IAA production and phosphate solubilization performed by native rhizobacteria in western Paraná

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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 *Falsibacillu*s (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: Biotecnology, 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 *Azoarcus*, *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 $FeCl_3$; 7,9 M H_2SO_4) (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, 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^{-1} of PO_4, 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), *Falsibaccilus* (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.

Table1. 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 $FeO_4P.2H_2O$)	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
Delftia (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	13.84	3.06
Enterobacter (194)	409.45	964.83	-	-
Enterobacter (203)	140.67	88.94	22.82	5.04
Delftia (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
Delftia (273)	118.28	118.33	39.99	8.83
Agrobacterium tumefaciens (292)	-	111.30	26.84	5.93
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
Coefficient of Variation (%)		36.00	34.11	

[1]Means followed by the same letter in the column do not differ significantly among isolates by the Scott-Knot test at 5% probability of error. [2]Number 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 (*Falsibaccilus)* 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.

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