Revista Geama

Article

Ciências Ambientais

Environmental Sciences

Quantification and characterization of putative diazotrophic bacteria from forage palm under saline water irrigation

Gabiane dos Reis Antunes[1], Beatriz Rodrigues Carvalho[2], Thaise Rosa da Silva[3], José Neuman Miranda Neiva[4], Gherman Garcia Leal de Araújo[5] & Paulo Ivan Fernandes-Júnior[6*]

* paulo.ivan@embrapa.br
Todos autores contribuíram de forma igualitária

Received: July 19, 2017
Accepted: July 28, 2017
Published: October, 2017

ABSTRACT

The aim of this study was to evaluate the density and phenotypical diversity of diazotrophic endophytic bacteria from the forage palm irrigated with different saline water depths. Opuntia stricta (IPA-200016) received five depths of saline water (L1: 80%. ETo; L2: 60%.ETo; L3: 40%; ETo; L4: 20%; ETo and, L5: 0% ETo, where ETo is the reference evapotranspiration). The roots were collected in the field, disinfected, grounded and serial diluted from 10-1 to 10-4. The total concentration of diazotrophic bacteria was determined by the most probable number method (MPN) and the isolated bacteria were characterized phenotipically. The concentration of bacteria found in forage palm roots ranged from 0.36 x 104 to 109.89 104 cells per gram of root, with highest occurrence on the 60 and 80% ETo. In the dendrogram of similarity it was possible to observe the formation of 24 phenotypic groups with 100% similarity. All bacteria presented similarity superior to 40%. Among these groups, 14 are rare groups, formed by only a single bacterial isolate. In the Semi-Arid conditions, the forage palm that receives the highest amount of saline water, presents a higher density of putative nitrogen-fixing endophytic bacteria with high phenotypic diversity.

Keywords: Microorganisms, Forage, Biossaline Agriculture, Inoculant

Introduction

Animal production is one of the most important economic activities in the Brazilian semi-arid region, responsible to warrant the food security of rural families and the generation of employment and income for the region. Due to the great variability of the forage availability among the rainy and dry periods, the productive performance of the cattle is low, mainly due to the decrease of forage availability during the dry period (ARAUJO et al., 2010). The low water availability in the region is constant and, for this reason, the interests to the biossaline agriculture is increasing.

The yields in the agricultural systems is strongly influenced by the soil microbial activity. The irrigation with saline water can increase the rates of exchangeable sodium in the soil which can affect negatively several biological, chemical and physical edaphic properties (ESPAÑA-GAMBOA et al., 2011). Although the addition of organic matter can reduce the negative effects of salinization (QADIR et al., 2007), as well as the plant genotypes, the bacterial community associated to the forage cactus (*Opuntia* spp. and *Nopalea* spp.) shows variation in the salt tolerance.

This bacteria can promote the plant growth by several mechanisms and the biological nitrogen fixation is the most studied. Several bacteria associated to the plants in saline soils are halo-tolerant and can promote the plant growth even under adverse conditions (GROVER et al., 2011).

1 Doutoranda em Zootecnia, Universidade Federal do Ceará, Fortaleza, Ceará, Brasil
2,3 Graduação em Ciências Biológicas, Universidade de Pernambuco, Petrolina, Pernambuco, Brasil
4 Universidade Federal do Tocantins, Araguaína, Tocantins, Brasil
5,6 Embrapa Semiárido, Petrolina, Pernambuco, Brasil

Nowadays, research efforts are been conducted to use the saline water resources as an alternative to reduce the water supply crisis, mainly in the arid and semi-arid environments. The irrigation with saline water can lead to high yields of forage palm (LIMA et al., 2015). But the impact of this irrigation systems on beneficial micro-organisms are not known.

Studies evaluating the infection of crop species by diazotrophic bacteria are scarce in the Brazilian Semi-arid region, mainly in the biossaline agricultural systems. The quantification and the characterization of the diazotrophic community are important principally to direct studies to the isolation, purification and selection of new diazotrophic bacteria (DÖBEREINER et al., 1995). In spite of its importance and adaptability to the biossaline agriculture systems, studies aiming the isolation and characterization of diazotrophic bacteria from forage cactus under saline water irrigation were not conducted in the Brazilian Semi-Arid. The aim of this study was evaluate the density, isolate and characterize phenotypically the putative diazotrophic bacteria isolated from roots of forage cactus (Opuntia stricta IPA-200016) irrigated with saline water in different irrigation depths.

Material and Methods

The harvest of forage palm was conducted at the Embrapa Semiárido (Petrolina, PE), at the Caatinga Experimental Field (09° 04'16"S, 40°19'05"W). The area was cropped with Opuntia stricta IPA-200016 (Mexican elephant ear type) for 14 months. The drip irrigation with four depths used were: L1: 100% ETo; L2: 75% ETo; L3: 50% ETo; L4: 25% ETo; e, L5: Control 0% ETo, where ETo is the reference evapotranspiration. All experimental area was supplied with bovine manure (3 ton ha^{-1}).

Three composite samples were collected in each irrigation depth. Each composite sample was performed of fine roots collected from three plants within the same irrigation regime. The samples were placed in plastic bags and transported to the Soil Microbiology Laboratory at the facilities of Embrapa Semiárido. The samples were storage at 10°C up to the processing.

The root samples were carefully washed with tap water and surface disinfected by immersion in NaClO (1% v/v) for 10 minutes followed by abundant wash with distilled and autoclaved water (DAW). An amount of 10 g of each sample were crushed with 90 mL of autoclaved NaCl (0.9% w/v) in a common blender, previously disinfected. The crushed samples were splitted in triplicates and serially diluted from 10^{-1} to 10^{-5} and 100 µL of each dilution were inoculated in tubes with 7 mL of BMGM semisolid medium (ESTRADA DE LOS SANTOS et al., 2001).

The tubes were incubated at 28°C for ten days and, after this period, the presence of the microaerophilic pellicule (MP) below the medium surface were observed. The data of the presence of MP in each dilution within were tabulated and used to calculate the density of putatively diazotrophic bacteria through the most probable number (MPN) method, according to Hungria and Araújo (1994). The quantitative data of the MPN of putative diazotrophic bacteria were analyzed by means of ANOVA followed by the Scott-Knott mean range comparison test. The data were analyzed using the Sisvar v 5.0 statistical package.

The tubes positive to the MP in the lowest concentrated samples were used to purification of the bacterial isolates. The samples were inoculated in Petri dishes with solid Dyg's medium (RODRIGUES NETO et al., 1986) and incubated in the dark at 28°C for four days. After the incubation period each colony with different morphological characteristics were purified in the same medium. To confirm the MP formation ability, pure cultures were inoculated in BMGM semisolid medium and incubated, as described above, for ten days. The isolates positive for MP formation were characterized according to their phenotypical characteristics in Dyg's medium.

For the phenotypic characterization, the bacterial isolates were streaked again in Dyg's medium and incubated, as described above, for four days. The phenotypic characteristics evaluated was the color, size (mm), elevation (flat or rised), shape (circular or irregular), presence of mucus (present or absent) and the transparency (translucent or opaque) of single colonies. The data was transformed in a binary matrix for construction of a similarity dendrogram applying the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) and the Bray-Curtis coefficient, using the PaSt statistical package (HAMMER et al., 2001).

Pure bacterial cultures were inoculated in liquid Dyg's medium with glycerol (25% v/v) and stored at the Culture Collection of Microorganisms with Agricultural Interests of Embrapa Semiárido (CMISA) at -80°C.

Results and Discussion

The total number of diazotrophic bacteria in roots of forage palm roots, estimated by means of MPN varied from 0.36 to 109.89 10^4 cells per grams (Table 1). The occurrence of total diazotrophic bacteria was higher at the irrigation depths with more saline water supply, mainly in the 80% ETo depth, with the average of 85.81 x 10^4 cells per gram of root (Figure 1).

The evaluation of plants from Caatinga already showed high colonization by potentially diazotrophic bacteria. High densities of putative diazotrophic bacteria was also found in roots of buffel grass (*Cenchrus ciliaris*) and andropogon (*Andropogon* sp.), Moreira et al. (2013) observed concentrations about 24 x 10^3 bacteria per gram of fresh roots in buffel grass and 46 x 10^3 bacteria per gram of fresh roots of *Andropogon* sp. Evaluating the concentration of potentially diazotrophic bacteria associated to Buffel grass in Paraíba state, Santos et al. (2013) observed concentrations about 4 to 44 x 10^3 bacteria per gram of roots. The authors affirmed that at the dry season, there are no potentially diazotrophic bacteria, differing from our results.

The plants of forage palm evaluated on the present study grew under constant irrigation. The availability of water was probably a contributing factor for the bacterial abundance. According to Baldani et al. (1999), the occurrence and activity of diazotrophic bacteria in soils and plant tissues are strongly influenced by abiotic factors, such as the soil humidity.

Moreira and Siqueira (2006) indicate that the water, along with the temperature, influence at the enzymatic activity in soils. The water availability is favorable to the microbial metabolism and bacterial proliferation. Studies conducted by Osaki and Netto (2009) and Lopes et al. (2011), indicated that in addition to the humidity, the quantity and quality of the organic matter in the soil are the main variables responsible for the dynamic of edaphic microorganisms.

All bacterial isolates that showed the pellicule formation were considered positive for diazotrophic capacity, and are putative diazotrophic bacteria. The nitrogenase enzymatic complex, present in all diazotrophic bacteria, is inhibited by high oxygen concentrations. This enzymatic characteristic is a paradox with the aerobic way of life of the bacteria and the microorganisms, and the bacteria developed several strategies to reduce the oxygen levels at rates feasible both for nitrogenese activity and bacterial primary metabolism (REIS; TEIXEIRA, 2005).

In the semisolid N free medium, diazotrophic associative bacteria reach a point in the medium column where the oxygen pressure allows both the bacterial development and the nitrogenase activity, forming a microaerophilic pellicule (biofilm) in the appropriated medium depth (BALDANI et al., 2014). In this case, the pellicule forming bacteria can be considered as potentially nitrogen-fixing bacteria, and were selected for further evaluations.

After the isolation, purification and confirmation of the pellicule formation ability, 58 bacterial isolates were isolated and characterized phenotypically. From the 58 isolates obtained in this study, 25 were obtained from the irrigation depth of 80% of ETo, and 18 from the 60% of ETo. At the similarity dendrogram, constructed from the data of the cultural diversity of the bacterial isolates in Dyg's medium, all bacterial isolates showed similarity about 40% (Figure 2). The isolates grouped in 24 clusters with 100% of similarity, which 14 were rare clusters, with only one isolate, indicating the high diversity of the culture collection. Isolating diazotrophic bacteria from "mandacaru" (*Cereus jamacaru*) from the Semi-arid region of Ceará state, Lima et al. (2015) observed the presence of nine different bacterial phenotypical groups. The higher number of phenotypical clusters observed in the present study indicated the great variability and genetic diversity of the bacterial isolates within our culture collection.

The table 2 shows the cultural characteristics of the bacterial isolates and was obtained from the similarity dendrogram. Regarding the phenotypical characteristics of the 58 isolates about 91.4% showed colony size higher than 2 mm. Bacteria showing colonies with heterogeneous aspect were 86.2% of the collection and the translucent cultures were a total of 93.1%. The color of bacterial cultures were yellow (44.8%), creamy (37.9%), white (8.6%), light pink (5.2%), green (1.7%) and brown (7.7%). Only two isolates did not produced mucus and the majority of the collection (93.1%) produced little mucus quantity.

According to Compant et al. (2010) the soil bacteria are attracted or inhibited by the plants by the root exudation. Plants that receive more water probably can release distinguish compounds allowing the colonization by different bacterial taxonomic and ecological clusters. Plant-bacterial interactions occur by means of several ecologic processes with divergent physical proximity. The endophytic bacteria, as the isolates in the present study, colonizes the inner part of plant tissues, where there is a favorable environment to promote the plant growth by several mechanisms, such as the BNF (BACON; HINTON, 2006). The plant growth promoting bacteria are a beneficial and heterogeneous group of microorganisms. In below-ground interactions, they are found in the rhizosphere, in the surface or inside the roots. They are able to increase the plant growth and also to protect them of biotic and abiotic stresses (GROVER et al., 2011; GLICK, 2012). The mechanisms that these microorganisms use to stimulate the plant growth are also strongly influenced by the environmental conditions, such as the irrigation regime applied (JOHRI, 2006; GROVER et al., 2011).

Around 1% of the plants are able to develop and reproduce in saline soils (MANOUSAKI; KALOGERAKIS, 2011). These distinguishable halophytes plants, are able to develop in soils with

more than 200 mmol dm^{-3} of NaCl (FLOWERS; COLMER, 2008). Among the plants with this desirable characteristic, the forage palm can be pointed out but the diversity and role of the microbial community associated to the forage palm under salinity conditions until remains unknown.

In the last few years, the scientific interest in the plant-microbe association is increasing worldwide. Studies about the composition and function of diazotrophic community colonizing several crops are been conducted, but for forage palm the results are still very scarse. To our knowledge this is the first report of the association of forage palm and diazotrophic bacteria under irrigation with saline water in Brazilian Semi-Arid.

Table 1. Most probable number (MPN) and confidence interval (95%) of the number of diazotrophic bacteria in roots of forage palm (*Opuntia stricta* IPA-200016).

Irrigation deepth	Sample	Confidence Interval		MPN
		Min	Max	
L0 1	AM1	9.12	139.55	$23.97 \times 10^{4\,b}$
L0 1	AM2	0.28	3.77	$0.91 \times 10^{4\,e}$
L0 1	AM3	0.22	2.76	$0.77 \times 10^{4\,e}$
L0 2	AM1	3.33	38.55	$9.32 \times 10^{4\,c}$
L0 2	AM2	0.535	4.95	$1.46 \times 10^{4\,e}$
L0 2	AM3	0.087	2.05	$0.36 \times 10^{4\,e}$
L20 1	AM1	17.83	240.76	$46.20 \times 10^{4\,b}$
L20 1	AM2	5.56	50.58	$14.93 \times 10^{4\,c}$
L20 1	AM3	8.65	64.06	21.47×10^{4b}
L20 2	AM1	9.12	139.55	$23.97 \times 10^{4\,b}$
L20 2	AM2	6.48	42.50	$15.87 \times 10^{-4\,d}$
L20 2	AM3	12.54	79.22	$29.17 \times 10^{4\,b}$
L40 1	AM1	9.12	139.55	$23.97 \times 10^{4\,b}$
L40 1	AM2	17.83	240.76	$46.20 \times 10^{4\,b}$
L40 1	AM3	12.54	79.22	$29.17 \times 10^{4\,b}$
L40 2	AM1	17.83	240.76	$46.20 \times 10^{4\,b}$
L40 2	AM2	9.12	139.55	$23.97 \times 10^{4\,b}$
L40 2	AM3	17.83	240.76	$46.20 \times 10^{4\,b}$
L60 1	AM1	8.65	64.06	$21.47 \times 10^{4\,b}$
L60 1	AM2	38.22	478.76	$109.84 \times 10^{4\,a}$
L60 1	AM3	9.12	139.55	$23.97 \times 10^{4\,b}$
L60 2	AM1	17.83	240.76	$46.20 \times 10^{4\,b}$
L60 2	AM2	8.65	64.06	$21.47 \times 10^{4\,b}$
L60 2	AM3	12.54	79.22	$29.17 \times 10^{4\,b}$
L80 1	AM1	38.22	478.76	$109.84 \times 10^{4\,a}$
L80 1	AM2	38.22	478.76	$109.84 \times 10^{4\,a}$

Cont.Table 1. Most probable number (MPN) and confidence interval (95%) of the number of diazotrophic bacteria in roots of forage palm (*Opuntia stricta* IPA-200016).

L80 1	AM3	38.22	478.76	109.84 x 10^{4} [a]
L80 2	AM1	17.83	240.76	46.20 x 10^{4} [b]
L80 2	AM2	12.54	79.22	29.17 x 10^{4} [b]
L80 2	AM3	38.22	478.76	109.84 x 10^{4} [a]

Averages with the same letter do not differ by the Scott-Knott range test (p<0.05)

Table 2. Phenotypic characteristics of the 24 clusters of putative diazotrophic bacterial isolates from forage palm (*Opuntia stricta* IPA-200016) clustered throughout their phenotypical characteristics.

Group (Number of isolates)[1]	CS[2]	CSh[3]	CA[4]	CT[5]	CC[6]	MP[7]	MQ[8]
G1(1)	>2	IR	HE	TR	BR	No	-
G2(1)	>2	IR	HE	TR	CR	Yes	MM
G3(1)	>2	IR	HO	OP	BR	No	-
G4(1)	>2	IR	HE	OP	BR	Yes	PM
G5(1)	<2	CI	HO	TR	BR	Yes	PM
6(1)	>2	CI	HO	OP	BR	Yes	MM
G7(1)	>2	CI	HO	OP	CR	Yes	PM
G8(1)	>2	IR	HO	TR	VE	Yes	PM
G9(2)	>2	IR	HO	TR	AM	Yes	PM
G10(1)	>2	CI	HO	TR	AM	Yes	PM
G11(3)	<2	CI	HE	TR	AM	Yes	PM
G12(1)	<2	IR	HR	TR	AM	Yes	PM
G13(7)	>2	CI	HE	TR	AM	Yes	PM
G14(6)	>2	IR	HE	TR	CR	Yes	PM
G15(1)	>2	CI	HE	TR	RO	Yes	MM
G16(2)	>2	CI	HE	TR	RO	Yes	PM
G17(7)	>2	IR	HE	TR	AM	Yes	MM
G18(1)	>2	CI	HE	TR	CR	Yes	MM
G19(3)	>2	CI	HE	TR	AM	Yes	PM
G20(1)	>2	IR	HE	TR	MA	Yes	PM
G21(1)	>2	IR	HE	TR	CR	Yes	PM
G22(9)	>2	IR	HE	TR	CR	Yes	PM
G23(3)	>2	IR	HE	TR	CR	Yes	MM
G24(2)	>2	CI	HE	TR	AM	Yes	MM

1Phenotypical cluster (number of bacterial isolates); 2CS – colony size (> 2 mm or <2 mm); 3CSh-colony shape (Ci: circular, Ir: irregular); 4CA-colony aspect (Ho: homogeneous, He: heterogeneous); 5CT-colony transparency (Op: opaque, Tr: translucent) 6CC – Colony color (Am: yellow; Br: white; Cr: creamy; Ro: light pink; Ve: green; Ma: brown) 7MP-Mucus production; 8 MQ-mucus quantity (MM: much mucus, PM: little mucus).

Figure 1. Densitry of putative diazotrophic bacteria in the roots of forage palm (*Opuntia stricta* IPA-200016) grown under the irrigation with diferent depths of saline water. L80: 80% ETo; L60: 60% ETo; L40: 40% ETo; L20: 20% ETo; e, L0: Control 0% Eto.

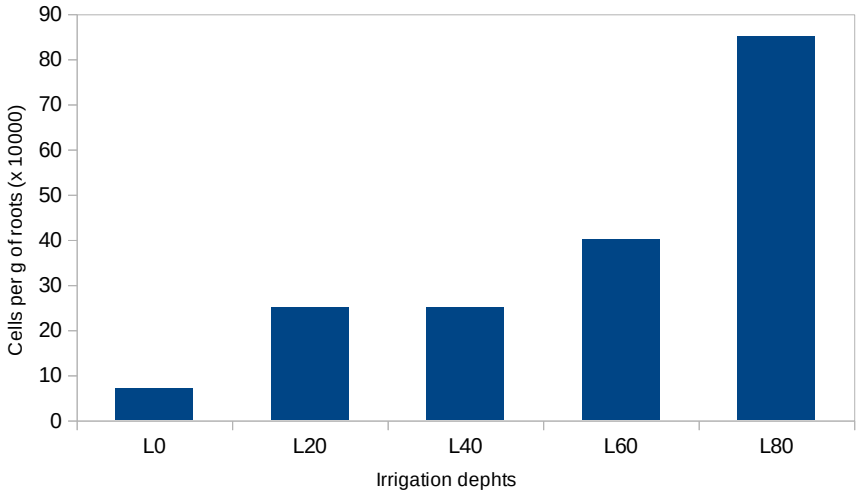
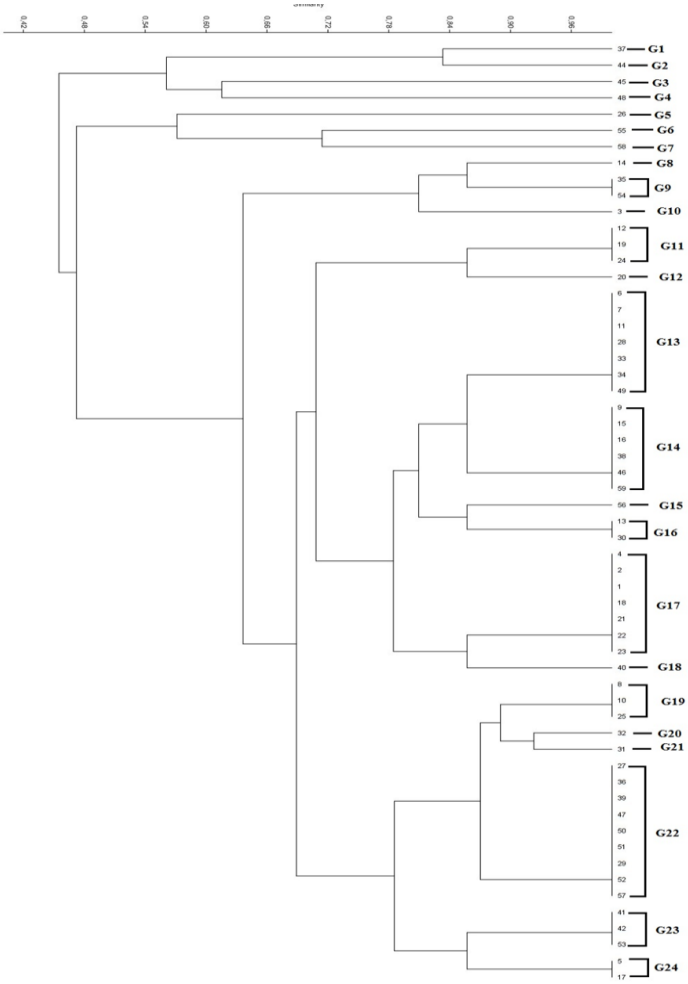


Figure 2. Similarity dendrogram based on the phenotypical characteristics of 58 putative diazotrophic bacteria obtained from forage palm (*Opuntia stricta* IPA-200016) irrigated with different amounts of saline water. UPGMA clustering method, Bray-Curtis similarity index.



Conclusions

The forage palm irrigated with saline water is densely colonized by a great diversity of putative diazotrophic endophytic bacteria in Brazilian Semi-Arid. The supply with saline water are favorable to the colonization of forage palm with the endophytic bacteria.

Acknowledgement

To the Brazilian Council for Scientific and Technological Development (CNPq), Brazilian Agricultural Research Corporation (Embrapa) and To the Coordination of Improvement of Higher Education Personnel (CAPES) for the financial support.

References

ARAUJO, G.G.L., VOLTOLINI, T.V., CHIZZOTTI, M.L., TURCO, S.H.N., CARVALHO, F.F.R. Water and small ruminant production. Brazilian Journal of Animal Science, Piracicaba, v. 39, 326-336, 2010.

BACON, C.W., HINTON, D.M. AND HINTON JR, A. Growth-inhibiting effects of concentrations of fusaric acid on the growth of Bacillus mojavensis and other biocontrol *Bacillus species*. Journal of Applied Microbiology v. 100, n.1, p.185-194, 2006

BALDANI, J.I.; AZEVEDO, M.S.; REIS, V.M.; TEIXEIRA, K.R.S.; OLIVARES, F.L.; GOI, S.R.; BALDANI, V.L.D. & DÖBEREINER, J. Fixação biológica de nitrogênio em gramíneas: avanços e aplicações. In: SIQUEIRA, J.O.; MOREIRA, F.M.S.; LOPES, A.S.; GUILHERME, L.R.G.; FAQUIN, V.; FURTINI NETO, A.E. & CARVALHO, J.G., eds. Inter-relação fertilidade, biologia do solo e nutrição de plantas. Viçosa, SBCS/UFLA/DCS p.621-666, 1999.

BALDANI, J.I.; REIS, V.M.; VIDEIRA, S.S.; BODDEY, L.H.; BALDANI, V.L.D. The art of isolating nitrogen-fixing bacteria from non-leguminous plants using N-free semi-solid media: a practical guide for microbiologists. Plant and Soil, v.384, p.413-431, 2014.

COMPANT, S.; CLEMENT, C.; SESSITSCH, A. Plant growth-promoting bacteria in the rhizo-and endosphere of plants: Their role, colonization, mechanisms involved and prospects for utilization. Soil Biology and Biochemistry, Oxford, v.42, p.669-678, 2010.

DOBEREINER, J.; BALDANI, V.L.D.; BALDANI, J.I. Como isolar e identificar bactérias diazotróficas de plantas não leguminosas. Brasília: Embrapa, SPI, 60p., 1995.

ESPAÑA-GAMBOA, E.; MIJANGOS-CORTES, J.; BARAHONA-PEREZ, L.; DOMINGUEZ-MALDONADO, J.; HERNÁNDEZ-ZARATE, G.; ALZATEGAVIRIA, L. Vinasses: characterization and treatments. Waste Management & Research, v.29, p.1235-1250, 2011.

ESTRADA DE LOS SANTOS, P.; BUSTILLOS-CRISTALES, R.; ABALLEROMELLADO, J. Burkholderia a genus rich in plant-associated nitrogen fixers with wide environmental and geographic distribution. Applied and Environmental Microbiology, v.67, p.2790-2798, 2001.

FLOWERS, T.J.; COLMER, T.D. Salinity tolerance in halophytes. New Phytol. v. 179, p.945-950, 2008.

GLICK, B.R. Plant growth-promoting bacteria: mechanisms and applications. Hindawi Publishing Corporation, Scientifica, 2012.

GROVER, M.; ALI, S.Z.; SANDHYA, V.; RASUL, A.; VENKATESWARLU, B. Role of microorganisms in adaptation of agriculture crops to abiotic stresses. World Journal of Microbiology and Biotechnology v. 27, n.5, p.1231-1240, 2011.

HAMMER, O.; HARPER, D. A. T.; RYAN, P. D. PAST: Paleontological statistics software package for education and data analysis. Palaeontologia Electronica, Boulder, v. 4, n. 1, p. 1-9, 2001.

HUNGRIA, M.; ARAUJO, R. Manual de métodos empregados em estudos de microbiologia agrícola. Brasília, Empresa Brasileira de Pesquisa Agropecuária, 542p., 1994.

JOHRI, B.N. Endophytes to the rescue of plants. Current Science v. 90, n.10, p.1315-1316, 2006.

LIMA, G. F. C.; RÊGO, M. M. T.; AGUIAR, E. M. I.; SILVA, J.G.M.; DANTAS, F.D.G.; GUEDES, F.X.; LÔBO, R.N.B. Effect of different cutting intensities on morphological characteristics and productivity of irrigated Nopalea forage cactus. In: International Congress on Cactus Pear and Cochineal, 8., 2013. Palermo: ISHS Acta Horticulturae, v. 1067. p. 253-258, 2015.

LIMA, J.V.L.; WEBER, O.B.L.; CORREIA, D.; SOARES, M.A.; SENABIO, J.A. Endophytic bacteria in cacti native to a Brazilian semi-arid region. Plant and Soil n.389: p.25–33, 2015.

LOPES, E. L. N. et al. Microbial biomass and soil chemical properties under different land use systems in northeastern Pará. Revista. Brasileira de Ciência do Solo, v. 35, n. 4, p. 1127-1139, 2011.

MANOUSAKI, E.; KALOGERAKIS, N. Halophytes present new opportunities in phytoremediation of heavy metals and saline soils. Industrial e

Engineering Chemistry Research, v. 50, p.656-660, 2011.

MISHRA, R. R.; PRAJAPATI, S.; DAS, J.; DANGAR, T. K.; DAS, N.; THATOI, H. Reduction of selenite to red elemental selenium by moderately halotolerant Bacillus megaterium strains isolated from Bhitarkanika mangrove soil and characterization of reduced product. Chemosphere, v.84, p.1231-1237, 2011.

MOREIRA, F. M. S.; SIQUEIRA, J. O. Microbiologia e Bioquímica do Solo. 2 ed. Lavras, UFLA, 2006, 729 p.

MOREIRA, F. T. A., SANTOS, D. R., SILVA, G. H., ALENCAR, L. S. Ocorrência De Bactérias do Gênero Azospirillum spp. associadas a gramíneas forrageiras no Semiárido Nordestino. HOLOS, Ano 29, Vol. 3. 2013.

OSAKI, F.; NETTO, S. P. Flutuação da população de bactérias sob floresta ombrófila mista e povoamento de Pinus taeda L. Floresta, v. 39, p. 845-852, 2009.

REIS, V.M.; TEIXEIRA, K.R.S. Fixação biológica do nitrogênio - Estado da arte. In: Aquino AM, Assis RL, editores. Processos biológicos no sistema solo-planta: ferramentas para uma agricultura sustentável. Brasília: Embrapa Informação Tecnológica, p.350-68, 2005.

RODRIGUES NETO, J.; MALAVOLTA, J.R.; VICTOR, O. Meio simples para isolamento e cultivo de Xanthomonas campestris pv. citri tipo B. Suma Phytopathologica, v.12, p.16, 1986.

SANTOS, M.C.M.; SANTOS, D.R.; BAKKE, O.A.; BAKKE, I.A. Ocorrência e Atividade de Bactérias Diazotróficas em Forrageiras cultivadas na região Semiárida no Brasil. Revista Caatinga, v. 26, n. 1, p. 27-34, 2013.

QADIR, M.; OSTER, J. D.; SCHUBERT, S.; NOBLE, A. D.; SAHRAWAT, K. L. Phytoremediation of sodic and saline-sodic soils. Advances in Agronomy, v.96, p.197-247, 2007.