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A N I N T E R N A T I O N A L J O U R N A L

Auxin Production by Endophytic Bacteria Isolated from Banana Trees

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ABSTRACT

This study aimed to evaluate the capacity for in vitro indole compound synthesis by endophytic bacteria and to compare the Salkowski colorimetric and high-performance liquid chromatography (HPLC) quantification methods. Initial screenings were performed using 10% tryptic soy agar (TSA) medium with and without L-tryptophan via the Salkowski colorimetric method in a completely randomized design with 40 treatments and three replications. In the second experiment, the five most promising isolates were used for the detection and quantification of indoles by HPLC in a completely randomized design with five treatments and three replications. Of the 40 isolates, 16 synthesized indole-3-acetic acid (IAA) in the presence of L-tryptophan, and only three synthesized IAA in the absence of this amino acid. The concentration of IAA determined by absorbance indicated that Agrobacterium tumefaciens isolate EB.07 showed a higher concentration of IAA in the presence of L-tryptophan (16.61 $\mu g\ mL^{-1}$). HPLC analysis revealed the production of the following four indole compounds by these bacteria: IAA, indole pyruvic acid (IPA), indole lactic acid (ILA), and indole-3-acetaldehyde (IAAld). Significant differences between the two estimation methods were observed for two of the five tested isolates: A. tumefaciens (EB.07) and Bacillus pumilus (EB.12). For both isolates, the Salkowski method indicated higher concentrations than those obtained with HPLC. Considering the obtained results, the Salkowski colorimetric method should be used in initial screenings, and HPLC should be used as a complementary method in the selection of isolates with the highest potential to synthesize indoles.

Key words: HPLC, Salkowski, L-tryptophan, PGPB, indole-3-acetic acid.

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INTRODUCTION

New areas of biotechnological exploration based on the isolation, characterization, and determination of microbial diversity have emerged and have had a great impact on the agricultural and industrial sectors. Several studies have reported the effects of endophytic bacteria on plant growth and development. These findings highlight the ability of bacteria to stimulate plant growth via nitrogen fixation[10,21], phosphorus solubilization[33], phytohormone production[9,13,30,38,], resistance induction, and anti-fungal and anti-bacterial activity[5,34].

The main auxin found in plants is indole-3-acetic acid (IAA), which is produced primarily in the apical meristem (bud) of the stem and is transported to the roots by parenchymal cells. According to Patten and Glick[27], in addition to the production of IAA in plant tissues, this compound is also synthesized by plant-associated bacteria, which, in turn, employ mechanisms that influence plant growth.

IAA is synthesized by certain bacteria via multiple distinct pathways[27]. Tryptophan-dependent IAA is synthesized via several pathways, which are generally named after an intermediate compound, *e.g.*, the indole-3-pyruvic acid (IPA) pathway, the indole-3-acetamide (IAM) pathway, the tryptamine pathway, and the indole 3-acetaldoxime (IAOx) pathway. Several authors have indicated that the main pathway for IAA production in bacteria is the IPA pathway, as observed in the genera *Bradyrhizobium*, *Azospirillum*, *Rhizobium*, *Azotobacter*, and *Enterobacter* as well as in cyanobacteria[1].

In addition to knowledge of biosynthetic pathways, the use and application of biochemical tests are important tools for evaluating the potential of endophytic bacteria. Some techniques allow the determination of the activity of enzymes that are used in these pathways while considering that the metabolism of these organic molecules produces end products that are detectable by analytical methods in the laboratory[8].

The Salkowski colorimetric method[17] has been widely used for the detection and quantification of bacterial auxins primarily because of its convenience, speed, specificity, and efficiency[16,32]. However, high-performance liquid chromatography (HPLC) has recently gained importance as a specific method, particularly for identifying compounds in a mixture. This technique enables the separation, identification, and quantification of compounds[31].

Several authors have evaluated these two methods for detecting the production of auxins and have reported that HPLC is more reliable than the colorimetric method because the latter generally tends to overestimate the results. According to Tsavkelova *et al.*[41], this effect may be due to the presence of other indoles (involved in the biosynthesis of auxins by bacteria), which accumulate in the culture medium and are more reactive with the Salkowski reagent than IAA alone.

Knowledge of the metabolic profile of different isolates of endophytic bacteria facilitates the selection of genotypes with high biotechnological potential. In turn, this information will expand the use of these microorganisms and the application of this knowledge for the development of products with high biological value, including bioinoculants and biostimulants.

In this context, the present study aimed to evaluate the indole compound production capacity of endophytic bacteria collected from banana roots and to compare the efficiency of the Salkowski colorimetric and HPLC quantification methods.

MATERIALS AND METHODS

Selection of isolates and preparation of bacterial suspensions

The endophytic bacteria used in this study were isolated and identified at the genus and species level by Souza *et al.*[36] (Table 1).

The isolates were maintained on tryptic soy agar (TSA) in test tubes for 24 hours at 28 °C. To obtain bacterial suspensions, the selected isolates were inoculated in tryptic soy broth (TSB) medium and maintained at 120 rpm using an automatic shaker at 28 °C for 48 hours.

The suspension was centrifuged for 15 minutes at 10,000 rpm to collect the bacterial cells. The pellet was resuspended in 0.85% saline solution under aseptic conditions in a laminar flow hood. The concentration of bacterial cells in the suspension was adjusted by using a spectrophotometer to read the absorbance at a wavelength (λ) of 540 nm and an optical density (OD) of 1.0.

Quantification of indoles via the Salkowski colorimetric method

IAA was quantified using the method described by Kuss *et al.*[20]. The volume of the bacterial suspension of each isolate was adjusted, and 0.250-mL aliquots of each suspension were inoculated in 10% TSA medium and incubated for 48 hours at 28 °C at 120 rpm in the absence of light. After this period, 2.0 mL of each homogenized culture was transferred to 2-mL Eppendorf tubes and centrifuged at 10,000 rpm for 10 minutes.

The supernatant was transferred to 15.0-mL vials, and 2.0 mL of Salkowski reagent was added[28]. The test tubes were incubated for 30 minutes in the dark. IAA was qualitatively measured by the presence of a pink coloration in the vial and quantitatively measured by reading the absorbance of the samples using a spectrophotometer at a wavelength of 530 nm.

The concentrations of IAA were determined by reading the absorption in a spectrophotometer using commercial concentrations of 1.0, 2.0, 4.0, 8.0, 16.0, 32.0, and 64.0 μg mL^{-1}, and the concentrations were normalized using a standard curve, which was used to generate an equation for determining the concentration of IAA in the samples in μg mL^{-1}.

Two standard curves were used to compare the colorimetric absorbance of IAA. To quantify IAA in the medium supplemented with L-tryptophan, the equation $Y = 0.0310 + 0{,}0114x$ ($R^2 = 97.00\%$) was used, where Y is the absorbance reading in the spectrophotometer and x is the concentration of IAA in μg mL^{-1}. To quantify IAA in the medium that was not supplemented with L-tryptophan, the equation $Y = 0.0508 + 0.0122x$ ($R^2 = 94.90\%$) was used.

Two assays were performed to determine the ability of bacteria to synthesize IAA. The first assay was used to quantify IAA in 10% TSA supplemented with L-tryptophan (10%), and the second assay was used to quantify IAA in 10% TSA medium without L-tryptophan.

The two assays were performed in a completely randomized design with 40 treatments (isolates of endophytic bacteria) and three replications.

Extraction, identification, and quantification of indole compounds via HPLC

The cultures of isolates EB.07, EB.12, EB.17, EB.78, and EB.98 with the highest capacity to synthesize IAA based on the Salkowski colorimetric method were selected for analysis via HPLC. For HPLC analysis, indoles were extracted according to the protocol established by Tsavkelova *et al.*[41], which consisted of vacuum concentration of 100 mL of the liquid culture supernatant of each isolate

using a lyophilizer to obtain a final volume of 10 mL. The pH was adjusted to 2.8 with 1N HCl and extracted three times with ethyl acetate (JT Baker, HPLC grade) (1:2 v:v) by vigorous shaking for 10 minutes. The following HPLC-grade indole standards were used: IAA, indole pyruvic acid (IPA), indole lactic acid (ILA), and indole-3-acetaldehyde (IAAld) (Sigma).

After separation of the two phases using a separating funnel, the ethyl acetate fraction was evaporated in a rotoevaporator coupled to a vacuum pump, whereas the solid phase was suspended in 500 µL of absolute methanol and centrifuged at 10,000 rpm for 10 minutes. HPLC was performed by injecting 10 µL of an aliquot in an ULTRA C_{18} reverse phase column (150 x 4.6 mm; Restek, Bellefonte, Pennsylvania, USA) with a particle size of 5 µm, connected to an SLC 10A VP HPLC apparatus (Shimadzu), and the absorbance was monitored using an UV-visible detector (model SPD M10A VP) at a wavelength of 254 nm.

The mobile phase consisted of water:acetonitrile:acetic acid (40:60:1), pH 2.8. The flow rate was 0.5 mL min^{-1} at a pressure of 7.5 MPa. The presence of IAA was confirmed by comparing the retention time of the commercial IAA and indoles. The eluates were quantified by comparing the weights and areas of the peaks using CLASS-VP software (Shimadzu).

The assay was performed in a completely randomized design with five treatments and three replications.

STATISTICAL ANALYSIS

The IAAs quantified by using the Salkowski colorimetric method and the indoles quantified via HPLC were subjected to analysis of variance. When the F-test indicated significance, the variables were subjected to the Scott-Knott test and Tukey's test at a probability of 5% using Sisvar software[14].

RESULTS AND DISCUSSION

Production of IAA determined via the Salkowski colorimetric method

The synthesis of IAA in 10% TSA medium supplemented with L-tryptophan was observed in 40% of the tested bacterial isolates, and of these isolates, three produced IAA via pathways that were alternatives to the tryptophan pathway. Among these isolates, 12 belonged to the genus *Bacillus*, and one isolate was identified for each of the following genera: *Agrobacterium*, *Micrococcus*, *Rhizobium*, and *Aneurinebacillus* (Table 1).

Table 1 - Identification of the isolates (genera and species), ability to synthesize IAA using the Salkowski reagent and the accession number in the GenBank database of endophytic bacteria associated with the "Prata Anã" banana, Janaúba, Minas Gerais, 2015.

Isolates	Genera/Species	IAA		Accession number GenBank
		With Trp	Without Trp	
EB. 01	*Bacillus pumilus*	+	-	HM006706.1
EB. 03	*Bacillus pumilus*	-	-	JF738118.1
EB. 05	*Bacillus pumilus*	-	-	HQ218993.1
EB. 06	*Bacillus subtilis*	-	-	JF926521.1
EB. 07	*Agrobacterium tumefaciens*	+	+	GU784794.1
EB. 11	*Bacillus* sp.	+	-	HQ218993.1
EB. 12	*Bacillus pumilus*	+	-	GQ917222.1
EB. 14	*Bacillus pumilus*	+	-	HQ218993.1
EB. 16	*Bacillus* sp.	+	-	AJ550463.1
EB. 17	*Bacillus* sp.	+	-	JF802184.1
EB. 18	*Bacillus* sp.	+	-	EU977790.1
EB. 20	*Bacillus* sp.	+	-	HQ256520.1
EB. 32	*Bacillus* sp.	-	-	HQ003450.1
EB. 33	*Bacillus* sp.	-	-	GQ34O503.1
EB. 39	*Streptomyces* sp.	-	-	FJ951435.1
EB. 42	*Bacillus* sp.	-	-	JN082266.1
EB. 45	*Lysinibacillus sphaericus*	-	-	JN215512.1
EB. 61	*Bacillus* sp.	-	-	AF332386.1
EB. 69	*Bacillus* sp.	-	-	GQ34O516.1
EB. 78	*Bacillus* sp.	+	+	EU977790.1
EB. 80	*Bacillus* sp.	-	-	EU972777.1
EB. 81	*Bacillus* sp.	+	-	HQ003450.1
EB. 83	*Bacillus* sp.	-	-	AF332386.1
EB. 84	*Bacillus subtilis*	+	-	HQ334981.1
EB. 86	*Arthrobacter* sp.	-	-	FJ477042.1
EB. 97	*Bacillus amyloliquefaciens*	-	-	AB301022.1
EB. 98	*Micrococcus luteus*	+	+	FJ380958.1
EB. 102	*Bacillus pumilus*	-	-	EU977790.1
EB. 106	*Rhizobium* sp.	+	-	AY693664.1
EB. 107	*Bacillus thuringiensis*	-	-	AM292316.1
EB. 108	*Rhizobium* sp.	-	-	AY693664.1
EB. 132	*Bacillus subtilis*	-	-	AY741264.1
EB. 135	*Bacillus pumilus*	-	-	EU977790.1
EB. 139	*Acetobacter* sp.	-	-	GU385849.1
EB. 141	*Lysinibacillus* sp.	-	-	GU172164.1
EB. 148	*Aneurinebacillus* sp.	+	-	AB112723.1
EB. 154	*Bacillus pumilus*	+	-	HQ334985.1
EB. 162	*Bacillus pumilus*	-	-	GQ917222.1
EB. 181	*Paenibacillus* sp.	-	-	HE577054.1
EB. 200	*Bacillus pumilus*	-	-	EU977790.1

With L-tryptophan: TSA supplemented with L-tryptophan (10%); Without L-tryptophan: TSA not supplemented with L-tryptophan; + Ability to synthesize IAA; – Inability to synthesize IAA.

Isolates EB.07, EB.78, and EB.98 (corresponding to *Agrobacterium tumefaciens*, *Bacillus* sp., and *Micrococcus luteus*, respectively) were the only isolates that synthesized IAA in the absence of L-tryptophan. These isolates also synthesized IAA in culture medium supplemented with L-tryptophan.

Our results indicated that most of the tested isolates synthesize IAA in medium supplemented with L-tryptophan and that most of these isolates belong to the genus

Bacillus. Patten and Glick[27] and Barazani and Friedman[7] reported that, in general, endophytic bacteria and rhizobacteria synthesize IAA via an L-tryptophan-dependent route. This amino acid can be found in soil, is produced by plants, and is exuded through their roots; therefore, IAA production via L-tryptophan is the most common pathway.

Approximately 80% of microorganisms isolated from the rhizosphere from various crops possess the ability to synthesize and release auxins as secondary metabolites. The secreted IAA interferes with the many plant developmental processes; furthermore, IAA acts as a reciprocal signaling molecule affecting gene expression in microorganisms[2].

Different authors have reported the incidence of the genus *Bacillus* associated with banana roots in India[18], Mexico[24], China[19], Kenya[26], and Brazil[36]. The works cited above indicate the existence of a close association between the mentioned genera and *Musa* spp.

Several species of the genus *Bacillus* promote plant growth via the synthesis of auxins (primarily IAA), the solubilization of poorly soluble phosphates, siderophore production, and the fixation of atmospheric nitrogen[4, 22, 23, 28, 29, 40].

Significant differences in the production of IAA were observed between the isolates in 10% TSA medium with and without L-tryptophan. Among the 16 isolates evaluated in medium supplemented with L-tryptophan, seven clusters were formed, and isolate EB.07 (*A. tumefaciens*) had the highest concentration of IAA, with an average production of 16.61 µg mL^{-1} (Fig. 1).

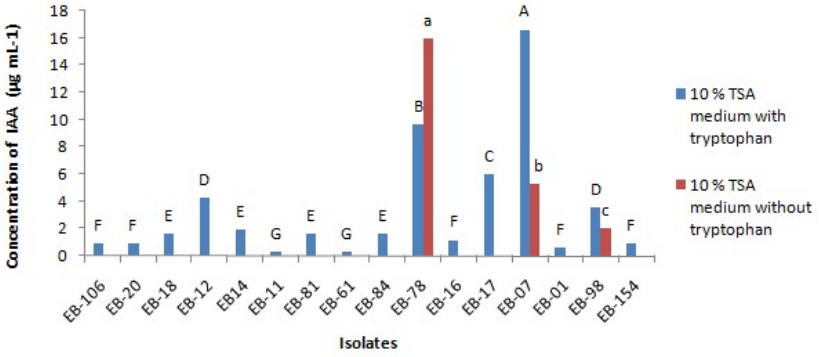

Figure 1 - Average concentrations of IAA (µg mL^{-1}) in 10% TSA medium with and without L-tryptophan by endophytic bacteria isolated from "Prata Anã" banana roots. The means followed by the same upper and lower case letters indicate they belong to the same group through the Scott-Knott test at a significance level at $P < 0.05$.

Agrobacterium tumefasciens, was one of the firsts plant-associated bacteria in which IAA biosynthesis was studied. In this specie and all other gall-inducing bacteria, the indole-3-acetamide (IAM) pathway is present and contribute to the virulence by producing high amounts of IAA[37]. Two genes have been characterized in *A. tumefasciens*, *iaaM* gene, responsible for converting tryptophan to IAM and *iaaH* gene, responsible for hydrolyzed IAM in IAA[42].

In dicotyledonous plants this specie can infect at wound sites by integrating their genes into the genoma of the plants. The overproduction of auxin and cytokinin by the transformed plant cells results in the typical crown gall[37]. Although, *A. tumefaciens* is described as phytopathogenic it has been reported in the literature as an endophytic bacteria that is asymptomatically associated with the roots of *Musa* spp.[36].

Mayer[25] reported that several variations in the Salkowski test are found in the literature regarding the detection of indole compounds produced by bacteria, including IAA[6]. Certain organisms, such as *Agrobacterium* sp. and *Bacillus* sp., can

promote plant growth by increasing the length of roots and the number of root hairs[27]. In addition, Tien *et al.*[39] confirmed that this effect is due to the microbial production of plant hormones or plant growth regulators.

The optimal tissue auxin concentration for elongation growth is approximately 10^{-5} to 10^{-6} parts per million. In the present study, isolate EB.07 produced 16.61 parts per million, corresponding to 1.6 million more than the optimal tissue auxin concentration.

Variations in the synthesis of IAA between the isolates cultured in 10% TSA medium without L-tryptophan were also observed. The IAA concentrations ranged between 2.1 µg mL^{-1} for isolate EB.98 (*M. luteus*) and 15.97 µg mL^{-1} for isolate EB.78 (*Bacillus* sp.) (Fig. 1).

Bacillus sp. isolate EB.78 synthesized IAA in media with and without L-tryptophan, corresponding to 4.12 µg mL^{-1} in the supplemented medium and 15.97 µg mL^{-1} in the unsupplemented medium. This result indicates that in addition to IAA synthesis via the L-tryptophan-dependent pathway, this isolate efficiently uses a pathway independent of L-tryptophan.

Tsavkelova *et al.*[40] reported that the amount of IAA produced via an L-tryptophan-independent pathway is negligible, *i.e.*, only a few bacterial species produce IAA through this pathway. However, those that use this pathway produced large amounts of IAA, with a variation between 7 and 43 µg mL^{-1}.

A previous study conducted with endophytic bacteria isolated from banana roots also demonstrated the same profile. Most of the tested isolates synthesized IAA in TSA medium supplemented with L-tryptophan (40%) and fewer than 20% synthesized IAA via an L-tryptophan-independent pathway in this medium[3].

Production of indole compounds determined by HPLC

Four indole compounds synthesized by endophytic bacteria were identified: IAA, IPA, ILA, and indole-3-acetic aldehyde (IAAld). All isolates synthesized IAAld, an intermediate of IAA biosynthesis, but in significantly lower amounts compared with the production of the other indole compounds (Fig. 2).

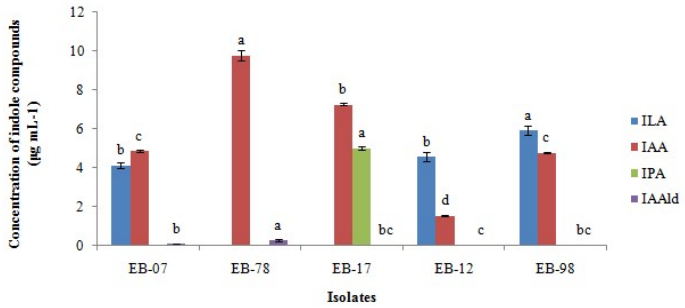

Figure 2 - Average concentrations (ug mL-1) of ILA, IAA, IPA, and IAAld synthesized by endophytic bacteria isolated from the roots of "Prata Anã" bananas. Vertical bars indicate the standard error of the mean. The means followed by different letters indicate significant differences using Tukey's test at a significance level of 5% ($P < 0.05$).

IAA and IPA presented a very similar retention time for the *M. luteus* isolate EB.98. In addition, the average concentration of IPA synthesized by *Bacillus* sp. isolate EB.17 was 1.0046 µg mL^{-1} (Fig. 2).

ILA was produced by three of the five evaluated isolates: *Agrobacterium tumefaciens* isolate EB.07, *Bacillus pumilus* isolate EB.12, and *M. luteus* isolate EB.98. The isolate EB.98 produced a significantly higher amount of ILA than the other isolates, with an average of 1.18 µg mL^{-1} (Fig. 2).

The most highly produced indole was IAA (Fig. 2). The highest concentration of IAA was produced by *Bacillus* sp. isolate EB.78, with an average of 1.95 µg mL^{-1}, whereas the lowest concentration was produced by *B. pumilus* isolate EB.12, with an average of 0.30 µg mL^{-1}.

At least five different pathways have been described for the synthesis of IAA, and most of them show similarity to those described in plants. However, some intermediates can differ in the following respects: (1) IAA formation by IPA and IAAld; (2) The conversion of tryptophan into IAAld may involve an alternative pathway; (3) IAA biosynthesis via IAM; (4) IAA biosynthesis that involves tryptophan conversion into indole-3-acetronitrile; and (5) The tryptophan independent pathway[27, 37, 40].

In beneficial bacteria that produce IAA, the main pathway is the IPA pathway. In this pathway, L-tryptophan is converted into IPA by an aminotransferase. Through a decarboxylase, IPA is converted to IAAld, which is converted to IAA[35].

Endophytic bacteria isolated from orchids were evaluated, and three indole compounds were determined: IAA was found in the greatest quantity, IPA was present in all tested isolates, and only two isolates produced ILA[15].

Comparison of the production of indoles determined via the Salkowski colorimetric method and HPLC

Significant differences were observed between the two methodologies used to measure the production o00f IAA by the five isolates grown in 10% TSA medium supplemented with L-tryptophan (Fig. 3). This result was likely due to the environmental and growth conditions to which the isolates were subjected during the experiments in Janaúba, Minas Gerais (Salkowski reagent), and in Jaboticabal, São Paulo (HPLC).

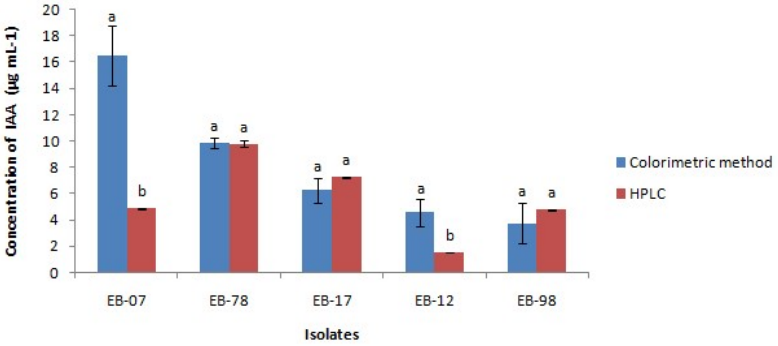

Figure 3 - Average concentrations of IAA (ug mL^{-1}) produced by isolates grown in 10% TSA medium supplemented with L-tryptophan and quantified using the Salkowski colorimetric method and HPLC. Vertical bars indicate the standard error of the mean, and means followed by separate lower case letters indicate significant differences using Tukey's test at a significance level of 5% ($P < 0.05$).

The comparison of the methods for quantifying IAA in 10% TSA medium supplemented with L-tryptophan indicated significant differences in two of the five evaluated isolates: *A. tumefaciens* isolate EB.07 and *B. pumilus* isolate EB.12. For both isolates, the Salkowski method indicated higher concentrations than the concentrations obtained by HPLC. For isolate EB.07, there was a four-fold difference between the two methods (Fig. 3). No significant differences were observed between the two methods for the *Bacillus* sp. isolate EB.78, *Bacillus* sp. isolate EB.17, and *M. luteus* isolate EB.98.

Crozier *et al.*[12] compared the synthesis of auxins in cultures of *Azospirillum* spp. and found differences greater than 50-fold for IAA between the two techniques. In this

same study, the authors showed that HPLC detected the production of IAA in isolates for which production was not detected by colorimetry.

The overestimation of the IAA concentration using the colorimetric method may be caused by the presence of other indoles (involved in the biosynthesis of auxins by bacteria) in the culture that are more reactive toward the Salkowski reagent than IAA alone[41].

HPLC exhibits high sensitivity, speed, performance, reliability, and promptness in the release of results. By contrast, the Salkowski method is based on the comparison of the color produced in a chemical reaction with a standard color, and the intensity of the color produced indicates the concentration of a particular analyte.

Considering the results obtained in the present study as well as previous investigations, we can infer that inaccurate data are likely to be obtained with the Salkowski reagent and that the use of complementary methods, such as HPLC, is consequently required.

However, it is important to note that the Salkowski colorimetric method, because of its practicality, speed, specificity, and efficiency, should be used in initial screenings aimed at selecting isolates with the potential to synthesize IAA. By contrast, HPLC, due to its high sensitivity, great cost, rapid delivery of results, and high efficiency, should be used as a complementary method in the selection of isolates with the greatest potential to synthesize indoles.

CONCLUSION

Four indole compounds were determined: IAA, IPA, ILA, and IAAld. Among the isolates, *A. tumefaciens* isolate EB.07 presents a greater ability to synthesize IAA *in vitro* in the presence of L-tryptophan, whereas *Bacillus* sp. isolate EB.78 synthesizes IAA more efficiently in an L-tryptophan–independent alternative pathway. Considering the methods for indole-compound estimation, both are useful and present valuable information for initial and complementary screenings of plant growth-promoting bacteria (PGPBs).

CONFLICTS OF INTEREST

The authors declare no conflicts of interest

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REFERENCES

1. Aguilar-Piedras JJ, Xiqui-Vásquez ML, García-García S, Baca BE. Producción de ácido indol-3-acético en Azospirillum. Rev Latinoam Microbiol. 2008;50:29-37.

2.Ahemad M, Kibret M. Mechanisms and application of plant growth promoting rhizobacteria: Current perspective. J of King Saud U Sci. 2014;26:1-20.

3.Andrade LF, Souza GLOD, Nietsche S, Xavier AA, Costa MR, Cardoso MAS, Pereira MCT, Pereira DFGS. Analysis of the Abilities of Endophytic Bacteria Associated with Banana Tree Roots to Promote Plant Growth. J of Microbiol. 2014;52:27-34.

4.Araújo FF, Guaberto LM, Silva IF. Bioprospecção de rizobactérias promotas de crescimento em Brachiaria brizantha. Rev Bras Zootecn. 2012;41:521-527.

5.Baldotto LEB, Baldotto MA, Olivares FL, Viana AP Bressan-Smith R.) Seleção de bactérias promotoras de crescimento no abacaxizeiro (Ananas comosus L. Merrill) cultivar Vitória durante a aclimatização. Rev Bras Cienc Solo. 2010;34:349-360.

6.Balota EL, Lopes ES, Hungria M, Dobereiner J (1995) Interações e efeitos fisiológicos de bactérias diazotróficas e fungos micorrízicos arbusculares na mandioca. Pesqui Agropecu Bras. 1995;30:1335-1345.

7.Barazani O, Friedman J. Effect of exogenously applied ltryptophanon allelochemical activity of plant growth-promoting rhizobacteria (PGPR). J Chem Ecol. 2000;26:344-349.

8.Blazevic DJ, Ederer GM. Principles of biochemical tests in diagnostic microbiology: techniques in pure and applied microbiology. New York, USA: Willey and Co.;1975.

9.Cassán F, Perrig D, Sgroy V, Masciarelli O, Penna C, Luna V. Azospirillum brasilense Az39 and Bradyrhizobium japonicum E109, inoculated singly or in combination, promote seed germination and early seedling growth in corn (Zea mays L.) and soybean (Glycine max L.). Eur J Soil Biol. 2009;45:28-35.

10.Cavalcante JJV, Vargas C, Nogueira EM, Vinagre F, Schwarcz K, Baldani JI, Ferreira PCG, Hemerly AS. Members of the ethylene signalling pathway are regulated in sugarcane during the association with nitrogen-fixing endophytic bacteria. J Exp Bot. 2007;58:673-686.

11.Costacurta A, Vanderleyden J. Synthesis of phytohormones by plant associated bacteria. Crit Rev Microbiol. 1995;21:1-18.

12.Crozier L, Arruda P, Jasmim JM, Monteiro AM, Sandberg G. Analysis of indole-3-acetic acid and related indoles in culture medium from Azospirillum lipoferum and Azospirillum brasilense. Appl Environ Microbiol. 1998;54:2833-2837.

13.Fagan EB, Medeiros SLP, Manfron PA, Casarolil D, Simon J, Dourado Neto D, Van Lier QJ, Muller L. Fisiologia da fixação biológica de nitrogênio em soja. Rev Fac Zootecn V Agron. 2007;14:89-106.

14.Ferreira DF. SISVAR: um programa para análises e ensino de estatística. Rev Cient Sym. 2008;6:36-41.

15.Galdiano Jr. RF, Pedrinho EAN, Castellane TCL, Lemos EGM. Auxin-producing bacteria isolated from the roots of cattleya walkeriana, an endangered brazilian orchid, and their role in acclimatization. Rev Bras Cienc Solo. 2011;35:729-737.

16.Glickmann E, Dessaux Y. A critical examination of the specificity of the Salkowsky reagent for indolic compounds produced by phytopathogenic bacteria. Appl Environ Microbiol. 1995; 61:793-796.

17.Gordon SA, Weber RP. Colorimetric estimation of indoleacetic acid. Plant Physiol. 1951;26:192-195.

18. Harish S, Kavino M, Kumar N, Saravanakumar D, Soorianathasundaram K, Samiyappan R. Biohardening with plant growth promoting rhizosphere and endophytic bacteria induces systemic resistance against banana bunchy top virus. Appl Soil Ecol. 2008;39:187-200.

19.Jie L, Zifeng W, Lixiang C, Hongming T, Patrik I, Zide J, Shining Z. Artificial inoculation of banana tissue culture plantlets with indigenous endophytes originally derived from native banana plants. Biol Control. 2009;51:427-434.

20.Kuss AV, Kuss VV, Lovato T, Flôres ML. Fixação de nitrogênio e produção de ácido indolacético in vitro por bactérias diazotróficas endofíticas. Pesqui Agropecu Bras. 2007;42:1459-1465.

21.Loiret FG, Grimm B, Hajirezaei MR, Kleiner D, Oetega E. Inoculation of sugarcane with Pantea sp. Increases amino acid contents in shoot tissues, serine, alanine, glutamine, and asparagine permit concomitantly ammonium excretion and nitrogenase activity of the bacterium. J Plant Physiol. 2009;166:1152-1161.

22.Luo S, Xu T, Chen L, Chen J, Rao C, Xiao X, Wan Y, Liu Y. Endophyte-assisted promotion of biomass production and metal-uptake of energy crop sweet sorghum by plant-growth-promoting endophyte Bacillus sp. SLS18. Appl Microbiol Biotechnol. 2012;93:1745-1753.

23. Manjula K, Podile AR. Increase in seedling emergence and dry weight of pigeon pea in the field with chitin-supplemented formulations of Bacillus subtilis AF 1. World J Microb Biot. 2005;21:1057-1062.

24.Martínez L, Caballero-Mellado J, Orozco J, Martínez-Romero E. Diazotrophic bacteria associated with banana (Musa spp.). Plant Soil. 2003;257:35-47.

25.Mayer AM. Determination of indole acetic acid by the Salkowsky reaction. Nature. 1958; 182:1670-1671.

26.Ngamau CN, Matiru VN, Tani A, Muthuri CW. Isolation and identification of endophytic bacteria banana production. Afr J Microbiol Res. 2012;6:6414-6422.

27.Patten CL, Glick BR. Bacterial biosynthesis of indole-3-acetic acid. Can J Microbiol. 1996;42:207-220.

28.Persello-Cartieaux F, Nussaume L, Robaglia C. Tales from the underground: Molecular plant-rhizobacteria interactions. Plant Cell Environ. 2003;26:189–199.

29.Phetcharat P, Duangpaeng A. Screening of endophytic bacteria from organic rice tissue for indole acetic acid production. Procedia Eng. 2012;32:177-183.

30.Reis Junior FB, Silva MF, Teixeira KRS, Urquiaga S, Reis VM. Identificação de isolados de Azospirillum amazonense associados a Brachiaria spp., em diferentes épocas e condições de cultivo e produção de fitormônio pela bactéria. Rev Bras Cienc Solo. 2004;28:103-113.

31.Rodrigues EP, Coelho MS, Soares CP, Vidal MS, Araújo JLS, Baldani JI. Identificação e quantificação de compostos indólicos no sobrenadante de Gluconacetobacter diazotrophicus, cultivada em meio liquido, por cromatografia líquida de alta eficiência (HPLC). Bol Pesq Desenv. 2009;52:1-25.

32.Sarwar M, Kremer RJ. Determination of bacterially derived auxins using a microplate method. Lett Appl Microbiol. 1995;20:282-285.

33.Shahab S, Ahmed N, Khan NS. Indol acetic acid production and enhanced plant growth promotion by indigenous PSBs. Afr J Agric Res. 2009;4:1312-1316.

34.Shiomi HF, Melo IS, Minhoni MTA. Seleção de bactérias endofíticas com ação antagônica a fitopatógenos. Sci Agr. 2008;9:535-538.

35.Solano BR, Barriuso J, Gutiérrez Mañero FJ. Physiological and Molecular Mechanisms of Plant Growth Promoting Rhizobacteria (PGPR). In: Plant-Bacteria Interactions: Strategies and Techniques to Promote Plant Growth (eds I. Ahmad, J. Pichtel and S. Hayat), Wiley-VCH Verlag GmbH & Co. Weinheim; 2008.

36.Souza SA, Xavier AA, Costa MR, Cardoso AMS, Pereira MCT, Nietsche S. Endophytic bacterial diversity in banana 'Prata Anã' (Musa spp.) roots. Genet Mol Biol. 2013;36:252-264.

37.Spaepen S, Vanderleyden J, Remans R. Indole-3-acetic acid in microbial and microorganism-plant signaling. FEMS Microbiol Rev. 2007;31:425–448.

38.Teixeira MA, Melo IS, Vieira RF, Costa FEC Harakava R. Microrganismos endofíticos de mandioca de áreas comerciais e etnovariedades em três estados brasileiros. Pesqui Agropecu Bra. 2007;42:43-49.

39.Tien TM, Gaskin MH, Hubbell DH. Plant growth substances produced by Azospirillum brasilense and their effect on growth of pearl millet (Pennisetum americanum L.). Appl Environ Microbiol. 1979;37:1016-1024.

40.Tsavkelova EA, Klimova SY, Cherdyntseva TA, Netrusov AI. Microbial producers of plant growth stimulators and their practical use: a review. Appl Biochem Micro. 2006;42:117–126.

41.Tsavkelova EA, Cherdyntseva TA, Botina SG, Netrusov AI. Bacteria associated with orchid roots and microbial production of auxin. Microbiol Res. 2007;162:69-76.

42.Zupan J, Muth TR, Draper O, Zambryski P. The transfer of DNA from Agrobacterium tumefaciens into plants: a feast of fundamental insights. Plant J. 2000;23: 11–28.

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