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Endophytic microorganisms from *Bauhinia monandra* leaves: Isolation, antimicrobial activities and interaction with galactose-specific lectin BmoLL

S. A. F. Ramos[1], L. C. N. Silva[1,3]*, M. T. S. Correia[1], J. M. Araújo[2] and L. C. B. B. Coelho[1]

[1]Departamento de Bioquímica, Centro de Ciências Biológicas, Universidade Federal de Pernambuco. Av. Moraes Rego S/ N, Cidade Universitária, Recife-PE 50000, Brazil.
[2]Departamento de Antibióticos, Centro de Ciências Biológicas, Universidade Federal de Pernambuco. Av. Moraes Rego S/ N, Cidade Universitária, Recife-PE 50000, Brazil.
[3]Functional Genomics, Department of Biology, Faculty of Science, University of Copenhagen, Ole Maaløes Vej 5, 2200, Denmark.

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Bauhinia monandra **leaves are well known hypoglycemic agent in Brazilian popular medicine from which a galactose specific lectin (BmoLL) has been purified in milligram quantities. In this work, the antimicrobial activity of endophytes isolated from *B. monandra* leaves and the ability of BmoLL to agglutinate the microorganisms were evaluated. After disinfection, leaves of *B. monandra* were fragmented and distributed in Petri plates leading to isolation of fungi (37 strains) and both Gram-positive (26 strains) and negative bacteria (6 strains). A preliminary antimicrobial assay revealed that 62% of bacterial strains were active, while no antagonist action was detected with fungi. However, only 2 strains were able to excrete the antimicrobial compounds: *Pseudomonas aeruginosa* UFPEDA598 inhibited *Aspergillus niger*, *Fusarium moniliform*, *Fusarium oxysporum*, *Micrococcus luteus, Staphylococcus aureus*, *Bacillus subtilis* and PE(24)C1 inhibited *Candida* sp. strains, *M. luteus* and *S. aureus*. On the other hand, BmoLL did not show any antimicrobial action, but it was able to agglutinate the active strain *P. aeruginosa* UFPEDA598. The BmoLL-*Pseudomonas* interaction could promote a defense against the attack of phytopathogenic microorganisms in plants through a *P. aeruginosa* metabolic production mechanism. The lectins/endophytes interaction could be a new line to unravel defense mechanisms against phytopathogenic organisms.**

Key words: Endophytes, *Bauhinia monandra* leaf lectin, antimicrobial activities, *Pseudomonas aeruginosa*, agglutination.

INTRODUCTION

Endophytic bacteria and fungi are organisms that could live in association with plants for most, if not all, their life cycles. They live within the intercellular spaces of plants, in tissues such as roots, stems and leaves (Hormazabal and Piontelli, 2009). They invade the vegetal in different stages of development, but do not cause symptoms of

diseases. Some fungi and bacteria can be isolated from a great variety of host plant families while growing under different ecological and geographical conditions. Other endophytes are apparently restricted to host species that occur within a particular plant family (Azevedo et al., 2000; Yuan et al., 2010). Endophytic microorganisms may confer advantage to the plant, and the benefits may be reciprocal, resulting in an enhanced symbiotic system for specific plant characteristics. Therefore, the use of endophytic bacteria and fungi opens up new areas of biotechnological explorations, which leads to the necessity to isolate and cultivate these organisms. Endophytes are used for biological control of various plant diseases, to enhance agronomic plant characteristics such as increased drought tolerances and nitrogen efficiency, as bioherbicides, and pharmaceutical agents (Bacilio-Jiménez et al., 2001; Audenaert et al., 2002; Vendan et al., 2010). Several endophytes can be found in a unique species and act in plant defense against pathogenic microorganisms (Omacine et al., 2001).

Lectins are carbohydrate binding proteins or glycol-proteins that participate in various metabolic processes in cells (Coriolano et al., 2014; da Silva and Correia, 2014). There is evidence that lectins act in the recognition between cells or cells and various carbohydrate-containing molecules; they may be involved in regulating physiological functions. Lectins seem to play an important role in defense mechanisms of plants against the attack of microorganisms, pests and insects. Fungal infection or wounding of the plant seems to increase lectin concentrations (Guan et al., 2008; Charungchitrak et al., 2011). In legumes, the role of lectins in the recognition of nitrogen-fixing bacteria *Rhizobium* genus, which have sugar-containing substances, has received a special attention (Antonyuk and Evseeva, 2006). Plant lectin functions have been speculated, among them; these proteins participate as a binding factor in the interaction between plants and microorganisms (Carlini and Grossi-de-Sá, 2002; Souza et al., 2011). Seeds, roots, flowers as well as leaves may contain considerable concentrations of lectins (Coelho and Silva, 2000; Silva et al., 2014); it is believed that in these organs, lectins could favor the symbioses with endophytic organisms (Antonyuk and Evseeva, 2006; Vershinina et al., 2012).

The genus, *Bauhinia* (Fabaceae) contains a number of ornamental species which are well distributed in the tropics. Some species are important for animal nutrition because of their high protein content; they have been used as forage, as human food, in folk medicine for the treatment of diabetes and as diuretic (Macedo et al., 2007; Souza et al., 2011). Leaves of *Bauhinia monandra* (pata-de-vaca, pulse) contain a relatively high concentration of a galactose-specific lectin (BmoLL); more than 2 mg of highly purified lectin has been obtained from 5 g of leaf powder (Coelho and Silva, 2000). BmoLL already showed insecticidal action against Pyralidae and Bruchidae larvae (Macedo et al., 2007). Previous studies have also reported the isolation and characterization of lectins from *B. purpurea* and *B. monandra*, seeds and roots (Souza et al., 2011). Although the interaction between lectins and phytopathogen microorganisms have been studied deeply (Gaidamashvili and Van Staden, 2002; Charungchitrak et al., 2011), there is still scarce literature concerning their interaction with plant endophytics. The present study reports the isolation and evaluation of antimicrobial activity of endophytic microorganisms obtained from *B. monandra* leaves, and the ability of BmoLL to agglutinate them.

MATERIALS AND METHODS

Isolation of endophytes

A total of 69 endophytes (bacteria and fungi) were isolated from *B. monandra* leaves. Sample collections were performed at the campus of the Universidade Federal de Pernambuco (Recife, State of Pernambuco, Northeast of Brazil). Leaves were washed (10 min) and disinfected (70%, v/v ethanol, 1 min; 5%, v/v, sodium hypochloride, 5 min; 70%, v/v ethanol, 30 s; washed twice in sterile distilled water, 1 min). A control consisted of the last wash. Fragments of tissue (5 mm) were distributed in Petri plates with distinct culture medium. Bacterial isolation was performed using different five media supplemented with cyclohexamide: Nutrient Agar (NA; meat extract 1 g, leaven extract 2 g, peptone 5 g, sodium chloride 5 g, agar 15 g and pH 7.2 - 7.4; distilled water 1000 mL); NA 50%; Czapek-agar medium (CZP; $NaNO_3$ 3 g; K_2HPO_4 1 g, $MgSO_4.7H_2O$ 0.5 g, KCl 0.5 g, $FeSO_4$ 0.01 g, sacarose 30 g, agar 15 g; distilled water 1000 mL), Triptic Soy Agar (TSA; MERCK, 4 g in 100 mL of distilled water) and Casein Starch Agar (CSA; agar 15 g, soluble starch 10 g, $K_2SO_4(2H_2O)$ 2 g, KNO_3 2 g, NaCl 2 g, casein 0.3 g, $MgSO_4(7H_2O)$ 0.05 g, $CaCO_3$ 0.02 g, $FeSO_3(7H_2O)$ 0.01 g, distilled water 1000 mL). For fungi isolation, Sabouraud agar (SAB; DIFCO, 6.5 g in 100 mL of distilled water) and potato dextrose agar medium (PDA; potato 200 g, dextrose 15 g, agar 17 g, distilled water 1000 mL) were both supplemented with tetracycline. Samples were incubated at 28°C for 5 to 20 days. The strains were stored at 4ºC, for short-term on mineral oil in freeze (-20°C).

Antimicrobial activity

To evaluate antimicrobial activity, two assays were accomplished with each isolated endophyte: one in solid (agar plug assay) and

*Corresponding author. E-mail: luisclaudionsilva@yahoo.com.br. Tel: +45 91110893 or +55 8194508770.

another in liquid culture medium (fermentation broth assay). In all assays, 11 microorganisms were used: *Staphylococcus aureus* (UFPEDA 01), *Micrococcus luteus* (UFPEDA 06), *Bacillus subtilis* (UFPEDA 16), *Candida albicans* (UFPEDA 1007 and UFPEDA 2224), *Aspergillus niger* (UFPEDA 2003), *Colletotrichum gramminicola* (UFPEDA 2403), *Fusarium moniliforme* (UFPEDA 2409), *Fusarium oxysporum* (UFPEDA 3505), *Candida* sp. (UFPEDA 1315 and UFPEDA 1316) (Table 1).

Agar plug diffusion assay (primary screening)

For the preparation of the agar plug, bacterial strains were cultivated in NA and TSA plates, while SAB and PDA plates were used to inoculate fungi. After incubation, agar plugs (5 mm) were placed on the agar medium seeded with test microorganisms. After incubation during 16-18 (bacteria), 24-48 (yeast) or 72-96 h (fungi), the inhibition diameter zones (IDZ) formed around the fungal agar plugs were measured. Isolates exhibiting significant activities were then subjected to secondary screening.

Assay with fermentation broth

The endophytic bacteria with highest spectrum activity in agar plug assay were cultured overnight (28°C, shaked at 180 rpm) in 50 mL of tryptic soy broth (TSB, DIFCO, 3 g in 100 mL of distilled water). Here, 2.5 mL of each pre-inoculum was cultured in a 250 mL Erlenmeyer flask containing 100 mL of M1 (soybean meal 1 g, glucose 1 g, $CaCO_3$ 0.1 g, NaCl 5 g and 100 mL of distilled water, pH 7.0) and TSB. The endophytic fungi were grown (48 h) in 50 mL of SAB as pre-inoculum and 100 mL of SAB and M1 as fermentation media, shaked at 180 rpm, 30°C. Aliquots of fermentation broth (30 µL) were added to Petri plates seeded with each pathogenic strain to be assayed in the respective culture media. All experiments were made in triplicate.

Assays with BmoLL

Purification of BmoLL

Fresh leaves of *B. monandra* were harvested from the Germplasm Bank of Medicinal Plants at the Experimental Station of Itapirema (Goiana, State of Pernambuco Brazil) or from ornamental trees in the cities of Olinda and Recife (State of Pernambuco, Northeast of Brazil). A sample of the collected material is archived as voucher specimen number 57462, IPA, at the herbarium "Dárdano de Andrade Lima" (Empresa Pernambucana de Pesquisa Agropecuária, Recife, Brazil). Petioles were removed from the fresh leaf material; the blades were well washed in tap water followed by distilled water, and allowed to dry at room temperature. Dried blades were powdered in a multiprocessor and extract (10% w/v) was obtained by overnight gentle shaking at 4°C in a 0.01 mol L^{-1} citrate phosphate buffer (pH 6.5) containing 0.15 mol L^{-1} sodium chloride (the selected buffer). This extract was passed through gauze and centrifuged at 11,270 g for 15 min to give preparation P1, which was submitted to 60% (w/v) ammonium sulphate fractionation (F 0-60%) by addition of solid salt. After 4 h at room temperature, the resuspended precipitate was dialyzed against distilled water, followed by the selected buffer (preparation P2). An affinity matrix was made by cross-linking refined gum guar (guaran; Sigma Chemical Co., St. Louis, MO, USA) with epichlorohydrin in a mixture of water: 2-propanol (Gupta et al., 1979). A sample (140 mg) of P2 containing BmoLL was applied to a 10 mL guar gel column. The affinity column was washed with buffer until the absorbance at 280 nm was zero, and then a galactose solution (0.05 mol L^{-1}) prepared in the buffer was used to irrigate the gel. The fractions with high activity were bulked and dialyzed with three changes of 0.01 mol L^{-1} citrate phosphate buffer (pH 6.5) containing 0.15 mol L^{-1} sodium chloride (1 mL/100 mL/h). Hemagglutinating activity (HA) was determined (Correia and Coelho, 1995); protein concentration was measured according to Lowry et al. (1951) and by absorbance at 280 nm. The material was stored at 20°C until used.

Assay of antimicrobial activity to lectin

A previous assay of antimicrobial activity to lectin (Ye and Ng, 2001) was carried out in Petri plates (100 x 15 mm) containing 10 mL of NA medium or 10 mL of SAB. Around a plug of bacterial or fungi (0.5 cm in diameter) grown previously in specific culture medium, at a distance of 1 cm away from it, were placed sterile blank paper disks of the same size. Aliquots (10 µL) containing 30 to 300 µg of BmoLL (in selected buffer) was added to a disk. The plates were incubated at 28°C for 24 h for bacteria, to analyze the development of microorganisms on plates. The pathogenic microorganisms were used in this assay, as well the endophytics bacteria strains PE(23)C1, PE(24)C1, UFPEDA598, PE(63)C1, PE(64)C1 and PE(67)C1 (chosen since they showed a good performance in primary antimicrobial screening).

Agglutination assay

To evaluate the agglutination activity of BmoLL, two Gram-positive (PE(17)C1 and PE(24)C1) and two Gram-negative (UFPE-DA598 and PE(65)C1) endophytics isolated from *B. monandra* leaves were used as test organisms. Additionally, *S. lutea* (Waksman), *P. aeruginosa* (IT 2633 and ATCC 27853), *E. coli* (ATCC 25922) and *S. aureus* (Waksman) were used as controls. The bacterial strains were cultured in TSB broth (50 mL) and incubated overnight under shaking (180 rpm) at 28°C. Aliquots (5 mL) were transferred to Erlenmeyers containing 100 ml^{-1} of medium and were incubated at 28°C (180 rpm). After 48 h, bacterial cells were centrifuged at 750 g, 7 min at 4°C, washed three times in NaCl 0.15 mol l^{-1}, two times in citrate-phosphate buffer 5% (v/v) pH 6.5 for 7 min at room temperature and resuspended in selected buffer. The turbid suspensions were adjusted to approximately 10^8 cells per mL^{-1}.

The agglutination assay was performed in microtitre plates (96 wells). In this assay, 50 µL of 0.15 mol L^{-1} NaCl, 50 µL of a bacterial suspension (10^8 cell mL^{-1}) and a serial dilution of 50 µL highly purified BmoLL or Con A (a comparison lectin) preparation (0.96 mg mL^{-1}) were mixed in the plate. The control did not contain lectin. Agglutination activity was observed and photographed after 24 h, using an OLYMPUS BH-2 microscope.

Inhibition assays were performed with a solution containing 100 µL of 100 mmol L^{-1} galactose in 0.15 mol L^{-1} sodium chloride mixed with 100 μL^{-1} of lectin preparation (0.96 mg mL^{-1}), and 50 µL of this mixture was distributed in the wells. After 15 min at room temperature, 50 µL of bacterial solution was added in a final volume of 100 µL. The result was recorded visually after 45 min at room temperature.

RESULTS

Isolation and antimicrobial screening of *B. monandra* endophytic microorganisms

A total of 69 endophytic strains were isolated from

Table 1. Antimicrobial activity of *Bauhinia monandra* leaves endophytes in NA medium.

Microorganism assayed	Endophytic in NA medium					
	PE23C1	PE24C1	UFPE-DA598*	PE63C1	PE64C17	PE67C1
Staphylococcus aureus (UFPEDA 01)[a]	--	+++	+++++	--	+	+++
Micrococcus luteus (UFPEDA 06)[a]	+++	++++	++++	--	+	--
Bacillus subtilis (UFPEDA 16)[a]	--	--	++++	--	--	--
Candida albicans (UFPEDA 1007)[a]	--	--	++	--	--	--
Aspergillus niger (UFPEDA 2003)[a]	--	--	+++	--	--	--
Candida albicans (UFPEDA 2224)[m]	++	+	--	--	+	--
Colletotrichum gramminicola (UFPEDA 2403)[a]	--	+++	+++++	+++	--	--
Fusarium moniliforme (UFPEDA 2409)[a]	--	--	+	++	--	+
Fusarium oxysporum (UFPEDA 3505)[m]	--	--	++	+	+	+++
Candida sp. (UFPEDA 1315)[a]	+	+	++++	--	+	--
Candida sp. (UFPEDA 1316)[a]	--	+++	+++	--	--	--

Inhibition zone: --, 0-5 mm; +, 5-10 mm; ++, 10-15 mm; +++, 15-20 mm; ++++, 20-25 mm; +++++, above 25 mm. [a] Collection from the Department of Antibiotics, [m] collection from the Department of Mycology, * *Pseudomonas aeruginosa*. NA: Nutrient Agar.

Table 2. Antimicrobial activity of *Bauhinia monandra* leaves endophytes in TSA medium.

Microorganisms	Endophytics in TSA medium					
	PE23C1	**PE24C1**	**UFPE-DA598***	**PE63C1**	**PE64C1**	**PE67C1**
Staphylococcus aureus (01)[a]	--	++++	++++	--	+	++
Micrococcus luteus (06)[a]	++++	++++	++++	--	+	++
Bacillus subtilis (16)[a]	--	--	+++++	--	+	+
Candida albicans (1007)[a]	--	--	++	--	--	--
Aspergillus niger (2003)[a]	--	--	+++	--	--	--
Colletotrichum gramminicola (2403)[a]	--	++++	+++++	+++	--	--
Fusarium moniliforme (2409)[a]	--	--	++	++	--	++
Fusarium oxysporum (3505)[m]	--	--	+++	+	+	+
Candida sp. (1315)[a]	--	+	+++	--	++	--
Candida sp. (1316)[a]	++	+	++++	--	--	--

Inhibition zone: --, 0-5 mm; +, 5-10 mm; ++, 10-15 mm; +++, 15-20 mm; ++++, 20-25 Inhibition zone: --, 0-5 mm; +, 5-10 mm; ++, 10-15 mm; +++, 15-20 mm; ++++, 20-25 mm; +++++, above 25 mm. [a] Collection from the Department of Antibiotics, [m] collection from the Department of Mycology, * *Pseudomonas aeruginosa*. TSA, Triptic Soy Agar (MERCK, 4 g in 100 mL of distilled water).

leaves of *B. monandra*, of which 32 were bacteria (Gram-positive: 26 strains; Gram-negative: 6 strains) and 37 fungi. The best medium was PDA for isolation of fungi, and NA for bacteria isolation (data not shown). These organisms were subsequently submitted to a preliminary antimicrobial screening on solid medium against 11 pathogenic microorganisms, where 62% of bacterial strains were active and displayed inhibition zone ranged from 5 to 25 mm. The best antimicrobial activity was found using NA plates (IDZ ranged from 20 to 30 mm). On the other hand, the endophytic fungi did not inhibit any pathogenic microorganism. Six strains showed the best activities as they inhibit more than two pathogenic microorganisms or showed one IDZ value higher than 15 mm (Tables 1 and 2). From those, two strains were selected for the fermentation assay (PE24C1 and UFPEDA 598), as they showed the best antimicrobial potential. The strain PE24C1 inhibited the growth of *S. aureus, M. luteus, C. albicans*, two strains of *Candida* sp. (isolated from clinical material) and *C. gramminicola.* The strain UFPEDA598 inhibited all tested microorganisms and it was identified as *P. aeruginosa* UFPEDA598 (King et al., 1954).

Endophytic efficiency in fermentation broth

Both strains which showed highest spectrum activity in plug agar (PE(24)C1 and *P. aeruginosa* UFPEDA598), were cultured in fermentation broth M1 and TSB and the

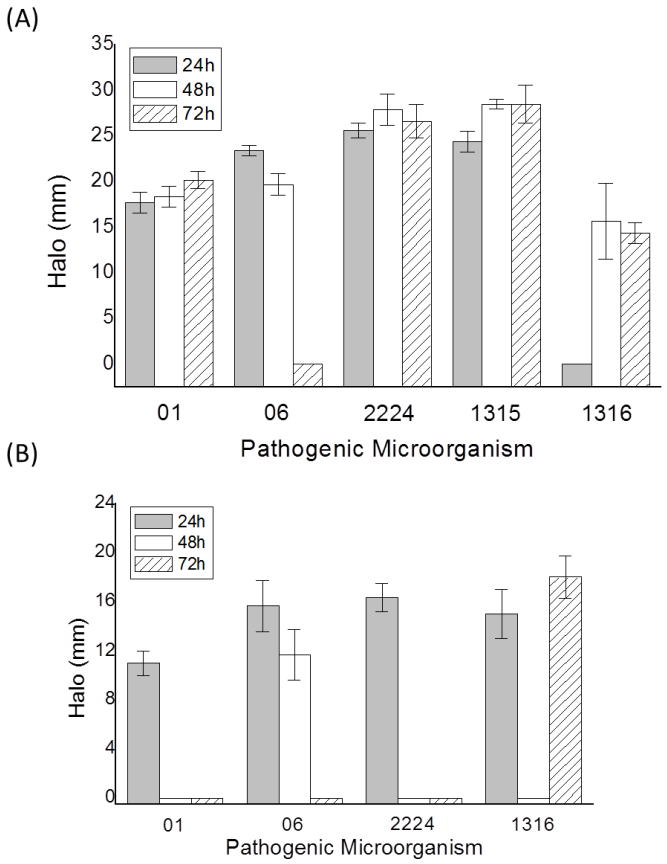

Figure 1. Antimicrobial activity of endophytic strain PE(24)C1 growth in medium M1 (A) and TSB (B). Microorganisms: *S. aureus* (UFPEDA 01), *M. luteus* (UFPEDA 06), *C. albicans* (UFPEDA 2224), *Candida* sp. (UFPEDA 1315 and UFPEDA 1316).

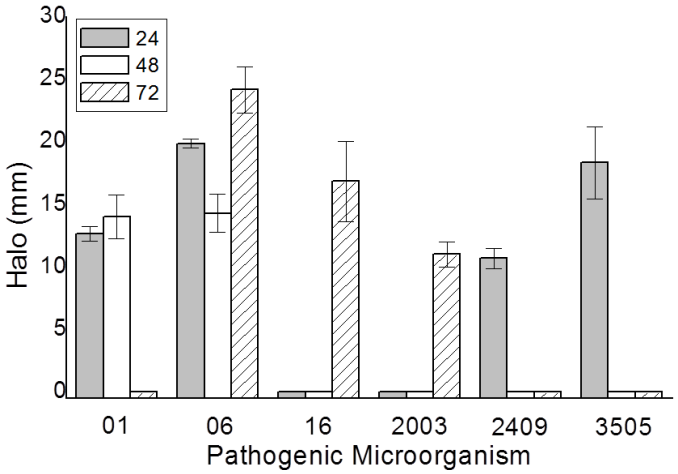

Figure 2. Antimicrobial activity of endophytic strain UFPEDA598 growth in medium M1. Microorganisms: *S. aureus* (UFPEDA 01), *M. luteus* (UFPEDA 06), *B. subtilis* (UFPEDA 16), *A. niger* (UFPEDA 2003), *F. moniliforme* (UFPEDA 2409) *F. oxysporum* (UFPEDA 3505).

antimicrobial activity was evaluated after 24, 48 and 72 h. PE(24)C1 displayed high activity in M1 (Figure 1A) and TS broth (Figure 1B). In M1 broth, the activity was found against the same microorganisms than in agar assay in all times, except *B. subtilis* and *Candida* sp. 1316, for them, no inhibition was observed at 72 or 24 h, respectively. When growth in TSB, PE(24)C1 only inhibited *S. aureus* and *C. gramminicola* (at 24 h), *B. subtilis* (24 and 48 h) and *Candida* sp. 1316 (24 and 72 h). On the other hand, *P. aeruginosa* UFPE-DA598 was only active in TSB, inhibiting fungi (*A. niger*, *F. moniliform* and *F. oxysporum*) and bacteria strains (*M. luteus, S. aureus* and *B. subtilis*) (Figure 2).

Assays with BmoLL

Agglutination assays were performed to investigate the possible interaction of BmoLL, a highly purified galactose specific lectin prepared in milligram quantities (Coelho and Silva, 2000), with endophytes. Bacterial agglutination was expressed as the degree of agglutinin-bacterial solution to the bottom of microtitration plate different from that of a bacterial control. Aggregation was observed visually after overnight incubation of plates clearly indicating the minimal concentration of agglutinins eliciting the bacterial aggregation (Figure 3A and B). Observation of plates revealed agglutination between BmoLL and *P. aeruginosa* UFPEDA598, with a titer of 16^{-1} (Figure 3A). No agglutination was detected in the inhibition assay containing BmoLL, 100 mM of galactose and endophytic. BmoLL did not show an inhibitory effect against six tested bacterial endophytes and negative results were also obtained with all tested microorganisms (data not shown).

DISCUSSION

Infusions of leaves from *B. monandra* are broadly used in popular medicine to treat diabetes and antioxidant activity was revealed in leaf preparations (Argolo et al., 2004). A galactose specific lectin was obtained in milligram quantities from leaves of *B. monandra* and termed *B. monandra* leaf lectin (BmoLL) (Coelho and Silva, 2000). *B. monandra* is an ornamental plant whose leaves are always very healthy. In fact, Macedo et al. (2007) detected an insecticidal activity of BmoLL against *Anagasta kuehniella* (Lepidoptera: Pyralidae), *Zabrotes subfasciatus* and *Callosobruchus maculatus* (Coleoptera: Bruchidae). Another galactose-specific lectin from *B. monandra* secondary roots (BmoRoL) also purified in milligram quantities, showed significant antifungal and

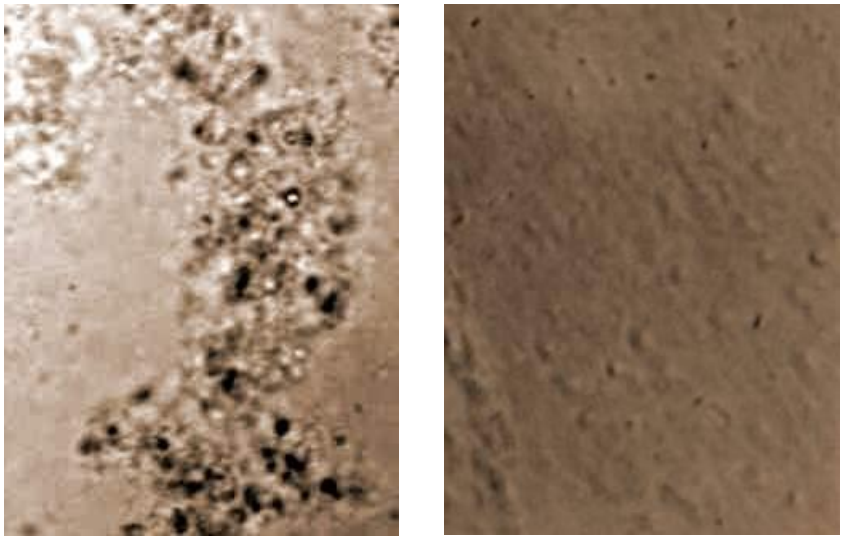

Figure 3. Agglutination of *P. aeruginosa* (UFPEDA598) by BmoLL. A. Suspension of bacteria with 50 µL of BmoLL; B. Suspension of bacteria without BmoLL.

termiticidal activities (Souza et al., 2011).

Endophytic microorganisms have been isolated from different plant species and they are reported as agents in biological control of plant diseases and plagues (Hormazabal and Piontelli, 2009; Vendan et al., 2010; Jin et al., 2014). Endophytes have potential applications in agriculture, industry and medicine (Brader et al., 2014). Bacterial (32) and fungi (37) strains of endophytics were isolated from *B. monandra* leaves, which were assayed for antimicrobial activity against 14 pathogenic micro-organisms. The endophytic strains assayed, with antagonism against phytopathogens, could validate the applicability of endophytic microorganisms as sources of new antibiotic production, or agents for biological control (Yuan et al., 2010). The performance of PE(24)C1 and *P. aeruginosa* (UFPE-DA598) strains against pathogenic microorganisms may allow the speculation of plant defense action by endophytic bacteria, and confirm the production of compounds with antibiotic function by endophyte microorganisms.

P. aeruginosa (UFPE-DA598) displayed antimicrobial activity against fungi *A. niger*, *F. moniliform* and *F. oxysporum*, as well as bacteria *M. luteus, S. aureus* and *B. subtilis*. Most strains of *P. aeruginosa* endophyte shows *in vitro* antagonism to fungi and bacteria. *Pseudomonas* strains have been extensively used in biological control, antibiotic production, rhizobacteria-mediated induced systemic resistance (ISR) or as a considerable tool to plant-defense mechanism evaluation (Nomura et al., 2005). Audenaert et al. (2002) mentioned that tomato with a patented strain, *P. aeruginosa* 7NSK2, induced systemic resistance by secondary metabolites production against *Botrytis cinerea*; this fungus attacks several species of cultivated plants of economic importance. Production of metabolite with antimicrobial activity, or competition for nutrients and exclusion from the ecological niche of colonizing microorganisms, has been suggested as a possible mechanism (Bacilio-Jiménez et al., 2001). It has been proposed that endophytically resident bacteria may be strategically available at the right place and at the right time for suppression of vascular wilt diseases and probably have the unique ability to survive inside plants with little or no microbial competition; the endophytic bacterium and its host plant can coevolve in a communicative and co-operative manner that leads to inhibiting and suppressing vascular (wilt) diseases (Antonyuk and Evseeva, 2006; Bright and Bulgheresi, 2010; Vershinina et al., 2012).

BmoLL did not show any inhibitory effect against six tested bacterial endophytes and negative results were also obtained with all tested microorganisms. Positive results have already been mentioned for other lectins, which agglutinated pathogen microorganisms (Charungchitrak et al., 2011; Souza et al., 2011) or symbiotic root endophytes (Antonyuk and Evseeva, 2006). Gaidamashvili and Van Staden (2002) worked with lectin-like proteins from medicinal plants of South African; they observed antibacterial effects against two Gram-positive bacteria pathogens, *S. aureus* and *Bacillus subtilis*. In roots of transgenic rice (*Oriza sativa* L. cv. Murasaki), the expression of two legume symbiotic lectin genes, psl and gs52, promoted rhizobial colonization (Sreevidya et al., 2005). N-Glycolylneuraminic acid-specific lectins from leaves of mulberry (*Morus alba*) showed anti-bacterial activity against *P. syringae pv mori* a phytopathogenic bacteria of mulberry leaves (Ratanapo

et al., 2001). The high concentration of BmoLL in *B. monandra* leaves allowed obtaining more than 2 mg of pure lectin from 5 g of leaf powder (Coelho and Silva, 2000). BmoLL (96 µg ml^{-1}) agglutinated only one endophytic bacterium: the strain UFPE-DA598, identified as *P. aeruginosa*. *Pseudomonas* strains have been reported to bind lectins (Boteva et al., 2005; Nomura et al., 2005); the agglutination activity allows speculating a possible relationship between BmoLL and endophytics from *B. monandra* leaves. Many plant lectins have interacted with several pathogens (Macedo et al., 2007; Souza et al., 2011); also, the symbiotic relationships between plants and root endophytic bacteria have been explored (Antonyuk and Evseeva, 2006). There is no plausible mechanism suggested for interaction of lectins with leaf endophytics. Plant–associated *Pseudomonas* are known to use one or more systems to regulate the production of antibiotics and secondary metabolites, exoprotease activity, and cell-surface features to contribute to their persistence on plants and effectiveness as biological control agents or potential pathogen (Nomura et al., 2005; Antonyuk and Evseeva, 2006).

The function of milligram quantities of BmoLL in plant leaves is unclear; this lectin could interact with endophytic microorganisms as an inhibitor, symbiotic or stimulating factor. Lectin functions in plants as carbohydrate binding proteins and regulatory molecules (Santos et al., 2014). Gao et al. (2003) showed that higher plants produce compounds that specifically stimulate or inhibit response in bacteria. The interaction between plants and microorganisms has been extensively explored, and a symbiotic mechanism in roots, through plant lectins, has been deeply studied (Antonyuk and Evseeva, 2006). Plant lectins have been involved in recognition of bacteria in roots (Sreevidya et al., 2005) or agglutination, *in vitro*, to phytopathogens (Macedo et al., 2007; Charungchitrak et al., 2011; Souza et al., 2011). The interaction between lectins and *Pseudomonas* strains has been extensively studied (Boteva et al., 2005). The potential interaction, BmoLL-*Pseudomonas* in leaves, could promote a defense against the attack of phytopathogenic microorganisms in plants through a *P. aeruginosa* metabolic production mechanism (since this strains was active against phytopathogenic strains). The interaction of plant lectins and endophytes could be a new line to unravel agricultural defense mechanisms against phytopathogenic organisms in biological control. In this way, metabolites of *B. monandra* endophytics are currently under investigation in our laboratory to search for antibacterial products.

Conflict of Interests

The authors have not declared any conflict of interests.

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