ABSTRACT
In search for a more sustainable agriculture, the use of microorganisms as a technology is increasingly being used by agriculture throughout the world. This is due to the fact that it minimizes the use of agricultural supplies reducing environmental costs and impacts, based on the beneficial and natural relationships between edaphic organisms and cultivated plants. The rhizobacteria habitat in the soil establishes biochemical relationships with the plants acting as plant growth promoters (PGPR). Many of these bacteria are producers of phytohormones and enzymatic compounds with the capacity to provide important nutrients for plants. In this context, the present work aimed to quantify the potential of indole-3-acetic acid (IAA) production and the phosphate solubilization of rhizobacteria from Western Paraná. Isolates grown in DYGS medium plus tryptophan were quantified by colorimetry for the production of IAA. Iron phosphate solubilization was carried out by inoculation in modified Pikovskaya medium (PKV) and quantified by colorimetry. The results were evaluated by the Scott-Knott test at 5% using the SASM-Agri program. The highest IAA production was observed with the addition of tryptophan to Erwinia (219); Enterobacter (302) and Salmonella (57). Isolates Falsibacillus (438) and 505 showed higher efficiency in the iron phosphate solubilization. Isolates Enterobacter (130), 438 and Enterobacter (151) were highlighted in both tests, being characterized as a great potential for use in biotechnological products.

Key words: Biotechnology, soil bacteria, phytohormones, plant growth promoter, rhizobacteria.

INTRODUCTION
Fertilizers represent the costliest supply in agricultural production systems, and their excessive use has led to contamination of groundwater rivers and soils (Sano et al., 2011). Technology and products that seek to mitigate the application of these products by developing more sustainable and economical agriculture have demonstrated that it is possible to reduce costs and increase productivity by using plant-growth-promoting bacteria (PGPR) (Kaschuk et al., 2010; Glick 2012; Rodrigues et al., 2013).

In soils, PGPRs act beneficially by interacting with plants improving their performance. These associative bacteria can act directly in the production of phytohormones, phosphate solubilization, nutrient mineralization and biological nitrogen fixation (BNF) (Egamberdieva et al., 2015). Many microorganisms have been reported as growth promoters in plants such as those of the genera Azorarcus, Azospirillum, Azotobacter, Arthrobacter, Bacillus, Clostridium, Enterobacter, Gluconacetobacter, Pseudomonas and Serratia (Sommer and Vanderleyden 2004; Beneduzi et al., 2013; Egamberdieva et al., 2015).

One of the main mechanisms to promote plant growth is the ability to stimulate the production of phytohormones. Synthesis of growth regulators such as auxins, gibberellins and cytokinins can act directly on the plant root expansion. This effect can improve the search for water and nutrients, as well as reduce or block the production of ethylene (Araújo and Guerreiro 2010; Balota 2017).
Production of auxins such as 3-indoleacetic acid (IAA) is performed by many rhizobacteria, and their synthesis occurs via different metabolic pathways. The main precursor of this compound is tryptophan (Tpr), which presents an independent route concentrating the production of IAA in tissues with high growth rate, such as apical meristems, young leaves, fruits and seeds (Taiz and Zeiger 2004; Florentino 2017). In the studies by Brzezinski et al. (2014), strains of *Azospirillum brasilense* were able to promote increase in the vigor of wheat seeds and in the aerial part of seedlings. Similarly, Dartora et al. (2013) observed gains in the initial development of plants inoculated with *Azospirillum e Herbaspirillum*.

Another mechanism used by rhizobacteria to promote plant growth is the phosphate solubilization. Among the various forms of action of these microorganisms in the increase of phosphorus availability to plants, the release of organic acids stands out the excretion of siderophores into inorganic phosphates and the production of enzyme phosphatases (Patino-Torres and Sanclemente-Reyes 2014; Balota 2017). Studies on this pathway have been relevant to research on tropical soils, since they present low levels of phosphorus due to high weathering and retention of their ions in iron and aluminum oxides. In these conditions, this macronutrient becomes unavailable to plants, one alternative being the association with phosphate solubilizing bacteria (PSB) to improve the absorption of these minerals (Chaves et al., 2013).

Arruda et al. (2013) demonstrated the great ability of native isolates for phosphate solubilization and plant growth promotion. In both cases, rhizobacteria were isolated from corn; in the first, among 173 isolates, about 56.5% had a positive effect in vitro. In the second, out of 292 isolates, 154 (52.7%) were found to be efficient in the solubilization of this nutrient. Pedrinho et al. (2010) also observed that, out of 58 bacteria obtained from corn roots, 27 (46.5%) presented a solubilization halo around the colony.

In this context, the present study aimed to evaluate the production of phytohormones (IAA) and phosphate solubilization performed by rhizobacteria in the western region of Paraná with different cultivation management, aiming at identifying strains with biotechnological potential for promoting growth.

### MATERIAL AND METHODS

#### Obtaining the isolates

For biochemical analyzes, 42 bacteria were selected from the culture collection of the biotechnology laboratory (LABIOTEC) of the Federal University of Paraná (UFPR) – Palotina Sector. These isolated were obtained from rhizospheric soils of 17 cultivated areas with different managements in the western region of Paraná state.

#### Determination of IAA production

The isolates were grown in DYGS tryptophan, until final concentration of 100μg.ml⁻¹. The tubes were incubated for 48 hours at 180 rpm at 20ºC in a shaker and centrifuged at 9000 rpm for 5 minutes to obtain the cell free extract. Then, the samples were quantified by the colorimetric method using the modified Salkowsky reagent (40 mM FeCl₃; 7,9 M H₂SO₄) (Sarwar and Kremer 1995). IAA production was calculated by absorbance readings in a spectrophotometer at 530 nm. Strains grown in DYGS medium without tryptophan were used as negative control. To build a standard curve, readings of increasing concentrations of commercial 3-indole acetic acid were used (0, 2, 4, 8, 10, 15, 20, 25 and 30 μg mL⁻¹).

The protein concentration was performed by the Bradford method (1976) for normalization of extracts concentration in absorbance readings at 595 nm. The IAA standard curve resulted in the equation y = 0,0052x + 0,0287, where “y” represents the amount of auxin secreted in the liquid culture. A Standard curve using bovine serum albumin (BSA) was determined for obtaining the total proteins by the Bradford method (1976). In this purpose, concentrations 0, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 μg mL⁻¹, respectively, were used and generated the equation y = 0,0041x – 0,0045. The absorbance data were replaced in the equation, and the x value obtained was reported as μg/ml.

#### Phosphate solubility capacity

To quantify iron phosphate solubilization by the spectrophotometric method, the molybdenum blue (Murphy and Riley 1962) was used. Aliquots of 100 μL of each sample, grown in liquid DYGS medium were inoculated in modified medium of Pikovskaya (1948), incubated for 7 days at 28ºC, under constant shaking.
of 1810 rpm. From the cells free extract, 1 mL was withdrawn and 2 mL of MWS (Molybdate Working Solution) was added, as well as 50 μL of ascorbic acid. The sample was shaken quickly and filtered for reading in the spectrophotometer at 660 nm. The experiments were carried out in triplicate and as control the PKV medium was used without inoculum addition. The data obtained were compared to the standard curve of phosphate at concentrations 0.0; 0.5; 1.0; 2.0; 3.0 and 4.0 mg/L of PO₄, respectively and, yielded the equation \( y = 0.125x + 0.0194 \).

**Statistical analysis**

The assays were analyzed by a completely randomized design with three replicates and submitted to variance analysis, the means were compared using the Scott-Knott group test with 5% significance level using the SASM-Agri software (Canteri et al., 2001).

**RESULTS AND DISCUSSION**

Out of 42 bacteria, IAA production was identified in 36 of them without TPR supplementation, and in 40 with TPR. The genus *Erwinia* (219) stood out, demonstrating a production of 1741.31 μM/mg in the presence of tryptophan.

Similar results were obtained for Tozlu et al. (2012), who performed the evaluation of the biological fixation efficiency of ten bacterial strains, the phosphate solubilization. The bacterial production capacity of IAA belonging to the genus Erwinia was higher (Table 1).

The IAA producing bacteria is related to the capacity of tolerance to the modification occurring in the edaphic environment, as pH, carbon availability, nitrogen and the tryptophan concentration (Mohite 2013). Among all isolates evaluated, 2 did not present IAA with and without tryptophan. There was also great variation in IAA production in the isolates in the presence of the amino acid (Table 1). Therefore, the average IAA production of all isolates in the absence of tryptophan were 289.86 μM / mg, lower value than that obtained in the presence of the precursor, 480.93 μM/mg.

The production of auxins, such as 3-indoleacetic acid (IAA), is a characteristic present in about 80% of the rhizobacteria. Therefore, some strains like *Azospirillum, Lipoferum* and *Azospirillum brasiliense*, have high capacity of this metabolite production from its precursor (Balota 2017). In addition, it was possible to note that some strains *Enterobacter* (24, 203), *Enterobacter agglomerans* (132), *Microbacterium* (220, 241, 317), *Falsibacillus* (446, 580) and 660, in the absence of tryptophan amino acid, demonstrated a higher production of IAA, pointing those isolates for further investigations. For Isolates 219 (*Erwinia*), 57 (*Salmonella bongori*) and 241 (*Microbacterium*), the presence of the precursor was determinant in the production of IAA, showing increases, respectively, of 369.61%, 126.40% and 402.58%.

According to Bar and Okon (1993) and Florentino (2017), tryptophan is the main metabolic pathway for the production of IAA. The amount of this precursor may interfere with the phytohormone synthesis since each genus of bacteria has an optimum concentration. Also, values outside this range can affect the enzymatic production efficiency. According to Bhattacharyya and Jha (2011), in recent studies, new biochemical routes are being used for the synthesis of auxins.

In relation to the phosphate solubilization capacity by the same bacterial strains, the results showed low concentrations. Reports by Prasanna et al. (2011) and Chaiharn and Lumyong (2011) describe bacteria with great capacity for phosphate solubilization. In the first study, *Enterobacter aerogenes* produced 825.8 mg.L⁻¹, whereas in the second, the isolate *Acinetobacter* showed a production of 334 mg.L⁻¹. In this study, the amount of solubilization considered effective for plants was observed only in strains 438 (*Falsibacillus*) (114.49 mg. L⁻¹), with an efficiency of 25.27% and in strain 505 (83.06 mg. L⁻¹), with an efficiency of 18.34%.

According to Guang-Can et al. (2008), most bacteria can solubilize phosphate from calcium phosphate, but not all of them can extract phosphate from other sources like iron or aluminum phosphate. Panda et al. (2016), evaluated phosphate solubilization from three different sources: iron, aluminium and calcium phosphates, in the latter the solubilization was higher than in the others.

In this research, *Enterobacter spp* (130), *Falsibacillus* (438) and *Enterobacter asburiae* (151) strains were the most efficient in both biochemical tests. Strain 130 (*Enterobacter spp.*) showed 38.42 mg.L⁻¹, an efficiency of 12.37% of iron phosphate solubilization, and 745.65 μM/mg of IAA production. Strain 438 was the one that obtained the highest phosphate solubilization, 114.49 mg.L⁻¹, with an efficiency of 25.27% and had 586.83 μM/mg of IAA production. Strain 151 showed 49.25 mg.L⁻¹ and efficiency of 10.87% of
phosphate solubilization and a production of 882.88 μM/mg of IAA.

Table 1. Quantification of the IAA production with and without tryptophan and phosphate solubilization efficiency of native rhizobacteria from the Western Region of Paraná.

| Isolates                        | Without tryptophan (µM/mg) | With tryptophan^1 (µM/mg) | Soluble phosphate^1 (mg.L^-1 de FeO.P.2H₂O) | Solubilization Efficiency (%) |
|---------------------------------|-----------------------------|---------------------------|---------------------------------------------|-------------------------------|
| Pantoea (10)                   | 209.00                      | 741.73                    | -                                           | -                             |
| Enterobacter (24)              | 641.17                      | 567.35                    | 12.78                                       | 2.82                          |
| Enterobacter (34)              | -                           | 1.73                      | -                                           | -                             |
| Enterobacter asburiae (42)     | 473.75                      | 726.15                    | 26.75                                       | 5.91                          |
| Salmonella bongori (57)        | 526.75                      | 1192.58                   | 21.86                                       | 4.83                          |
| 59^2                           | 483.38                      | 901.93                    | -                                           | -                             |
| Microbacterium (103)           | -                           | -                         | 25.15                                       | 5.55                          |
| Delfta (109)                   | 117.67                      | 683.42                    | -                                           | -                             |
| Enterobacter (120)             | 436.57                      | 501.38                    | 17.98                                       | 3.97                          |
| Enterobacter (130)             | 377.13                      | 745.65                    | 38.42                                       | 12.37                         |
| Enterobacter asburiae (151)    | 498.91                      | 882.88                    | 49.25                                       | 10.87                         |
| Enterobacter asburiae (142)    | 432.63                      | 443.67                    | 17.25                                       | 3.81                          |
| Enterobacter agglomerans (132) | 390.64                      | 338.00                    | 19.12                                       | 4.22                          |
| Enterobacter (152)             | 511.00                      | 927.92                    | -                                           | -                             |
| Enterobacter (194)             | 409.45                      | 964.83                    | -                                           | -                             |
| Enterobacter (203)             | 140.67                      | 88.94                     | 22.82                                       | 5.04                          |
| Delfta (208)                   | 210.89                      | 508.00                    | 13.79                                       | 3.04                          |
| Erwinia (219)                  | 363.07                      | 1741.31                   | 15.97                                       | 3.53                          |
| Microbacterium (220)           | 477.91                      | 449.60                    | 23.40                                       | 5.17                          |
| Microbacterium paraoxydans (232)| -                           | -                         | -                                           | -                             |
| Microbacterium (241)           | 468.22                      | 167.21                    | 23.99                                       | 5.29                          |
| 255^2                          | 432.31                      | 477.96                    | 56.92                                       | 12.56                         |
| Enterobacter (265)             | 264.96                      | 344.98                    | 32.52                                       | 7.18                          |
| Delfta (273)                   | 118.28                      | 118.33                    | 39.99                                       | 8.83                          |
| Agrobacterium tumefaciens (292)| 111.30                      | 26.84                     | -                                           | -                             |
| Enterobacter asburiae (299)    | 257.67                      | 537.81                    | 32.50                                       | 7.17                          |
| Pantoea anatis (300)           | 120.71                      | 309.15                    | 7.05                                        | 1.56                          |
| Enterobacter (302)             | 392.00                      | 1259.81                   | 41.50                                       | 9.16                          |
| Microbacterium (317)           | 47.91                       | 28.84                     | -                                           | -                             |
| Enterobacter (326)             | 330.67                      | 850.57                    | 32.93                                       | 7.27                          |
| Falsibacillus (438)            | 230.73                      | 586.83                    | 114.49                                      | 25.27                         |
| Falsibacillus (446)            | 313.78                      | 41.24                     | -                                           | -                             |
| Bacillus (454)                 | -                           | 122.21                    | -                                           | -                             |
| 456^2                          | 59.50                       | 161.87                    | 16.52                                       | 3.65                          |
| 471^2                          | 67.31                       | 338.29                    | -                                           | -                             |
| 482^2                          | 70.23                       | 74.29                     | -                                           | -                             |
| 493^2                          | 149.68                      | 284.05                    | 21.65                                       | 4.78                          |
| 505^2                          | 143.67                      | 234.05                    | 83.06                                       | 18.34                         |
| 522^2                          | 49.18                       | 96.84                     | -                                           | -                             |
| Pseudomonas (535)              | 130.69                      | 423.13                    | -                                           | -                             |
| Falsibacillus (580)            | 269.17                      | 200.00                    | -                                           | -                             |
| 660^2                          | 107.55                      | 59.60                     | 26.40                                       | 5.83                          |

^1Means followed by the same letter in the column do not differ significantly among isolates by the Scott-Knot test at 5% probability of error. ^2Number of isolates which do not have DNA sequencing.

De Souza et al. (2018), evaluating the potential of bacteria of the genus Enterobacter in the promotion of plant growth, observed positive results regarding the production of IAA (27 μM/mg), which resulted in gains in soybean seedling growth. In the research by Assumpção et al. (2010), the production of 31.7 μM/mg of IAA by bacteria of the same genus and with the capacity of phosphate solubilization was found, but without promoting soybean plants growth.
In this context, the present work was able to evaluate the bacteria from the western region of Paraná in two promoting plant growth factors, developing a better understanding of plant-bacterial interactions, allowing the continuity of the search for new bacteria with biotechnological potential for plant-growth promotion.

CONCLUSIONS

The isolates *Erwinia (Enterobacter soli)* (219), *Enterobacter* (302) and *Salmonella bongori* (57) were identified as the most efficient for the production of IAA. For the phosphate solubilization, the strain 438 (*Falsibacillus*) was the most efficient, followed by strain 505. *Enterobacter* (130), Falsibacillus (438) and *Enterobacter asburiae* (151) isolates were the most efficient in both biochemical testes performed.

REFERENCES

Assumpção LC, Lacava PT, Dias ACF, De Azevedo JL and Menten JO (2010) Diversidade e potencial biotecnológico da comunidade bacteriana endofítica de sementes de soja. Pesquisa Agropecuária Brasileira 44: 503-510.

Araújo FF and Guerreiro RT (2010) Bioprospecção de isolados de Bacillus promotores de crescimento de milho cultivado em solo autoclavado e natural. Ciência e Agrotecnologia 34: 837-844.

Arruda L, Beneduzzi A, Martins A, Lisboa B, Lopes C, Bertolo F, Passaglia LMP and Vargas LK (2013) Screening of rhizobacteria isolated from maize (*Zea mays* L.) in Rio Grande do Sul State (South Brazil) and analysis of their potential to improve plant growth. Applied Soil Ecology 63: 15-22.

Balota EL (2017) Importância da Microbiota do Solo Balota EL Manejo e Qualidade Biológica do Solo. Mecenas, Londrina, p. 57-84.

Bar T and Okon Y (1993) Tryptophan conversion to indole-3-acetic acid via indole-3-acetamide in *Azospirillum brasilense* Sp7. Canadian Journal of Microbiology 39: 81-86.

Beneduzzi A, Moreira F, Costa PB, Vargas LK, Lisboa BB, Favreto R, Baldani JI and Passaglia LMP (2013) Diversity and plant growth promoting evaluation abilities of bacteria isolated from sugarcane cultivated in the South of Brazil. Applied Soil Ecology 4: 94-104.

Bhattacharyya PN and Jha DK (2011) Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. World Journal of microbiology and biotechnology 28: 1327-1350.

Bradford M (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72: 248-254.

Brzezinski CR, Zucareli C, Henning FA, Abati J, Prando AM and Henning AA (2014) Nitrogênio e inoculação com Azospirillum na qualidade fisiológica e sanitária de sementes de trigo. Revista de Ciências Agrárias/Amazonian Journal of Agricultura land Environmental Sciences 57: 257-265.

Canteri MG, Althaus RA, Virgens Filho JS, Giglioti EA and Godoy CV (2001) SASM - Agri: Sistema para análise e separação de médias em experimentos agrícolas pelos métodos Scott - Knott, Tukey e Duncan. Revista Brasileira de Agrocomputação 1: 18-24.

Chaiharn M and Lumyong S (2011) Screening and optimization of indole-3-acetic acid production and phosphate solubilization from rhizobacteria an imedat improving plant growth. Current microbiology 62: 173-181.
Chaves DP, Zucareli C and Oliveira Junior A (2013) Fontes de fósforo associadas à inoculação com Pseudomonas fluorescens no desenvolvimento e produtividade do milho. Semina: Ciências Agrárias 34: 57-42.

Dartora J, Marini D, Guimarães VF, Pauletti DR and Sander G (2013) Germinação de sementes e desenvolvimento inicial de plântulas de milho e trigo inoculadas com estirpes de Azospirillum brasilense e Herbaspirillum seropedicae. Global Science and Technology 6: 3.

Egamberdieva D, Shrivastava S and Varma A (2015) Plant-Growth-Promoting Rhizobacteria (PGPR) and Medicinal Plants. SoilBiology 42: 1-16.

Florentino LA (2017) Inoculação de bactérias produtoras de ácido 3-indol acético em plantas de alface (Lactuca sativa L.). Revista Colombiana de Ciências Hortícolas 11: 89-96.

Guang-Can TAO, Shu-Jun TIAN, Miao-Ying CAI and Guang-Huii XIE (2008) Phosphate-Solubilizing and Mineralizing Abilities of Bacteria Isolated from Soils. Pedosphere 18: 515-523.

Glick BR (2012) Plant Growth – Promoting Bacteria: Mechanisms and Applications. Scientific, p.1-16.

Kaschuk G, Alberton O and Hungria M (2010) Quantifying effects of diferente agricultural land uses on soil microbial biomass and activity in Brazilian biomes: inferences to improve soil quality. Plant and Soil 338: 467-481.

Mohite B (2013) Isolation and characterization of indole acetic acid (IAA) producing bacteria from rhizospheric soil and its effect on plant growth. Journal of soil science and plant nutrition 13: 638-649.

Murphy J and Riley JP (1962) A modified single solution method for the determination no phosphate in natural waters. Analytica chimica acta 27: 31-36.

Panda B, Rahman H and Panda J (2016) Phosphate solubilizing bacteria from the acidic soils of Eastern Himalayan region and their antagonistic effect on fungal pathogens. Rhizosphere 2: 62-71.

Patino-Torres CO and Sanclemente-Reyes OE (2014) Los microorganismos solubilizadores de fósforo (MSF): uma alternativa biotecnológica para uma agricultura sustentável. Entramado 10: 288-297.

Pedrinho EAN, Galdiano Junior RF, Campanharo JC, Alves LMC and Lemos EGDM (2010) Identificação e avaliação de rizobactérias isoladas de raízes de milho. Bragantia 69: 905-911.

Pikovskaya RI (1948) Mobilization of phosphorus in soil in connection with vital activity of some microbial species. Mikrobiologiya 17: 362-370.

Prasanna A, Deepa V, Murthy PB, Deecaraman M, Sridhar R and Dhandapani P (2011) Insoluble phosphate solubilization by bacterial strains isolated from rice rhizosphere soils from Southern India. International Journal of Soil Science 6:134-141.

Rodrigues LFOS, Guimaraes VF, Silva MB, Pinto Junior AS, Klein J and Costa ACPR (2013) Características agronômicas do trigo em função de Azospirillum brasilense, ácidos húmicos e nitrogênio em casa de vegetação. Revista Brasileira de Engenharia Agrícola e Ambiental 18: 31-37.

Sano EE, Santos CCM, Silva EM and Chaves JM (2011) Fronteira agrícola do oeste baiano: considerações sobre os aspectos temporais e ambientais. Geociências 30: 479-489.

Sarwar M and Kremer RJ (1995) Determination of bacterially derived auxins using a microplate. Method. Letters in Applied Microbiology 20: 202-205.

Sommers E and Vanderleyden J (2004) Rhizosphere bacterial signaling: A love parade beneath our feet. Critical Reviews Microbiology 30(4):205-240.
Souza R, Mendonça EAF, Biz AR and Soares MA (2018) Potencial Agronômico de Bactérias Endofíticas de Echinodorus scaber Rataj (macrophyllus) em Plântulas de Soja. Ensaios e Ciência: C. Biológicas, Agrárias e da Saúde 21: 187-193.

Taiz L and Zeiger E (2004) Fisiologia Vegetal. 3ª ed. Artmerd Editora, Porto Alegre, 719p.

Tozlu E, Karagoz K, Babagil GE, Dizikisa T and Kotan R (2012) Effect of Some Plant Growth Promoting Bacteria on Yield, Yield Components of Dry Bean (Phaseolus vulgaris L. cv. Aras 98). Atatürk Üniversitesi Ziraat Fakultesi Dergisi 43: 101-106.

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