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Auxin Production and Detection of the Gene Coding for the Auxin Efflux Carrier (AEC) Protein in *Paenibacillus polymyxa*

Fabio Faria Da Mota[1], Eliane Aparecida Gomes[2], and Lucy Seldin[1]*

[1]*Instituto de Microbiologia Prof. Paulo de Góes, Universidade Federal do Rio de Janeiro, Centro de Ciências da Saúde, Bloco I, Ilha do Fundão, CEP 21941-590, Rio de Janeiro, Brazil*
[2]*EMBRAPA/CNPMS, Sete Lagoas, Minas Gerais, Brazil*

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Different species of *Paenibacillus* are considered to be plant growth-promoting rhizobacteria (PGPR) due to their ability to repress soil borne pathogens, fix atmospheric nitrogen, induce plant resistance to diseases and/or produce plant growth-regulating substances such as auxins. Although it is known that indole-3-acetic acid (IAA) is the primary naturally occurring auxin excreted by *Paenibacillus* species, its transport mechanisms (auxin efflux carriers) have not yet been characterized. In this study, the auxin production of *P. polymyxa* and *P. graminis*, which are prevalent in the rhizospheres of maize and sorghum sown in Brazil, was evaluated. In addition, the gene encoding the Auxin Efflux Carrier (AEC) protein from *P. polymyxa* DSM36^T was sequenced and used to determine if various strains of *P. polymyxa* and *P. graminis* possessed this gene. Each of the 68 *P. polymyxa* strains evaluated in this study was able to produce IAA, which was produced at concentrations varying from 1 to 17 μg/ml. However, auxin production was not detected in any of the 13 *P. graminis* strains tested in this study. Different primers were designed for the PCR amplification of the gene coding for the AEC in *P. polymyxa*, and the predicted protein of 319 aa was homologous to AEC from *Bacillus amyloliquefaciens*, *B. licheniformis*, and *B. subtilis*. However, no product was observed when these primers were used to amplify the genomic DNA of seven strains of *P. graminis*, which suggests that this gene is not present in this species. Moreover, none of the *P. graminis* genomes tested were homologous to the gene coding for AEC, whereas all of the *P. polymyxa* genomes evaluated were. This is the first study to demonstrate that the AEC protein is present in *P. polymyxa* genome.

***Keywords*:** auxin, auxin efflux carrier protein, indole-3-acetic acid, *Paenibacillus polymyxa*, *Paenibacillus graminis*

Paenibacillus is a widely distributed bacterial genus that is found in various plant rhizospheres. Different species of *Paenibacillus* are considered to be plant growth-promoting rhizobacteria (PGPR) because they can induce plant growth by antagonizing and repressing soilborne pathogens (Beatty and Jensen, 2002; Selim *et al*., 2005; Von Der Weid *et al*., 2005), fixing atmospheric nitrogen and making it available to its host plant (Seldin *et al*., 1984; Berge *et al*., 2002; Von Der Weid *et al*., 2002; among others), inducing plant resistance to diseases following root colonization (Timmusk and Wagner, 1999), and/or producing a wide variety of secondary metabolites such as auxins, which can regulate plant growth (Lebuhn *et al*., 1997; Nielsen and Sorensen, 1997; Bent *et al*., 2001; Çakmakçi *et al*., 2007).

The primary naturally occurring auxin excreted by bacteria and fungi is indole-3-acetic acid (IAA) (Patten and Glick, 1996; Maor *et al*., 2004; Nassar *et al*., 2005). Although several different IAA biosynthesis pathways have been described in bacteria (Patten and Glick, 1996), the transport mechanisms of auxin (auxin efflux carriers-AEC) have not yet been fully characterized (Spaepen *et al*., 2007). It has been suggested that IAA produced by microorganisms participates in microbe-plant interaction (Lambrecht *et al*., 2000) during the early stages of plant colonization (Maor *et al*., 2004; Spaepen *et al*., 2007). In general, microorganisms isolated from the rhizosphere and rhizoplane of various crops show higher production of auxins than those that are isolated from root-free soil (Patten and Glick, 1996; Arshad and Frankenberger, 1998). Similarly, Rossi *et al*. (1984) found that auxin-like compounds were more abundant in the rhizosphere soil of maize than in non-rhizosphere soil, especially during seeding emergence.

P. polymyxa is one of the best studied PGPR within the genus *Paenibacillus.* Strains belonging to this species have been isolated from the rhizospheres of wheat, barley, white clover, perennial ryegrass, crested wheatgrass, lodgepole pine, green bean, garlic, banana tree, sugarcane, lemon grass, sorghum, maize, and many other plants. Due to its broad host range, its ability to form endospores, and its ability to produce different types of antibiotics, *P. polymyxa* has the potential to be a commercially useful biocontrol agent. Although the plant growth-promoting activity of *P. polymyxa* has been the subject of numerous studies (Von Der Weid *et al*., 2000; Bent and Chanway, 2002; Ryu *et al*., 2005), the mechanisms of root colonization are not well understood. In addition, although auxin production has been described

* To whom correspondence should be addressed.
(Tel) 55-21-2562-6741; (Fax) 55-21-2560-8344
(E-mail) lseldin@micro.ufrj.br or lucy@seldin.com.br

in different *P. polymyxa* strains, such as L6, Pw-2 (Bent *et al*., 2001) and E681 (Jeong *et al*., 2006), the auxin transport mechanisms (efflux) have not yet been characterized.

In Brazil, another *Paenibacillus* species, *P. graminis* (Berge *et al*., 2002), is prevalent in the rhizospheres of maize and sorghum as well as in Cerrado soil (Da Mota *et al*., 2005; Vollú *et al*., 2006; Coelho *et al.*, 2007). Although all *P. graminis* strains studied to date have been found to fix atmospheric nitrogen (Berge *et al*., 2002; Vollú *et al*., 2006), other characteristics that contribute to plant growth, such as phytohormone production, have not yet been evaluated. Therefore, this study was conducted to evaluate the auxin production by different strains of *P. polymyxa* and *P. graminis*. Moreover, the gene coding for the Auxin Efflux Carrier (AEC) protein from the type strain of *P. polymyxa* was sequenced and used to determine if different strains of *P. polymyxa* and *P. graminis* contained this gene. Finally, the predicted AEC protein of *P. polymyxa* was phylogenetically compared to previously characterized AEC proteins.

Materials and Methods

Bacterial strains and growth conditions

Sixty seven strains of *Paenibacillus polymyxa* and 12 strains of *P. graminis* previously isolated from four maize cultivars (Da Mota *et al*., 2002; Vollú *et al*., 2006) and their type strains ($DSM36^T$ and $RSA19^T$, respectively) were used in this study (Table 1). All strains were grown in one-half strength TSB medium at 27°C for 72 h using the method described by Bent *et al*. (2001). The cultured strains were then stored aerobically at room temperature on TSB agar slants supplemented with 1% $CaCO_3$ (w/v).

Bacterial production of auxin

All 81 *Paenibacillus* strains were grown in 3 ml of TSB medium for 48 h at 32°C, after which 100 μl of these cultures were inoculated in triplicate in 2 ml of one-half strength TSB medium in 24 well plates. Uninoculated wells were used as controls. The plates were then maintained at 27°C in the dark in a rotary shaker (100 rpm) as described by Bent *et al*. (2001). Next, the samples were assayed under conditions that were optimized for the detection of IAA (Gordon and Weber, 1951). Briefly, the samples were cultured for 72 h and the optical density (OD) at 630 nm of 100 μl of each sample was then evaluated using ELISA plates and a spectrophotometer (Stat Fax 2100, Awareness Technology Inc., USA), with all measurements being conducted in triplicate. The level of auxin present in the culture supernatants was then estimated colorimetrically using Salkowski's reagent (1.875 g of $FeCl_3 \cdot 6H_2O$, 100 ml H_2O, and 150 ml of H_2SO_4 at 96% of purity) following the method described by Tang and Bonner (1947). To produce a standard curve, the optical density of triplicate samples of varying concentrations of IAA (0, 1, 10, 20, 30, 40, 50, 60, and 100 μg/ml) diluted in one-half strength TSB medium was measured as described above.

Preparation of genomic DNAs

Total DNA was extracted from all strains using the method described by Seldin and Dubnau (1985). The concentration of DNA was quantified spectrophotometrically using a Gene

Table 1. Strains used in this study and their origin and/or reference

Species	Origin/Reference	Strains
Paenibacillus polymyxa	Type strain; DSMZ	$DSM36^T$
	Rhizosphere of maize[a], cultivar CMS 04; Da Mota *et al*. (2002)	PM04-1, PM04-2, PM04-3, PM04-4, PM04-5, PM04-6, PM04-7, PM04-8, PM04-9, PM04-10, PM04-11, PM04-12, PM04-14, PM04-15, PM04-16, PM04-17
	Rhizosphere of maize, cultivar CMS 11; Da Mota *et al*. (2002)	PM11-1, PM11-2, PM11-3, PM11-4, PM11-5, PM11-6, PM11-7, PM11-8, PM11-9, PM11-10, PM11-11, PM11-12, PM11-13, PM11-14, PM11-15, PM11-16, PM11-17, PM11-18, PM11-21, PM11-22
	Rhizosphere of maize, cultivar CMS 22; Da Mota *et al*. (2002)	PM22-2, PM22-4, PM22-5, PM22-6, PM22-7, PM22-8, PM22-9, PM22-11, PM22-16
	Rhizosphere of maize, cultivar CMS 36; Da Mota *et al*. (2002)	PM36-1, PM36-2, PM36-3, PM36-4, PM36-5, PM36-6, PM36-7, PM36-9, PM36-10, PM36-11, PM36-12, PM36-13, PM36-14, PM36-15, PM36-16, PM36-17, PM36-18, PM36-19, PM36-20, PM36-21, PM36-22, PM36-23
Paenibacillus graminis	Type strain. Rhizosphere of maize, Ramonville-Saint-Agne, France; Berge *et al*. (2002)	$RSA19^T$
	Rhizosphere of maize, cultivar CMS 04; Vollú *et al*. (2006)	MC04.01, MC04.06, MC04.13, MC04.16, MC04.21
	Rhizosphere of maize, cultivar CMS 11; Vollú *et al*. (2006)	MC11.05
	Rhizosphere of maize, cultivar CMS 22; Vollú *et al*. (2006)	MC22.02, MC22.7, MC22.19
	Rhizosphere of maize, cultivar CMS 36; Vollú *et al*. (2006)	MC36.22, MC36.24, MC36.31

[a] Different maize cultivars were sown in Cerrado soil, Sete Lagoas, MG, Brazil.

Quant apparatus (Pharmacia Biotech, Sweden).

Primers design, PCR amplification, cloning, and gene sequencing of the AEC protein of *P. polymyxa*

A gene walking approach using the primers presented in Fig. 1 was used to obtain the complete sequence of the gene coding for AEC in *P. polymyxa*. The first set of primers used in this study: 168IR reverse (gi:50812173: 3798926-3798939); 5'-AGCGGATCAAGAAT-3' and 168RS forward (gi:50812173: 3799809-3799796); 5'-ATTGCAACAGGAAG-3' were designed based on conserved regions of the auxin efflux carrier gene (NP_391585) and the upstream gene (NP_391586, coding for a malate oxidoreductase) in the genome of *Bacillus subtilis* strain 168 (GenBank accession no. NC_000964 - gi|50812173|). These primers were used to amplify the genomic DNA of *P. polymyxa* DSM36^T in a reaction mixture (50 µl) comprised of the following: 1 µl of template DNA (corresponding to approximately 15 ng), 10 mM Tris-HCl; pH 8.3, 10 mM KCl, 2.5 mM $MgCl_2$, 0.2 mM of each dNTPs, 1.25 U of *Taq* polymerase, and 0.2 µM of each primer. This mixture was then subjected to a hot start at 94°C for 5 min, followed by 30 cycles of 94°C (1 min), 40°C (1 min) and 72°C (2 min), with a final extension at 72°C for 10 min. The PCR product was then sequenced and used to design a new primer: DSM36IF; 5'-GGTTTTGGGAAG CTTGTTC-3', based on the partial sequence of *P. polymyxa* DSM36^T (Fig. 1, corresponding to the position 50812173: 3799012-3798994 in *B. subtilis*). This primer was then used in a PCR reaction under low stringency (36°C as the annealing temperature) and the same reaction mixture and conditions described above to obtain the remaining sequence of the gene coding for AEC (Fig. 1, fragment B). Negative controls (without template DNA) were included with each batch of reactions, and the PCR products were visualized by 1% agarose gel electrophoresis followed by staining with ethidium bromide. The fragments obtained in the PCR reactions were purified using a QIAquick gel extraction kit (QIAGEN, Germany). After purification, the DNA fragments were cloned using the pGEM-T easy vector according to the instructions provided by the manufacturer (Promega, USA). After transformation of *Escherichia coli* JM109 competent cells, clones were picked and the size of their inserts were confirmed by PCR using M13f; 5'-TAAAACGACGGCCAG-3' and M13r; 5'-CAGGAAACAGCTATGAC-3' primers. The

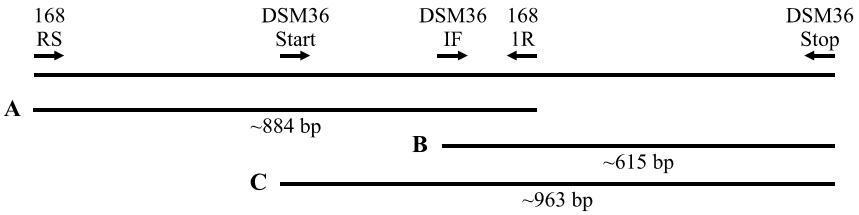

Fig. 1. Primers designed in this study. *B. subtilis* 168 strain gi|50812173| was used as the reference sequence.

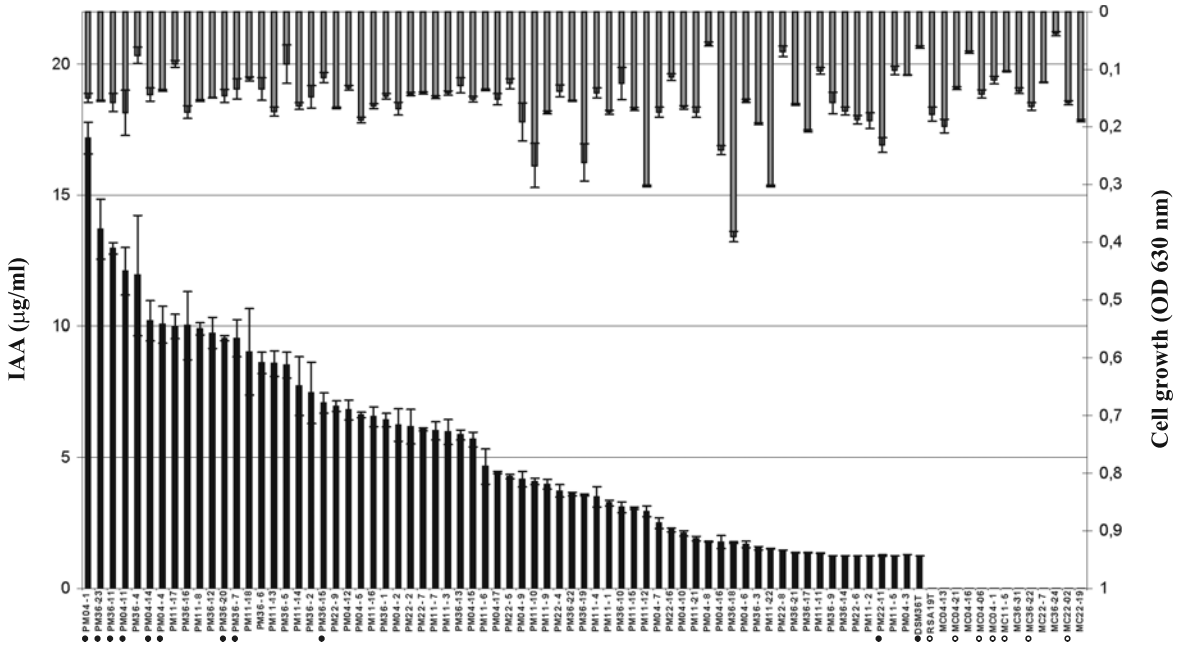

Fig. 2. Growth of *P. polymyxa* and *P. graminis* cells in one-half strength TSB (optical density at 630 nm, gray bars) and indole-3-acetic acid production (µg/ml, black bars) after incubation for 72 h at 27°C. Strains marked with dark (*P. polymyxa*) and white (*P. graminis*) bullets correspond to those used as the template for the PCR reaction using DSM36IF forward and DSM36Stop reverse primers.

clones were sequenced using M13f and M13r primers and an ABI Prism 3100 automatic sequencer (Applied Biosystems, USA).

Characteristics of the AEC protein of *P. polymyxa* and molecular phylogeny

The obtained nucleotide sequence was analyzed using Easy-Gene 1.0 (Larsen and Krogh, 2003) and GeneMark.hmm (version 2.6m, Besemer *et al*., 2001) to find the ORFs and allow conceptual translation of the amino acid sequence. The predicted amino acid sequence of the AEC protein was then analyzed using the Expasy Compute pI/Mw tool to estimate the molecular weight and pI (http://www.expasy.org/tools/pi_tool.html) and PSORTb 2.0.4 to predict the subcellular localization of the AEC protein (Gardy *et al*., 2005). In addition, HMMTOP2 (Tusnády and Simon, 2001) and InterProScan 4.3 (http://www.ebi.ac.uk/InterProScan/) were used to predict the number of transmembrane helices and to identify the conserved domains in the AEC protein, respectively. Furthermore, a Blast P analysis was performed using the non-redundant GenBank database available through the NCBI home page (www.ncbi.nlm.nih.gov/blast). Protein sequences showing high homology to the *Paenibacillus* sequence were aligned using CLUSTAL X (Thompson *et al*., 1997) and a maximum-parsimony phylogenetic tree with 1,000 replicates of bootstrap was constructed using MEGA 4 software (http://www.megasoftware.net/).

Detection of the gene coding for AEC

The primers DSM36IF forward and DSM36Stop reverse; 5'-TTATAAGAGRAGYGARATGAGYAA-3' were used to amplify approximately 615 bp of the gene coding for AEC (Fig. 1, fragment B) from two *Paenibacillus* species. Genomic DNA from 12 strains of *P. polymyxa* and 7 strains of *P. graminis* (shown in Fig. 2) were used as templates and the amplification conditions were as follows: a hot start at 94°C for 5 min, followed by 30 cycles of 94°C (1 min), 53°C (1.5 min) and 72°C (30 sec), with a final extension at 72°C for 5 min. Each reaction mixture (50 μl) contained 1 μl of template DNA (corresponding to approximately 15 ng), 10 mM Tris-HCl (pH 8.3), 10 mM KCl, 2.5 mM $MgCl_2$, 0.2 mM of each dNTPs, 1.25 U of *Taq* polymerase, and 0.2 μM of each primer. Negative controls (without DNA) were included with each batch of amplifications, and the PCR products were visualized by 1% agarose gel electrophoresis followed by staining with ethidium bromide.

Preparation of the probe for the gene coding for AEC and hybridization conditions

A probe for the AEC gene (about 1 kb) was generated by PCR using the primers DSM36Start forward; 5'-TTGYACT TGCWTAMGCAAAGT-3' and DSM36Stop reverse, which were both designed for this study (Fig. 1, fragment C). The amplification conditions were as follows: a hot start at 94°C for 5 min, followed by 30 cycles of 94°C (1 min), 36°C (1 min) and 72°C (2 min), with a final extension at 72°C for 10 min. All reaction mixtures (50 μl) contained approximately 100 ng of template (genomic DNA of strain DSM36^T), 10 mM Tris-HCl; pH 8.3, 10 mM KCl, 2.5 mM $MgCl_2$, 0.2 mM of each dNTPs, 1.25 U of *Taq* polymerase, and 0.2 μM of each primer. Negative controls (without DNA) were included in all amplifications, and PCR products were visualized by 1% agarose gel electrophoresis followed by staining with ethidium bromide. The PCR product obtained was digested with *Eco*RI restriction enzyme and then DIG (digoxigenin-11-dUTP) labeled using a DIG DNA labeling and detection kit, following the manufacturer's protocols (Roche Molecular Biochemicals, Germany). Genomic DNA samples were digested with 10 to 20 U of *Eco*RI per μg of DNA at 37°C for 16 h, after which they were subjected to electrophoresis in 0.8% agarose in Tris-borate-EDTA buffer (Sambrook *et al*., 1989) at 2 V/cm for 16 h at room temperature. Bacteriophage lambda DNA digested with *Hin*dIII and labeled with DIG was used as the molecular weight marker. After separation, the digested DNAs were loaded onto positively charged nylon membranes using a previously described method (Sambrook *et al*., 1989). The conditions presented in the manual for DIG nucleic acid detection kit (Roche) were used for prehybridization and hybridization. The nylon membrane was subjected to stringent washing steps after hybridization at 65°C for 16 h. Signals were detected using a CSPD-based chemiluminescence detection kit (Roche), following the manufacturer's recommendations.

Results

Production of auxins by *Paenibacillus* strains

All 68 *P. polymyxa* strains evaluated in this study (Table 1) were able to produce IAA at concentrations varying from 1 to 17 μg/ml (Fig. 2). Strain PM04-1 was found to produce the highest amount of IAA, whereas the type strain produced the lowest amount. Conversely, auxin was not produced by any of the 13 *P. graminis* strains tested (Fig. 2).

Sequencing of the gene coding for AEC in *P. polymyxa* DSM36^T

The 168RS forward and 168IR reverse primers designed in this study were used for the PCR amplification of genomic DNA from *P. polymyxa* DSM36^T. Only a single band of approximately 900 bp was visualized by gel electrophoresis. This PCR product was cloned, sequenced and analyzed using Blast X, which revealed that the product corresponded to a partial sequence of the gene coding for the auxin efflux carrier (AEC) and the upstream part of the malate oxidoreductase coding gene. To obtain the remaining sequence of the gene coding for AEC, a specific primer based on the *Paenibacillus* sequence previously obtained was designed. PCR amplification using only the DSM36IF primer and low-stringency conditions produced three bands of approximately 800 bp, 1200 bp, and 1300 bp (data not shown). These three bands were purified, cloned and sequenced as described above. Blast X analysis of the sequences revealed that the 800 bp band corresponded to the remainder of the gene sequence coding for the AEC. The two partial sequences of the gene coding for the AEC were then assembled using the Bioedit software, which gave the full nucleotide sequence (EU215788).

Nucleotide and protein sequence analyses

The complete nucleotide sequence was analyzed to identify

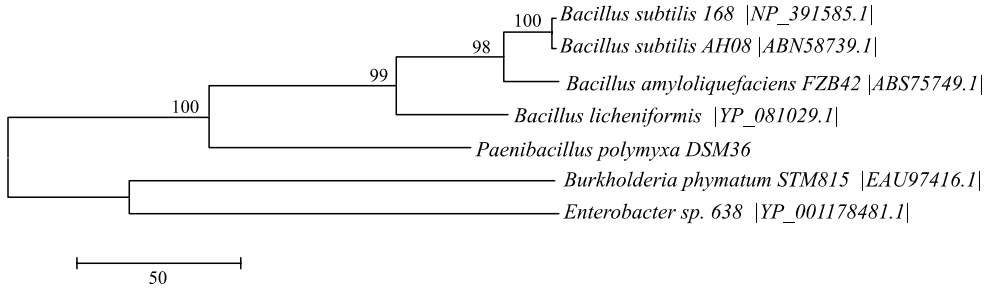

Fig. 3. Unrooted Maximum Parsimony tree based on *Bacillus*, *Paenibacillus*, *Enterobacter*, and *Burkholderia* proteins homologous to the Auxin Efflux Carrier. Bootstrap values are shown as a percentage (1,000 replicates).

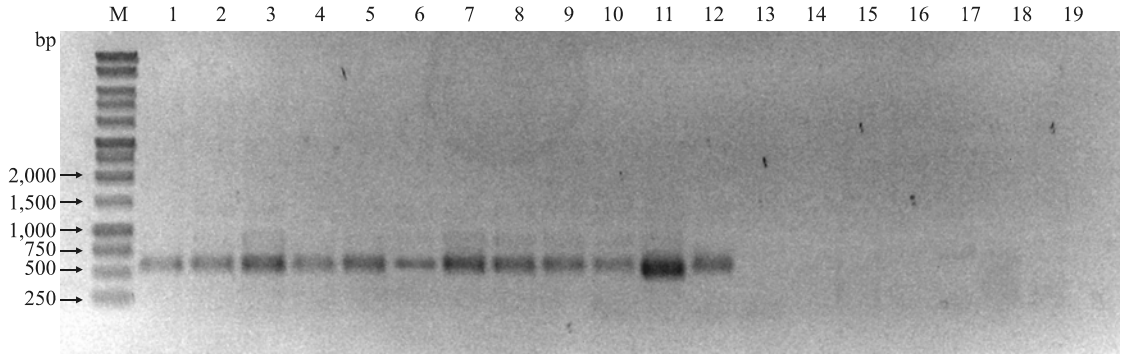

Fig. 4. Agarose gel electrophoresis of PCR products of approximately 615 bp of the Auxin Efflux Carrier (AEC) protein coding gene obtained using the DSM36IF and DSM36Stop primers and genomic DNA from *P. polymyxa* (1, PM04-1; 2, PM36-23; 3, PM36-11; 4, PM04-11; 5, PM04-14; 6, PM04-4; 7, PM36-16; 8, PM36-20; 9, PM36-7; 10, PM36-15; 11, PM22-11; 12, DSM36^T) and *P. graminis* (13, RSA19^T; 14, MC04.21; 15, MC04.6; 16, MC04.1; 17, MC11.5; 18, MC36.22; 19, MC22.02). M, Molecular weight marker (1 kb DNA ladder, Promega).

the open reading frames of the gene coding for AEC using GeneMark.hmm and EasyGene. The nucleotide sequence of this gene was also translated to an amino acid sequence. The theoretical molecular weight (34,177.99 Da) and the isoelectric point (9.74) of the AEC protein were estimated based on the 319 amino acids using the ExPASy Server. Cell localization of the AEC protein using HMMTOP2 revealed that it is a cytoplasmic transmembrane protein (with 9.99 score) with at least 8 internal helices. In addition, a Blast P search of the GenBank database (09/18/2007) demonstrated that the predicted protein (319 aa) of *P. polymyxa* DSM36^T exhibits significant homology to the AEC proteins from *Bacillus amyloliquefaciens*, *B. licheniformis*, and *B. subtilis.* All these sequences have the same domain structure, identified as IPR004776, TIGR00946, pfam03547, or COG0679R. Finally, the phylogenetic tree constructed with Blast P closest sequences showed that *Paenibacillus* AEC protein is closer (78% protein identity) to *B. licheniformis* than to other *Bacillus* species. However, differences could be observed between the sequences obtained from *Paenibacillus* and *Bacillus* (Fig. 3).

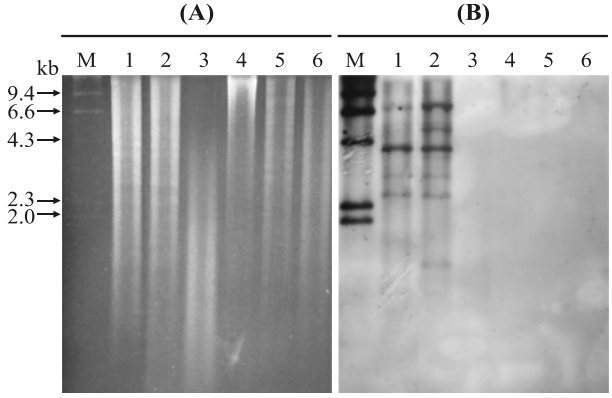

Fig. 5. (A) Agarose gel electrophoresis of DNAs digested with *Eco*RI from *P. polymyxa* strains 1, PM04.11; 2, PM11.05 and from *P. graminis* strains 3, RSA19^T; 4, MC04.06; 5, MC36.22 and 6, MC22.19. (B) DNA hybridization patterns obtained using DIG-labeled Auxin Efflux Carrier (AEC) protein coding gene from *P. polymyxa* DSM36^T as a probe. M corresponds to phage lambda DNA digested with *Hin*dIII and labeled with digoxigenin.

PCR detection of the gene coding for AEC in *Paenibacillus* strains

All 12 strains of *P. polymyxa* tested (PM04-1, PM36-23, PM36-11, PM04-11, PM04-14, PM04-4, PM36-16, PM36-20, PM36-7, PM36-15, PM22-11, and DSM36^T) produced a PCR fragment (about 615 bp) corresponding to the gene coding for AEC when the DSM36IF and DSM36Stop primers were used (Fig. 4). Conversely, this band was not observed after PCR amplification of the genomic DNA of seven

strains of *P. graminis* evaluated (RSA19^T, MC04.21, MC04.6, MC04.1, MC11.5, MC36.22, and MC22.02) (Fig. 4), which suggests that *P. graminis* does not possess this gene.

DNA hybridization of *P. polymyxa* and *P. graminis* using the gene coding for AEC as a probe

To corroborate the data obtained by PCR amplification using the AEC primers, different strains of *P. polymyxa* and *P. graminis* were evaluated in hybridization assays using the gene coding for AEC as a probe. Although the two *P. polymyxa* DNAs tested (PM04.11 and PM11.05) showed a very similar pattern of homology to the probe, none of the *P. graminis* tested (RSA19^T, MC04.06, MC36.22, and MC22.19) showed homology to the gene coding for AEC (Fig. 5).

Discussion

It is well known that IAA production contributes to colonization efficiency as well as to the growth and survival of bacteria on their host plants (Vandeputte *et al*., 2005). Shishido *et al*. (1999) have already demonstrated that *P. polymyxa* strain Pw-2 is able to invade spruce (*Picea* sp.) root tissues following soil inoculation, and to ultimately colonize the stem vascular tissue without causing visible symptoms of disease. In lodgepole pine (*Pinus contorta*), the same strain significantly stimulated root-biomass accumulation and total root elongation after 12 weeks of inoculation (Bent *et al*., 2001). Although most of free-living rhizobacteria colonize only the surface of the roots and remain vulnerable to competition with other rhizosphere microorganisms (Cocking, 2003), the plant still receives benefits such as nitrogen fixation (Holl *et al*., 1988; Holl and Chanway, 1992; Petersen *et al*., 1996) and the production of plant growth-regulating substances (Lebuhn *et al*., 1997; Nielsen and Sorensen, 1997; Jeong *et al*., 2006). In the aforementioned studies, different crop species were used for the isolation and characterization of *Paenibacillus* strains. However, most of these studies were on plants from temperate regions. In tropical countries, where unfavorable climatic conditions are prevalent, making their ecosystems unique, the application of *Paenibacillus* strains to seed as a PGPR could be a successful approach. Because strains can form endospores, they can resist a range of environmental stress conditions like high temperatures, dryness or heavy rainfalls, usually present in tropical countries.

Two species of *Paenibacillus* were used in the present study: *P. polymyxa* and *P. graminis*. These species are closely related, showing 93% of similarity in 16S rRNA (Da Mota *et al*., 2004), and both species harbor nitrogen-fixing strains. Various strains have been isolated from the rhizosphere of maize and other plant species in Brazil (Da Mota *et al*., 2002; Vollú *et al*., 2006); however, their ability to produce auxins and their potential to invade and colonize internal tissues of plants have not been evaluated to date. Therefore, this study was conducted to determine the ability of various strains of *P. polymyxa* and *P. graminis* to produce indole-3-acetic acid (Table 1). All of the *P. polymyxa* strains but none of the *P. graminis* strains tested were able to produce IAA under the conditions used in this study. Furthermore, although some strains of *P. polymyxa* produced only low amounts of IAA, strain PM04-1 produced a high level of indole-3-acetic acid (17 μg/ml). This result is similar to the amount of IAA produced by *P. polymyxa* strain Pw-2 (18 μg/ml), which was shown to enhance growth of the lodgepole pine (Bent *et al*., 2001). Other studies have demonstrated that *P. polymyxa* is capable of colonizing the root tip and the intercellular spaces outside of the vascular cylinder of *Arabidopsis thaliana* and barley (*Hordeum vulgare*) (Timmusk *et al*., 2005). Moreover, auxin production and plant growth increment (foliar fresh weight and total leaf area) have also been reported in *A. thaliana* that has been colonized by *P. polymyxa* E681 (Jeong *et al*., 2006). Therefore, strain PM04-1 and other strains isolated from the rhizosphere of different cultivars of maize sown in Cerrado soil are potential candidates for promoting growth under tropical conditions. However, strains of *P. graminis* that were found to be prevalent in sorghum rhizospheres do not seem to produce IAA and can only be considered as PGPR because of their ability to fix nitrogen (Berge *et al*., 2002; Vollú *et al*., 2006).

Although several different IAA biosynthesis pathways have been described in bacteria (Patten and Glick, 1996), the transport mechanisms (efflux) of auxin are not well characterized (Spaepen *et al*., 2007). Recently, a protein showing high homology with Auxin Efflux Carriers from plants [transporter classification (TC) No. 2.A.69] was detected in the *Bacillus amyloliquefaciens* FZB42 genome (Chen *et al*., 2007). Secretion of indole-3-acetic acid and growth promotion of plants have been attributed to this strain and other *Bacillus* species (Domenech *et al*., 2006; Chen *et al*., 2007; Idris *et al*., 2007). Although the AEC family has been described in bacteria, archaea and eukaryotes, only plant AEC members have been characterized to date. This study demonstrates for the first time the presence of a gene coding for the AEC family protein in auxin-producing *P. polymyxa* strains and its absence from non-producing *P. graminis* strains, which suggests that the AEC family protein might play an important role in auxin secretion in *Paenibacillus*. More studies should be conducted to help understand the function of AEC proteins in the genus *Paenibacillus*.

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