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Bioprospecting and plant growth-promoting bacteria tolerant to salinity associated with *Atriplex nummularia* L. in saline soils

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This study aimed to bioprospect and select halotolerantes bacteria and promoting plant growth associated with the plant *Atriplex nummularia* L. in saline soils. For bioprospecting of bacteria, samples were collected in five niches and two field experiments located in Serra Talhada and Ibimirim, Pernambuco, Brazil. After collecting the material it was performed the isolation and selection of bacteria based on plant growth promotion mechanisms. 107 bacterial salt tolerant isolates were obtained in which the population density of bacteria was higher in the rhizosphere (10^7 CFU g^{-1} soil), the cultivated soil (10^6 CFU g^{-1} soil) and uncultivated soil (10^5 CFU g^{-1} soil). For the solubilization rate of inorganic phosphate was obtained 65 and 25% positives isolated in 0 to 5% NaCl concentration, respectively. For the characteristics of biological fixation nitrogen, indole acetic acid production, exopolysaccharides and quorum sensing molecule, reached up to 87 percent; 100; 83.33 and 96.66% of the bacteria, respectively. Therefore, the bacterial isolates UAGAt 89 and UAGAt 101 expressed greater tolerance to salinity when analyzed in relation to the characteristics that promote plant growth, making it promising for future studies in order to contribute to the development of *Atriplex* plants and rehabilitation of soil affected by salts.

Key words: Halophytes, quorum sensing, exopolysaccharide, phytoremediation

INTRODUCTION

Salinity is an abiotic factor that negatively affect crop yields worldwide, especially in arid and semiarid regions (Silini-Chérif et al., 2012). Salinization occur due to inadequate irrigation management and excessive fertilization, contributing to the increase in areas with high concentrations of salts in the soil. The improvement of these soils is important to adopt recovery techniques and, among those, there is the possibility of using halophytes

with phytoremediation function which *Atriplex nummularia* L. is highlighted by its adaptability to salinity and water deficits (Souza et al., 2011; Santos et al., 2013).

On the other hand, excess salts in the soil may or may not interfere effectively in microbial communities depending on tolerance to salts thereof. Thus, select isolates that support this type of stress, as well as bacterial isolates bioprospect with growth-promoting characteristics associated with salty environments provides a possible alternative strategy to improve plant growth and would also benefit the biological and chemical soil characteristics (Upadhyay et al., 2012; Damodaran et al., 2013).

In addition, sustainable agriculture is important to find technologies that increase efficiency and reduce the use of chemical fertilizers, therefore, a viable alternative is the halotolerantes bacteria with plant growth promotion. Thus, microorganisms can act expressing different mechanisms, such as inorganic phosphate solubilization, nitrogen fixation, synthesis of phytohormones, exopolysaccharide, as well as the expression of quorum sensing molecules. These processes are performed by different species of bacteria with the ability to solubilize inorganic phosphate in the soil, leaving it available for the plant, and also include the conversion of atmospheric nitrogen into ammonia, production of auxins able to exert function in the regulation of plant growth, protection against plant stress such as salinity, drought and high temperatures (Pereira et al., 2012; Dawwam et al., 2013). These actions of microorganisms decrease the use of chemical fertilizers, representing an economic benefit and minimizing the impacts of fertilizers on the environment (Salamone et al., 2012).

Despite being the largest soil microbial biodiversity of the planet, studies on the biotechnological potential of salt tolerant bacteria associated to plants in saline soils are still scarce (Flores-Fernández et al., 2010). Mapelli et al. (2013) point out that the influence of these bacteria on plant growth has been recognized in conventional and extreme habitats, where the ability of bacteria to facilitate the adaptation of plants and promote growth and productivity has been proven.The extremes to which these microorganisms survive trigger an intense curiosity of the scientific community in the understanding of the physiology of these organisms.

However, the major driving force behind these studies is the biotechnological potential of these mechanisms and expressions from bacteria (Ramadoss et al., 2013). Thus, the interactions of soil, plant and microorganisms play a vital role in the mobilization of nutrients and substances,positively influencing the yield of crops can contribute in growing *Atriplex* and aid in recovery from two saline soils. In this context, this study was conducted in order to bioprospect and to select salt tolerant bacteria and promoter of plant growth associated with the *A. nummularia* L. in saline soils.

MATERIAL AND METHODS

Study material

For bioprospecting of bacteria, samples were collected in five niches: 1) soil area without *Atriplex* cultivation (control) (0-20 cm depth); 2) soil cultivated with *Atriplex* collected at a distance of 1 meter from the plant; 3) soil under cultivation of *Atriplex*, collected in the rhizosphere of plants; 4) Roots of *Atriplex* plants; 5) Leaves of *Atriplex* plants. The samples were collected in two field experiments with *A. nummularia* L. grown in plots in the irrigated areas of Serra Talhada and Ibimirim in the Pernambuco state, Brazil (Table 1). Soon after the collected, the samples were placed in box containing ice and transported to the Laboratory of Genetics and Microbial Biotechnology (LGBM) of the Academic Unit of Garanhuns - Rural Federal University of Pernambuco, for the isolation of bacteria and further analysis.

Isolation of bacteria

The isolation of endophytic and rhizosphere bacteria was performed according to the methodology proposed by Araujo (2010). The number of colony forming units (CFU) per gram of soil and fresh plant tissue was estimated by counting grown colonies, resulting in the population density of the bacteria on solid medium (TSA - Tripcase Soy Agar), plus 5% NaCl. Then purification was made by bacterial strains depletion technique of striae, which were subsequently stored at -20°C, and then performed in vitro plant growth promotion analysis: inorganic phosphate solubilization, biological nitrogen fixation, synthesis indole acetic acid, exopolysaccharide production, and expression of the quorum sensing molecule.

Solubilization inorganic phosphate

In order to evaluate inorganic phosphate solubilization were used 107 bacterial isolates: 65 isolates in the Serra Talhada area. The bacteria were inoculated onto solid medium containing insoluble calcium phosphate (Verma et al., 2001) supplemented with 0 to 5% NaCl with three replications. The plates were incubated at 28°C and readings were taken at 3, 6 and 10 days after inoculation.

The presence of clear area around the bacterial colonies indicated the solubilization of phosphate. Thus, it was calculated the solubilization index (SI), expressed by the average diameter ratio of solubilization halo by the average diameter of the colony halo (Berraquero et al., 1976).

Biological fixation nitrogen

From the phosphate solubilization test in the absence of NaCl (0%) 71 bacteria were selected for N2 fixation test *in vitro*. The bacteria

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Table 1. Chemical characterization of the soil used in the isolation of bacteria.

Attribute		Serra Talhada			Ibimirim		
		SNC[1]	SWC[2]	RS[3]	SNC[1]	SWC[2]	RS[3]
pH water (1: 2.5)		8.15	9.04	9.4	7.3	7.6	7.6
Ca^{2+}	($cmol_c\ kg^{-1}$)	6.45	4.99	5.31	26.32	36.62	17.1
Mg^{2+}	($cmol_c\ kg^{-1}$)	0.15	0.12	0.13	0.64	0.57	0.37
Na^{+}	($cmol_c\ kg^{-1}$)	10.72	9.93	5.98	7.77	8.32	3.19
K^{+}	($cmol_c\ kg^{-1}$)	0.53	0.49	0.67	0.72	0.82	0.51
P	($mg\ kg^{-1}$)	6.05	8.04	6.81	11.75	9.85	16.87
CEes	($dS\ m^{-1}$)	41.59	38.73	13.69	59.89	54.44	44.29
Ca^{2+}	($mmol_c\ L^{-1}$)	270.32	217.6	160.8	438.5	229.8	148.1
Mg^{2+}	($mmol_c\ L^{-1}$)	44.66	19.66	3.83	991.66	904.16	383.33
Na^{+}	($mmol_c\ L^{-1}$)	160.08	88.6	13.39	263.45	238.62	127.45
K^{+}	($mmol_c\ L^{-1}$)	0.41	0.44	0.15	0.76	0.85	0.67
COT	($dag\ kg^{-1)}$	0.86	0.82	0.78	1.83	1.36	1.28

[1]SNC- Soil no cultivation; [2]SWC- Solo with cultivation; [3]RS- Rhizosphere soil of *Atriplex* plants.

were inoculated into test tubes containing 10 mL of culture medium NFb (5 g L^{-1} of malic acid; 0.5 g L^{-1} of K_2HPO_4; 0.2 g L^{-1} of $MgSO_4.7H_2O$; 0.1 g L^{-1} of NaCl; 0.01 g L^{-1} of $CaCl_2.2H_2O$; 4 mL L^{-1} of Fe-EDTA (solution 1.64%); 2 mL L^{-1} of bromothymol blue (0.5%); 2 mL L^{-1} micronutrients solution, (0.2 g L^{-1} of $Na_2MoO_4.2H_2O$; 0.235 g L^{-1} of $MnSO_4.H_2O$; 0.28 g L^{-1} of H_3BO_3; 0.008 g L^{-1} of $CuSO_4.5H_2O$); 1.8 g L^{-1} of agar; and pH 6.8) semisolid without nitrogen, with two replicates incubated at 28 °C and evaluated after seven days of growth. The positive result was characterized qualitatively by film formation or bacterial growth halo of clear color, within the culture medium, indicating fixation capacity of nitrogen (Dobereiner et al., 1995).

Synthesis of indole acetic acid

In the selection and quantification of indole acetic acid production (IAA), positive bacteria were used with respect to inorganic phosphate solubilization. Bacterial isolates were evaluated in vitro by means of calorimetric and specific method which characterizes the production of phytohormone (Crozier et al., 1988). The experiment was conducted with three replications and the positive result was characterized by the formation of pink color. The samples were evaluated in a spectrophotometer, which measures the absorbance at 530 nm. To convert readings was used a standard curve (Barbosa, 2010) from an IAA solution of different concentrations.

Production exopolysaccharide

For the selection and production of exopolysaccharides (EPS), we used the methodology proposed by Kavamura (2012). Bacterial isolates were inoculated in a modified solid culture (2% yeast extract, 1.5% K_2HPO_4, 0.02% $MgSO_4$, $MnSO_4$ 0.0015%; 0.0015% FeSO4 0.003% $CaCl_2$; 0.0015% NaCl, 1.5% agar), plus 10% sucrose and grown for 24 hours at pH 7.5 at 28°C.

The experiment was performed in triplicate using 30 bacterial isolates originating from both environment described above, characterized positive for inorganic phosphate solubilization (1% NaCl), biological nitrogen fixation and indole acetic acid synthesis. The production of EPS was visually characterized by the presence or absence of halo production thereof, characterized as positive or negative, and then rated according to the size of the halo EPS produced was made a classification (+ for halo halo with little production- <10 mm in diameter; ++ for halo with average production - halo> 10 <14 mm; +++ for halo halo with great production-> 14 mm).

Molecule expression quorum sensing

The identification of bacteria producing the quorum sensing molecule (N-acyl homoserine lactone - AHL) was performed by *Agrobacterium tumefaciens* biosensor AHLs in bioassays with the test bacteria. *A. tumefaciens* NT1 was inoculated vertically on Petri dishes containing LB (Luria Bertani) + X-gal (10 µg mL^{-1}) (5-bromo-4-chloro-3-indolyl-beta-D galacto-pyranoside). Bacterial isolates were inoculated transversely to A. tumefaciens, containing the promoter TraR (TRAG: LacZ fusion gene), forming a complex which regulates the expression of the LacZ operon. In the presence of AHLs, these TraR bind to the promoter activating the expression of the lacZ gene, encoding the enzyme β-galactosidase, which breaks the molecule X-gal, making the blue cell (Gold et al., 2013). Thus, after inoculation at 28°C for 48 h, the observation of A. tumefaciens colonies with blue pigment indicated by the production of AHLs bacterial isolates In this test, we evaluated 30 positive bacterial isolates as the inorganic phosphate solubilization (1% NaCl), biological nitrogen fixation and indole acetic acid synthesis. The experiment was performed in duplicate.

Statistical analysis

The results for the density of bacterial isolates were subjected to analysis of variance F-test, for up to 5% of significance and means were compared by Tukey test. For the average index of inorganic phosphate solubilization, production of acid indole acetic and exopolysaccharides was applied Scott-Knott test up to 5% probability, using the statistical program, Sisvar 5.3. Frequency data were subjected to relative chi-square (χ^2) to confirm the influence of the factors area and bacterial colonization niche on the distribution of phosphate solubilizing isolates, biological nitrogen fixers and producers of IAA.

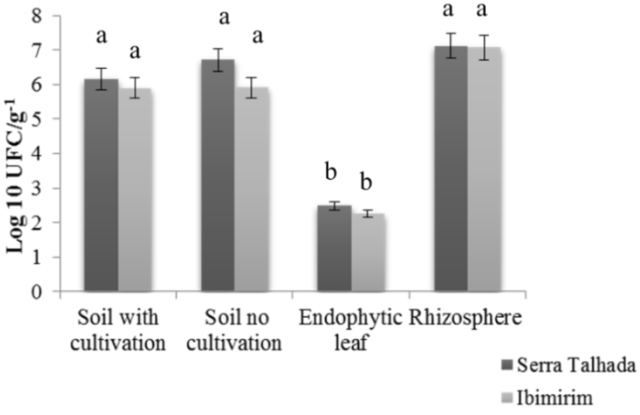

Figure 1. Population density of the bacterial community (Log10 CFU g^{-1} soil or g^{-1} fresh plant tissue) in two areas cultivated with *Atriplex nummularia* L. Columns with same letters within each area, does not differ statistically between the niches (Tukey's test,$p < 0.05$).

RESULTS

Isolation of bacteria

107 bacterial isolates were obtained from the two areas of study. Thus, it was observed that the bacterial density in both areas and niches present variation 10^2 to10^7 CFU g^{-1} soil or g^{-1} fresh plant tissue (CFU- colony forming units). There was a significant difference ($p<0.05$) between niches of the areas studied, observing that the population density of bacteria in the rhizosphere soil, soil with and without cultivation of *Atriplex* were statistically similar, differing from endophytic leaves niche (smaller population of bacteria isolated) (Figure 1). The rhizosphere provided average value of 10^7 CFU g^{-1} soil for the two areas studied; followed with 10^6 and 10^5 CFU g^{-1} soil, soil with and without cultivation in the areas of Serra Talhada and Ibimirim. It is worth noting that the ground without the cultivation of *Atriplex*, with cultivation and rhizosphere soil associated with plants, they are salinized (Table 1) and therefore, bacteria in these environments formed groups tolerance to this stress factor in each environment studied.

Solubilization of inorganic phosphate

Among 107 bacterial strains evaluated, 65% were able to solubilize inorganic phosphate in the absence of NaCl and 25% at concentration of 5% NaCl, indicating tolerance of these microorganisms to salinity. Consequently, as higher salt concentrations, lower the mechanism of inorganic phosphate solubilization (SIF), as well was noted at 5% NaCl. However, does not inhibit this activity, confirming the existence of solubizer bacteria salt-tolerant of phosphate associated with *Atriplex*.

The inorganic phosphate solubilization index (SI) differs among the bacterial strains tested (Table 2), as the days of cultivation and NaCl concentrations. Similarly to inorganic phosphate solubilization index (SI) by the bacterial isolates in relation to absence and presence of NaCl (5%), there was only significant difference for isolated UAGAt89 (without soil cultivation) and UAGAt101 (soil with cultivation), resulting with higher values in the concentration of 5% NaCl with 3.91 and 4.10 for SI, respectively.

For the three days of cultivation it is clear that in the absence of NaCl, after 3 days of culture, isolated UAGAt19 (without soil cultivation-Serra Talhada) had the highest SI (9.53) (Table 2). After 6 days of culture in the absence of NaCl, it was observed a greater number of isolated solubilizers, when compared after 3 days of cultivation, especially isolated UAGAt34, root endophytic area Serra Talhada, with a maximum value of 9.73 (SI). After 10 days of culture in both areas, there was the largest SI (5.57) for isolated UAGAt01 soil without soil cultivation of Serra Talhada (Table 2).

With the assessment of the bacteria at 5% NaCl in 3 days of culture, it was observed that there was no solubilization of inorganic phosphate by the bacterial isolates for any of the areas and niches, while at 6 days of culture, had solubilization, for 15% of the isolates, specially UAGAt101 (soil cultivation without-Ibimirim) and UAGAt02 (soil cultivation without-Serra Talhada) with SI of 4.10 and 3.48, respectively. After 10 days of cultivation, there was an increase in the solubilization rate of the cultured bacteria at this concentration of NaCl to afford 21.5% of positive isolates under these conditions. However, bacteria that significantly expressed the highest value of SI was UAGAt89 (6.0) associated with soil cultivation without of Ibimirim area (Table 2). Phosphate solubilization ratio in the cultivation time in the absence and presence of NaCl (5%) it is perceived that the 6 days of cultivation, no significant difference from the isolated, while the 10 days of cultivation the SI isolates were higher in the absence of NaCl comparing with the concentration of 5% NaCl.

Biological nitrogen fixation

For biological nitrogen fixation (BNF), among 71 bacterial isolates evaluated, 87% were able to grow in culture medium without nitrogen source, indicating potential for fixation nitrogen biological in vitro, with emphasis on high frequency, bacteria niche root endophytic (Serra Talhada) and leaf (Ibimirim).

By analyzing the BNF of bacteria bioprospected area Serra Talhada, exceeding 27.9% was observed relative frequency for endophytic bacteria in the root niche, however, endophytic leaves had lower frequency (13.94%) in for positive isolates. Thus, there was a variation of this growth promotion mechanism between

Table 2. Index inorganic phosphate solubilization of isolated bacterial salt-tolerant associated with *Atriplex nummularia* L. due to the absence and presence of NaCl at different time evaluation.

Isolated	Niche	0% of NaCl			5% of NaCl		
		3 days	6 days	10 days	3 days	6 days	10 days
UAGAt 01	SNC	-	1.38Ac[a]	5.57Aa[a]	-	-	3.62Ab[b]
UAGAt 02	SNC	2.41Ac	2.31Ac[a]	2.26Ac[a]	-	3.48Aa[a]	-
UAGAt 06	SNC	-	1.84Ac[a]	2.31Ac[a]	-	-	-
UAGAt 08	SNC	1.88Ac	2.51Ac[a]	2.63Ac[a]	-	-	-
UAGAt 09	SNC	1.83Ac	2.51Ac[a]	2.63Ac[a]	-	-	-
UAGAt 13	SNC	1.87Ac	2.80Ac[a]	-	-	-	-
UAGAt 14	SNC	1.59Ac	1.57Ac[a]	2.01Ac[a]	-	1.42Ac[a]	
UAGAt 15	SNC	1.90Ac	2.14Ac[a]	1.96Ac[a]	-	-	-
UAGAt 19	SNC	9.53[a]	-	-	-	-	-
UAGAt 21	SNC	1.16Ac	1.94Ac[a]		-	-	1.60Ac[b]
UAGAt 22	SNC	-	2.44Ac[a]	-	-	1.14Ac[a]	-
UAGAt 23	SWC	1.52c	-	-	-	-	-
UAGAt 25	SWC	1.89Ac	2.91Ac[a]		-	1.50Ac[a]	1.93Ac[b]
UAGAt 33	ER	2.67Ac	1.92Ac[a]	-	-	-	1.45Ac[b]
UAGAt 34	ER	6.82Ab	9.73Aa[a]	-	-	-	-
UAGAt 35	ER	2.17Ac	-	-	-	-	1.59Ac[b]
UAGAt 36	ER	2.04Ac	-	-	-	-	1.48Ac[b]
UAGAt 37	ER	2.15Ac	1.68Ac[a]	-	-	1.13Ac[a]	
UAGAt 38	ER	-	1.99Ac[a]	-	-	-	1.64Ac[b]
UAGAt 39	ER	2.72c	3.82b[a]	2.92b[a]	-	-	-
UAGAt 40	ER	3.80c	4.83b[a]	4.19b[a]	-	-	-
UAGAt 41	ER	3.75c	4.59b[a]	4.82a[a]	-	-	-
UAGAt 42	ER	2.06c	1.96c[a]	-	-	-	-
UAGAt 43	ER	3.47c	4.36b[a]	3.75b[a]	-	-	-
UAGAt 45	ER	2.43c	2.76c[a]	2.88b[a]	-	-	-
UAGAt 51	EL	-	2.19Ac[a]	1.73Ac[a]	-	-	1.65Ac[b]
UAGAt 53	EL	-	2.02c[a]	-	-	-	1.686c[b]
UAGAt 54	EL	2.04Ac	1.92Ac[a]	-	-	1.10Ac[a]	-
UAGAt 61	EL	-	2.35c[a]	2.90b[a]	-	-	-
UAGAt 63	EL	-	-	-	-	-	1.67c[b]
UAGAt 69	RS	1.74Ac	-	-	-	2.50Ab[a]	1.68Ac[b]
UAGAt 71	RS	1.95c	1.94c[a]	-	-	-	-
UAGAt 72	RS	-	1.45c[a]	-	-	-	-
UAGAt 73	RS	2.12c	2.52c[a]	-	-	-	-
UAGAt 75	RS	1.84c	2.09c[a]	-	-	-	-
UAGAt 76	RS	-	2.68c[a]	-	-	-	-
UAGAt 77	RS	-	3.03Ac[a]	-	-	2.79Ab[a]	1.98Ac[b]
UAGAt 89	SNC	-	2.33Bc[a]	3.916Bb[a]	-	1.81Ac[a]	6.02Aa[b]
UAGAt 92	SNC	-	1.88Ac[a]	2.59Ac[a]	-	-	2.26Ac[b]
UAGAt 93	SNC	-	1.93Ac[a]	1.90Ac[a]	-	-	-
UAGAt 94	SNC	-	1.79Ac[a]	1.77Ac[a]	-	-	-
UAGAt 95	SNC	1.47c	1.75c[a]	-	-	-	-
UAGAt 98	SWC	1.85c	2.09c[a]	-	-	-	-
UAGAt 99	SWC	-	2.20c[a]	2.36c[a]	-	-	-
UAGAt 101	SWC	-	1.89Bc[a]	3.15Bb[a]	-	4.10Aa[a]	4.2Ab[b]
UAGAt 104	SWC	-	-	1.08c[a]	-	-	-
UAGAt 107	SWC	-	1.836c[a]	-	-	-	-
UAGAt 108	SWC	1.37Ac	1.43Ac[a]	-	-	1.68Ac[a]	
UAGAt 113	EL	1.67Ac	1.94Ac[a]	-	-	-	-

Table 2. contd.

UAGAt 114	EL	1.59Ac	2.49Ac[a]	-	-	2.41Aa[a]	-
UAGAt 115	EL	-	1.18c[a]	-	-	-	-
UAGAt 119	EL	1.64Ac	1.87Ac[a]	-	-	-	1.72Ac[b]
UAGAt 120	EL	-	1.87Ac[a]	-	-	-	1.53Ac[b]
UAGAt 122	EL	1.83Ac	1.69Ac[a]	-	-	-	1.42Ac[b]
UAGAt 123	EL	-	1.83Ac[a]	2.17Ac[a]	-	-	1.47Ac[b]
UAGAt 124	EL	1.62Ac	1.91Ac[a]	-	-	-	1.28Ac[b]
UAGAt 125	EL	-	1.46Ac[a]	1.62Ac[a]	-	-	1.33Ac[b]
UAGAt 126	EL	-	1.28c[a]	-	-	-	-
UAGAt 127	EL	1.17Ac	-	-	-	-	1.61Ac[b]
UAGAt 128	EL	-	-	1.97c[a]	-	-	-
UAGAt 133	RS	-	1.54Ac[a]	-	-	1.61Ac[a]	-
UAGAt 135	RS	-	1.37Ac[a]	-	-	1.49Ac[a]	-
UAGAt 140	RS	2.54Ac	1.69Ac[a]	-	-	1.92Ac[a]	-
UAGAt 141	RS	-	1.88Ac[a]	-	-	2.59Ab[a]	2.04Ac[b]
C.V		54.48					

Means with same uppercase letter in the line comparing the rate of solubilization by bacterial isolates compared to NaCl concentrations (0 and 5%); Lowercase letters in the column comparing the rate of solubilization by bacterial isolates in the three culture times - 3, 6 and 10 days; Letters in exponential line compare the solubilization index by bacterial isolates in relation to time and the concentrations of NaCl. Same letter do not differ at 5% probability by Scott-Knott's test. (SNC- soil no cultivation; SWC- soil with cultivation; ER- endophytic root; EL: endophytic leaf; RS: rhizosphere soil); (UAGAt 01-UAGAt 77: Isolates of Serra Talhada; UAGAt 89-UAGAt 141: Isolates of Ibimirim)

the different niches associated with the plant *Atriplex*. For Ibimirim area, different responses were observed, evidencing significant value in endophytic niche leaves (42.85%), where in the rhizosphere percentage of 6.28% was observed in the relative frequency of FNB. It is observed that Serra Talhada excelled in relation to Ibimirim, with values of 60.55 and 39.43%, respectively.

Synthesis of indole acetic acid

Regarding of synthesis of indole acetic acid (IAA), it was observed that 100% of the tested isolates were able to synthesize this phytohormone. The values of the IAA production varied from 61.10 to 1.0 μgmL^{-1} corresponding to the UAGAt21 bacteria (soil with crop - Serra Talhada) and UAGAt119 (endophytic leaf-Ibimirim) respectively and a significant difference between bacteria evaluated (Table 3). Production of IAA in Serra Talhada show 31.57% positive isolates for endophytic root niche, especially in relation to the studied environments. For samples arising Ibimirim, isolated endophytic leaf shown positive relative frequency of 63.15%. In generally, 100% of the isolates producing IAA, (66.66%) are Serra Talhada and (33.33%) to Ibimirim. Thus, it is possible to point the most potential of endophytes isolated for mechanism of plant growth promotion in Serra Talhada.

Production of exopolysaccharides

Among the 30 bacterial isolates studied, 83.33% were positive to the test and was possible to confirm the production of EPS by bacteria tolerant to salt associated with *Atriplex* (Figure 2). Given the classification described in Table 4, it is observed that five isolates (16.66%) were negative for the production of EPS, while 13.33% have provided little production, 10% medium production and 60% optimum production of EPS. Difference also was observed between isolates according to their EPS production (Table 5). The isolated UAGAt89, from the soil without cultivation of area Ibimirim provided the highest average value, with the halo of producing 60.33 mm, differing from the other, except UAGAt90 and UAGAt128.

In addition, the isolates showed greater diameter halo originating from the soil without cultivation for endophytic leaf of Ibimirim area, which confirms the classification result shown in Table 4.

Molecule expression quorum sensing

It was found 29 positive bacterial isolates tested for production of the quorum sensing (AHL) molecule because bacterium *A. tumefaciens* colonies showed blue pigmentation (Figure 3) while only isolate belonging to UAGAt77 rhizosphere from Serra Talhada, did not express the production of this molecule. For that, it can be highlighted the high rate (96.66%) of isolates producing the AHL molecule, a fact which shows the adaptation of these isolates to the saline environment under *Atriplex* cultivation.

Table 3. Production of indole acetic acid (IAA) in vitro by salt-tolerant bacterial isolates associated with *Atriplex mummularia* L.

Isolated bacterial	Niche	IAA µg mL^{-1}
UAGAt 01	SNC	3.82[e]
UAGAt 02	SNC	15.38[d]
UAGAt 06	SNC	3.13[e]
UAGAt 08	SNC	1.36[e]
UAGAt 09	SNC	3.04[e]
UAGAt 12	SNC	4.42[e]
UAGAt 13	SNC	3.73[e]
UAGAt 14	SNC	18.35[d]
UAGAt 15	SNC	11.67[d]
UAGAt 19	SWC	7.57[e]
UAGAt 21	SWC	61.10[a]
UAGAt 22	SWC	6.15[e]
UAGAt 23	SWC	21.72[d]
UAGAt 25	SWC	53.25[a]
UAGAt 27	SWC	4.42[e]
UAGAt 34	SWC	41.64[b]
UAGAt 33	ER	2.14[e]
UAGAt 35	ER	42.59[b]
UAGAt 36	ER	2.05[e]
UAGAt 37	ER	3.22[e]
UAGAt 38	ER	24.62[c]
UAGAt 39	ER	11.88[d]
UAGAt 40	ER	21.80[d]
UAGAt 41	ER	16.67[d]
UAGAt 42	ER	1.23[e]
UAGAt 43	ER	14.77[d]
UAGAt 45	ER	39.19[b]
UAGAt 51	EL	1.83[e]
UAGAt 53	EL	31.77[c]
UAGAt 54	EL	2.40[e]
UAGAt 61	EL	27.41[c]
UAGAt 63	EL	14.95[d]
UAGAt 66	RS	1.79[e]
UAGAt 74	RS	4.38[e]
UAGAt 77	RS	7.14[e]
UAGAt 93	RS	9.94[d]
UAGAt 71	RS	3.34[e]
UAGAt 73	RS	11.45[d]
UAGAt 75	RS	10.72[d]
UAGAt 89	SNC	2.91[e]
UAGAt 90	SNC	6.79[e]
UAGAt 92	SNC	21.76[d]
UAGAt 95	SNC	2.40[e]
UAGAt 99	SWC	1.23[e]
UAGAt 101	SWC	1.49[e]
UAGAt 107	SWC	2.39[e]
UAGAt 113	EL	6.19[e]
UAGAt 114	EL	2.52[e]
UAGAt 115	EL	1.10[e]
UAGAt 119	EL	1.01[e]

Table 3,contd.

UAGAt 120	EL	1.10[e]
UAGAt 122	EL	1.58[e]
UAGAt 123	EL	1.96[e]
UAGAt 124	EL	1.58[e]
UAGAt 125	EL	16.33[d]
UAGAt 126	EL	15.25[d]
UAGAt 127	EL	5.63[e]
UAGAt 128	EL	42.68[b]

(Coefficient of Variation: 23.84%) Same letters in the columns do not differ by the Scott-Knott's test at 5% probability. (SNC- (Soil without cultivation; SWC- Solo with cultivation; ER- Endophytic root; RS- Rhizosphere soil; EL: Endophytic leave of *Atriplex* plants).

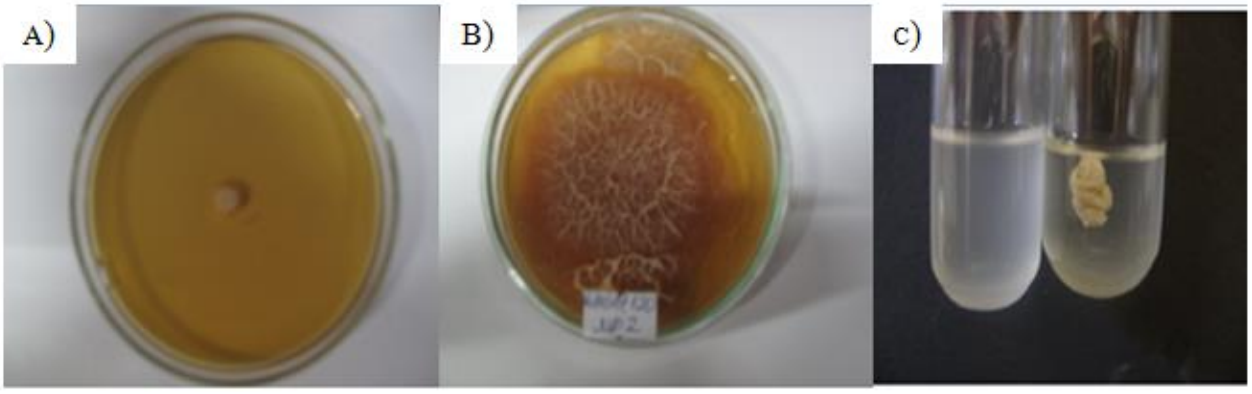

Figure 2. Negative bacterial isolate (A); Isolated positive bacterial (B); Confirmation of EPS production (negative: cloudy / pipe on the left; positive: precipitate / all right) (C).

Table 4. Classification of exopolysaccharide (EPS) by bacterial isolates possibly plant growth promoters associated with *Atriplex*.

Production of exopolysaccharides							
Isolated bacterial	**Little**	**Mean**	**Optimum**	**Isolated bacterial**	**Little**	**Mean**	**Optimum**
UAGAt 08	-	-	-	UAGAt 71			+++
UAGAt 09			+++	UAGAt 75			+++
UAGAt 14	+			UAGAt 77		++	
UAGAt 21		++		UAGAt 89			+++
UAGAt 22	-	-	-	UAGAt 90			+++
UAGAt 25		++		UAGAt 92			+++
UAGAt 33			+++	UAGAt 93	+		
UAGAt 35			+++	UAGAt 95	-	-	-
UAGAt 37			+++	UAGAt 99			+++
UAGAt 51			+++	UAGAt 101			+++
UAGAt 53	+			UAGAt 107			+++
UAGAt 54	+			UAGAt 114			+++
UAGAt 63			+++	UAGAt 122			+++
UAGAt 66	-	-	-	UAGAt 125			+++
UAGAt 69	-	-	-	UAGAt 128			+++

Table 5. Halos of exopolysaccharide production by bacterial isolates possibly plant growth promoters associated with *Atriplex*.

Isolated bacterial	Niche	Halo de EPS (mm)
UAGAt 09	SNC	16.78^{g}
UAGAt 14	SNC	7.46^{g}
UAGAt 21	SNC	10.65^{g}
UAGAt 25	SWC	10.86^{g}
UAGAt 33	ER	23.26^{e}
UAGAt 35	ER	27.76^{d}
UAGAt 37	ER	20.30^{e}
UAGAt 51	EL	18.05^{f}
UAGAt 53	EL	9.83^{g}
UAGAt 54	EL	7.72^{g}
UAGAt 63	EL	39.75^{c}
UAGAt 71	RS	29.83^{d}
UAGAt 75	RS	31.63^{d}
UAGAt 77	RS	10.67^{g}
UAGAt 89	SNC	60.33^{a}
UAGAt 90	SNC	57.08^{a}
UAGAt 92	SNC	18.42^{f}
UAGAt 93	SNC	8.00^{g}
UAGAt 99	SWC	41.00^{c}
UAGAt 101	SWC	25.66^{e}
UAGAt 107	SWC	50.38^{b}
UAGAt 114	EL	14.12^{f}
UAGAt 122	EL	17.67^{e}
UAGAt 125	EL	53.96^{b}
UAGAt 128	EL	60.28^{a}
C.V (%)	14.54	

Similar letters in the same columns do not differ according to Scott-Knott's test at 5% probability. (Soil no cultivation; SWC- Solo with cultivation; ER- Endophytic root; RS- Rhizosphere soil; EL: Endophytic leave of *Atriplex* plants).

DISCUSSION

Isolation of bacteria

The microbial community may be vary depending on the plant species and soil type, since these organic compounds influence the quantity and quality of exudates, which, in turn, will select or favor specific nutritional groups and organisms in the rhizosphere soil (Ahemad and Kibret, 2014).Similar results were found by Santos (2010), studying the bioprospecting of bacteria associated with *Atriplex nummularia* L. in saline-sodic soil in Pesqueira, Wasteland of Pernambuco (Brazil), revealing significant amount of population density in the rhizoplane niche (UFC 10^{9} g^{-1} soil). Mapelli et al. (2013) studied bacteria associated with rhizosphere of Salicornia plant in hypersaline soils, detected values from 10^{4} a 10^{10} CFU g^{-1} soil for population density, bigger those found in this study. These authors also report that the interaction with the plant and the presence of root exudates may account for the higher abundance of halotolerantes bacteria detected in the rhizosphere.

Regarding to salt tolerant bacteria associated with *A. nummularia* L. in this research, some factors must have influenced the population density of these bacteria, such as climate change, plant species, and the type of soil in which the plant is being grown, giving conditions to varying results.

Solubilization of inorganic phosphate

Several factors can influences this mechanism. Thus, by the attributes shown in Table 1, the soil cultivation without Ibimirim area has higher electrical conductivity (59.89 dS m^{-1}), being more saline than the soil cultivation without area Serra Talhada (41.59 dS m^{-1}). These data may explain the predominance of larger SI area Serra Talhada when tested in the absence of NaCl, while a concentration of 5%, the bacteria isolated from Ibimirim area stood out this characteristic. This can be justified by the possible formation of more bacteria groups adapted to each environment, promoting change in SIF. Many microorganisms are able to solubilizing inorganic phosphate, but their transformative capacity can associate to the ecological conditions, including soil characteristics and vegetation. Regarding the expression of SIF in salinity conditions associated with *Atriplex* it can be explained by the fact that adversely affect salt growth and proliferation of microbial cells, resulting in a loss of efficiency of growth promotion mechanism, which may be variable among bacterial species. Thus, bacteria appears to adapt constantly their physiology to changes in physic and chemical factors of the environment, including the accumulation of osmoprotectors expressing adaptive behaviors to achieve osmotic adjustment and to ensure the stability of certain active protein (Chérif-Silini et al., 2013).

In another research, Nakbanpote et al. (2013) addressing tolerance of bacteria to salinity and plant growth-promoting, SIF bacteria observed in 8% NaCl conditions, confirming tolerance to salinity of these microorganisms. Regarding to more expressive values for the SI (9.73) were found in this study with bacteria tolerant to salinity associated with plants of *Atriplex*, indicating the biotechnological potential of salt-tolerant isolated. It can be noticed that the inorganic phosphate solubilization rates found with bacteria associated with Atriplex this study provide potential for promoting plant growth, which indicates the efficiency of these micro-organisms coming from saline soils, contributing to plant growth.

However, for the enhancement of inorganic phosphate solubilization process, there is still need for further knowledge of these micro-organisms associated with halophytes, as the Atriplex in saline soils, for this

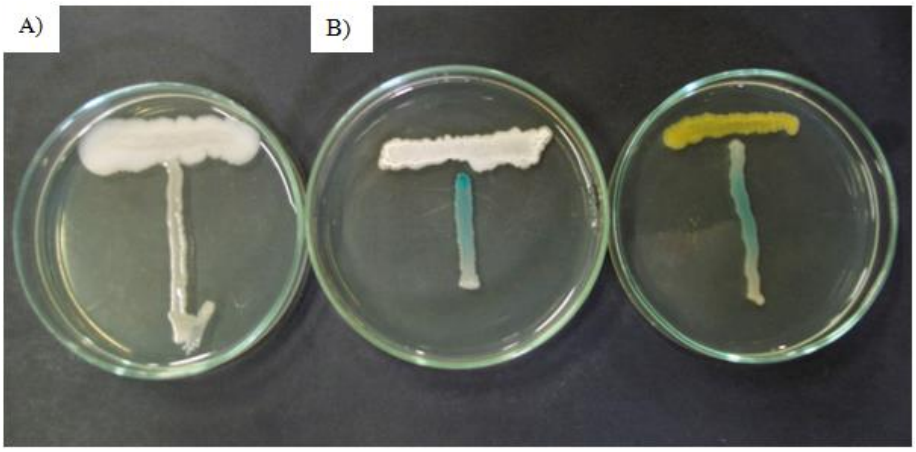

Figure 3. Absence of blue staining (negative) (A); Presence of indicator blue staining of quorum sensing molecule expression (AHL) (B). *A. tumefaciens* is peaked in the vertical and horizontal test bacteria.

process to be safely indicated in phytoremediation of soils and in sustainable agriculture.

Biological nitrogen fixation

The existence of nitrogen-fixing bacteria associated with different niches and areas is very variable. Santi et al. (2013), report that endophytic bacteria can have advantages over the rhizospheric bacteria, because they colonize the interior of the plant tissues and consequently can be established in niches providing more appropriate conditions for effective nitrogen fixation, suffering less competition the bacteria present in the soil, possibly, excreting part of the nitrogen, occurring the transfer of fixed nitrogen to the plant. This may have occurred with the endophytic root tested in this study, especially in the FNB mechanism.

In research carried out by Santos (2010) is mentioned the existence of salt-tolerant nitrogen-fixing bacteria associated with plants of *Atriplex*, where there was a greater number of bacteria with this feature in endophytic root niche, a result similar to that found in soil Serra Talhada. Pereira et al. (2012), studying endophytic bacteria of sugarcane in relation to salinity, they observed the NFB in the tested strains, detaching the importance of exploring bacteria tolerant to salinity so that they can be used as inoculants, in order to minimize the use of chemical fertilizers and thus the salinization increasing.

Synthesis of indole acetic acid

Regarding to stronger association of *Atriplex* roots with bacterial community may be related to the compounds exuded by the roots, rich system sugars, polysaccharides, phenolic compounds and aliphatic attracting these microorganisms, with consequently greater interaction bacteria and plant. Thus, there are better conditions for IAA phytohormone synthesis (Compant et al., 2010; Ahemad and Kibret, 2014). Kuklinsky-Sobral et al. (2004) report that the habitat associated with the plant is a dynamic environment, which may occur interference of several factors on composition of the bacterial community, which colonizes the niches associated to the plants. Microorganisms can select a different pathway depending on the environment and can reveal interesting results.

According to other results obtained by Jha et al. (2011) about salt-tolerant bacteria and plant growth-promoting associated with *Salicornia brachiata*, was observed that all isolates tested produced IAA in amounts ranging from 30 to 100 µg mL^{-1}. In another study, Sgroy et al. (2009) evaluated endophytic bacteria associated to the halophyte *Prosopis strombulifera* and found the highest values for IAA production of 2.2 µg mL^{-1}. In this context, the findings of this study are significant for the production of IAA by bacteria salt-tolerant with dependent pathway of tryptophan.

Production of exopolysaccharides

The observation of great production of exopoly-saccharides is more visible in bioprospected bacteria of Ibimirim area. Therefore, it is because bacteria are associated with more stressful environments in relation to soil salinity and these microorganisms can to express more sharply, in order to protect the plant against raised stress (Qurashi et al., 2012). Thus, it is believed that bacteria have been induced to produce EPS in large quantities, since the accumulation or formation of this substance tends to increase from the moment in which the salt stress or other stress is expressed most

pronouncedly the environment. Thus, the bacteria tend to produce this substance in order to protect the plant against stress causes, such as saline strees, drought and heat stress, benefiting crop growth and development, especially in saline soils, where *Atriplex* is grown.

On the production of EPS Qurashi et al. (2012) studied this mechanism under influence of salt stress and reported increase of EPS production at higher salinity levels promote formation of biofilm and protects the plant by maintaining a water layer around the cells, contributes significantly to the improvement of soil fertility and plant growth. Ashraf et al. (2005), evaluating producing bacteria EPS, the observed an increase in soil aggregation around the roots of inoculated wheat plants, grown in saline soil, positively affecting their physico-chemical characteristics. The authors also realized that to offset the stress imposed by salinity, the production of exopolysaccharides are significant strategies to help in the metabolism of tolerance to salts by bacteria adapted to this environment.

Molecule expression quorum sensing

Associating with the results found in this work to ready the literature, it was noticed a higher percentage of bacteria producing the quorum sensing (AHL) molecule. Leite et al. (2014) studied bacteria salt-tolerant associated to sugar cane and analyzing the production of quorum sensing among 102 isolates, 49% positive isolate were found.

Bhattacharyya and Choudhury (2008) report that environmental conditions often change rapidly and the bacteria need to respond very quickly to this change in order to survive. These responses can be different, including adaptation, availability of nutrients and defense against other microorganisms. However, quorum sensing communication helps bacteria to coordinate their behavior face to the adverse environmental conditions.

Thus, the importance of detecting quorum sensing, especially the AHL molecule, the adaptation of microorganisms in general and particularly in salty environments, is indispensable. However, the role of this molecule in microbial biosphere is still relatively unknown, requiring further studies for biotechnology in agriculture purposes.

Conclusions

In general, bacteria tolerant to salinity associated with plant *A. nummularia* L., provided plant growth promoting confirmed in the areas studied (Serra Talhada and Ibimirim). The study indicates high levels of SIF at a concentration of 5% NaCl by isolated UAGAt 89 (without soil cultivation-Ibimirim) and UAGAt 101 (soil with cultivation-Ibimirim). The endophytic bacterial isolates stood out with NFB and production of IAA. It is worth noting that tolerant to salt bacteria have potential for EPS production and expression of N-acyl homoserine lactone molecule by the communication system quorum sensing.

Thus, the bacterial isolates UAGAt89 and UAGAt101 expressed greater tolerance to salinity, making it promising for future studies and may contribute to the development of *Atriplex* plants and soil reclamation affected by salinity.

Conflict of Interests

The authors have not declared any conflict of interest.

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