Isolation and Partial Characterization of Antagonistic Peptides Produced by *Paenibacillus* sp. Strain B2 Isolated from the Sorghum Mycorrhizosphere

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Paenibacillus sp. strain B2, isolated from the mycorrhizosphere of sorghum colonized by *Glomus mosseae*, produces an antagonistic factor. This factor has a broad spectrum of activity against gram-positive and gram-negative bacteria and also against fungi. The antagonistic factor was isolated from the bacterial culture medium and purified by cation-exchange, reverse-phase, and size exclusion chromatography. The purified factor could be separated into three active compounds following characterization by amino acid analysis and by combined reverse-phase chromatography and mass spectrometry (liquid chromatography-mass spectrometry and mass spectrometry-mass spectrometry). The first compound had the same retention time as polymyxin B₁, whereas the two other compounds were more hydrophobic. The molecular masses of the latter compounds are 1,184.7 and 1,202.7 Da, respectively, and their structure is similar to that of polymyxin B₁, with a cyclic heptapeptide moiety attached to a tripeptide side chain and a fatty acyl residue. They both contain threonine, phenylalanine, leucine, and 2,4-diaminobutyric acid residues. The peptide with a molecular mass of 1,184.7 contains a 2,3-didehydrobutyryne residue with a molecular mass of 101 Da replacing a threonine at the A2 position of the polymyxin side chain. This modification could explain the broader range of antagonistic activity of this peptide compared to that of polymyxin B.

Over the last decade the increase in problems linked to the use of pesticides, such as the emergence of fungicide-resistant pathogens, has stimulated the search for alternative methods to prevent or at least limit the use of chemical pesticides. Several studies have reported the possibility of using one or more living microorganisms to inhibit the growth or metabolic activity of deleterious microorganisms. The control mechanisms involved are very diverse; they can be based on competition in the rhizosphere for nutrients or on the production of iron-chelating siderophores, antibiotics, and cell wall-degrading enzymes (8, 11, 13, 18, 30, 36).

Bacillus spp. and related genera, which are very common soil bacteria (2), have been identified as potential biological control agents as they produce a wide range of cyclic lipopeptide antibiotics active against various microorganisms (3, 6, 15, 20, 32, 39). The antifungal compounds that have been identified include the iturin and bacillomycin families produced by *Bacillus subtilis* and fusaricidin produced by *Paenibacillus polymyxa* (previously *Bacillus polymyxa*, reclassified by Ash et al. [1]) (4, 5, 7, 10, 19, 23, 28, 31, 40). Antibacterial compounds, including the polymyxins produced by *P. polymyxa* and mactacin (polymyxin M) produced by *Paenibacillus kobensis* M, have also been identified (26, 34, 38), and antiviral compounds produced by *Bacillus* sp., such as satabacins and satazolin, have been isolated (22). A major group of peptide antibiotics pro-

duced by some strains of *P. polymyxa* belongs to the polymyxin-colistin-circulin family (34, 38). The general structure of polymyxins includes a cyclic heptapeptide moiety attached to a tripeptide side chain with a fatty acyl residue on the N-terminal amino group. Polymyxins can be distinguished from each other by differences in amino acid or fatty acid composition (29, 35).

The gram-positive bacterium *Paenibacillus* sp. strain B2, which was previously isolated from the mycorrhizosphere of *Sorghum bicolor* inoculated with *Glomus mosseae*, has been shown to possess extracellular cellulolytic, proteolytic, chitinolytic, and pectinolytic enzyme activities (9). This bacterium exhibits antagonistic activity against many important soilborne fungal pathogens, but it stimulates root colonization by arbuscular mycorrhizal fungi (9). In addition *Paenibacillus*, as an endospore-forming bacterium, can resist desiccation, heat, UV irradiation, and organic solvents and can flourish in agricultural soils, making it well suited for biocontrol applications (4, 26). In this paper we report purification and partial characterization of the antagonistic factor produced by *Paenibacillus* sp. strain B2.

MATERIALS AND METHODS

Bacterial strain. The *Paenibacillus* sp. strain B2 used in this study was isolated from the mycorrhizosphere of *S. bicolor* inoculated with *G. mosseae* (Nicol & Gerd) Gerdmann & Trappe BEG 12 and was identified from its analytical profile index and by 16S rRNA gene analysis (9).

Chemicals and biochemicals. Luria-Bertani (LB) medium, agar, polymyxin B sulfate, diaminobutyric acid, pronase, and benomyl were purchased from Sigma, and CM-Trisacryl was purchased from Biosepra (Process Division of Ciphergen, France).

Growth conditions. *Paenibacillus* sp. strain B2 was stored in 20% glycerol at –80°C. Starter cultures were prepared by inoculation of LB agar incubated at

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27°C for 24 h. The following four media were tested for antagonistic factor production: LB medium, Difco minimal broth Davis (DMBD) containing 50% glucose, and potato dextrose broth. All media were prepared as recommended by Difco (12). The liquid media were incubated at 27°C and 125 rpm for 48 h. In order to monitor production of the antagonistic factor in the different media, the following bioassay (top agar technique) based on *Escherichia coli* strain MRAP2 was developed. Three milliliters of molten LB medium containing 0.7% agarose was seeded with 100 µl of a suspension of *E. coli* strain MRAP2 (optical density at 600 nm, 0.5) and spread onto a petri dish (90 by 90 mm) containing 32 ml of LB medium. Sample wells were punched out of the agar with a sterile Pasteur pipette, and 1 µl of a test compound was placed in each well. The plates were incubated at 37°C for 24 h, and zones of inhibition (ZOI) were measured.

Purification of antagonistic factor. *Paenibacillus* sp. strain B2 was grown in 1 liter of DMBD in a 5-liter Erlenmeyer flask at 27°C and 125 rpm for 20 h, the time at which the end of the stationary phase was reached. The liquid culture medium was then centrifuged at 14,000 rpm for 30 min, filtered through 0.2-µm filters, and heated (110°C, 10 min), and the clarified culture medium was applied to a CM-Trisacryl column (2 by 10 cm) equilibrated in 25 mM Tris-HCl (pH 8.5). A 100-ml NaCl gradient (0 to 0.5 M) in the same buffer was used to elute the active components at a flow rate of 1 ml min⁻¹. One-milliliter fractions were collected and tested for antagonistic activities as described above. The active fractions were pooled, diluted three times with Milli-Q water, and applied to another CM-Trisacryl column (2 by 5 cm). Active compounds were eluted with 30 ml of 0.1 M NH₄OH at pH 11. Three-milliliter fractions were collected and tested. The CM-Trisacryl-purified material was concentrated and further purified with a C₁₈ Sep-Pack cartridge (Waters, Milford, Mass.) by following the manufacturer's protocol. Active compounds were eluted from the cartridge in 100% methanol. The methanol was evaporated under reduced pressure, and the residue was redissolved in Milli-Q water. Two hundred microliters of the Sep-Pack-purified material was applied to a fast protein liquid chromatography Superdex 75 HR 10/30 gel filtration column (Amersham) that was equilibrated in 0.01 M Tris-HCl (pH 8.5) containing 0.15 M NaCl at a flow rate of 1 ml min⁻¹. Peptides were detected by measurement of the absorbance at 230 nm. One-milliliter fractions were collected, and 0.2-ml aliquots were assayed for antagonistic activity. The active fractions were desalted using Sep-Pack C₁₈ cartridges. After evaporation of the methanol, the antagonistic factor was obtained as a white powder that was soluble in methanol, ethanol, and water. It could be heated to 100°C for 1 h or autoclaved at 121°C for 20 min, and it could also be stored at 4°C without any loss of activity. It was redissolved in Milli-Q water (0.25 mg/ml) before use. Samples of this material are referred to below as Superdex-purified material.

Antagonistic activity of the purified strain B2 factor. The Superdex-purified material was tested against phytopathogenic microorganisms (*Pseudomonas viridiflava* DSM 11124, 50337, and 50338, *Erwinia carotovora*, and *Fusarium acuminatum* DSM 62148) and nonpathogenic microorganisms (*E. coli*, *Pseudomonas fluorescens* strain C7R12, *P. fluorescens*, and *Fusarium solani* [Lascaux caves, Lot, France]) using the top agar technique for bacteria, as described above. Potato glucose agar was used for *F. solani* and *F. acuminatum*. One hundred-microliter portions of different concentrations of the antagonistic factor were placed in wells containing 32 ml of potato glucose agar on which 500 µl of a fungal spore suspension (10⁵ spores/ml) had previously been spread. The plates were incubated at 37°C for *E. coli*, at 27°C for other bacteria, and at 25°C for fungi.

Serial dilutions of the Superdex-purified material were prepared in a sterile 12-well plate (flat-bottom multiwell tissue culture plate with low-evaporation lid) in order to determine the MIC, defined as the minimal peptide concentration that inhibited bacterial or fungal growth, using the following liquid media: LB broth (*E. coli*, *P. fluorescens*, and *E. carotovora*), tryptic soy broth (*P. viridiflava*), and potato glucose broth (*F. solani* and *F. acuminatum*). A commercial fungicide, benomyl, was used as a positive control treatment against fungi. Polymyxin B was used as a positive control against bacteria.

Physicochemical properties of antagonistic substances. (i) **TLC.** Ten microliters (40 µg) of Superdex-purified material was spotted onto a thin-layer chromatography (TLC) plate (Silica Gel 60 F₂₅₄; 20 by 20 cm; layer thickness, 0.25 mm; Merck). Chromatograms were developed up to a height of 11 cm using methanol-*n*-butanol-25% ammonia-chloroform (14:4:9:12, vol/vol/vol/vol) as the mobile phase. The plates were dried at room temperature and then at 100°C for 1.5 h. The plates were immersed in 0.2% ninhydrin (ethanol solution) for 15 min and dried again at 100°C for 5 min (21). Five hundred microliters (2,000 µg) of Superdex-purified material was spotted onto a TLC plate, and the conditions described above were used to develop the chromatograms. Bands visualized by ninhydrin were excised by grating with a razor; the powder was transferred into Eppendorf tubes, and the compounds were solubilized in methanol. The identity

TABLE 1. Comparison of the MICs in different liquid media of the *Paenibacillus* sp. strain B2 antagonistic factor and of a commercial sample of polymyxin B against plant-pathogenic and nonpathogenic microorganisms

Microorganism	MICs (µg/ml)	
	Strain B2 antagonistic factor ^a	Polymyxin B ^b
Gram-negative bacteria		
<i>Escherichia coli</i>	2.22	0.25
<i>Pseudomonas viridiflava</i> DSM 11124	0.60	0.08
<i>P. viridiflava</i> DSM 50337	0.60	0.08
<i>P. viridiflava</i> DSM 50338	0.60	0.08
<i>Pseudomonas fluorescens</i> (Lascaux caves, Lot, France)	2.22	0.60
<i>P. fluorescens</i> C7R12	2.22	2.22
<i>Erwinia carotovora</i>	6.67	0.25
Gram-positive bacteria		
<i>Streptomyces argenteolus</i> ATCC 1109 (B15)	20	— ^c
<i>Streptomyces griseolus</i> ATCC 11796 (B22)	20	—
<i>Arthrobacter crystallopoietes</i> SR1	20	—
<i>A. crystallopoietes</i> X80738 (SR5)	20	—
Fungi		
<i>Fusarium solani</i> (Lascaux caves, Lot, France)	2.60	2.60
<i>Fusarium acuminatum</i> DSM 62148	8.00	2.60

^a Superdex-purified sample.

^b Commercial sample.

^c —, no inhibition at concentrations up to 100 µg/ml.

of the active compounds was confirmed by comparing the inhibition zone with the bands on a TLC plate visualized by ninhydrin.

(ii) **Amino acid analysis.** Superdex-purified material (2 mg) was hydrolyzed in 0.2 ml of 6 N HCl (100°C, 16 h). The hydrolysate was evaporated under reduced pressure and redissolved in 0.2 ml Milli-Q water. Ten-microliter aliquots of the hydrolysate were spotted onto TLC plates (precoated cellulose 300-25 thin-layer 0.25-mm cellulose MN300; Macherey-Nagel) and analyzed by two-dimensional chromatoelectrophoresis in order to separate the different amino acids. Electrophoresis was performed in pyridine-acetic acid-water (1:5:94, vol/vol/vol) for 1 h at 300 V. Chromatography was performed in butanol-acetic acid-water-pyridine (4:1:2:1, vol/vol/vol/vol). Amino acids were visualized with ninhydrin, and the results were compared with the results obtained with samples of commercial polymyxin B or E. The same hydrolysate was used to quantify amino acids using precolumn derivatization with ortho-phthalaldehyde (OPA)/9-fluorenylmethyl-oxycarbonyl chloride (Fmoc) and fluorescence detection (16, 17).

(iii) **Pronase digestion.** The antagonistic factor was treated with pronase (0.05 mg/ml) at 37°C for 30 min; samples (5 µl) were then assayed for antagonistic activity.

Separation and identification of the active constituents of the antagonistic factor. (i) **HPLC.** Twenty microliters of Superdex-purified material (250 ng/µl in H₂O) was applied to a C₁₈ high-performance liquid chromatography (HPLC) column (YMC-Pack Pro C₁₈; 5 µm; 250 by 2.0 mm [inside diameter]) equilibrated with a mixture of 0.01 M trifluoroacetic acid and acetonitrile (77:23, vol/vol), using a flow rate of 0.2 ml min⁻¹. Elution of polymyxins was monitored at 215 nm using isocratic conditions. The solvent was removed by evaporation using a Speed Vac apparatus, and active fractions were located by the bioassay. Several runs were combined to test the antagonistic activity of the different fractions.

(ii) **MS.** Liquid chromatography-mass spectrometry (MS) analysis was performed using the conditions that were used for HPLC. Full MS acquisition over the mass range from 500 to 1,500 was performed online with UV detection at 215 nm. MS was performed with an LCQ ion trap mass spectrometer (Finnigan MAT, San Jose, CA) equipped with an electrospray interface operated in the positive-ion mode (28). MS-MS spectra were acquired with a collision energy of 40%.

RESULTS

Purification of the antagonistic factor. Of the four culture media tested, DMBD turned out to be the best medium to

FIG. 1. Reverse-phase HPLC purification of *Paenibacillus* sp. strain B2 antagonistic factor. (A) Analysis of a commercial polymyxin B sample (Sigma). (B) Analysis of the Superdex-purified factor (5 µg) on a C₁₈ HPLC column. Elution of polymyxins was monitored at 215 nm using isocratic conditions (0.01 M trifluoroacetic acid—acetonitrile [77:23]).

optimize the production of the antagonistic factor. The optimal temperature for bacterial growth was 30°C, while production of the antagonistic factor was maximal at 27°C. Growth on DMBD at 27°C for 20 h was therefore used throughout this work. The antagonistic factor was detected in DMBD after 9 h of bacterial growth, corresponding to one-half of the exponential growth phase, and the maximum level was reached at the end of the stationary phase.

The antagonistic factor could be concentrated easily from the culture medium on a cation-exchange column, but high salt concentrations or a very high pH was required for elution, indicating that the compound was probably strongly basic. After further purification using the C₁₈ Sep-Pak cartridge and elution in methanol, the antagonistic factor was spotted onto a TLC plate and compared with polymyxin B and polymyxin E.

Ninhydrin detection revealed bands with R_f values of 0.60, 0.73, and 0.79 for the active compounds, 0.68, 0.74, and 0.80 for polymyxin B, and 0.69 and 0.74 for polymyxin E. The three ninhydrin-positive bands for *Paenibacillus* sp. strain B2 were eluted with methanol, and antagonistic activity, as measured by the *E. coli* bioassay, was detected in each of the three bands, indicating that the antagonistic factor was in fact a mixture of active compounds. Following further purification by gel filtration on a Superdex column, antagonistic activity was detected in fractions corresponding to a molecular mass of about 1 kDa, suggesting that it could be a peptide or a mixture of peptides. No reduction in antagonistic activity was detected after incubation with pronase, as previously reported for cyclic peptides (14). However, the antagonistic activity was completely lost after strong acid hydrolysis, and several amino acids could be

FIG. 2. Analysis of the *Paenibacillus* sp. strain B2 antagonistic factor by liquid chromatography-MS. (A) Base peak chromatogram corresponding to the UV chromatograms shown in panel B. (B) Full MS spectra of the two most active peaks (peaks 2 and 6).

detected in the hydrolysate by two-dimensional chromatoelectrophoresis. Threonine, phenylalanine, leucine, and an unknown amino acid were the major amino acids revealed after two-dimensional chromatoelectrophoresis. The unknown amino acid was subsequently identified as diaminobutyric acid. Amino acids in the hydrolysate were quantified by HPLC after precolumn derivatization with OPA and FMOC. A commercial sample of polymyxin B was hydrolyzed and analyzed in the same conditions. The analysis revealed that there were similar molar amounts of threonine, phenylalanine, and leucine in the antagonistic factor and polymyxin B. Diaminobutyric acid could not be accurately quantified using this technique since it gave two peaks, as previously observed in the case of γ -aminobutyric acid (17), but the intensity of these two peaks was almost identical to that of the antagonistic factor and polymyxin B samples.

Antagonistic activity of the Superdex-purified antagonistic factor. Antagonistic activity of the Superdex-purified material was detected against all the gram-negative and gram-positive bacteria tested and the two species of *Fusarium* (Table 1). The MICs indicated that the antagonistic factor was as effective as commercial polymyxin B (2.22 and 2.60 $\mu\text{g/ml}$, respectively) in the case of *P. fluorescens* strain C7R12 and *F. solani*. In the case of *E. coli*, *P. viridiflava*, *P. fluorescens* (Lascaux caves, Lot, France), *E. carotovora*, and *F. acuminatum*, however, the antagonistic factor was far less active. In the case of *F. acuminatum* and *F. solani*, the MICs of the antagonistic factor were lower (8.0 and 2.6 $\mu\text{g/ml}$, respectively) than those of benomyl, the commercial fungicide used as control (12 $\mu\text{g/ml}$ and >100 $\mu\text{g/ml}$). For the gram-positive bacteria, no inhibitory effect was observed in the presence of 100 $\mu\text{g/ml}$ polymyxin B, whereas 20 $\mu\text{g/ml}$ of the antagonistic factor was enough to stop the growth of the four gram-positive bacteria tested (Table 1).

HPLC-electrospray ionization MS analysis. Comparison of the amino acid compositions of the hydrolysates of the Superdex-purified factor and of commercial samples of polymyxin B indicated that the antagonistic factor was sufficiently purified to be analyzed by HPLC. A commercial polymyxin B sample containing both polymyxin B₁ and B₂ was used for comparison

(Fig. 1A). The Superdex-purified material was separated into more than 10 peaks (Fig. 1B), and the main compounds eluted later than polymyxin B₁. The corresponding base peak chromatogram is shown in Fig. 2A. Full MS spectra of the main compounds (compounds 1 to 6 in Fig. 1B) gave molecular weights of 1,202.7 for compounds 1, 3, 5, and 6 and 1,184.7 and 1,202.5 for compounds 2 and 4, respectively. Full MS spectra are shown for compounds 2 and 6 in Fig. 2B. Antagonistic activity was detected in three of the HPLC fractions. The first fraction with an antagonistic activity had the same retention time (25.33 min) as polymyxin B₁ but was less active (ZOI against *E. coli*, 0.5 cm^2) and corresponded to a minor peak on the chromatogram (Fig. 1B, peak b). This fraction was active only against gram-negative bacteria and *F. acuminatum* (data not shown). The two other fractions, which had retention times of 34.16 min. and 48.44 min and corresponded to peaks 2 and 6 in Fig. 2A, were much more active, with ZOIs against *E. coli* of 2.4 and 2.08 cm^2 , respectively. These two fractions were active against all the microorganisms listed in Table 1, including gram-positive bacteria (data not shown). The electrospray ionization MS-MS spectra of these two compounds were analyzed in detail by comparing them with the spectra of polymyxin B₁ and B₂ (Fig. 3 and 4).

(i) Peak 2 ($[\text{M}+\text{H}]^+$, 1,185.7 Da). The MS-MS spectrum of peak 2 and the first and second series of product ions identified in the spectrum are shown in Fig. 3. The first loss of 241.1, yielding the product ion with m/z 945.3 in the first series and the ion with m/z 241.1 in the second series, corresponds to fatty acid (FA)—2,4-diaminobutyric acid (Dab) and indicates that the fatty acyl moiety has the elemental composition $\text{C}_9\text{H}_{17}\text{O}$. The fatty acid residue of this molecule is probably the same as that in polymyxin B₁ (6-methyloctanoyl), but the molecular mass, 1,184.7 Da, is 18 Da less than that of polymyxin B₁. In the “A2” position of the side chain (FA-Dab-A2-), threonine is replaced by another molecule with a molecular mass of 101 Da (a residual mass of 83 Da with possible -NH₂ and -COOH groups) which could be 2,3-didehydrobutyryne (Dhb), obtained by dehydration of threonine. The other moiety of the molecule is probably the same as that of polymyxin B₁.

FIG. 3. (A) MS-MS spectrum and the first and second series of product ions acquired for peak 2, the result of isolation and collisional activation with a collision energy of 40% in ion trap of [M + 2H]²⁺ with *m/z* 593.5. The mass differences are indicated above the arrows. (B) Proposed structure of the corresponding molecule. Leu, leucine; Phe, phenylalanine; Thr, threonine; Moa, 6-methyloctanoic acid.

(ii) **Peak 6** ([M+H]⁺, 1,203.7 Da). The mass of the peak 6 peptide (Fig. 4) is identical to that of polymyxin B₁, and the MS-MS spectra are almost identical, although the retention times differ considerably. The first and second series of product ions (*m/z* 241.0 and 963.4) in the mass spectrum of the doubly charged protonated molecular ion [(M + 2H)]²⁺ 602.5 correspond to [FA+Dab]⁺ and [(M+H)-(FA+Dab)]⁺ ions, indicating that the mass of the fatty acid residue is identical to that of 6-methyloctanoic acid, the fatty acid moiety of polymyxin B₁.

DISCUSSION

The antagonistic factor of *Paenibacillus* sp. strain B2 was active against several gram-negative and gram-positive bacteria and two pathogenic soilborne fungi. Purification of this antagonistic factor showed that it is composed of a mixture of polymyxins or polymyxin-related peptides. Data from the par-

tial characterization of two of these peptides confirmed their broad antagonistic spectrum and their activity against gram-positive bacteria, in contrast to polymyxin B. Although the MICs of the purified peptides were not determined, they are probably higher than those determined for the Superdex-purified factor since the amounts of the antagonistic factor and of commercial polymyxin B were normalized on a weight basis in these bioassays.

A large part of the antagonistic activity in the purified factor is due to the presence of three peptides, although more than 10 peaks could be separated by HPLC. The first active fraction, which had the same retention time as polymyxin B₁, was difficult to characterize as it corresponded to a minor peak in the chromatogram. The mass spectrum of the peptide present in the peak 6 fraction was almost identical to that of polymyxin B₁, but its retention time was much longer. One possible explanation for this is that the compound is composed of poly-

FIG. 4. (A) MS-MS spectrum and the first and second series of product ions acquired for peak 6, the result of isolation and collisional activation with a collision energy of 40% in ion trap of $[M + 2H]^{2+}$ with m/z 602.5. The mass differences are indicated above the arrows. (B) Proposed structure of the corresponding molecule. Leu, leucine; Phe, phenylalanine; Thr, threonine; FA, fatty acid.

myxin B₁ bound to another molecule, making it more hydrophobic. However, this peptide is active against gram-positive bacteria, in contrast to polymyxin B₁, indicating that the two molecules are different. Another possible explanation is that the two peptides differ only by the fatty acid moiety. Since the mass spectrum indicates that the fatty acid has the same mass as (+)-6-methyloctanoic acid, it could be a (+)-6-methyloctanoic acid isomer. Hydrophobicity of the fatty acid moiety of polymyxins is known to affect their biological activity (35).

The mass spectrum of the peptide in the peak 2 fraction indicates that this compound has a molecular mass of 1,184.7 Da, 18 Da less than the molecular mass of polymyxin B₁. Furthermore, the threonine residue typical of polymyxins is replaced by an unusual amino acid with a molecular mass of 101 Da, related to Dhb, whereas the other moiety of the molecule appears to be identical to polymyxin B₁. Many peptide antibiotics produced by *Bacillus* spp. and related genera contain unusual amino acids (25). It is possible that the activity of

the *Paenibacillus* sp. strain B2 peptide against gram-positive bacteria is related to the presence of the unusual Dhb-related amino acid (Dhb) or to the loss of one threonine molecule (24). Because of the difference between these peptides and polymyxin B, we propose that the peptides should be grouped under the name paenimyxin.

Gram-negative bacteria have generally been found to be more sensitive to polymyxins than gram-positive bacteria (26, 35), whereas only a few studies on yeasts have reported antagonistic activity of these antibiotics against fungi (33, 35). Our results show that polymyxin B also has an antifungal activity against *Fusarium* spp. Polymyxins increase the permeability of the outer membrane of gram-negative bacteria by binding to the bacterial lipopolysaccharide (27, 37), but their mode of action against gram-positive bacteria or fungi has not been extensively studied. *Paenibacillus* sp. strain B2 has antagonistic activity against all the bacteria and pathogenic soilborne fungi tested so far, but it is inactive against the mycorrhizal fungus *G.*

mosseae and even stimulates its development within roots (9). Further work is necessary to understand the biochemical basis of the resistance of symbiotic fungi to *Paenibacillus* sp. strain B2. The use of this bacterial strain as a biocontrol agent to increase plant protection, in combination with arbuscular mycorrhizal fungi, therefore seems to be a promising approach for controlling some plant diseases, while the use of chemical intrants in agriculture is limited.

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