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Lozada, Jorge Avelino Rodriguez; Silveira, Klever Cristiano; Silva, Libério Junio da; Baldotto, Marihus Altoé; Baldotto, Lílian Estrela Borges

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Selection of diazotrophic bacteria isolated from wastewater treatment plant sludge at a poultry slaughterhouse for their effect on maize plants[1]

*Jorge Avelino Rodriguez Lozada[2], Klever Cristiano Silveira[2] , Libério Junio da Silva[2], Marihus Altoé Baldotto[2], Lílian Estrela Borges Baldotto[2]**

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ABSTRACT

The economic and environmental costs of nitrogen fertilization have intensified the search for technologies that reduce mineral fertilization, for example atmospheric nitrogen-fixing (diazotrophic) bacteria inoculation. In this context, the present study addressed the isolation and quantification of diazotrophic bacteria in the sludge from treated wastewater of a poultry slaughterhouse; a description of the bacteria, based on cell and colony morphology; and an assessment of growth and N content of maize plants in response to inoculation. Sixteen morphotypes of bacteria were isolated in six N-free culture media (JMV, JMVL, NFb, JNFb, LGI, and LGI-P). The bacteria stained gram-positive, with 10 rod- and six coccoid-shaped isolates. To evaluate the potential of bacteria to promote plant growth, maize seeds were inoculated. The experiment consisted of 17 treatments (control plus 16 bacterial isolates) and was carried out in a completely randomized design with six replicates. The experimental units consisted of one pot containing two maize plants in a greenhouse. Forty-five days after planting, the variables plant height, leaf number, stem diameter, root and shoot fresh and dry weight, and N content were measured. The highest values were obtained with isolate UFV L-162, which produced 0.68 g total dry matter per plant and increased N content to 22.14 mg/plant, representing increments of 74 and 133%, respectively, compared with the control. Diazotrophs inhabit sludge from treated wastewater of poultry slaughterhouses and can potentially be used to stimulate plant development and enrich inoculants.

Key words: *Zea mays* L.; plant growth-promoting bacteria; nitrogen; solid waste.

RESUMO

Seleção de bactérias diazotróficas isoladas de estação de tratamento de efluentes de abatedouro de aves e seu efeito em plantas de milho

Os custos econômico-ambientais gerados pela fertilização nitrogenada têm incrementado o interesse em tecnologias que diminuam a aplicação de fertilizantes minerais, como por exemplo, a inoculação de bactérias fixadoras de nitrogênio atmosférico, também denominadas diazotróficas. Nesse contexto, o presente trabalho objetivou: (i) isolar e quantificar bactérias diazotróficas do lodo gerado em estação de tratamento de efluentes de abatedouro de aves; (ii) caracterizar as bactérias de acordo com a morfologia celular e morfologia da colônia e (iii) avaliar o crescimento e conteúdo de N em plantas de milho em resposta à inoculação. Foram isolados 16 morfotipos de bactérias nos seis meios de cultivo sem adição de nitrogênio JMV, JMVL, NFb, JNFb, LGI e LGI-P. As bactérias apresentaram coloração gram positiva, sendo dez isolados com formato de bastonete e seis com formato de cocos. Para avaliar o potencial de promoção do crescimento de plantas realizou-se a inoculação em sementes de milho. O experimento, conduzido em casa-de-vegetação, consistiu de 17 tratamentos (controle e 16 isolados bacterianos), em delineamento inteiramente casualizado, com seis repetições

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[2] Universidade Federal de Viçosa, Campus Florestal, Rodovia LMG 818, km 06, 35690-000, Florestal, Minas Gerais, Brasil. jarodriguezlo28@gmail.com; klevercris@gmail.com; liberiojunior@gmail.com; marihus@ufv.br; lilian.estrela@ufv.br
*Corresponding author: lilian.estrela@ufv.br

e a unidade experimental foi um vaso contendo duas plantas de milho. Aos 45 dias após o plantio, foram mensuradas as variáveis: altura da planta, número de folhas, diâmetro do caule, matéria fresca e seca da raiz e parte aérea e conteúdo de N. Os maiores valores foram obtidos com o isolado UFV L-162, proporcionando matéria seca total de 0,68 g/planta e um conteúdo de N de 22,14 mg/planta, representando incrementos em relação ao controle de 74% e 133%, respectivamente. Conclui-se que bactérias diazotróficas habitam o lodo das estações de tratamento de efluentes de abatedouro de aves e possuem potencial para uso como estimulantes do desenvolvimento vegetal e enriquecimento de inoculantes.

Palavras-chave: *Zea mays* L.; bactérias promotoras do crescimento de plantas; nitrogênio; resíduos sólidos.

INTRODUCTION

The high economic and environmental costs resulting from nitrogen fertilization have stimulated research for strategies to reduce mineral fertilization without compromising crop yields (Guimarães, 2011). Besides plant breeding with a view to developing cultivars more adaptable to low fertility soils and unfavorable agro-environmental conditions, biotechnological studies with plant growth-promoting bacteria (PGPB) have also focused on the biodiversity and bioprospection of new microorganisms (Mitter *et al*., 2013).

Plant growth-promoting bacteria stimulate plant growth by direct mechanisms, e.g., nitrogen fixation, phosphate solubilization, and synthesis of growth regulators, and by indirect mechanisms such as the production of siderophores and allelochemicals, biological control, and induction of local and systemic resistance (Hallmann *et al*., 1997).

Biological nitrogen fixation (BNF) is one of the actions of PGPB and is performed by means of bacteria known as diazotrophs, which are free-living or associated with plant tissues (Hallmann *et al*., 1997; Baldotto *et al*., 2011). The process of BNF is essential to transform N_2, a stable and abundant molecule in the atmosphere that cannot be directly used by plants, into the inorganic form NH_3 and, subsequently, into organic and inorganic forms which can be used in biological systems. The reduction reactions from N_2 to NH_3 are mediated by microorganisms containing the enzyme nitrogenase (Kim & Rees, 1994).

Knowledge about the relationship between nitrogen-fixing bacteria and maize plants is extremely interesting, since maize is one of the three most important crops in Brazil and around the world. In addition to its economic aspect, this crop is vitally important in social and political contexts (Oliveira *et al*., 2007). Interactions between PGPB and the maize environment have been demonstrated in several studies and by various authors (Kappes *et al*., 2013; Baptista *et al*., 2011; Ikeda *et al*. 2010), with promising results in the search for economic and sustainable fertilization systems.

Nitrogen-fixing bacteria have been isolated from different sources, for example, from plant organs (Grayston *et al*., 1998), soils (Nóbrega *et al*., 2004), and organic residues (Aguiar, 2012). The sludge generated in wastewater treatment plants of poultry slaughterhouses has high microbial diversity (Bettiol & Fernandes, 2004) and can also harbor diazotrophs. This sludge is defined as a mixture of substances that generally contains high amounts of mineral colloids and particles from decomposed organic matter suspended in the aqueous medium (CONAMA, 2006).

Given the above, this study addressed the isolation of diazotrophic bacteria from sludge of treated wastewater from a poultry slaughterhouse and quantification in different culture media; the characterization of the bacteria, based on cell and colony morphology; and the selection of bacterial isolates promoting maize growth in a greenhouse.

MATERIAL AND METHODS

Collection and characterization of sludge samples

Samples of poultry slaughterhouse sludge, a waste (composted of blood, viscera, tissues, feathers, and bones) generated in the effluents from treatment plants of the activated sludge/extended aeration type, were provided by the company Francap SA (Francap, 2015). Samples were collected from the sludge generated at the end of the treatment process and transported to the Campus Florestal of the Universidade Federal de Viçosa (UFV), where microbiological analysis were carried out in 2013 and 2014. The chemical properties of the sludge were determined as: N = 9.55, P = 1.21, K = 0.45, Ca = 1.35, Mg = 0.18, S = 0.77, and CO = 18.72 dag/kg; Zn = 517, Fe = 1908, Mn = 114, Cu = 342, and B = 8.3 mg/kg; pH = 6.7; and C/N = 1.96.

Isolation and quantification of diazotrophs

The diazotrophic bacteria were isolated as described by Döbereiner *et al*. (1995). Samples of 10 g sludge were diluted in 90 mL saline solution (0.85 g L^{-1} NaCl) and from this dilution (10^{-1}), serial dilutions were performed to 10^{-6}

dilution. Aliquots of 100 μL of the different dilutions were transferred in triplicate to glass bottles containing 5 mL of semi-solid N-free culture media JNFb, NFB, LGI, LGI-P, JMV, and JMVL. The formation of a typical aerotaxic film on the surface of the medium incubated for seven days in a growth chamber at 30 °C was considered a positive result. The bacteria in the culture medium were counted by the most probable number (MPN) technique, using the McCrady table for three replicates per dilution. The results were log-transformed, and the mean and standard error of the mean were calculated for each treatment.

Cell characterization and bacterial colonies

After isolation, the bacteria were grown in liquid DYGS medium for 24 h at 30 ºC with agitation at 120 rpm and placed on Petri plates containing solid DYGS medium (Döbereiner *et al*., 1995). The trays were maintained in a bacteriological incubator at 30 °C for seven days. The resulting colonies were characterized based on the cell characteristics (shape and gram staining) and the characteristics of the colonies (shape, color, size, elevation, edge, surface, and mucus) (Perin, 2003; Lozada *et al.*, 2017). Each bacterial isolate was named by the following scheme: UFV L-ABC, in which UFV = Federal University of Viçosa; L = sludge; A = isolation culture medium (1 = JMV, 2 = JMVL, 3 = NFb, 4 = JNFb, 5 = LGI, 6 = LGI-P); B = dilution; and C = number of the isolate in the collection.

Selection for nitrogen-fixing, maize growth-promoting bacteria

The bacterial selection experiment consisted of 17 treatments, 16 bacterial isolates, and one control (no inoculation), performed in a greenhouse in a completely randomized design with six replicates, based on experimental units of one pot containing two maize plants.

To obtain the pre-inoculum, bacteria were grown in 5-mL liquid DYGS medium for 24 h at 30 °C and 120 rpm. Then, the pre-inoculum was poured into Erlenmeyer flasks containing 200 mL liquid DYGS medium. The flasks were shaken for 24 h at 120 rpm at 30 °C to obtain the inoculum. Inoculation consisted of immersion of maize seeds (*Zea mays* L. variety AG1051) for 2 h in the flask, followed by application of the bacterial medium to the substrate. The control was immersed in autoclaved liquid DYGS. Subsequently, the seeds were transferred to 0.7-dm^3 plastic pots containing soil. The soil used was collected near the UFV-CAF dryer (geographic coordinates: 19º87’53.91’’ S and 44º42’26.55'’ W), which is classified as subsurface horizon of Dystrophic Red Latosol (Embrapa, 2013), whose chemical analysis presented: pH: 4.1; P: 6.8 and K: 78 mg/dm^3; Ca: 0.6, Mg: 0.3, Al: 1.4, H+Al: 7.59, SB: 1.10, $CEC_{(t)}$: 2.50, and $CEC_{(T)}$: 8.69 $cmol_c/dm^3$; V: 13; and m: 56%. Liming and fertilization were not carried out to perform the selection of diazotrophic bacteria in dystrophic environment. The pots were kept in greenhouse, monitored daily throughout the experiment, and the irrigations aimed at maintaining the field capacity between 80 and 100%. The other variables, such as weed control, phytosanitary treatments, and luminosity, were controlled and maintained equal and constant for all treatments.

Forty-five days after planting, the plants were harvested for the measurement of the variables: plant height (PH), stem diameter (SD), number of leaves (NL), root fresh weight (RFM), shoot fresh weight (SFM), total fresh weight (TFM), root dry matter (RDM), shoot dry matter (SDM), and total dry matter (TDM), determined by oven-drying by forced-air ventilation at 65 °C for 72 h, followed by weighing on a precision scale.

Nitrogen concentrations were determined by the Kjeldahl method in the Soil Analysis Laboratory Viçosa Ltd., in three replicates per treatment (replicates with highest TDM) after sulfuric acid digestion of leaves and roots. The N contents were estimated by multiplying the TDM content by the N concentration.

Statistical analysis

The data were subjected to analysis of variance using program R, and means were compared by the Tukey test at 5% probability.

RESULTS AND DISCUSSION

Isolation and quantification of diazotrophs

The bacterial isolation using different N-free culture media resulted in the isolation of 16 diazotrophic strains from sludge of treated wastewater from poultry slaughterhouses (Table 1).

In a comparison of the number of isolates of diazotrophs from poultry slaughterhouse sludge (n = 16) with the results of Bergamaschi *et al*. (2007), consisting of 76 strains detected in sorghum plants, or with that of Ikeda *et al*. (2010), who isolated 217 endophytic bacteria from maize plants, the number was lower in the present study. This may be explained by effect of symbiosis among plant, bacteria, and soil (Grayston *et al*., 1998). Other studies corroborated this hypothesis, since in biogeographic studies of bacteria cultured from plants such as maize (Roesch *et al*., 2005; Gomes *et al*., 2010), rice (Ferreira *et al*., 2003), and sugarcane (Perin, 2003), a higher number of N-fixing bacteria was found in roots than in shoots, or in environments involving interaction with soil.

The highest amount of diazotrophs was observed in the JMV medium (log MPN/g sludge = 7.15) and lowest in the JMVL medium (log MPN/g sludge = 6.46) (see Table 1). These results are similar to the findings of Milani *et al*. (2011) in sugarcane. These authors isolated bacteria from soil, roots, and leaves, in amounts ranging from 5.46 to

7.28 log MPN/g plant. In grasses and sugarcane, respectively, Reis *et al*. (2000) and Perin (2003) obtained similar results regarding the number of bacteria in poultry slaughterhouse sludge. The large number of these bacteria in the sludge can be explained by the type of treatment of the activated sludge, in which a continuous injection of air allows the proliferation of aerobic microorganisms (Sobrinho, 1983), e.g., N-fixing bacteria.

A current fact in research for prospective diazotrophs is the variability of results in relation to the richness and abundance of species. Low richness and high abundance were found in the poultry slaughterhouse sludge. In other cases, for example, in an investigation of tropical fruits (Santos, 2008), both species richness and abundance were high and season-related.

In the study by Melloni *et al*. (2004), 36 diazotrophic bacterial strains were isolated from soil recovered after mining. The authors found a wide variation in the log MPN per g of soil, ranging from 0 to 12. The authors attributed the low values to the low content of organic matter in the soil, as well as to the low levels of K and P and low base saturation. This result is contrary to our findings, since the microbial abundance in sludge from poultry slaughterhouses was high, which can be attributed in part, to the high organic matter content. In this sludge, microorganisms find nutritional conditions favorable for growth. Our results indicate that diazotrophs naturally inhabit sludge from wastewater treatment plants of poultry slaughterhouses in a number exceeding 4.6 x 10^7 cells per gram of sludge.

Cell characterization and bacterial colonies

Of the 16 samples analyzed (Table 2), the colony diameter of 50% of the bacterial isolates was equal to or greater than 1 mm. In terms of color, hues varied from milky-white to milky yellow. The other morphological characteristics of the colonies were as follows: 100% had a round shape and smooth surface; 75% entire and 25% undulated edges; and for the top, the colonies were 50, 30, and 20% for lense, flat, and convex, respectively.

The cell characterization (Table 3) showed that from all isolates stained gram-positive, 62.5% were rod-shaped and 37.5% coccoid-shaped. This was similar to the results of Faria *et al*. (2009), in which 77 and 61% of strains isolated from sewage sludge from two treatment plants were gram-positive. In contrast, Nóbrega *et al*. (2004) found that the 72 diazotrophic isolates from bacterial soil recovered after mining were gram-negative bacteria and with higher phenotypic variability. In summary, diazotrophic gram-positive bacteria with a rod shape predominate in the sludge generated in the wastewater treatment plants of poultry slaughterhouses, forming colonies with different morphological characteristics.

Test for selection of nitrogen-fixing bacteria in maize plants

Diazotrophic inoculation in *Zea mays* plants had a positive effect on the variables RFM, TFM, and N content compared with the control (Table 4). For the variables PH, SD, NL, SFM, SDM, RDM, and TDM, there was no difference between control and inoculation treatments (Table 4).

Similar results for plant height were verified by Lana *et al*. (2012), evaluating the responses of maize to *Azospirillum* inoculation associated with nitrogen fertilization. Dartora *et al*. (2013) also verified that maize plant height was not influenced by the inoculation with *A. brasilense* and *H. seropedicae*; however, contrary to the result shown here, the author noticed a positive effect of diazotrophic inoculation on the basal diameter of the stem.

Among 16 diazotrophic isolates evaluated, only three (UFVL-162, UFVL-163, and UFVL-164) differed from control in some variable (Table 4). The absence of response of maize plant to the inoculation of 13 isolates may be related to several factors such as the vulnerability of these microorganisms to the environment (Moreira *et al*., 2010) and absence of interaction with the host plant genotype (Roesch *et al*., 2005).

The bacterial isolate UFVL-163 induced the highest accumulation of TFM, with an increase of 27% over the control. These data confirmed the results of Perin *et al*. (2003), in which the bacterial species *Herbaspirillum seropedicae* and *Azospirillum brasilense* increased TFM of maize plants by 2 to 28%. Maize is frequently used for silage production,

Table 1: Number of diazotrophic bacterial isolates per culture medium with the respective dilution and MPN (average of three replications followed by the mean standard error)

Culture medium	Number of isolates	Dilution	log MPN/g of sludge
JMV	4	10^{-6}	7.15 ± 0.00
JMVL	2	10^{-6}	6.46 ± 0.29
NFB	4	10^{-6}	6.95 ± 0.15
JNFB	3	10^{-5}	6.48 ± 0.09
LGI	1	10^{-6}	7.11 ± 0.03
LGI-P	2	10^{-6}	7.04 ± 0.00

MPN - most probable number.

as it has better qualities for feed than other species. The use of PGPB in maize destined for silage can be a viable alternative means of producing feed for animal species raised in confined and semi-confined systems or for periods with low forage production (Matte, 2014).

Inoculation with isolate UFV-164 increased RDM by 91% compared with that of the control. This result was better than the cumulative root biomass increase of 3 to 17% reported by Perin *et al*. (2003) in studies with *Zea mays* L. after inoculation with diazotrophs (*H. seropedica* and *A. brasilense*). By increasing root biomass production, the plants can become more efficient in absorbing nutrients and water from the soil. Consequently, this is reflected in greater shoot biomass, better plant health, and higher yield.

The highest N contents in maize plants were induced by inoculation with isolate UFVL-162, corresponding to an increase of 134% over the control. An increase of 41% in maize N content was observed by Obando *et al*. (2013) after inoculation with Azotobacter. Similarly, Reyes *et al*. (2008) observed an 88% increase in N content in maize plants inoculated with rhizobacteria.

The present work aimed to select the diazotrophic bacteria in a dystrophic environment, the same strategy used by Inagaki (2014) when evaluating the influence of soil pH on the colonization of maize plants by *A. brasilense* and *H. seropedicae*. The author concluded that acid pH (4.5, 5.0, and 5.5) had no influence on the population of these two diazotrophic bacteria in maize plants evaluated at 41 days after sowing. However, Baldotto *et al*. (2012) observed that the population of diazotrophic bacteria in maize plants evaluated at 30 days after sowing can be favored with the application of 25 to 75% of NPK recommended dose.

This study demonstrated the colonization capacity of diazotrophic bacteria isolated from environments of residues such as treated sludge from a poultry slaughterhouse. This sludge can be applied to environmental restoration processes and as raw material for manufacturing substrates and biofertilizer (Santos *et al*., 2014), and can be exploited as a source of microbial diversity for studies of bacteria with potential for degrading organic substances (Lozada *et al*., 2017) and plant growth-promoting bacteria. In conclusion, bacterial isolates from sludge of poultry slaughterhouses, in particular UFVL-162, UFVL-163, and UFVL-164, promote the growth and development of maize plants.

Table 3: Cell characterization of bacterial isolates from treated sludge of poultry slaughterhouse wastewater

Isolate	Shape	Gram staining
UFV L-161	Rod	+
UFV L-162	Rod	+
UFV L-163	Rod	+
UFV L-164	Rod	+
UFV L-261	Coccoid	+
UFV L-262	Coccoid	+
UFV L-361	Coccoid	+
UFV L-362	Coccoid	+
UFV L-363	Coccoid	+
UFV L-364	Rod	+
UFV L-451	Rod	+
UFV L-452	Rod	+
UFV L-453	Rod	+
UFV L-562	Coccoid	+
UFV L-661	Rod	+
UFV L-662	Rod	+

Table 2: Characterization of colonies of bacterial isolates from treated sludge of poultry slaughterhouse wastewater in specific media

Isolate	Color	Size	Top	Shape	Edge	Surface	Mucu
UFV L-161	Milk-white center	<1 mm	Lense	Round	Straight	Smooth	+
UFV L-162	Clear white center, white and translucent edge	<1 mm	Flat	Round	Undulated	Smooth	+
UFV L-163	Milk-white center, white and translucent edge	1 mm	Lense	Round	Straight	Smooth	+
UFV L-164	White center, white and translucent edge	1 mm	Flat	Round	Straight	Smooth	+
UFV L-261	Milk-white center	1 mm	Lense	Round	Undulated	Smooth	+
UFV L-262	Milky yellow center	>1 mm	Convex	Round	Undulated	Smooth	+
UFV L-361	Milk-white center	<1 mm	Lense	Round	Straight	Smooth	+
UFV L-362	Milk-white center	>1 mm	Convex	Round	Straight	Smooth	+
UFV L-363	White center	1 mm	Flat	Round	Straight	Smooth	+
UFV L-364	Clear white center, translucent edge	>1 mm	Lense	Round	Straight	Smooth	+
UFV L-451	Milk-white center, translucent edge	<1 mm	Flat	Round	Undulated	Smooth	+
UFV L-452	White center. translucent edge	<1 mm	Lense	Round	Straight	Smooth	+
UFV L-453	Milk-white center, white and translucent edge	>1 mm	Convex	Round	Straight	Smooth	+
UFV L-562	Clear white center, translucent edge	<1 mm	Flat	Round	Straight	Smooth	-
UFV L-661	Clear white center, clear edge	<1 mm	Flat	Round	Straight	Smooth	-
UFV L-662	Clear white center, clear edge	<1 mm	Convex	Round	Straight	Smooth	-

Identification of bacterial strains: UFV name followed by the characters that indicate sludge (L), isolation culture medium (1 = JMV, 2 = JMVL, 3 = NFb, 4 = JNFb, 5 = LGI, 6 = LGI-P), dilution, order.

Table 4: Characteristics of *Zea mays* growth in response to diazotrophic inoculation

Treatments	PH cm	SD mm	NL unit	RFM g	SFM g	TFM g	SDM g	RDM g	TDM g	N CONT mg/plant
Control	14.10 ab	2.45 ab	4 abcde	1.62 b	4.00 ab	5.62 bc	0.10 a	0.28 abc	0.39 ab	9.48 b
UFV L-161	17.53 ab	2.75 ab	6 ab	2.29 ab	4.13 ab	6.42 abc	0.14 a	0.45 ab	0.60 ab	14.68 ab
UFV L-162	17.24 ab	3.08 ab	6 ab	2.18 ab	4.74 a	6.92 abc	0.16 a	0.51 a	0.68 a	22.14 a
UFV L-163	20.35 a	3.33 ab	6 a	3.04 ab	4.13 ab	7.17 a	0.20 a	0.28 abc	0.49 ab	15.17 ab
UFV L-164	19.51 a	3.37 ab	6 ab	3.10 a	3.95 ab	7.05 ab	0.23 a	0.31 abc	0.55 ab	13.50 ab
UFV L-261	18.76 ab	3.50 a	5 abcd	2.20 ab	3.31 ab	5.51 abc	0.16 a	0.24 bc	0.41 ab	11.28 ab
UFV L-262	20.15 a	2.65 ab	5 abcd	2.08 b	3.00 ab	5.08 abc	0.17 a	0.20 bc	0.38 ab	10.17 b
UFV L-361	17.84 ab	2.58 ab	4 bcde	1.71 b	3.62 ab	5.33 bc	0.11 a	0.14 c	0.25 b	8.07 b
UFV L-362	13.45 ab	2.43 b	4 de	1.7 b	3.10 ab	4.8 c	0.10 a	0.25 abc	0.36 ab	7.96 b
UFV L-363	17.54 ab	2.93 ab	4 de	1.95 b	4.62 a	6.57 abc	0.13 a	0.23 bc	0.36 ab	9.12 b
UFV L-364	13.95 ab	2.68 ab	4 cde	1.93 b	3.40 ab	5.33 bc	0.15 a	0.32 abc	0.48 ab	12.96 ab
UFV L-451	12.08 b	2.50 ab	3 e	1.93 b	4.19 ab	6.02 abc	0.17 a	0.18 c	0.35 ab	9.57 b
UFV L-452	18.95 ab	3.04 ab	5 abcd	2.41 ab	4.11 ab	6.52 abc	0.17 a	0.29 abc	0.47 ab	10.73 b
UFV L-453	17.58 ab	3.18 ab	5 abcd	2.44 ab	3.75 ab	6.19 abc	0.16 a	0.26 abc	0.42 ab	11.21 ab
UFV L-562	17.62 ab	2.84 ab	5 abcd	1.97 b	3.10 ab	5.07 bc	0.15 a	0.30 abc	0.41 ab	8.280 b
UFV L-661	15.45 ab	2.77 ab	4 abcde	2.04 b	4.18 ab	6.22 abc	0.15 a	0.18 c	0.34 ab	4.63 b
UFV L-662	15.16 ab	3.92 ab	6 abc	2.19 ab	2.55 b	4.474 c	0.16 a	0.34 abc	0.50 ab	11.98 ab
RMS	14.83	0.266	0.52	0.228	0.935	1.681	0.015	0.0168	0.028	13.696
MSD	7.90	1.059	1.48	0.98	1.986	2.662	0.251	0.2661	0.344	7.599
CV (%)	22.8	17.6	15.1	22.0	25.6	21.8	76.9	45.3	37.4	32.9

PH - plant height; SD - stem diameter; NL - number of leaves; RFM - root fresh matter; SFM - shoot fresh matter; TFM - total fresh matter; RDM - root dry matter; SDM - shoot dry matter; TDM - total dry matter; N CONT - nitrogen content; RMS - residual mean square; MSD - minimum significant difference; CV - coefficient of variation.

Averages followed by the same letter do not differ by the Tukey test ($p > 0.05$; $n = 6$).

CONCLUSIONS

Diazotrophs naturally inhabit sludge from wastewater treatment plants of poultry slaughterhouses, are predominantly gram-positive bacteria with a rod shape, and form colonies with different morphological characteristics.

Bacterial isolates from sludge of poultry slaughterhouses, in particular UFVL-162, UFVL-163, and UFVL-164, promote the growth and development of maize plants and can potentially be used to enrich inoculants and biofertilizers or in agricultural systems with low inputs or low fertility.

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REFERENCES

Aguiar KP (2012) Prospecção de bactérias promotoras do crescimento vegetal associadas a vermicompostos. Master Dissertation. Universidade Estadual do Norte Fluminense Darcy Ribeiro, Campos dos Goytacazes. 86p.

Baldotto LEB, Olivares FL & Bressan-Smith R (2011) Structural interaction between GFP-labeled diazotrophic endophytic bacterium *Herbaspirillum seropedicae* RAM10 and pineapple plantlets 'Vitória'. Brazilian Journal of Microbiology, 42:114-125.

Baldotto MA, Baldotto LEB, Santana RB & Marciano CR (2012) Initial performance of maize in response to NPK fertilization combined with *Herbaspirillum seropedicae*. Revista Ceres, 59:841-849.

Baptista RB, Mauri R & Baldani VLD (2011) Tolerância de estirpes de bactérias diazotróficas a Al^{3+}, para inoculação em milho (*Zea mays* L.). Revista de Ciência da Vida, 31:36-46.

Bettiol W & Fernandes SAP (2004) Efeito do lodo de esgoto na comunidade microbiana e atributos químicos do solo. Jaguariúna, Embrapa Meio Ambiente. 6p. (Comunicado Técnico, 24).

Bergamaschi CBLFW, Roeschr L, de Quadros PD & Camargo FA (2007) Ocorrência de bactérias diazotróficas associadas a cultivares de sorgo forrageiro. Ciência Rural, 37:727-733.

CONAMA (2006) Conselho Nacional do Meio Ambiente. Resolução Nº. 375 de 29 de Agosto de 2006. Available on: <www.mma.gov.br/port/conama/res/res06/res37506.pdf>. Accessed on October 28th, 2015.

Dartora J, Guimarães VF, Marini D & Sander G (2013) Nitrogen fertilization associated to inoculation with *Azospirillum brasilense* and *Herbaspirillum seropedicae* in the maize. Revista Brasileira de Engenharia Agrícola e Ambiental, 17:1023-1029.

Döbereiner J, Baldani VLD & Baldani JI (1995) Como isolar e identificar bacteìrias diazotroìficas de plantas naÞo leguminosas. Seropeìdica, Embrapa Agrobiologia. 60p.

Embrapa - Empresa Brasileira de Pesquisa Agropecuária (2013) Sistema Brasileiro de Classificação de Solos. 3ª ed. Rio de Janeiro, Centro Nacional de Pesquisa em Solos. 353p.

Faria CMDR, Campos VP, de Souza RM, Moreira FMS, de Moura B, de Oliveira Ribeiro L & Farias MV (2009) Isolamento caracterização de bactérias do lodo de esgoto com potencial antagonismo a nematoides. Ambiência, 2:247-256.

Ferreira JS, Sabino DCC, Guimarães SL, Baldani JI & Baldani, VLD (2003) Seleção de veículos para o preparo de inoculante com bactérias diazotróficas para arroz inundado. Agronomia, 37:06-12.

Francap (2015) Organizações Francap S/A. Available at: http://www.francap.ind.br/meioambiente.php. Accessed on October 28th, 2015.

Guimarães SL, Bonfim-Silva EM, Kroth BE, Moreira JFC & Rezende D (2011) Crescimento e desenvolvimento inicial de *Brachiaria decumbens* inoculada com *Azospirillum* spp. Enciclopédia Biosfera, 7:286-295.

Gomes L, Perin L, Pereira G & Zilli J (2010) Bactérias diazotróficas endofíticas em cultivares de milho em área de cerrado e mata no estado de Roraima. Boa Vista, Embrapa Roraima. 35p. (Documentos 43).

Grayston SJ, Wang S, Campbell CD & Edwards AC (1998) Selective influence of plant species on microbial diversity in the rhizosphere. Soil Biology and Biochemistry, 30:369-378.

Hallmann J, Quadt-Hallmann A & Mahaffee WF (1997) Bacterial endophytes in agricultural crops. Canadian Journal of Microbiology, 43:895-914.

Ikeda AC, Hungria M, Steffens M, Glienke C, Kava-Cordeiro V, Bassani L & Galli-teresawa VL (2010) Caracterização morfofisiológica e genética de bactérias endofíticas isoladas de raízes de diferentes genótipos de milho (*Zea mays* L.). In: Congresso Brasileiro de Genética, Guarujá. Resumos, Embrapa. p.51.

Inagaki AM (2014) Bactérias promotoras de crescimento de plantas na cultura do milho submetido a diferentes níveis de pH do solo. Dissertação de Mestrado. Universidade Estadual do Oeste do Paraná, Marechal Cândido Rondon. 79p.

Kappes C, Arf O, Arf MV, Ferreira JP, Bem EDA, Portugal JR & Vilela RG (2013) Inoculação de sementes com bactérias diazotróficas e aplicação de nitrogênio em cobertura e foliar em milho. Semina: Ciências Agrárias, 34:527-538.

Kim J & Rees DC (1994) Nitrogenase and biological nitrogen fixation. Biochemistry, 33:389-397.

Lana MC, Dartora J, Marini D & Hann JEH (2012) Inoculation with Azospirillum, associated with nitrogen fertilization in maize. Revista Ceres, 59:399-405.

Lozada JAR, Silveira KC, Silva LJ, Baldotto MA & Baldotto LEB (2017) Prospecting for sludge bacteria from a poultry slaughterhouse, with potential for degrading organic substances. Semina: Ciências Agrárias, 38:1209-1216.

Matte L (2014) Inoculante com *Azospirillum brasilense* combinado com níveis de nitrogênio na cultura de milho para silagem. Dissertação de Mestrado. Universidade Camilo Castelo Branco, Descalvado. 48p.

Milani KML, Machineski O & Balota EL (2011) Ocorrência e isolamento de bactérias diazotróficas associadas à cana de açúcar. Enciclopédica Biosfera, 7:1345-1351.

Mitter B, Brader G, Afzal M, Compant S, Naveed M, Trognitz F & Sessitsch A (2013) Advances in elucidating beneficial interactions between plants, soil, and bactéria. Advances in Agronomy, 121:381–445.

Melloni R, Nóbrega RSA, Moreira FMS & Siqueira JO (2004) Densidade e diversidade de bactérias diazotróficas endofíticas em solos de mineração de bauxita em reabilitação. Revista Brasileira de Ciência do Solo, 28:85-93.

Moreira FFMS, Silva K, Nóbrega RSA & Carvalho F (2010) Bactérias diazotróficas associativas: Diversidade, ecologia e potencial de aplicações. Comunicata Scientiae, 1:174-99.

Nóbrega RSA, Moreira FMS, Siqueira JO & Lima S (2004) Caracterização fenotípica e diversidade de bactérias diazotróficas associativas isoladas de solos em reabilitação após a mineração de bauxita. Revista Brasileira de Ciência do Solo, 28:269-279.

Obando M, Rivera D & Bonilla R (2013) Respuesta fisiológica a la fertilización por *Azotobacter chroococcum* AC1 y fertilización nitrogenada de síntesis sobre el maíz (*Zea mays* L.) en invernadero. Biotecnologia, 17:11-22.

Oliveira MSS, Roel AR, Arruda EJ & Marques AS (2007) Eficiência de produtos vegetais no controle da lagarta do cartucho-do-milho *Spodoptera frugiperda* (JE Smith, 1797) (Lepidoptera:Noctuidae). Semina: Ciência e Agrotecnologia, 31:326-331.

Perin L (2003) Ecologia e diversidade de isolados de *Gluconacetobacter diazotrophicus* associados à cultura da cana-de-açúcar (*Saccharum* spp.). Dissertação de Mestrado. Universidade Federal Rural do Rio de Janeiro, Seropédica. 74p.

Perin L, Silva MD, Ferreira JS, Canuto EL, Medeiros AFA, Olivares FL & Reis M (2003) Avaliação da capacidade de estabelecimento endofítico de estirpes de *Azospirillum* e *Herbaspirillum* em milho e arroz. Agronomia, 37:47-53.

Reis VM, Baldani JI, Baldani VLD & Döbereiner J (2000) Biological dinitrogen fixation in Gramineae and Palm trees. Critical Reviews in Plant Science, 19:227-247.

Reyes I, Alvarez L, El-Ayoubi H & Valery A (2008) Selección y evaluación de rizobacterias promotoras del crecimiento en pimentón y maíz. Bioagro, 20:37-48.

Roesch LFW, Camargo FADO, Selbach PA, Sa LSD & Passaglia LM (2005) Identificação de cultivares de milho eficientes na absorção de nitrogênio e na associação com bactérias diazotróficas. Ciência Rural, 35:924-927.

Santos ST (2008) Biogeografia de bactérias culturáveis associadas às fruteiras tropicais. Tese de Doutorado. Universidade Estadual do Norte Fluminense Darcy Ribeiro, Campos dos Goytacaes. 92p.

Santos FE, Kunz SH, Caldeira MV, Azevedo CH & Rangel OJ (2014) Características químicas de substratos formulados com lodo de esgoto para produção de mudas florestais. Revista Brasileira de Engenharia Agrícola e Ambiental, 18:971-979.

Sobrinho PA (1983) Estudo dos fatores que influenciam no processo de lodos ativados - determinação de parâmetros de projeto para esgotos predominantemente domésticos. Revista DAE, 132:49-55.