza Govern., Egypt. A. Pots 40 cm diameter were filled with saline soil (10Kg/pot) were used.15 seeds were sown in each pot, after thinning six plants in each pot were left. The water content of the soil in each pot was adjusted to 70% of WHC with tap water. Single or mixed cells of bacterial strains were prepared and mixed with sterilized vermiculite (20% moisture) then adhesion using sticker such as Arabic gum (20%) to give homogenized inoculums. This experiment was conducted using a randomized complete blocks design with three replicates.

The treatments were laid out as the following:

1- control (full N,P,K)
2- *Azospirillum brasilense* HQ678675(A)
3- *Azospirillum brasilense*HQ678676 (B)
4- Azospirillum brasilense HQ678677 (C)
5- *Bacillus polymyxa*JQU15993(D)
6- *Bacillus polymyxa* JQU15994 (E).
7- *Bacillus polymyxa* JQU15995(F)
8- Mixture of *Azospirila* (A+B+C)
9-Mixture of *Bacilla* (D+E+F)
10-Mixture of six strains.

Samples were taken for analysis after 45 and 75 days of sowing.

Growth parameters including plant height (cm.), shoot dry weight (g) and root dry weight (g) of maize plants were determined.

Fertilization

The recommended doses of chemical fertilizers (N,P,K) were used according to the recommendation of Egyptian Ministry of Agriculture. Nitrogen fertilizer was added at the rate of 120 Kg N/ Fed as ammonium nitrate (33.5%N) divided into equal doses and added with the second and third irrigation. Phosphorus fertilizer was applied in the form of calcium super phosphate (15.5% P_2O_5) at the rate of 100 Kg P /fed during soil preparation before sowing. Potassium fertilizer treatments were added as Potassium Sulphate (48% K_2O) intotwo equal doses with first and second irrigation.

Determination of chlorophyll and enzymatic activity.

Chlorophyll A, chlorophyll B and carotinoids content were determined according **Adams *et al*., (1997),** dehydrogenase activity in the rhizosphere were determined according to **Skujins and Burns., (1976).** Moreover, nitrogenase activity was determined according to **Somasegaran and Hoben (1994).**

Determination of free Proline content

Free Proline was determined in shoot dry weight after 45 days according to the method of **(Bates *et al*., 1973).**

Estimation of total carbohydrates content

The carbohydrate content in dried leaves samples was determined using the method described by(**Dubois *et al*., 1956).**

Determination of Macroelements

Total nitrogen (% N), total phosphours (% P) and Total potassium (% K) in addition to the crude protein (%) in seeds were determined according to **Black (1982).** 100 seeds weight (g) were also measured.

All the recorded data were subjected to statistical analysis of variance, and the means were compared using the" Least Significant Difference (L.S.D)" test at the 5%, as described by **Snedecor and Cochran (1980).**

3. Results and Discussion

Nitrogenase activity and Indole acetic acid (IAA) production of endophytic bacteria:

Eighteen entophytic bacteria were isolated from roots of maize grown in saline soil in Sahl El-Hussania (Fig 1). All isolates were able to reduce acetylene and produce IAA. Maximum N_2 ase - activity and IAA production were quantified with isolate *Ba* 5 (55.59 µmole C_2H_4/ml/h and 29.61 mg/L respectively), followed by isolate *Azo* 18and*Azo* 13 (50.61 and48.81 µmoleC_2H_4/ml/h respectively) and IAA production were detected (24.21 and 21.01 mg /l-1 respectively). While IAA production by nitrogen-fixing isolates *Ba* 8 and *Azo* 12 were comparatively low (7.45 and 2.22 mg/L respectively).In the present study 18 entophytic bacteria isolates were purified from roots of maize and divided into two groups. 11 isolates were belonged to the genus *Bacillus*. While 7 isolates showed the characteristics of the genus *Azospirillum*. The most common nitrogen fixing bacteria reported as endophytes in sugarcane has been *Herbaspirillum spp*and less often *Azosprillum spp*. **(Baldani *et al*., 2002).** IAA the most common auxin functions as important signal molecule in the regulation of plant development (**Usha Rani *et al.*, 2012).** Nitrogenase genes are the only know example of such highly conserved prokaryotic translated genes and also Nitrogen- Fixations are of the major mechanisms utilized by PGPRs for the plant growth promotion **(Baldani *et al*., 1997).** The results are harmony with **(Muhammad *et al*., 2011)** who isolated nitrogen fixing endophytes from roots of wheat plant and found that all of isolates producing IAA in growth medium. Also **Mirza*et al*., (2001)** isolated endophytic bacteria from roots of sugarcane and found all of isolates produced phytohormone indoleacetic acid (IAA) in pure culture and this IAA production was enhanced in growth medium containing tryptophan. The accordance to our results many researchers reported that Bacillus and Azosprillum are most specifically noted for their N2 – fixing ability and besides, they have the ability to produce different growth hormones (IAA and other axons, such as gibberellins and cytokines **(Jen. Hshuan, 2006).**

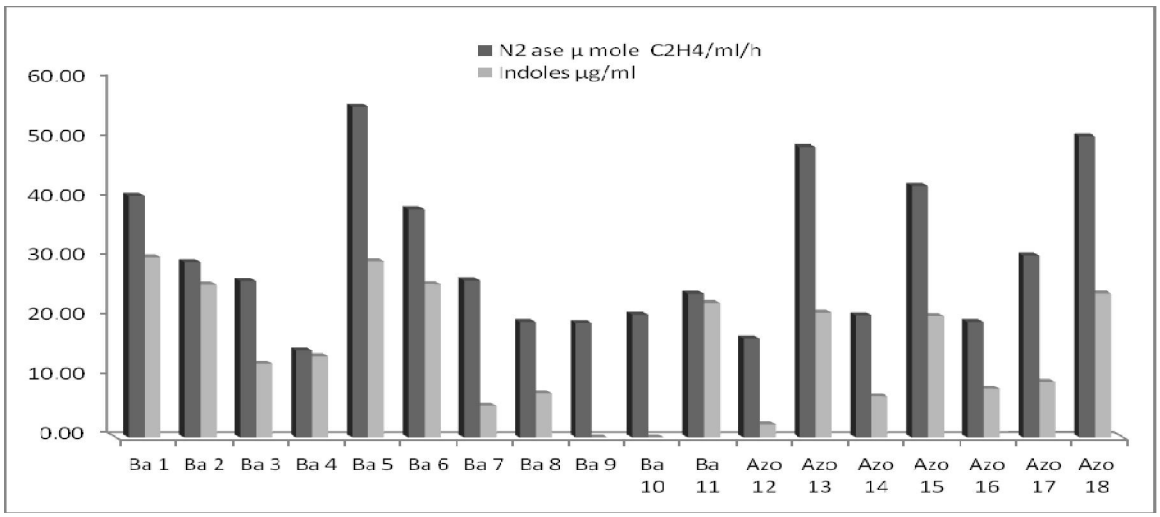


Fig (1): Nitrogenase activity and indole production by endophytic bacteria isolated from saline soil cultivated with maize plants

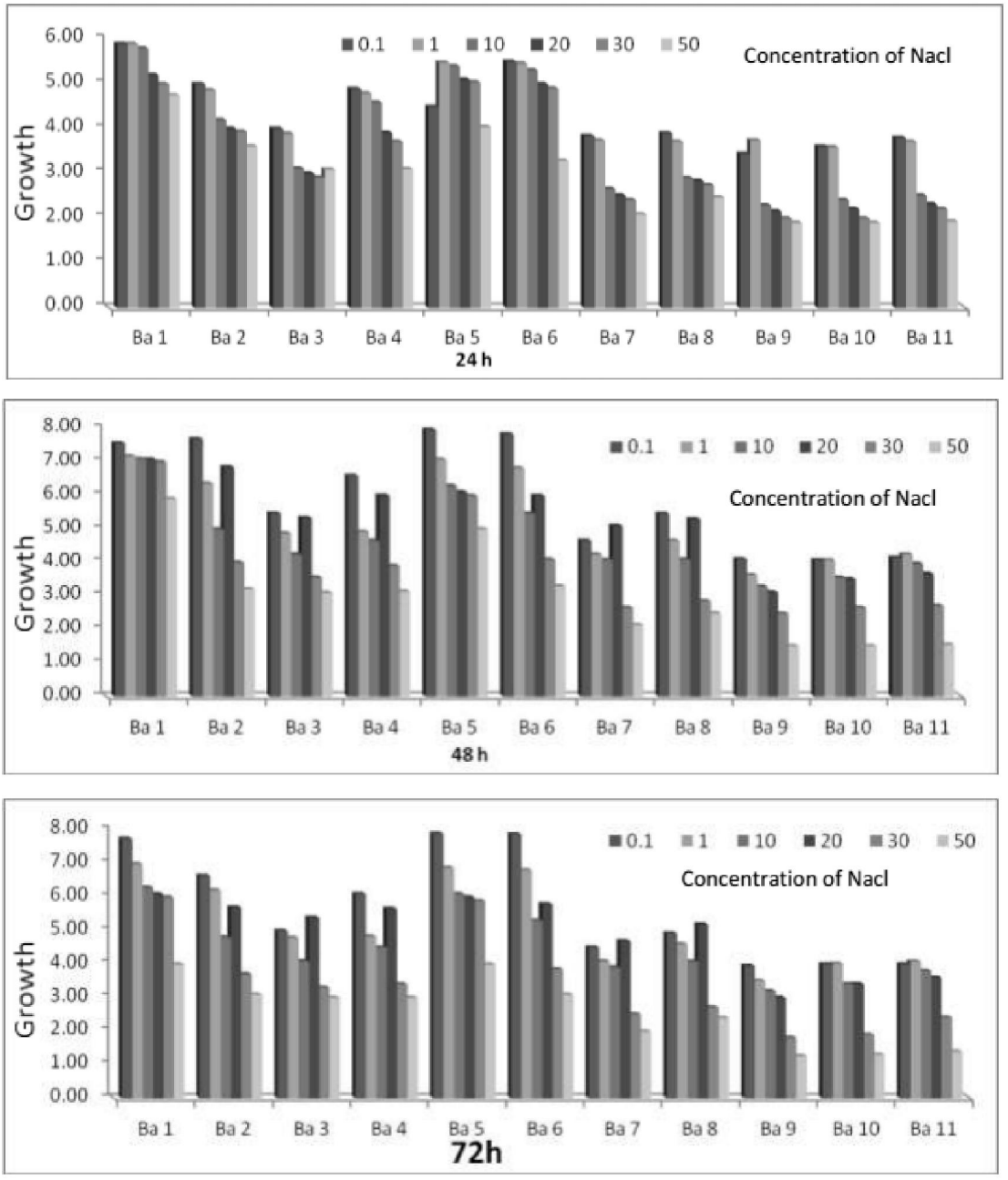


Fig (2) Effect of different conecntrations of Nacl on growth of Bacillus isolates after incubation periods (24,48 and 72 hours)

***In vitro* studies on salt tolerance endophytes.**

Total of 18 different isolates of *Bacillus* and *Azospirillum* were screened to evaluate their tolerance under various concentrations (0.1, 1.0,10, 20, 30,50 m M/l) of sodium chloride, nitrogenase activity and growth were presented in Figs (2, 3,4 and 5). It was observed that the growth and nitrogenase activity generally decreased with increasing salt concentration. *Azo* 18, *Azo* 13, *Ba* 1 and *Ba* 5 were survived at 50 m M NaCl and fixed nitrogen after 48 h from incubation followed by isolate *Azo* 15, *Ba* 6 and *Ba* 2. Isolate *Azo* 12 revealed maximum growth up to 10 m M concentration after 24h from incubation. On the other hand isolate *Ba* 9 revealed maximum growth and fixed nitrogen up to 20 m M concentration after 48 h from incubation. Optimum growth of *Ba*9 at 20 m M concentration was after 48 h from incubation. There was a decline in growth of *Ba*9 and *Azo*12 with increasing sodium salt up to 20 m M. These results are in harmony with **Mohan and Saranya (2015)** and

Upadhay *et al.* (2009) who isolated nitrogen fixing endophytes from the roots of wheat plants and found that these isolates were tolerant to 8% Nacl. In this study, the entophytic isolates were moderately tolerant up to 50 m M salt. **Tripathi *et al.* (2002)** found that there was a high association between soil salinity and the distribution of Azosprillum genotypes.

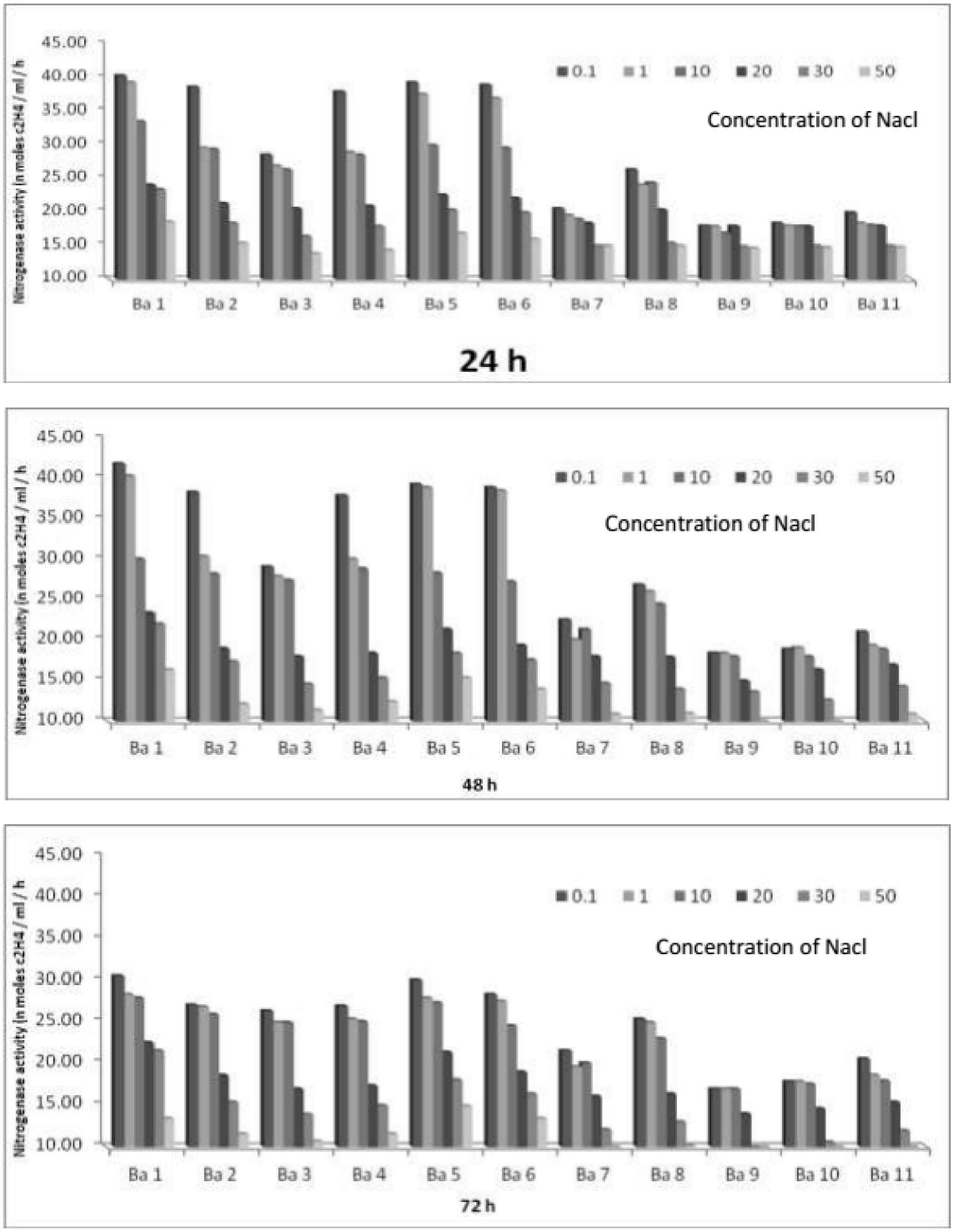


Fig (3) Effect of different conecntrations of Nacl on nitrogenase activiity (n mole c_2H_2/ml/h) of Bacillus isolates after incubation periods (24,48 and 72 hours).

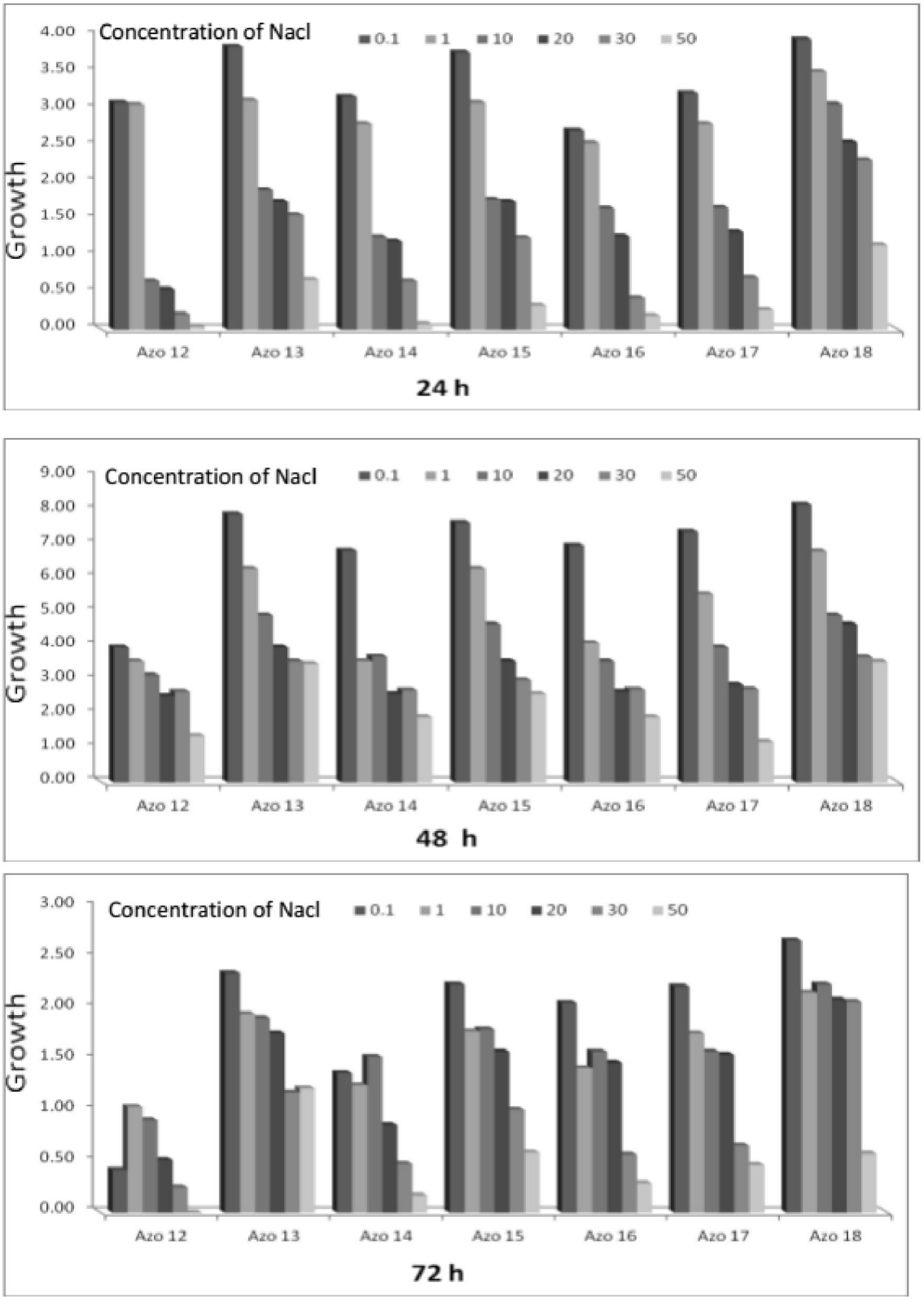
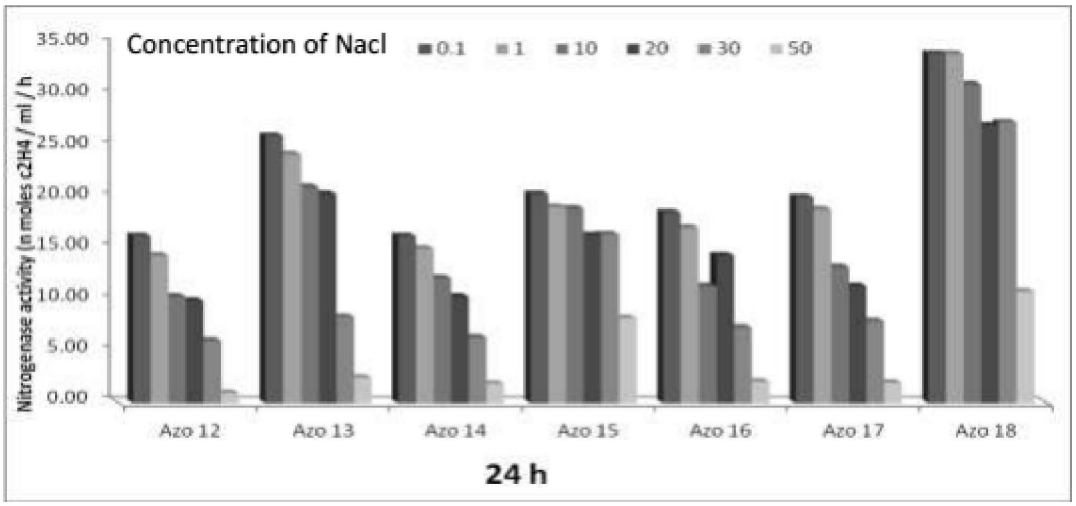
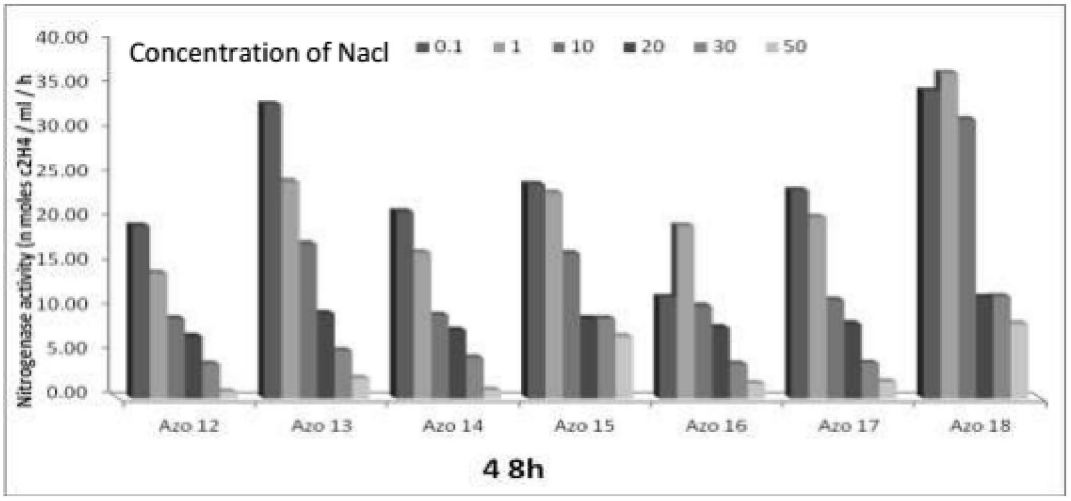


Fig (4): Effect of different conecntrations of Nacl on growth of Azospirllum isolates after incubation periods (24,48 and 72 hours)





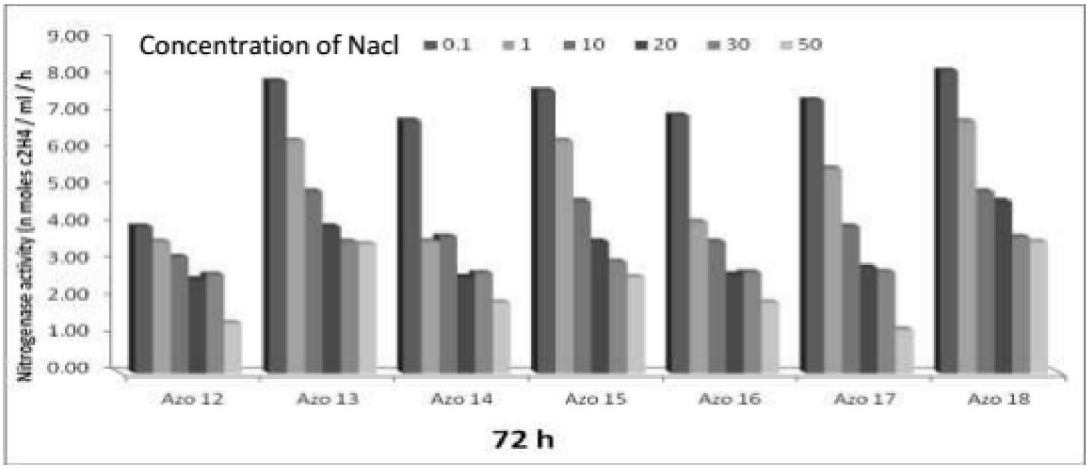


Fig (5): Effect of different conecntrations of Nacl on nitrogenase activiity (n mole c_2H_2//ml/h) of Azospirllum isolates after incubation periods (24,48 and 72 hours)

In growth room experiment, 18 endophytic bacterial isolates were tested as inoculants for maize plant grown in plastic pots filled with sterilized saline (Table 2).

Table (2): *In-vitro* testing enzymatic activity and growth parameters of the endophytic bacteria isolates in the rhizosphere of Zea mays seedlings after 30 days from sowing during seasons 2012.

No. of isolates	Enzymatic activity		Root Colonization (%)	growth parameters					Vigor index
	N_2-ase activity (m mole C_2H_4/g soil/h)	Dehydrogenase activity (µg TPF/g soil/day)		Germination (%)	Root D.W (g/plant)	Root V. (cm^3)	Root length (cm)	Shoot length (cm)	
Ba 1	45.92	131.77	70	80	0.52	4.00	24.33	35.00	47.46
Ba 2	14.44	103.45	40	70	0.40	3.50	21.33	25.67	32.90
Ba 3	13.31	93.14	50	77	0.23	2.90	19.33	23.00	36.14
Ba 4	12.31	94.11	60	78	0.39	3.00	21.00	25.33	36.14
Ba 5	53.85	159.19	70	90	0.44	4.00	23.33	27.67	45.90
Ba 6	19.97	115.66	70	88	0.40	4.00	23.00	27.60	44.53
Ba 7	10.49	65.83	70	80	0.19	2.70	18.67	21.17	31.87
Ba 8	09.41	48.31	20	86	0.22	2.71	19.33	22.01	45.55
Ba 9	05.45	40.70	60	88	0.17	2.00	11.80	16.33	24.65
Ba 10	02.99	40.31	20	77	0.17	2.00	16.80	16.50	25.64
Ba 11	04.31	46.31	10	79	0.18	2.00	19.67	17.00	28.97
Azo 12	05.27	44.25	30	78	0.31	1.50	11.16	14.67	20.15
Azo 13	41.31	71.35	100	90	0.55	4.50	24.63	33.67	52.47
Azo 14	10.10	44.22	40	88	0.35	2.90	11.17	25.00	31.83
Azo 15	38.19	64.98	70	90	0.40	3.50	24.03	29.40	48.09
Azo 16	11.11	56.61	60	78	0.40	3.00	18.37	26.00	34.61
Azo 17	27.68	64.27	60	70	0.40	3.00	25.60	27.00	36.82
Azo 18	47.81	100.26	100	77	0.55	4.50	25.60	41.45	51.61
Control *Bacillus*	01.40	30.27	0	70	0.07	0.17	9.57	12.23	15.26
Control Azospirillum	01.20	37.82	0	88	0.03	0.11	4.33	8.00	10.85
LSD at 0.05	**0.329**	**0.455**			0.008	0.022	0.029	0.095	

Nitrogenase activity, dehydrogenase activity and growth parameters besides vigor index were recorded. The highest N_2-ase and dehydrogenase activities were recorded with isolates *Ba*5 being 53.85 m mole C_2H_4/g soil/h and 159.19 µg TPF/g soil/day, respectively followed by isolate *Ba*1, *Azo*18 recorded 45.92m mole C_2h_4/g soil and 131.77 µg TPF/g soil/day. Where's the treatment with isolate *Ba* 10 recorded the lowest N_2-ase and dehydrogenase activity 02.99 m mole C_2h_4 /g soil and 40.31 µg TPF/g soil/day respectively compared to control and the other treatment. **Barua *et al.* (2012)** reported that pure cultures of diazotrophs obtained were efficiently exhibited nitrogenase activity and dehydrogenase activities under 1 % salinity stress. Similar results were reported by **Soumitra *et al.* (2007)** who found out of 20 endophytic diastrophic strains were isolated from roots of *L. Sindicus*, strains showed considerable in acetylene reduction and dehydrogenase activity. Among the 18 isolates tested for their efficacy to improve the vigor index of maize seedlings under saline soil, isolate *Azo*13 showed the highest plant-growth-enhancing activity and colonization percentage and recorded 90 % germination with a vigor index of 52.47 while vigor index of *Azo*18 and *Azo*15 also obtained higher germination percentage of > 80 % and vigor index 51.61 and 48.09 respectively. Some of isolates did not have any effect on the growth of maize seedlings like isolates *Azo*12 and *Ba*9 (Table 2) these isolates were discarded. Seed germination and early seedling growth are the most salt-sensitive plant growth stages under environmental stresses, because the seedling root is in direct contact with soil and is affected by many soil changes, including salt stress **Jamil *et al.* (2006)**. The use of endophytic bacteria for increasing the growth of plants in saline soil under pot experiment **(Egamberdieva, 2012)** in tomato and cucumber has been earlier established in various studies. **Chakraborty *et al.* (2011)** also isolated salt-tolerant bacteria and tested for germination under laboratory conditions. As observed, the presence of salinity reduced the plant growth under pot culture studies in rice for vigor index, the treatment with isolates *Ba*1, *Ba*5, *Azo*13 and *Azo*18 relieved the maize plants from stress and exhibit higher vigor index compared to plants treated with other isolates and untreated control. Similar results were also reported by **Munns (2002)** mentioning suppression of plant growth under saline stress, which may be due to water congestion in the surface soil and also the effect of sodium (Na+) and carbonates in the rhizosphere.

Potential of identification isolates:

The most tolerant endophytic isolates to sodium chloride and had higher vigor index were identified by 16S rDNA and tested in sperm sphere model to investigate the colonization patterns on the roots of maize. Seeds of maize were sterilized and sown in mineral medium, after 7 days from sowing. Plants were inoculated with endophytes under aseptic conditions. After 24hr from inoculation of 1ml 2, 3, 5 triphenyltetrazolium chloride (TTC) was added to observe the presence of colonization on roots as in (Fig 6).

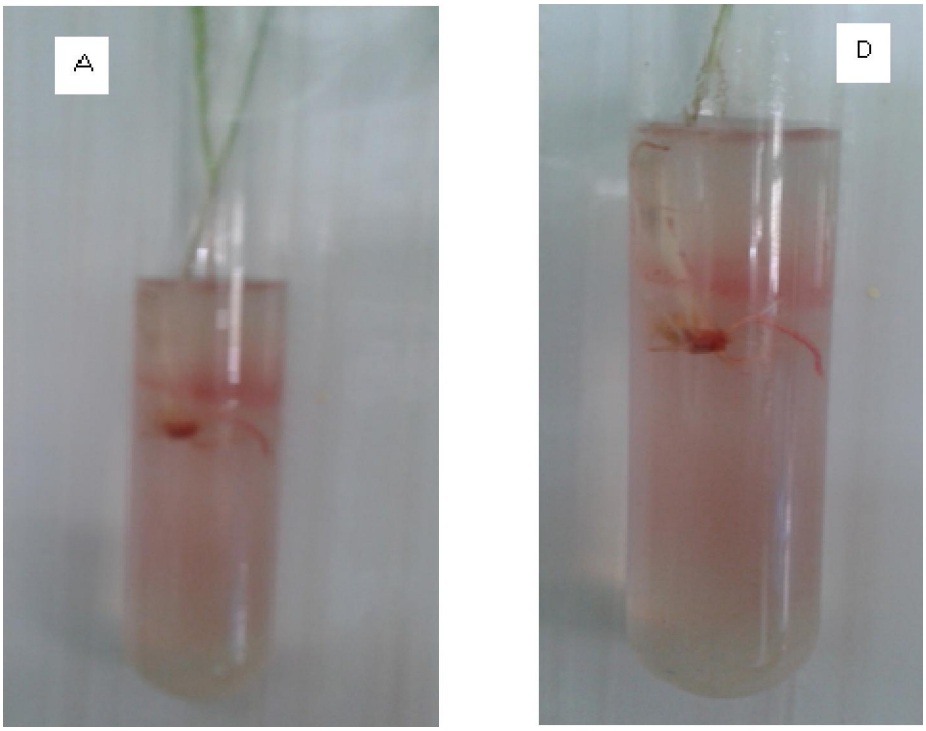


Fig (6): Colonization of maize roots by *Azosprillumbrasilense* HQ678675(A) and *Bacillus*polymxa JQU15993 (D) grown in spermosphere tubes.

The appearance red color due to reduction of TTC to TPF by bacteria, while uninoculated maize was colorless. It is known that the existence of bacterial species can affect positively on the root development **(Lopez-Bucio *et al.*, 2007)** root colonization by rhizosphere bacteria is associated with biofilm formation (**Bais *et al*., 2004; Ramey *et al*., 2004; Reva *et al*., 2004).** The strain *Azospirillum braislense* is able to promote the growth of maize, similar results were reported by **Idirs *et al*. (2007)**. The attachment of another diastrophic endophyte, *Herbaspirillum seropedicae*, to root surfaces of maize depends on LPS (liposaccharide) **(Balsanelli *et al.*, 2010)**.Additionally, rhizobacteria maybe specifically attracted to roots through chemo taxis, pattern of colonization was probably due to the distribution of receptor structures on the plant root surface, relating to sites of greater root exudation **(Brimecombe *et al*., 2001).**

Transmission electron microscopy:

Transmission electron microscopy (TEM) showed that the epidemics and cortex were colonized by different types of *Azosprillum brasilense* (A) and *Bacillus polymxa* (D) Figs (7 and 8). Bacteria were found attached to the cortex of maize root. Presence of rod-shaped bacteria covering the root surface, bacteria were apparently adhered to the root epidermal cells and particularly accumulated in the basal portion of the root hairs. The pattern showed that bacteria entering the roots and *Bacillus polymyxa* mainly localized within intercellular spaces of the parenchyma cells these results are in agreement with **Katherrine *et al.* (2008)** who reported that observations of roots, stems and leaves of inoculated rice plantlets by electron microscopy revealed *B. ururiensis* colonization predominantly on root hair zones, demonstrating endophytic colonization primarily through the endodermis, followed by spreading into xylem vessels, a possible pathway leading to aerial parts. Another explanation by **Su-Jung and Robert (2005)** who reported that the observation with transmission electron microscopy showed considerable alterations of root cells including vesiculation, partial cell wall degradation, and cytoplasm disorganization. The interaction between the extracellular substances of bacteria and cell wall components of the host cell appeared without damage the cell wall, these results are in agreement with **Su-Jung and Robert (2005).**

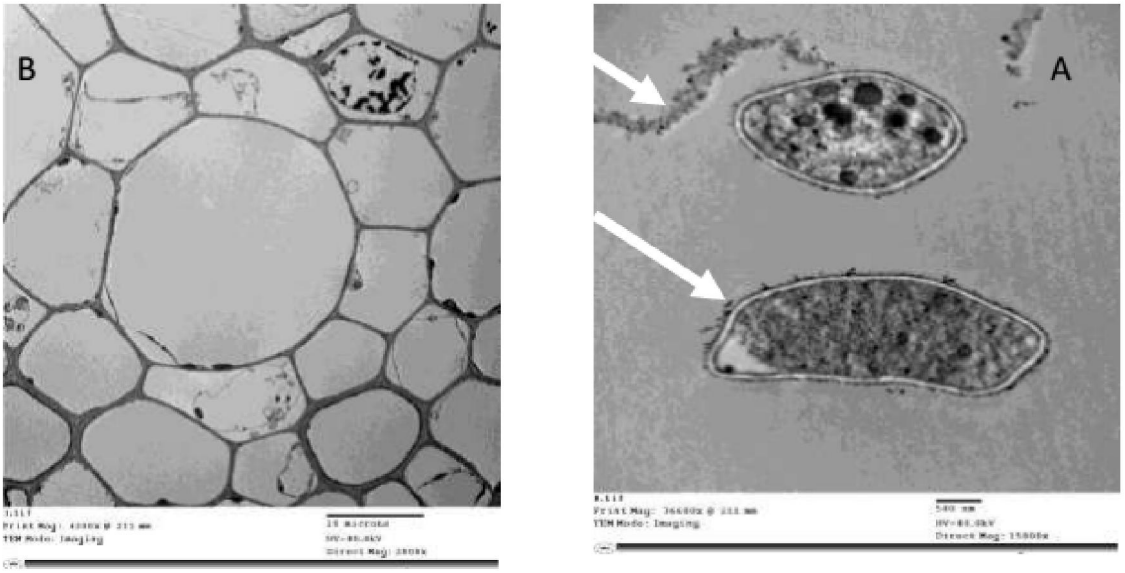


Fig (7): TEM micrographs displaying the polar flagellum of *A. brasilense* HQ678675strains (A) and uninoculated roots (B) under gnotobiotic conditions

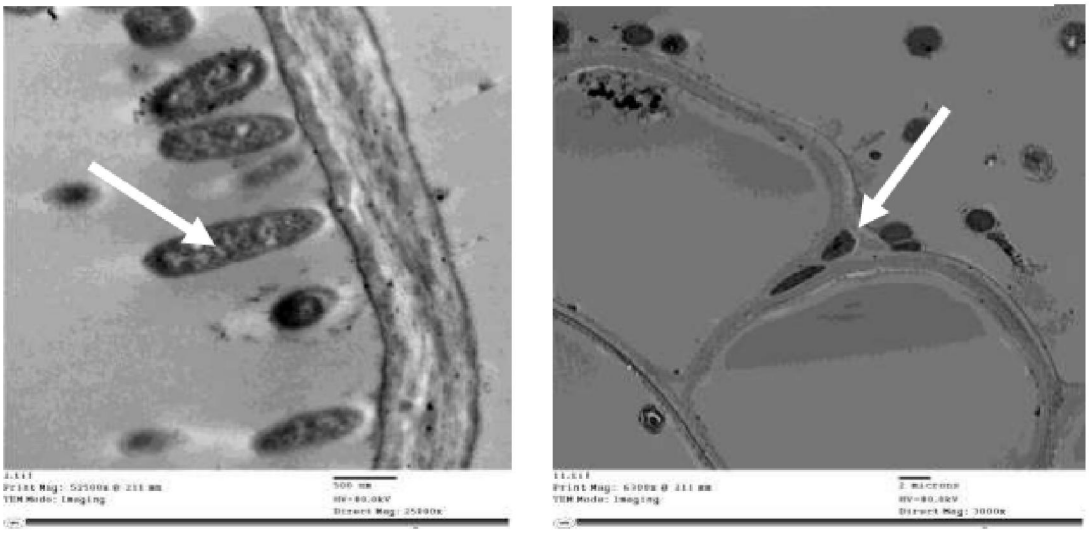


Fig (8): TEM of maize root colonization (root hair zones) by *Bacillus polymxa* JQU15993 living with the parenchymal cell wall

Growth parameters:

Effect of inoculation with six strains of entophytic bacteria on plant height (cm), shoot dry weight (g) and root dry weight(g) of maize plants grown in saline soil at 45 and 75 days after sowing are shown in Table (3).

Inoculation maize plants with bacterial of strains resulted in significant increase in plant height, shoot dry weight and root dry weight after 45 and 75 days respectively. Mixture of all treatment exhibited the optimum height 130.71 and 180.00 cm, shoot dry weight 19.41 and 31.46 g/ plant and root dry weight 20.41 and 36.51 g/plant at 45 and 75 days respectively whereas control and other treatments recorded less results. These treatments exhibited highest significant of growth parameters compared to other treatments followed by treatment with mixed of *bacillus* and treatment with mixed of *Azospirillum* treatment, being (115.21,161.66cm) (18.42, 29.46g/plant) and (19.00,33.61g/plant) with treatment mixed *bacillu* and recorded (105.11,152.88cm), (16.71,28.51g/plant) and (17.61,32.41g/plant) after 45 and 75 days from sowing. Treatment with *Azospirillum* (C) recorded the lowest growth parameters compared to other treatment and uninoculated. **Khatoon *et al.* (2010)** concluded that

salinity had adverse effect on growth of maize. NaCl concentrations at germinating stage could have much adverse effects on maize than later stages of growth. The inoculation response was comparatively higher at the early growth stages (vegetative and early flowering stages), compared with the other investigated phenophases. This could be related with the root nutrient exchanges that become more intense during vegetative growth **Zamfirache (2005)** Because rhizobacteria mediates soil minerals and nutrients uptake **Sylvia(1999)** PGPR's influence is more visible during this stage. Similar results were obtained by **Usman *et al.* (2012).** Salinity reduced the growth parameters, the reduction included shoot, root, dry weight etc and yet. **Saharan and Nehra (2011)** found that Plant Growth Promoting Rhizobacteria (PGPR) are naturally occurring as soil bacteria that aggressively colonize plant roots and benefit plant by providing growth promotion and biomass production through direct effects on root and shoot growth therefore, they could alleviate salinity stress. **Bano *et al.*,(2013)** found that *Azospirillum* showed promising effect and can be a potent inoculants for maize that can help the crop to endure limited water availability.

Table (3): Effect of inoculation with entophytic bacteria on growth parameters of maize plants grown in saline soils at 45 and 75 days after sowing during seasons 2012.

Treatments / Days after sowing	plant height (c m)		Shoot. Dry. w t. (g/plant)		Root.dry.w t (g/plant)	
	45 d	75 d	45 d	75 d	45 d	75 d
Uninoculated	45.00	60.11	11.08	13.00	10.80	12.00
Azospirillum brasilense A	75.77	149.13	16.82	26.41	14.31	24.00
Azospirillum brasilense B	72.11	133.32	16.02	23.31	13.01	23.91
Azospirillum brasilense C	65.22	127.44	15.77	23.00	13.00	23.90
Bacillus polymyxa E	95.22	147.33	18.06	27.09	16.31	26.30
Bacillus polymyxa F	75.41	139.32	16.28	26.00	14.30	21.42
Bacillus polymyxa G	75.11	134.66	17.02	15.11	13.31	20.91
Mixture of *Azospirillum*	105.11	152.88	16.71	28.51	17.61	32.41
Mixture of *Bacillus*	115.21	161.66	18.42	29.46	19.00	33.61
Mixture of all	130.71	180.00	19.41	31.46	20.41	36.61
LSD at 0.05	1.429	0.611	0.039	0.251	0.324	0.073

Photosynthetic pigments, enzymatic activity and Proline content:

Chlorophyll a, b, carotinoids, enzymatic activity and proline content are shown in table (4). Generally results showed decreased of photosynthetic pigments and enzymatic activity in bacterial un inoculated treatment (control). However inoculation with endophytic bacterial strains significantly increased the pigments and enzymatic activity compared to uninoculated treatment (control). The chlorophyll a content was decreased compared to the content of chlorophyll b, where chlorophyll a content reached 4.86, 8.06 mg/g dry weight after 45 and 75 days of sowing respectively with treatment mixture of all cultures. While chlorophyll b recorded 3.81, 6.70 mg/g dry weight respectively with treatment using mixture of all cultures. Mixture of bacterial cultures caused significant increase in chl a contents being 95.16% higher than control. Next to, it was significant increased chl a up to 81.79% with treatment of mixture of bacillus more than control. Carotinoids content were 1.87 and 2.43 mg/g dry weight with treatment mixed of culture as compared with control. Salinity reduced carotinoids in all treatment. However, inoculation with endophytic bacteria increased carotinoids content up to 63.39% and 80.84% after 45 and 57 days of sowing respectively with treatment mixture of *bacillus*. The increase of chl a, b and carotinoids content of maize as increased photosynthetic leaf area of plant even at salinity by exopolysaccharides producing endophytic bacteria **(Marcelis and Van Hooijdonk., 1999).** Similar results were reported by **Nemat *et al.*, (2012)** who reported that endophytic bacteria increased significantly chl a,b and carotinoids content compared with uninoculated control on maize plants grown in saline soil.

Nitrogenase activity in soil depends on ecological conditions in association with the specific nitrogen fixation capabilities of certain microorganisms **(Entry *et al.*, 1996).** Data in Table 4 showed significant increase in nitrogenase activity up to 94.55% over control after 75 days from sowing with treatment inoculated with *Azospirillum* A while the treatment with inoculated *Azospirillum* C recorded the lowest nitrogenase activity(20.21 µmole C_2H_4 /g soil /h) compared to other treatments and control after 75 days from sowing. **Dilfuza and Zulfiya (2008)**

suggested that soil containing greater organic matter will have higher nitrogenase activity. Dehydrogenase activity is an oxidsoreductase, which is only present in viable cells. This enzyme has been considered as a sensitive indicator of soil quality and it has been proposed as a valid biomarker to indicate changes in total microbial activity due to changes in soil management **(Rolda *et al.*, 2005)**. Data in Table (4) show that salinity reduced dehydrogenase activity, but the inoculation with endophytic bacteria enhanced the dehydrogenase activity compared to control. The increase of dehydrogenase activity as shown in table (4) with mixture of all strains more than using the strain individually relied on the viability of these microorganisms and the existence in high population that could colonized the rhizosphere, which led to increase in CO2 evolution and carbonic acids formation that decreased soil pH and consequently increases mineral absorption and enhances plant growth **Omer and Ismail (2002).** Treatment with mixture of all cultures recorded the highest significant dehydrogenase activity compared to other treatment and control being 74.11 and 80.11 µTPF/g soil/day after 45 and 75 days after sowing respectively, followed by treatment of mixture *bacillus* and treatment of mixture *Azosprillum* which recorded 45.88 and 36.88 µ TPF /g soil/ day respectively after 45 days from sowing. While the treatment with *Bacillus* A recorded the lowest dehydrogenase activity compared to other treatment and control being 34.44 µ TPF/g soil/ day after 75 days from sowing. Similar results were also reported by **Hanaa and Omar (2010)** who reported that dehydrogenase activity was significantly inhibited by increasing salinity in the rhizosphere of wheat plants uninoculated with nitrogen fixing bacteria.As shown in Table (4) proline content increased under salinity stress which was a response to stress. Moreover, Proline increased with uninoculated treatment. Proline assay showed that the inoculation with endophytic bacteria significantly decreased Proline content compared to control. The lowest content determined with treatment using mixture of all cultures of endophytic bacteria being 8.11 (mg/g d.w) while the highest content 13.16 (mg/g d.w) was determined with un inoculated treatment. Treatment with mixture of *Bacillus polymyxa* recorded significant decrease of Proline content being 9.06 mg/g d.w compared to control. Similar finding were reported by **Upadhyay *et al.*, (2011 and 2012)**. The proline content in leaves decreased when inoculated plant with endophytic bacteria. In other word, salinity and PGPRs and their interaction had significant effect on proline content in maize plant. Generally, results of this research demonstrated that inoculation of PGPRs benefits plants under saline conditions. It seems that the beneficial effects of PGPRs are more remarkable for stressed plants. The Proline content could be maintain the growth of bacterial up to higher salinity level because it may act as a mediator of osmotic adjustment protects macromolecules during dehydration and serve as a hydroxyl radical scavenger **(Miller and Wood, 1996).**

Table (4): Effect of bacterial inoculation with endophytic bacteria on chlorophyll a, chlorophyll b, carotinoids, nitrogenase and dehydrogenase activity of maize plants grown in saline soil at 45 and 75 days after sowing during seasons 2012.

Treatments / Days after sowing	Chl.a (mg/g dry weight)		Chl.b (mg/g dry weight)		Cartenoids (mg/g dry weight)		Nitrogenase activity (µ mole C_2H_4/g soil/h)		Dehydrogenase activity (µg TPF/g soil/day)		Proline (mg /g d. w)
	45 d	75 d	45 d	75 d	45d	75d	45 d	75 d	45 d	75 d	45 d
Uninoculated	0.51	0.39	0.19	0.60	0.41	0.85	6.21	3.73	16.41	2o.11	13.16
Azospirillum brasilense A	1.56	4.33	2.21	4.41	0.86	1.45	36.11	68.56	36.08	46.11	11.16
Azospirillum brasilense B	1.39	3.26	2.09	3.84	0.62	1.11	18.61	20.41	25.31	44.41	11.33
Azospirillum brasilense C	1.31	3.05	1.42	3.11	0.61	0.76	18.41	20.21	20.63	37.31	11.60
Bacillus polymxa D	1.27	2.41	1.29	2.81	0.51	0.77	16.41	29.41	17.66	34.44	12.10
Bacillus polymxa E	1,58	4.04	2.44	4.28	0.82	1.41	21.41	32.71	30.16	46.01	10.41
Bacillus polymxa F	1.51	3.26	2.37	4.04	0.70	1.41	19.61	31.56	25.31	44.41	10.61
Mixeture of *Azospirillum*	2.11	4.90	3.00	5.00	1.01	1.88	26.11	30.17	36.88	51.41	9.33
Mixture of *Bacillus*	2.80	5.68	3.10	5.10	1.12	2.14	25.66	48.16	45.88	60.41	9.06
Mixture of all	4.86	8.06	3.81	6.70	1.87	2.43	22.06	38.57	74.11	80.11	8.11
LSD at 0.05	0.011	0.028	0.009	0.031	0.009	0.010	1.007	0.129	0.206	0.180	1.864

Yield components and Macroelements

Table (5)show the effect of inoculation with endophytic bacterial strain on total carbohydrate, crude protein, N%,P%, k% of grains and 100-grain weight (g) of maize under salinity stress. Inoculation with endophytic bacteria showed higher grains protein-content compared to control. The differences in carbohydrates among treatments were limited as saline stress reduced total carbohydrates and protein in uninoculated plant (control) whereas inoculated treatment with entophytic bacteria improved plant carbohydrate content, crude protein compared to control (Table 5). Treatment using mixture of strains significantly improved total carbohydrates, crude

protein being 62.41(mg/g) and 4.81% respectively. Also mixture of *Bacillus* and mixture of *Azospirillum* strains improved plant total carbohydrates, and crude protein compared to treatment using *Bacillus* or *Azospirillum*. Reduction in total carbohydrates under salt stress condition were recorded by **Abd El-Ghany *et al.* (2015).** The crude protein and carbohydrate enhancement is related to a higher relative increases in nitrogen fixation due to PGPR inoculation, compared to control plants. Therefore the increase of grains protein content could be related to enhancement of physiological activities of maize plants and subsequent to maize growth. Similar effects were also reported and confirmed by many authors **(Zafar *et al.*, 2014)** and **Marius *et al*. (2013).** N%, P% and K% and 100-grain (g) of maize plants were significantly increased due to bacterial inoculation. Thus, N concentration in shoots was 0.77, 0.75 and 0.75 % respectively with mixture of all strains, mixture of *Bacillus* and *Azosprillum*. The same trend was observed in shoot P concentration 0.30, 0.28 and 0.27 % and shoot K (1.94, 1.76 and 1.74%) respectively while the treatment using *Azosprillum* C recorded the lowest concentration of N,P and K% compared to other treatments and control. This means that PGPRs strains could alleviate the effect of salinity stress in maize. **Han and Lee (2005)** also observed increase in N, P and K concentration under salinity stress due to inoculation with PGPR. Similarly, **Vivas *et al*., (2003)** reported that N,P and K concentration in lettuce inoculated by *Bacillus sp* under stress conditions were increased by about 5, 70 and 50 % respectively over control. **Adesemoye *et al*., (2009)** reported that inoculation with PGPR of maize plant under salinity stress increased shoot nitrogen and phosphorus. Similar results were obtained by **Babalola (2010).** Also endophytic bacteria significantly improved 100-grain weight, compared to control. The treatment with mixture of all strains recorded 37.12 g compared to control (16.61 g), followed by treatment using mixture of *Bacillus* (31.11g) and mixture of *Azosprillum* (28.41 g) respectively. **Muhammad Zafar *et al.* (2014)** reported that PGPR strains alone or in mixture improved yield parameters and attained promising results as compared to control. The results are strongly supported by the work of several researchers **(Belimov *et al*., 2002; Zahir *et al*., 2009).** As we observed, inoculation with endophytic bacteria under salinity stress was highly effective in improving growth and yield of maize plant.

Table (5): Effect of bacterial inoculation with endophytic bacteria on yield component and N, P, K percentage of maize plants grown in saline soil during seasons 2012.

Treatments	Total carbohydrate (mg/g)	Crude protein (%)	100-grain weight (g)	N %	P %	K %
Control	30.41	3.75	16.61	0.6o	0.20	1.51
Azospirillum brasilense A	50.31	4.62	26.01	0.74	0.24	1.69
Azospirillum brasilense B	50.00	4.44	20.01	0.71	0.23	1.67
Azospirillum brasilense C	48.61	4.63	20.31	0.74	0.22	1.60
Bacillus polymxa D	56.31	4.50	19.00	0.72	0.26	1.73
Bacillus polymxa E	55.21	4.38	25.31	0.70	0.25	1.69
Bacillus polymxa F	58.31	4.38	22.21	0.70	0.24	1.65
Mixture of *Azospirillum*	58.31	4.69	28.41	0.75	0.27	1.74
Mixture of *Bacillus*	59.41	4.69	31.11	0.75	0.28	1.76
Mixture of all	62.41	4.81	37.12	0.77	0.30	1.94
LSD at 0.05	0.393	0.022	0.026	0.040	0.011	0.009

Conclusion

The current study succeeded to isolate 18 endophytic bacterial isolates from roots of maize plants grown in saline soil. Screening of the obtained isolates on the basis of salt tolerance and N_2-fixation was done. The selected isolates were identified by 16S rDNA sequence. Colonization pattern of the selected strains on roots of maize plants show the ability of *Azospirillum brasilense* HQ678675 and *Bacillus polymyxa* JQU15993 to endophytically colonize maize plants by using transmission electron microscopy. Growth of Maize plants was significantly improved due to inoculation with the selected strains endophytic bacteria such as shoot, root dry weight and improved vigor index as well as increasing photosynthetic pigments (chlorophyll a, b and carotinoids) besides to enzymatic activity. Endophytic inoculation enhanced the protein content and yield component of maize.

Reference

1. Abd El-Ghany, TM.: Masrahi,YS.; Mohamed, A. Al Abboud, Alawlaqi, MM and Nadeem, I.

Elhussieny. (2015).Maize (Zea Mays L.) Growth and Metabolic Dynamics with Plant Growth Promoting Rhizobacteris under Salt Stress, Plant Pathol. Microb. 6 (9):1443-1450.
2. Abdul-Baki, AA. and Anderson, JD.(1973). Vigour determination in soybean seed by multiple criteria. Crop Sci. 13:630–633.
3. Adams, B,; Adams, W andGarce, S. (1997). Physiology of light tolerance in plants. Hort. Rev. 18: 215-246.
4. Adesemoye, AO.; Torbert, HA and Kloepper, JW. (2009). Plant growth promoting rhizobacteria allow reduced application rates of chemical fertilizers. Microb Ecol. 58:921–929.
5. Babalola, OO.(2010). Beneficial bacteria of agricultural importance. Biotechnol Lett 32:1559–1570.
6. Bais, HP.; Fall, R and Vivanco, JM. (2004). Biocontrol of *Bacillus subtilis* against infection of Arabidopsis roots by *Pseudomonas syringae* is facilitated by biofilm formation and surfactin production. Plant Physiol. 134: 307 – 319.
7. Baldani, JI.; Reis, VM.;Baldani, VLD and Döbereiner, J.(2002). A brief story of nitrogen fixation in sugarcane – reasons for sucess in Brazil. Func Plant Biol. 29:417-423.
8. Baldani,Vld.; Oliveira, E.; Balota, E.; Baldani, JI.; Kirchhof, G and Dobereiner, J.(1997). *Burkholderia brasilensis sp*. nov., uma nova espécie de bactéria diazotrófica endofitica. An Acad Bras Cienc 69: 116-126.
9. Baldani, J I.; Caruso, L.; Baldani, V L D.;Goi, S and Dobereiner, J.(1997).Recent advances in BNF with non-legume plants. Soil Biol. Biochem. 29:911–922.
10. Balsanelli, E.; Serrato, RV.; de Baura, V.; Sassaki, G.; Yates, MG.; Rigo, LU.; Pedrosa, FO.;de Souza, EM and Monteiro, RA.(2010). *Herbaspirillum seropedicae* rfbB and rfbC genes are required for maize colonization. Environ. Microbiol. 12: 2233-2244.
11. Bano, Q.; Ilyas, N.; Bano, A.; Zafar, N.; Akram, A and Hassan, F. (2013). Effect of *Azospirillum* inoculation on maize (Zea mays L.) under drought stress. Pak. J. Bot., 45: 13-20.
12. Barua, S.; Sudipta, T.; Ashis, C.; Sagarmoy, G andKaly, C. (2012).Characterization and crop production efficiency of diazotrophic bacterial isolates from coastal saline soils.V167 (2): 20 – 33.
13. Bates,LS.; Waldrem, RP and Tear, ID. (1973). Rapid determination of free proline for water stress studies. Plant Soil.39: 205-207.
14. Black, pp. (1982). The Black Scholar: Journal of Black Studies and Research Volume 13, Issue 2-3, 1982.
15. Belimov, AA.; Safranova, VI.;Mimura, T.(2002). Response of spring rape (*Brassica napus*) to inoculation with PGPR containing ACC-deaminase depends on nutrient status of plant. Can. J. Microbiol.48:189–199.
16. Bozzola, JJ and Russell, LD.(1999).Electron Microscopy, Second Edition. Sudbury, MA: Jones and Bartlett Publishers,Sudbury, MA.
17. Brimecombe, MJ.; Leij, FAD and Lynch, JM.(2001). The effect of root exudates on rhizosphere microbial populations. In: The Rhizosphere. Pinton, R., Varanini, Z., and Nannipieri, P., eds. Marcel Dekker, Inc., New York, pp.95–140.
18. Chakrabarti, N and Mukherji, S. (2003). Alleviation of NaCl stress by pretreatment with phytohormones in Vigna radiate. Biol. Plant, 46:589–594.
19. Chakraborty, AP.; Dey, P.; Chakraborty, B.; Chakraborty, U and Roy, S. (2011). Plant growth promotion and amelioration of salinity stress in crop plants by a salt- tolerant bacterium. Recent Res Sci Technol., 3:61–70.
20. Compant, S.;Reiter, B.; Sessitsch,A.; Nowak,J.; Clément, C andAit Barka, E. (2005). Endophytic colonization of Vitis vinifera L. by plant growth promoting bacterium *Burkholderia sp*strains PsJN. Appl. Environ. Microb. 71, 1685–1693.
21. Difco Manual. (1985). Dehydrated culture media and reagents for microbiology, Laboratories incorporated Detroit, Michigan, 48232 USS, 621.
22. Dilfuza Egamberdlevaand Zulfiya Kucharova. (2008). Cropping Effects on Microbial Population and Nitrogenase Activity in Saline Arid Soil Turk. J. Biol. 32:85-90.
23. Dobereiner, J.; Marrial, LE and Nery, M. (1976). Biological distribution of *Azospirillum lipoferum. Beijerink. Canadian*.J. Microbio., 22: 1464-1473.
24. Dubois,M.; Gilles, KA.;Hamilto,JK.;Rebers, PA and Smith, F. (1956).Colorimetric method for determination of sugars and related substances. Anal. Chem. 28:350-356.
25. Egamberdieva, D. (2012). *Pseudomonas chlororaphis*: a salt tolerant bacterial inoculants for plant growth stimulation under saline soil conditions. Acta Physiol. Plant., 34:751–756.
26. Entry, JA.; Mitchell, CC and Backman, CB. (1996). Influence of management practices on soil organic matter, microbial biomass and cotton yield in Alabama´s "Old rotation". Biology and Fertility of Soils, 23: 353-358.
27. Fan Ben.; Xiao Hua Chen.; Anto Budiharjo.; Wilfrid Bleiss, Joachimvater and Rainer Borriss. (2011). Efficient colonization of plant roots by the plant growth promoting bacterium *Bacillus*

amyloliquefaciens FZB42, engineered to express green fluorescent protein. Elsevier. Journal of biotechnology. vol. 151 hlm: 303 -311.
28. Gilickmann,E and Dessaux, Y.(1995). A critical examination of the specificity of the Salkowski reagent for indolic compounds produced by phytopathogenic bacteria Appl. Environ. Micobiol. 61(2), 793-796.
29. Gouzou, L.; Burtin, G.; Philippy, R.; Bartoli, F and Heulin, T.(1993). Effect of inoculation with *Bacillus polymyxa* on soil aggregation in the wheat rhizosphere: preliminary examination. Geoderma, 56: 479-490.
30. Hallmann, J.; Quadt-Hallmann, A.; Mahaffee, WF and Kloepper, JW.(1997). Bacterial endophytes in agricultural crops Canadian Journal of Microbiology. 43(10): 895-914.
31. Han, HS and Lee, KD.(2005). Physiological Responses of Soybean Inoculation of *Bradyrhizobium japonicum* with PGPR in Saline Soil Conditions Research Journal of Agriculture and Biological Sciences 1(3): 216-221.
32. Hanaa, A Abo-Kora and Omar, MNA. (2010). Evaluation of the potential of two plant Growth Promoting Rhizobacteria (PGPR) on the increase of wheat productivity under salinity regimes. Egypt J. Microbiol. Special Issue,13th Conf. of Microbiol., pp. 137-151.
33. Hino,S and Wilson, PW. (1958). Nitrogen fixation by a facultative bacillus. J. Bacterial. 75, 403.
34. Idris, EE.; Iglesias, DJ.; Talon\, M and Borriss, R.(2007). Tryptophandependent production of indole-3-acetic acid (IAA) affects level of plant growth promotion by *Bacillus amyloliquefaciens* FZB42. Mol. Plant. Microbe teract. 20: 619 – 626.
35. Jamil,M.; Lee, DB.;Jung, KY.; Ashraf, M.;Lee, SC and Rhal, ES. (2006).Effect of salt (NaCl) stress on germination and early seedling growth of four vegetables species. J. Cent. Eur Agric 7:273–282.
36. Jen-Hshuan, Chen. (2006). The combined use of chemical and organic fertilizers and/or biofertilizer for crop growth and soil fertility. International workshop on Sustained Management of the Soil-Rhizosphere System for Efficient Crop Production and Fertilizer Use 16 ñ 20. p:1-10.
37. Katherrine, A Mattosi.; Vanial, M Padua.; Alexandre Romeiro, Leticiaf Hallack.; Biancac Neves.; Teciam Ulissesi.; Claudiaf Barros.; Adriane, RTodeschinil.;Jose, O Previato and Lucia Mendon, A-Prevlato. (2008). Endophytic colonization of rice *(Oryza sativa L.*) by the diazotrophic bacterium Burkholderia kururiensis and its ability to enhance plant growth Anais da Academia Brasileira de Ciências.80 (3): 477-493.
38. Khatoon, T.; Hussain,K.; Majeed, A.;Nawaz, K and Farrukh, Nisar M. (2010).Morphological variations in maize (Zea mays L) under different levels of Nacl at germinating stage. Appl. Sci. J,8:1294-1297.
39. Lopez-Bucio, J.; Campos-Cuevas, JC.; Hernandez-Calderon, E.; Velasquez-Becerra, C.; Farias-Rodriguez, R.; Macias-Rodriguez LI and Valencia-Cantero, E.(2007).*Bacillus megaterium* rhizobacteria promote growth and alter root-system architecture through an auxin- and ethylene-independent signaling mechanism in Arabidopsis thaliana. Mol. Plant. Microbe Interact. 20: 207 – 217.
40. Lumyong, S.; Lumyong, P.;McKenzie, EHC and Hyde, K. (2002). Enzymatic activity of endophytic fungi of six native seedling species from Doi Suthep-Pui Nathional Park, Thailand. Can J Microbiol 48:1109–1112.
41. Malmquist,A.; Welander.T.; Moone,E.; Ternstrom, A.; Molin, G and Stenstrom,I.(1994). Ideonella dechlorans.gen.nov,a new bavterium capable of growing a naerobically with chlorate as a nelectron a cceptor. Syst.A ppl. Microbial.17:58-64.
42. Marcelis,LFM and Hooijdonk, HV. (1999).Effect of salinity on growth, water use and nutrient use in radish *(raphanus sativus L.).* Plant and Soil. 215: 57-64.
43. Marius,S.; Neculai, M.;Vasile, Sand Marius, M. (2013). Effects of inoculation with plant growth promoting rhizobacteria on photosynthesis, antioxidant status and yield of runner bean. Romanian Biotechnological Letters 18: 8132-8143.
44. Miller, KJ and Wood, JM. (1996). Osmoadaptation by rhizosphere bacteria. Annual Review of Microbiology 50, 101±136.
45. Mirza, MS.; Ahmad, W.; Latif, F.;Haurat, J.; Bally, R.; Normand, P and Malik, KA.(2001). Isolation, partial characterization, and effect of plant growth promoting bacteria (PGPB) on micro-propagated sugarcane in vitro. Plant Soil 237: 47-54.
46. Mohan, V and Saranya devi, K. (2015). Selection of Potential Isolates of Plant Growth Promoting Rhizobacteria (PGPR) in Conferring Salt Tolerance under In Vitro Journal of Academia and Industrial Research (JAIR) Volume 4, Issue 3 August.
47. Muhammad Arslan Ashraf.; Mahmood Rasool and Muhammad Sajjad Mirza. (2011).Nitrogen Fixation and Indole Acetic Acid Production

Potential of Bacteria Isolated from Rhizosphere of Sugarcane (*Saccharum officinarum L.*). Advances in Biological Research 5 (6): 348-355.
48. Muhammad, Zafar-ul-Hye.; Hafiz, Muhammad Farooq.; Zahir, Ahmad Zahir.; Mubshar Hussain and Amjad Hussain. (2014). Application of ACC-deaminase Containing Rhizobacteria with Fertilizer Improves Maize Production under Drought and Salinity Stress. International J, Of, Agriculture & Biology. ISSN Print: 1560–8530; ISSN Online: 1814–9596.
49. Munns, R.(2002).Comparative physiology of salt and water stress. Plant Cell Environ. 25:239–250.
50. Nemat, M Awad.;Azza, S H.; Turky Magdi T.; Abdelhamid, Magdy Attia (2012).Ameliorate of Environmental Salt Stress on the Growth of Zea mays L. Plants By Exopolysaccharides Producing Bacteria Journal of Applied Sciences Research, 8(4): 2033-2044.
51. Omer, J and Ismail, H.(2002). Complementation of diazotrophs and yeast as plant growth promoting agents for wheat plants, Egypt. J. Agric. Res., 80, 29-40.
52. Piper, GS. (1950). "Soil and Plant Analysis". Inter. Sci. Publisher Inc. New York. USA.
53. Pirttila, A.; Joensuu, P.; Pospiech, H.; Jalonen, J and Hohtola, A (2004).Bud endophytes of Scots pine produce adenine derivatives and other compounds that affect morphology and mitigate browning of callus cultures. Physiol Plant 121: 305–312.
54. Prabhat, JHA and Ashok Kumar.(2009). Characterization of Novel Plant Growth Promoting Endophytic Bacterium *Achromobacter xylosoxidans* from Wheat Plant Microb Ecol.58:179–188.
55. Ramey, BE.;Koutsoudis, M.; Bodman, SBV and Fuqua, C. (2004). Biofilm formation in plant-microbe associations. Curr. Opin. Microbiol. 7, 602 – 609.
56. Reva, ON.;Dixelius, C.; Meijer, J and Priest, FG. (2004). Taxonomic characterization and plant colonizing abilities of some bacteria related to Bacillus amyloliquefaciens and *Bacillus subtilis*. FEMS Microbiol. Ecol. 48, 249 – 259.
57. Rolda, JR.; Salinas-Garcia, JR.; Algualcl,MM and Diaz, E Caravacaf.(2005). Soil enzyme activities suggest advantages of conservation tillage practices in sorghum cultivation under subtropical conditions. Geoderma 129, 178, 2.
58. Saharan,BS and Nehra,V.(2011).Plant Growth Promoting Rhizobacteria: A Critical Review. Life Sci. and MedicineRes., 2: 1-30.
59. Sharma, PK.; Sarita, S and Prell, J. (2005). Isolation and characterization of an endophytic bacterium related to *Rhizobium/Agrobacterium* from wheat *(Triticum aestivum L.)* roots. Curr Sci.89:608–610.
60. Shulz, B and Boyle, C.(2005). The endophyte continuum. Mycological Recearch 109;661-686.
61. Skujins,J and Burns, RG. (1976). Extracellular enzymes in soil. Crit. Rev. Microbiol.4(4):383-421.
62. Snedecor, GW and Cochran, WG. (1980). Statistical Methods. Determination of total carbohydrates. Methods in Microbiology 7thed. Iowa State Univ. Press, Ames., Iowa, U.S.A.
63. Somasegaranm, P and Hoben, HJ. (1994). "Hand book for rhizobia." Springer-Verlag. New York. U.S.A. pp.79-158.
64. SoumitraPaul Chowdhury.; Michael Schmid.; Anton Hartmann and Anil Kumar Tripathi1. (2007). Identification of Diazotrophs in the Culturable Bacterial Community Associated with Roots of Lasiurus sindicus, a Perennial Grass of Tharp Desert, India. Microbial Ecology.Volume 54: 82–90.
65. Strausberg,SI.(1983).Turbidity interferes with accuracy in heavy metal concentrations: Industrial Wastes, v. 29, no. 2, p. 16-21.
66. Strobel, G.; Daisy, B.; Castillo, U and Harperm, J.(2004). Natural products from endophytic microorganisms. J Nat Prod. 67: 257-268.
67. Su-Jung, Kim and Robert,J Kremer. (2005). Scanning and transmission electron microscopy of root colonization of morningglory (Ipomoea spp.) seedlings by rhizobacteria. Sympiosis.39, 117–124.
68. Sylvia, D.M; Fuhrmann, jj; Hartel, PG; Zuberer, DA. (1999). Principles and applications of soil microbiology, Editor, Prentice Hall Inc., Upper Saddle River, New Jersey.
69. Thomas-Bauzon, D.; Weinhard, P.; Villecourt, P and Balandreau.(1982).The spermosphere model. I. Its use in growing, counting, and isolating N2 fixing bacteria from the rhizosphere of rice, Can. J. Microbiol., 28, 922.
70. Thompson, DC.; Clarke, BB and Kobayashi, DY. (1996). Evaluation of bacterial antagonist for reduction of summer patch symptoms in Kentucky blue grass. Plant Dis. 80:856–862.
71. Tripathi,AK.;Nagarajan, T.; Verma,SC and Le Rudulier, D.(2002). Inhibition of biosynthesis and activity of nitrogenases in *Azospirillum brasilense* Sp7 under salinity stress. Curr Microbiol 44: 363–367.
72. Usha, RM and Arundhathi, Reddy G.(2012). Screening of rhizobacteria containing plant growth promoting (PGPR) traits in rhizosphere soils and their role in enhancing growth of pigeon pea. A. J. Biotechnol. 11(32):8085-8091.

73. Upadhyay,SK.; Singh, DP and Saikia,R. (2009). Genetic Diversity of Plant Growth Promoting Rhizobacteria Isolated from Rhizospheric Soil of Wheat Under Saline Condition. Curr. Microbiol. 59:489 496.
74. Upadhyay, SK.; Singh.; JS.; Saxena,AK and Singh, DP. (2012).Impact of PGPR inoculation on growth and antioxidant status of wheat under saline conditions. Plant Biol 14(4):605–11.
75. Upadhyay, SK.; Singh, JS and Singh, DP. (2011). Exopolysaccharide-producing plant growth promoting rhizobacteria under salinity condition. Pedosphere., 2: 214-222.
76. Usman, M.; Haq.; AU.; Ahsan, T.;Amjad, S.; Riast.Z and Umar, M. (2012). Effect of NaCl on Morphological Attributes of Maize *(Zea mays L.*). World J. of Agri. Sci., 8(4) 381-384.
77. Vivas, A.; Marulanda, A.; Ruiz-Lozano, JM.; Barea, JM Azcon, R. (2003). Influence of a Bacillus sp. on physilogical activities of two *arbuscular mycorrhizal* fungi and on plant response to PEG-induced drought stress. Mycorriza. 13, 249-256.
78. Watanabe, I and Barraquio, W. (1979). Low levels of fixed nitrogen required for isolarion of free-fiving N_2-fixing organisma from rice roots. Mature (London), 277: 565-566.
79. Weisberg, WG.;Barns, SM.; Pelletier,BA and Lane, DJ.(1991). 16S ribosomal DNA amplification for phylogenetic study. J. Bacteriol. 173, 697–703.
80. Zafar, ul-Hye M.;Nasir, A andZahir, ZA. (2014). Rhizobacterial inoculation integrated with mineral fertilizers promote maize productivity in saline-sodic soil subjected to compactness. Pak J Agric Agril Engg Vet Sci 30:43-53.
81. Zahir, ZA.; Ghani, U and Naveed, M.(2009). Comparative effectiveness of *Pseudomonas* and *Serratia sp*. containing ACC-deaminase for improving growth and yield of wheat (*Triticum aestivum* L.) under salt-stressed conditions. Arch Microbiol. 2009;191:415–424. [PubMed].
82. Zamfirache, MM. (2005). Fiziologie vegetala, Editor, Editura Azimuth, Iasi, 2005.

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