GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Endophytic bacteria from banana cultivars and their antifungal activity

A. Souza[1], J.C. Cruz[2], N.R. Sousa[2], A.R.L. Procópio[1] and G.F. Silva[2]

[1]Laboratório de Biotecnologia e Recursos Naturais,
Universidade do Estado do Amazonas, Manaus, AM, Brasil
[2]Laboratório de Biologia Molecular, Embrapa Amazônia Ocidental,
Manaus, AM, Brasil

Corresponding author: G.F. Silva
E-mail: gilvan.silva@embrapa.br

Genet. Mol. Res. 13 (4): 8661-8670 (2014)
Received July 10, 2013
Accepted December 2, 2013
Published October 27, 2014
DOI http://dx.doi.org/10.4238/2014.October.27.6

ABSTRACT. Endophytic microorganisms consist of fungi, bacteria, and actinomycetes that play important roles in the process of plant adaptation to the environment. Currently, the natural associations between microorganisms and plant species are being explored for a large number of biotechnological applications. In this study, 122 endophytic bacteria were isolated from 5 cultivars of *Musa* spp from the state of Amazonas (Brazil). Four strains were selected because they exhibited antagonistic activities against *Fusarium oxysporum* f. sp *cubense* and *Colletotrichum guaranicola*, with inhibitions ranging from 19 to 30% and 27 to 35%, respectively. Phylogenetic analysis of the 16S rDNA regions of these bacteria with antifungal activity showed that they are phylogenetically related to 3 different species of *Bacillus* - *B. amyloliquefaciens*, *B. subtilis* subsp *subtilis*, and *B. thuringiensis*.

Key words: *Bacillus*; *Fusarium oxysporum* f. sp *cubense*; Endophytic; Biological control; *Musa* spp

INTRODUCTION

Endophytes are present in plant tissues and do not cause damage to the plant or produce external structures that emerge from the plant tissues (Azevedo and Araujo, 2007). In general, endophytes are fungi and bacteria that play important roles in the environmental adaptation process of plants (Mendes and Azevedo, 2007).

Plants are considered complex microecosystems in which different niches can be exploited by an extensive variety of microorganisms, including endophytes (Azevedo et al., 2000). Studies of plant/microorganism interactions and the role of these microorganisms in plants have indicated their utility for a large number of applications, such as the biological control of numerous diseases (Zheng et al., 2011; Ceballos et al., 2012), promotion of plant growth (Hallmann et al., 1997; Coombs et al., 2004; Montañez et al., 2012), and bioremediation of contaminated environments (Ma et al., 2011).

Endophytes have been isolated from different parts of plants, such as roots, stems, nodes, leaves, and fruits, and from a wide variety of plants, including many that are of agricultural interest such as grapevines (Bell et al., 1995), cotton (Quadt-Hallmann et al., 1997), rice (Stolzfus et al., 1997), tomatoes (Pillay and Nowak, 1997), maize (Araújo et al., 2000), wheat and sorghum (Zinniel et al., 2002), potatoes (Reiter et al., 2003), banana plants (Weber et al., 2007), and sugar cane (Lira-Cadete et al., 2012).

In banana plants, an analysis of the response of endophytic bacterial communities in plantlets derived from tissue cultures infected with a *Fusarium oxysporum* f. sp *cubense* (*FOC*) race 4 pathogen showed that antagonist endophytic bacterial communities tended to increase in *FOC*-inoculated banana plants, indicating that the presence of the pathogen played a role in the selection of antagonists (Lian et al., 2008). Endophytic bacteria isolated from older banana leaves exhibited higher percentages of antagonistic activity against *Mycosphaerella fijiensis*, which affected the morphology of the mycelia and ascospores (Ceballos et al., 2012).

Currently, natural associations between microorganisms and plant species are being explored for their potential use in a large number of biotechnological applications such as drug discovery. Strobel et al. (2004) noted that endophytic actinomycetes that were associated with medicinal plants, especially in the tropics, could be a rich source of functional metabolites. Endophytes have proven to be rich sources of new natural compounds possessing broad spectra of biological activities and high levels of structural diversity (Pimentel et al., 2011). Thus, the aim of this study was to isolate and select endophytic bacteria with antifungal activity from cultivars of *Musa* spp that were grown in the State of Amazonas, Brazil.

MATERIAL AND METHODS

Plant sources

Endophytic bacteria were isolated from 5 cultivars of banana - BRS Conquista (genotype BB), Pinsangue Seilão (AAA), FHIA 18 (AAAB), and Pacovan (AAB) - for which samples were obtained from the experimental area of Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA Amazônia Ocidental) Manaus, AM, and cultivar Maçã (AAB), which was collected at a crop in Manacapuru, Amazonas.

Bacterial isolation

Leaf tissue samples for each cultivar were collected at random from healthy adult plants, stored at room temperature, and processed within 24 h after collection. Initially, samples were washed in water, cut into pieces, and subjected to sterilization procedures with 70% ethanol, followed by disinfection with a solution of 3% sodium hypochlorite for 1 min, another wash with 70% ethanol, and 2 washes in sterile distilled water.

Endophytic bacteria were isolated from 20 fragments of 4 to 5 mm, inoculated in tryptic soy broth, and incubated for up to 2 days at 28°C. The bacterial colonies were isolated from leaf fragments and then deposited in the Amazon Biotechnology Center (CBA) collection.

Antagonistic activity tests

In vitro antagonistic activity tests were performed against *FOC* and *Colletotrichum guaranicola*, which are pathogens of banana and guarana (*Paullinia cupana* var. *sorbilis*) plants, respectively. Endophytic bacteria were evaluated by the paired culture technique. Mycelial discs of 5 mm that were obtained from each fungus were placed on Petri dishes containing enriched potato dextrose agar medium (200 g/L potato, 20 g/L dextrose, and 15 g/L agar) and incubated at 28°C for 3 days. Bacteria were inoculated, and incubation continued at 28°C for a further 6 days for *FOC* and 8 days for *C. guaranicola*. After this period, the average diameters of the pathogens and antagonistic colonies were determined using a caliper.

DNA extraction

Bacterial DNA was extracted according to a previously modified protocol by Sun et al. (2008). Quantitation was performed with a spectrophotometer (NanoDrop-Thermo) and 0.8% agarose gel.

Molecular identification by 16S rDNA sequencing

16S rDNA from M10, M28, and PS6 isolates was amplified using primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3'), while the primers V3F (5'-CCTACGGGAGGCAGCAG-3') and V3R (5'-ATTACCGCGGCTGCTGG-3') were used for the M05 isolate under the following polymerase chain reaction (PCR) conditions: 4 min at 94°C; 30 cycles of 30 s at 94°C, 1 min at 60°C, and 1 min at 72°C; and a final extension step of 10 min at 72°C. Reactions were carried out with 10X buffer (500 mM KCl, 100 mM Tris-HCl, pH 8.4, 1% Triton X-100), 2 mM $MgCl_2$, 0.2 mM of each dNTP, 50 ng DNA, 0.5 mM of each primer, and 1 U Taq polymerase (Phoneutria). The PCR products were purified with PEG8000 and sequenced using primers 27F, 1492R, V3F, and V3R.

Data analysis

Triplicate data from the antagonistic activity tests were subjected to analysis of variance and the Tukey test ($P < 0.01$). The percentage of inhibition was calculated based on the ratio between the average inhibition and the average growth of the control.

Phylogenetic analysis was performed by comparing 16S rDNA sequences from the isolates M05, M10, M28, and PS6 with different species deposited in the National Center for Biotechnology Information database (http://www.ncbi.nlm.nih.gov). The sequences were aligned with multiple sequence comparison by log-expectation, and the phylogenetic analysis based on the dataset alignment was performed with maximum likelihood (ML) by Tamura and Nei (1993) with the aid of MEGA5 (Tamura et al., 2011). The clade stability of the tree resulting from ML analysis was assessed by bootstrap analysis with 1000 replicates.

RESULTS

One hundred twenty-two endophytic bacteria were isolated. Twenty-nine samples were isolates from the Maçã cultivar, 23 were isolates of the Pacovan cultivar, 9 isolates were from the BRS Conquista cultivar, and 55 isolates were obtained from the FHIA 18 cultivar (Table 1). Of the 5 cultivars used for the isolation of endophytic bacteria, only the Pisangue Seilão and Maçã cultivars had bacteria with potential use for an *in vitro* biological control.

Table 1. List of isolates obtained from each cultivar identified using file numbers of the Amazon Biotechnology Center (CBA) collection.

Maçã	Collection No.	FHIA18	Collection No.	FHIA18	Collection No.	Pacovan	Collection No.	BRS Conquista	Collection No.	Pisangue Seilão	Collection No.
M01	CBA-1869	FH6B	CBA-1716	FH14A	CBA-1702	P1	CBA-1748	BRS3	CBA-1739	PS6*	CBA-1731
M02	CBA-1870	FH7C	CBA-1722	FH9B	CBA-1730	P1A	CBA-1804	BRS12	CBA-1745	PS9	CBA-1732
M03	CBA-1871	FH14	CBA-1701	FH1A	CBA-1706	P2A1	CBA-1806	BRS10	CBA-1741	PS11	CBA-1734
M04	CBA-1872	FH5A1	CBA-1711	FH5A	CBA-1712	P2B	CBA-1807	BRS10A	CBA-1742	PS6A	CBA-1737
M05*	CBA-1873	FH12C	CBA-1691	FH6C	CBA-1717	P2B1	CBA-1803	BRS10C	CBA-1744	PS11A	CBA-1735
M06	CBA-1874	FH11E1	CBA-1688	FH9	CBA-1728	P3A	CBA-1761	BRS6	CBA-1733	PS6A1	CBA-1738
M07	CBA-1875	FH9A1	CBA-1693	FH11C	CBA-1762	P6	CBA-1750	BRS12F	CBA-1747		
M08	CBA-1876	FH14C	CBA-1705	FH11D	CBA-1764	P8	CBA-1751	BRS10B	CBA-1743		
M09	CBA-1877	FH12B	CBA-1690	FH10C	CBA-1685	P11B	CBA-1758	BRS12.1	CBA-1746		
M10*	CBA-1878	FH10B1A	CBA-1683	FH8	CBA-1724	P11E	CBA-1765				
M11	CBA-1879	FH5	CBA-1710	FH12D	CBA-1694	P13	CBA-1769				
M12	CBA-1880	FH11	CBA-1686	FH8B	CBA-1726	P13D	CBA-1774				
M13	CBA-1881	FH10B1	CBA-1681	FH6D	CBA-1719	P2A	CBA-1805				
M14	CBA-1882	FH6C1	CBA-1718	FH11E	CBA-1668	P14A	CBA-1775				
M15	CBA-1883	FH11E1A	CBA-1689	FH14A1	CBA-1703	P14B	CBA-1777				
M16	CBA-1884	FH7B	CBA-1721	FH1D	CBA-1709	P14C	CBA-1779				
M17	CBA-1885	FH13	CBA-1695	FH7A	CBA-1720	P14D1	CBA-1781				
M18	CBA-1886	FH10B	CBA-1680	FH8A	CBA-1725	P14E	CBA-1782				
M19	CBA-1887	FH13A	CBA-1696	FH13D1	CBA-1700	P15B	CBA-1784				
M20	CBA-1888	FH10B1	CBA-1681	FH11	CBA-1686	P15C	CBA-1786				
M21	CBA-1889	FH13B	CBA-1697	FH10	CBA-1677	P15D1	CBA-1787				
M22	CBA-1890	FH13D	CBA-1699	FH5A1A	CBA-1713	P17E	CBA-1797				
M23	CBA-1891	FH18C	CBA-1746	FH16E	CBA-1749	P18A	CBA-1798				
M24	CBA-1992	FH11B	CBA-1727	FH1B	CBA-1707						
M25	CBA-1993	FH14B	CBA-1704	FH1C	CBA-1708						
M26	CBA-1994	FH9A	CBA-1729								
M27	CBA-1995	FH7D	CBA-1723								
M28*	CBA-1996	FH9C	CBA-1682								
M29	CBA-1897	FH10F	CBA-1714								

*Bacteria that exhibited antagonistic activity against *Fusarium oxysporum* f. sp *cubense* and *Colletotrichum guaranicola*.

Antagonistic activity against *FOC* and *C. guaranicola* was detected in 4 isolates: 3 Maçã cultivar isolates (M05, M10, and M28) and 1 Pisangue cultivar isolate (PS6). The antagonistic activity data demonstrated differences in inhibition efficiency among isolates. Iso-

late M28 showed the highest inhibition of *C. guaranicola* (35%), followed by M05 (32%), PS6 (30%), and M10 (27%). For *FOC*, isolate PS6 showed the highest inhibition rate (30%), followed by M05 (28%), M28 (24%), and M10 (19%) (Figures 1 and 2).

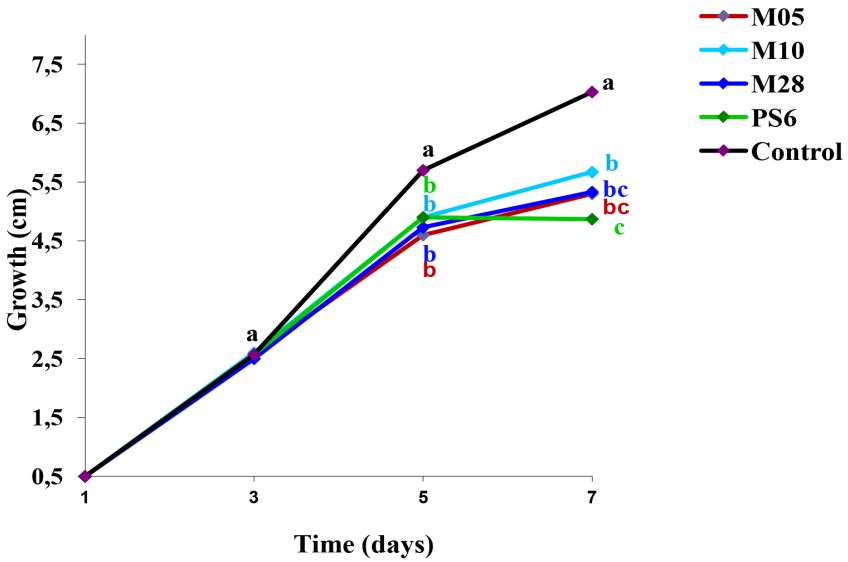

Figure 1. *In vitro* antagonistic activity test of endophytic bacteria against *Fusarium oxysporum* f. sp *cubense* in a direct challenge. Averages followed by the same letter do not differ by the Tukey test (P < 0.01%). M05, M10, and M28: isolates from the Maçã cultivar; PS6: isolate of the Pisangue Seilão cultivar.

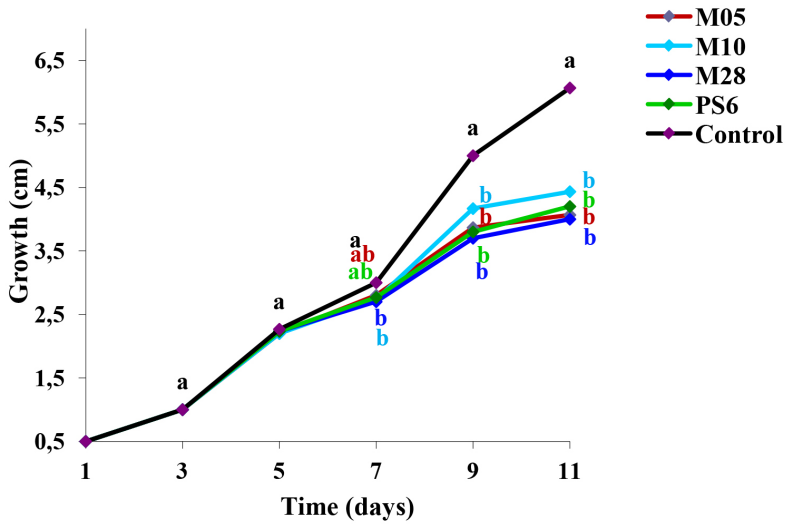

Figure 2. *In vitro* antagonistic activity test of endophytic bacteria against *Colletotrichum guaranicola* in a direct challenge. Averages followed by the same letter do not differ by the Tukey test (P < 0.01%). M05, M10, and M28: isolates from the Maçã cultivar; PS6: isolate of the Pisangue Seilão cultivar.

Phylogenetic analysis of the 16S rDNA of the 4 bacteria that exhibited antifungal activity revealed that these isolates corresponded to 3 different species of *Bacillus*. PS6 and M10 demonstrated higher correlation to *B. amyloliquefaciens*, M28 was most highly correlated to *B. subtilis* subsp *subtilis* (Figure 3), and the bacterium M05 was closely related to *B. thuringiensis* (Figure 4).

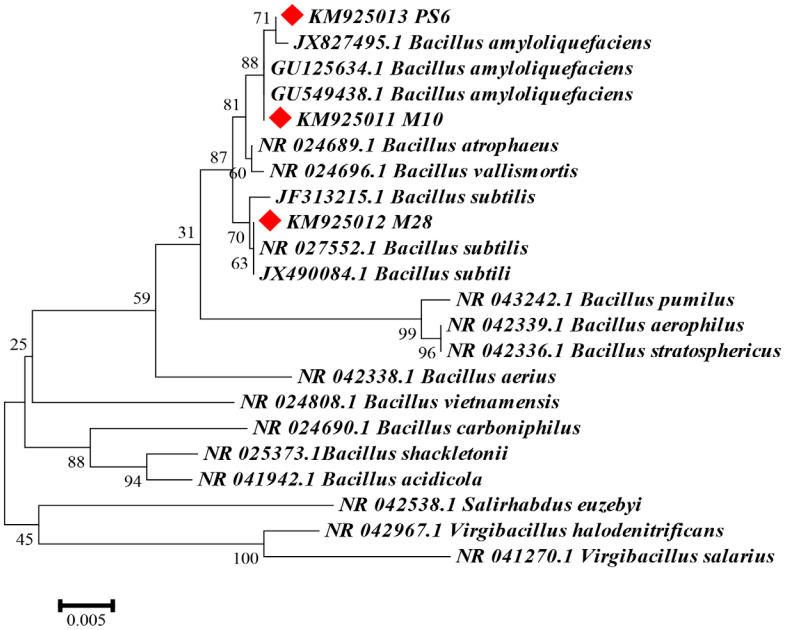

Figure 3. Phylogenetic relationships between endophytic bacteria based on partial 16S rDNA sequences by the maximum likelihood method described by Tamura and Nei (1993) performed using 1000 bootstrap replicates.

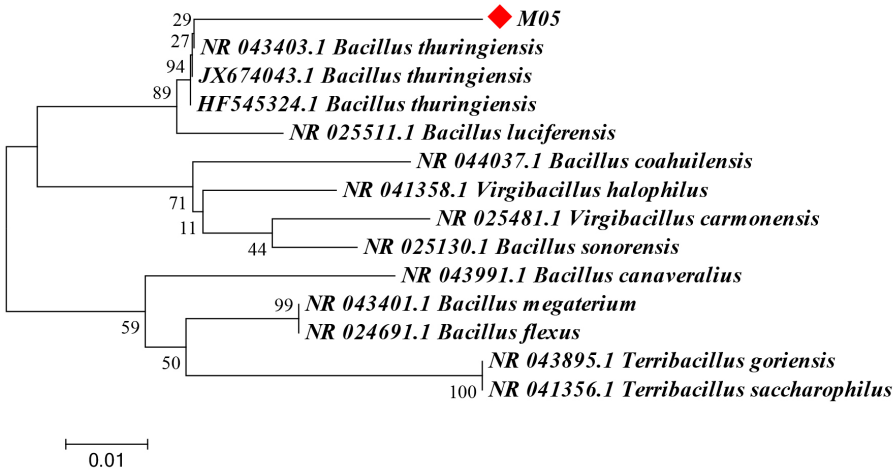

Figure 4. Phylogenetic relationships of endophytic bacteria based on partial 16S rDNA sequences by the maximum likelihood method described by Tamura and Nei (1993) performed using 1000 bootstrap replicates.

DISCUSSION

The 4 endophytic bacteria with antifungal activity isolated in this study were obtained from 2 banana cultivars that were grown in fields without high disease pressure. The ratio of the number of isolates with antagonistic activity to the total number of isolated bacteria (4/122) was relatively lower than that found by Wang et al. (2012), who obtained 6 antagonistic bacteria of 57 bacterial strains that were isolated from the rhizospheres of healthy banana plants that were grown in a heavily wilted and diseased field. A greater number of endophytes with antagonistic activity have been observed in plants subjected to biotic stress (Lian et al., 2008).

In recent years, several endophytic bacteria with potential use in biological control or growth promotion were isolated from different banana cultivars. Weber et al. (2007) isolated bacterial endophytes from *Musa* spp, specifically from Pacovan and Prata cultivars in the state of Ceará (northeast of Brazil), and they identified bacteria with potential use in biocontrol and biofertilization. Thomas et al. (2008) identified the presence of culturable and non-culturable endophytes and analyzed the ubiquitous and intense association between endophytes and bananas, including their quiescent survival in apparently clean tissue cultures.

The 4 isolates with antagonistic activity against *FOC* and *C. guaranicola* obtained in this study were characterized by sequencing the 16S rDNA region. Results indicated that the isolates were phylogenetically related to 3 species of the genus *Bacillus*. Isolates PS6 and M10 grouped with *B. amyloliquefaciens* (88% bootstrap), while isolate M28 showed higher correlation to *B. subtilis* subsp *subtilis* (Figure 3).

Bacillus spp have been described as the most promising biological control agents, and several studies have demonstrated the potential of this genus for the control of pathogens in different plant cultures (Wulff et al., 2002; Kildea et al., 2008; Arguelles-Arias et al., 2009; Pérez-García et al., 2011).

Recently, *B. amyloliquefaciens* isolated from the rhizospheres of healthy banana plants in a field highly contaminated with *FOC* exhibited an ability to promote the growth of banana plants and antagonistic activity against *FOC* through the production of antifungal lipopeptides and volatile compounds (Wang et al., 2012). Research conducted by Yuan et al. (2012) also showed that *B. amyloliquefaciens* produced at least 11 volatile compounds capable of reducing mycelial growth and inhibiting the germination of *FOC* pathogen spores. Some strains of *B. amyloliquefaciens* are also important plant growth-promoting rhizobacteria and can be used as bioorganic fertilizers to promote growth (Yuan et al., 2013). Isolated strains from activated sludge from a polluted river have also been evaluated for their potential in water remediation to remove nitrite-N and ammonia-N (Xie et al., 2013). Moreover, virucidal effects against herpes simplex virus 1 were detected with high concentrations of subtilosin, the cyclical antimicrobial peptide produced by *B. amyloliquefaciens* (Torres et al., 2013).

Isolate M28 (Table 1), which showed the highest inhibition rate against *C. guaranicola*, was identified as *B. subtilis* (Figure 3). Strains of *B. subtilis* have been used as biocontrol agents for diseases caused by fungi and bacteria (Zeriouh et al., 2011). *B. subtilis* also demonstrated *in vitro* activity against various species of *Fusarium*, and the component responsible for the antifungal activity was identified as fengycin, a lipopeptide commonly produced by *B. subtilis* (Rebib et al., 2012).

With the robust support of the bootstrap analysis, isolate M05 was determined to be phylogenetically closely related to *B. thuringiensis* (Figure 4). In most cases, *B. thuringiensis*

has been associated with the biological control of insects, because it is capable of causing toxicity that is 24 times higher than that by an insecticide (Konecka et al., 2012); however, this species has also been shown to have activity against microorganisms. Mojica-Marín et al. (2008) revealed not only the antagonistic potential of *B. thuringiensis* against *Rhizoctonia solani* of the pepper plant but also an increase in the growth of seedlings associated with antagonistic strains. In addition, Liu et al. (2012) demonstrated by recombination of the cry1E gene that *B. thuringiensis* was able to control pests and showed antifungal activity against 6 different phytopathogens. This demonstrates the potential of the *Bacillus* genus in the production of compounds with biotechnological potential and use as biological control agents for diverse cultures.

The percentages of inhibition obtained with the 3 *Bacillus* species that were identified in this study were within expectations because the inhibition efficacies obtained for both bacteria and yeast strains used for biological control vary based on the organism tested. Analysis of the antagonistic activity of *B. subtilis* against *F. oxysporum* ranged from 25 to 34%, and the activity was 100% against *Botryodiplodia theobromae* (Swain and Ray, 2009). When using rhizosphere and endorhiza fungi against *Verticillium dahliae*, inhibitions were shown to range from 8.58 to 69.78% (Zheng et al., 2011).

ACKNOWLEDGMENTS

Research supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa do Estado do Amazonas (FAPEAM), and Empresa Brasileira de Pesquisa Agropecuária (Embrapa).

REFERENCES

Araújo JM, Silva AC and Azevedo JL (2000). Isolation of endophytic actinomycetes from roots and leaves of maize (*Zea mays* L.). *Braz. Arch. Biol. Technol.* 43: 447-451.

Arguelles-Arias A, Ongena M, Halimi B, Lara Y, et al. (2009). *Bacillus amyloliquefaciens* GA1 as a source of potent antibiotics and other secondary metabolites for biocontrol of plant pathogens. *Microb. Cell Fact.* 8: 63.

Azevedo JL and Araujo WL (2007). Diversity and Applications of Endophytic Fungi Isolated from Tropical Plants. In: Fungi: Multifaceted Microbes (Ganguli BN and Deshmukh SK, eds.). CRC Press, Anamaya Publishers, Boca Raton, 189-207.

Azevedo JL, Maccheroni WJ, Pereira JO and Araújo WL (2000). Endophytic microorganisms: a review on insect control and recent advances on tropical plants. *Electron. J. Biotechn.* 3: 40-65.

Bell CR, Dickie GA, Harvey WLG and Chan JWYF (1995). Endophytic bacteria in grapevine. *Can. J. Microbiol.* 41: 46-53.

Ceballos I, Mosquera S, Angulo M, Mira JJ, et al. (2012). Cultivable bacteria populations associated with leaves of banana and plantain plants and their antagonistic activity against *Mycosphaerella fijiensis*. *Microb. Ecol.* 64: 641-653.

Coombs JT, Michelsen PP and Franco CMM (2004). Evaluation of endophytic actinobacteria as antagonists of *Gaeumannomyces graminis* var. *tritici* in wheat. *Biol. Control* 29: 359-366.

Hallmann J, Quadt-Hallmann A, Mahaffee WF and Kloepper JW (1997). Bacterial endophytes in agricultural crops. *Can. J. Microbiol.* 43: 895-914.

Kildea S, Ransbotyn V, Khan MR and Fagan B (2008). *Bacillus megaterium* shows potential for the biocontrol of *Septoria tritici* blotch of wheat. *Biol. Control* 47: 37-45.

Konecka E, Baranek J, Hrycak A and Kaznowski A (2012). Insecticidal activity of *Bacillus thuringiensis* strains isolated from soil and water. *Scientific World J.* 2012: 710501.

Lian J, Wang Z and Zhou S (2008). Response of endophytic bacterial communities in banana tissue culture plantlets to *Fusarium* wilt pathogen infection. *J. Gen. Appl. Microbiol.* 54: 83-92.

Lira-Cadete L, Farias ARB, Souza AP and Costa DP (2012). Variabilidade genética de bactérias diazotróficas associadas a plantas de cana-de-açúcar capazes de solubilizar fosfato inorgânico. *Biosci. J.* 28: 122-129.

Liu Q, Roh JY, Wang Y, Choi JY, et al. (2012). Construction and characterisation of an antifungal recombinant *Bacillus thuringiensis* with an expanded host spectrum. *J. Microbiol.* 50: 874-877.

Ma Y, Prasad MN, Rajkumar M and Freitas H (2011). Plant growth promoting rhizobacteria and endophytes accelerate phytoremediation of metalliferous soils. *Biotechnol. Adv.* 29: 248-258.

Mendes R and Azevedo JL (2007). Valor Biotecnológico de Fungos Endofíticos Isolados de Plantas de Interesse Econômico. In: Micologia: Avanços no Conhecimento. (Maia LC, Malosso E and Yano-Melo AM, eds.). Ed. Universitária da UFPE, Recife, 129-140.

Mojica-Marín V, Luna-Olvera HA, Sandoval-Coronado CF and Pereyra-Alférez B (2008). Antagonistic activity of selected strains of *Bacillus thuringiensis* against *Rhizoctonia solani* of chili pepper. *Afr. J. Biotechnol.* 7: 1271-1276.

Montañez A, Blanco AR, Barlocco C and Beracochea M (2012). Characterization of cultivable putative endophytic plant growth promoting bacteria associated with maize cultivars (*Zea mays* L.) and their inoculation effects *in vitro*. *Appl. Soil Ecol.* 58: 21-28.

Pérez-García A, Romero D and de Vicente A (2011). Plant protection and growth stimulation by microorganisms: biotechnological applications of Bacilli in agriculture. *Curr. Opin. Biotechnol.* 22: 187-193.

Pillay VK and Nowak J (1997). Inoculum density, temperature, and genotype effects on *in vitro* growth promotion and epiphytic and endophytic colonization of tomato (*Lycopersicon esculentum* L.) seedlings inoculated with a *Pseudomonas* bacterium. *Can. J. Microbiol.* 43: 354-361.

Pimentel MR, Molina G, Dionisio AP, Marostica Junior MR, et al. (2011). The use of endophytes to obtain bioactive compounds and their application in biotransformation process. *Biotechnol. Res. Int.* 2011: 576286.

Quadt-Hallmann A, Hallmann J and Kloepper JW (1997). Bacterial endophytes in cotton: location and interaction with other plant-associated bacteria. *Can. J. Microbiol.* 43: 254-259.

Rebib H, Hedi A, Rousset M and Boudabous A (2012). Biological control of *Fusarium* foot rot of wheat using fengycin-producing *Bacillus subtilis* isolated from salty soil. *Afr. J. Biotechnol.* 11: 8464-8475.

Reiter B, Burgmann H, Burg K and Sessitsch A (2003). Endophytic nifH gene diversity in African sweet potato. *Can. J. Microbiol.* 49: 549-555.

Stolzfus JR, So R, Malarvithi PP and Ladha JK (1997). Isolation of endophytic bacteria from rice and assessment of their potential for supplying rice with biologically fixed nitrogen. *Plant Soil* 194: 25-36.

Strobel G, Daisy B, Castillo U and Harper J (2004). Natural products from endophytic microorganisms. *J. Nat. Prod.* 67: 257-268.

Sun L, Qiu F, Zhang X, Dai X, et al. (2008). Endophytic bacterial diversity in rice (*Oryza sativa* L.) roots estimated by 16S rDNA sequence analysis. *Microb. Ecol.* 55: 415-424.

Swain MR and Ray RC (2009). Biocontrol and other beneficial activities of *Bacillus subtilis* isolated from cowdung microflora. *Microbiol. Res.* 164: 121-130.

Tamura K and Nei M (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Mol. Biol. Evol.* 10: 512-526.

Tamura K, Peterson D, Peterson N, Stecher G, et al. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* 28: 2731-2739.

Thomas P, Swarna GK, Roy PK and Patil P (2008). Identification of culturable and originally non-culturable endophytic bacteria isolated from shoot tip cultures of banana cv. Grand Naine. *Plant Cell Tiss. Org.* 93: 55-63.

Torres NI, Noll KS, Xu S, Li J, et al. (2013). Safety, formulation, and *in vitro* antiviral activity of the antimicrobial peptide subtilosin against herpes simplex virus type 1. *Probiotics. Antimicrob. Proteins* 5: 26-35.

Wang B, Yuan J, Zhang J and Shen Z (2012). Effects of novel bioorganic fertilizer produced by *Bacillus amyloliquefaciens* W19 on antagonism of *Fusarium* wilt of banana. *Biol. Fertil. Soils* 49: 435-446.

Weber OB, Muniz CR, Vitor AO and Freire FCO (2007). Interaction of endophytic diazotrophic bacteria and *Fusarium oxysporum* f. sp. *cubense* on plantlets of banana "Maça". *Plant Soil* 298: 47-56.

Wulff EG, Mguni CM, Mortensen CN and Keswani CL (2002). Biological control of black rot (*Xanthomonas campestris* pv. *campestris*) of brassicas with an antagonistic strain of *Bacillus subtilis* in Zimbabwe. *Eur. J. Plant Pathol.* 108: 317-325.

Xie F, Zhu T, Zhang F, Zhou K, et al. (2013). Using *Bacillus amyloliquefaciens* for remediation of aquaculture water. *Springerplus.* 2: 119.

Yuan J, Raza W, Shen Q and Huang Q (2012). Antifungal activity of *Bacillus amyloliquefaciens* NJN-6 volatile compounds against *Fusarium oxysporum* f. sp. cubense. *Appl. Environ. Microbiol.* 78: 5942-5944.

Yuan J, Ruan Y, Wang B, Zhang J, et al. (2013). Plant growth-promoting rhizobacteria strain *Bacillus amyloliquefaciens* NJN-6-enriched bio-organic fertilizer suppressed *Fusarium* wilt and promoted the growth of banana plants. *J. Agric. Food Chem.* 61: 3774-3780.

Zeriouh H, Romero D, Garcia-Gutierrez L, Cazorla FM, et al. (2011). The iturin-like lipopeptides are essential components

in the biological control arsenal of *Bacillus subtilis* against bacterial diseases of cucurbits. *Mol. Plant Microbe Interact.* 24: 1540-1552.

Zheng Y, Xue QY, Xu LL and Xu Q (2011). A screening strategy of fungal biocontrol agents towards *Verticillium* wilt of cotton. *Biol. Control* 56: 209-216.

Zinniel DK, Lambrecht P, Harris NB, Feng Z, et al. (2002). Isolation and characterization of endophytic colonizing bacteria from agronomic crops and prairie plants. *Appl. Environ. Microbiol.* 68: 2198-2208.