Article

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Characterization of actinomycetes antagonistic to *Phytophthora fragariae* var. *rubi*, the causal agent of raspberry root rot

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Eleven actinomycete strains that were previously shown to protect raspberry *(Rubus strigosus)* plants against *Phytophthora* infection were characterized. All were shown to belong to the genus *Streptomyces*. Two strains (EF-34 and EF-76) grew at 4, 15 and 30°C on V8 agar between pHs 5 to 9. Seven strains including EF-34 and EF-76 had both the ability to hydrolyze *Phytophthora* cell walls and to inhibit *Phytophthora* growth at 15°C between pHs 5 to 9. All actinomycetes inhibited the growth of *P. fragariae* var. *rubi* and of *Pythium ultimum*. The growth of other fungal species and of Gram-negative bacteria was inhibited only in the presence of three strains (EF-14, EF-72, and EF-76). The eleven antagonistic actinomycetes were classified into four groups with regard to their resistance to various pesticides used to protect raspberry crops. Strain EF-76 was further characterized. This strain was identified as *Streptomyces hygroscopicus* var. *geldanus*, and it was shown to produce geldanamycin, a known antibiotic.

[Caractérisation d'actinomycètes antagonistes au *Phytophthora fragariae* var. *rubi* causant un pourridié des racines chez le framboisier]

Onze souches d'actinomycètes ayant la capacité de protéger les plants de framboisiers *(Rubus strigosus)* contre les infections causées par les *Phytophthora* ont été caractérisées. Il a été montré que toutes les souches appartenaient au genre *Streptomyces*. Deux souches (EF-34 et EF-76) croissaient à 4, 15 et 30°C sur un milieu V8 agar dont le pH avait été ajusté entre 5 et 9. Sept souches dont EF-34 et EF-76 pouvaient hydrolyser les parois cellulaires de *Phytophthora* et inhiber la croissance du champignon à 15°C et à des pH variant entre 5 et 9. Toutes les souches inhibaient la croissance du *P. fragariae* var. *rubi* et du *Pythium ultimum*. La croissance d'autres espèces fongiques et de bactéries à Gram négatif n'était inhibée qu'en présence de trois souches (EF-14, EF-72 et EF-76). Les onze actinomycètes antagonistes ont été classés en quatre groupes selon leur résistance à divers pesticides utilisés pour protéger les cultures de framboisiers. La souche EF-76 a été caractérisée plus en détail. Cette souche a été identifiée comme étant le *Streptomyces hygroscopicus* var. *geldanus*, et produisait l'antibiotique geldanamycine.

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INTRODUCTION

Since 1987, *Phytophthora* spp. have been reported to be the main causal agents of raspberry root rot in several countries such as Australia (Washington, 1988), Chile (Latorre and Munoz 1993), France (Nourrisseau and Baudry 1987), Germany (Duncan *et al.* 1987), the United Kingdom (Montgomerie and Kennedy 1991), the United States (Wilcox 1989) and Canada (Thibodeau 1991). Metalaxyl is the only product authorized in Canada to control *Phytophthora* in raspberry (*Rubus strigosus* Michx.) crops. Unfortunately, it has been demonstrated that *P. fragariae* Hickman could develop resistance against metalaxyl (Nickerson 1990). Biological control appears to be a promising avenue to reduce raspberry root rot incidence. Indeed, we have recently isolated eleven actinomycete strains that had both the ability to lyse *Phytophthora* cell walls and the ability to inhibit the growth of *Phytophthora* spp. These actinomycetes, when introduced into the rhizosphere of raspberry plants grown under controlled conditions, were found to be efficient in reducing root rot disease (Valois *et al.* 1996).

Actinomycetes are currently used to control plant diseases. For example, *Streptomyces* strain 5406 has been used in China for more than 30 years now to protect cotton crops against soilborne pathogens (Yin *et al.* 1965). More recently, Kemira Oy has developed a biofungicide that contains living cells of *S. griseoviridis* Anderson, Erlich, Sun and Burkholder to protect crops against *Fusarium* and *Alternaria* infections (Lahdenperä *et al.* 1991).

There is accumulating evidence that diseases caused by fungus-like protoctistan *Phytophthora* and *Pythium* species could also be controlled by actinomycetes. Soil inoculation with specific streptomycete strains could significantly reduce damages caused by *Pythium* or *Phytophthora* species in ornamental (Bolton 1978, 1980; Malajczuk 1983), legume (Filnow and Lockwood 1985) and horticultural productions (Crawford *et al.* 1993; Sutherland and Papavizas 1991; Turhan and Turhan 1989).

In spite of some success, biological control is not widely used commercially. This is due in part to the inconsistent performance of biocontrol agents in the field. Weller (1988) suggested a multitude of factors that could account for inconsistent performance of biocontrol agents in field trials. A better knowledge of the physiological properties of the antagonistic agents would surely help to predict the behavior of the biocontrol agents in the agro-environment.

The antagonistic agents described by Valois *et al.* (1996) have not been used in field trials yet. However, we plan to test their efficiency to control root diseases caused by *Phytophthora* spp. on different crops such as raspberry, strawberry (*Fragaria chiloensis* (L.) *var. anassa* (Duchesne)) and balsam fir (*Abies balsamea* (L.) Mill.). To determine the environmental conditions that might influence the efficiency of the antagonistic actinomycetes and to optimize the chances for success in future field trials, we analyzed (1) the effects of pHs, temperatures and pesticides on growth of the antagonistic strains and, (2) the effects of temperatures and pHs on the production of hydrolytic enzymes and antibiotics by the antagonistic streptomycetes. In addition, one of the antagonistic actinomycetes, strain EF-76, was further characterized with regard to antibiotic production and taxonomic identity.

MATERIALS AND METHODS

Phenotypic characterization of the actinomycetes

The ability of the antagonistic actinomycetes to grow at various temperature (4, 15 and 30°C) and pH (5, 7 and 9) conditions was tested by inoculating the strains on V8 agar (Ribiero 1978). The growth of the actinomycetes was periodically recorded. Colony and spore color was observed after 10 d of growth at 30°C on Yeast Malt Extract (YME) (Pridham *et al.* 1956-1957). To observe sporulation, bacteria were inoculated and grown around a circular microscope cover slip obliquely inserted into YME plates. After sporulation, the cover slip

was removed and the attached aerial mycelium and spore chains were observed under a light microscope at 100 X total magnification. The procedure of Becker *et al.* (1964) was followed for the hydrolysis of whole cells for diaminopimelic acid analysis. Diaminopimelic acid isomers were separated by thin-layer chromatography according to Staneck and Roberts (1974).

Effect of different chemicals on bacterial growth

Chemicals most commonly used in Canada to protect raspberry crops against pathogens, pests or weeds (benomyl, captan, dichlobenil (Casoron), napropamide (Devrinol), ferbam, metalaxyl and simazine (Sidanex)) were added to V8 agar at a concentration of 10 mg L^{-1} (Santelmann 1977). A spore suspension (1 x 10^6) of each actinomycete strain was added to the center of the plates containing one of the chemicals. The plates were incubated for 10 d at 30°C and bacterial growth was then recorded. All tests were repeated three times.

Antagonistic properties of actinomycetes

The ability of the antagonistic actinomycetes to hydrolyze *Phytophthora* cell walls was tested at 15°C on Mycelium agar (Valois *et al.* 1996) adjusted at different pHs (5, 7 and 9). The presence of clear zones around colonies was recorded after 10 d of incubation.

The ability of the antagonistic strains to inhibit the growth of *P. fragariae* var. *rubi* and other fungi (*Pythium ultimum* Trow, *Aspergillus niger* Van Tieghem, *Penicillium chrysogenum* Thom, *Penicillium italicum* Wehmer, *Phycomyces blackesleeanus* Kunze, *Sclerotinia* sp., *Rhizoctonia solani* Kühn and *Rhizopus stolonifer* Lind) was tested onto a V8 plate as follows. The V8 plates were adjusted to pH 5 for all fungi except *Phytophthora*. In the latter case, the V8 plates were adjusted at various pHs (5, 7 or 9). Approximatively 1 x 10^6 spores of each actinomycete were inoculated in the center of each V8 plate and incubated for 2 d. A piece of 1 cm^2 of V8 agar bearing a 7-day-old culture of one of the fungi was then placed at 2 cm from the actinomycete inoculation spot. Fungal growth inhibition was recorded after 5-10 d of incubation at 22-25°C for all fungi but *P. fragariae* var. *rubi* that was incubated at 15°C.

Inhibition spectrum of the antagonistic actinomycetes was also determined for Gram-positive bacteria (*Bacillus cereus* Frankland and Frankland, *Staphyloccus aureus* Rosenbach and *Streptococcus faecalis* Andrewes and Horder), Gram-negative bacteria [*Acinetobacter calcoaceticus* Beijerinck (Baumann, Doudoroff and Stanier), *Citrobacter freundii* Braak (Werkman and Gillen), *Enterobacter aerogenes* Kruse (Hormaeche and Edwards), *Escherichia coli* Migula (Castellani and Chalmers), *Klebsiella oxytoca* Flügge (Lantrop), *Proteus vulgaris* Hauser and *Pseudomonas fluorescens* Trevisan (Migula)] and yeasts (*Saccharomyces cerevisiae* Mayen em Rees and *Schizosaccharomyces octosporus* Linder) as follows. Approximatively 1 x 10^6 spores of each actinomycete were inoculated in the center of a Nutrient Agar (NA) plate and incubated for 2 d at 30°C. NA plates were then covered with an overlay of soft NA (0.7%) previously inoculated with bacteria or yeasts (approximatively 10^5 cfu mL^{-1}). Growth inhibition was recorded after 2-5 d of incubation at 30°C.

Purification and identification of the antibiotic produced by strain EF-76 and by *S. hygroscopicus* var. *geldanus* ATCC 55256

For antibiotic production, the strain EF-76 and ATCC 55256 were grown with shaking (300 rpm) for 96 h at 30°C in 4-L flasks containing 1 L of medium YGM+ (King *et al.* 1991). Cultures were centrifuged at 5000 *g* for 15 min and the supernatants were filtered through paper (Whatman #1). The filtrates were extracted three times with an equal volume of chloroform. The three chloroform fractions were pooled and were then concentrated on a Büchi Rotavapor R-114 (Büchi Laborariums Technik AG., Switzerland) with a water bath adjusted at 35°C. The resulting material was then dissolved in dichloromethane and separated by thin layer chromatography on glass plates precoated with 0.5 mm silica gel 60F-250, using

chloroform:methanol (95:5) as migration solvents. A yellow product with a Rf of 0.47 was eluted from the silical gel using chloroform:methanol (80:20). It showed an antibiotic activity against *P. fragariae* var. *rubi*. Mass spectrum and peak matching (HRMS) of the purified antibiotic was determined at 70 eV on a Micromass ZAB-1F spectrometer. The ^1H (300 MHz) NMR spectra were recorded on a Brücker AC-300 instrument (with chloroform as internal standard).

DNA-DNA hybridization

Total DNA from *Streptomyces* strains was isolated according to Hopwood *et al.* (1985). The DNA reassociation experiments were performed according to Stall *et al.* (1994) as modified by Paradis *et al.* (1994). Briefly, DNA of *Streptomyces hygroscopicus* var. *geldanus* De Boer and Dietz strain ATCC 55256 were labeled by incorporating tritiated dCTP into sheared genomic DNA using the random primer method. Sheared DNA of strain EF-76 and of *Streptomyces scabies* Thaxter (Lambert and Loria) strain EF-46 was hybridized with labeled DNA of ATCC 55256. DNA reassociation was carried out for 24 h at 64°C. After reassociation, 300 U of nuclease S1 were added and the tubes were incubated for 1 h at 50°C. DNA was incorporated onto Whatman GF/C glass fiber filters. Radioactivity on the filters was recorded with a Beckman LS7000 scintillation counter. The hybridization values for pairs of strains were means of the values from two experiments; for each experiment, two hybridization reactions were performed.

RESULTS AND DISCUSSION

Phenotypic characterization of the antagonistic actinomycetes

All antagonistic strains produced a non fragmented, yellow to brown mycelium forming *Retinaculiaperti*, *Rectiflexibiles* or *Spirales* spore chains which appeared as white or gray-black masses on the colonies (Table 1). The cell walls of all these strains contained the LL-diaminopimelic acid isomer. Based on their morphological properties and the presence of the LL-diaminopimelic acid isomer in their cell walls, the 11 antagonistic strains could be associated with the genus *Streptomyces*.

The temperature range of growth of the antagonistic strains is an important parameter to evaluate since *Phytophthora* infections on raspberry occur at low temperatures (between 5 and 20°C with an optimum temperature of 15°C) (Thibodeau 1991). In our first screening procedure (Valois *et al.* 1996), the antagonistic strains were selected for both their ability to hydrolyze *Phytophthora* cell walls and to inhibit *Phytophthora* growth at 15°C. However, *Phytophthora* infections occur at lower temperature. Four strains appear promising as biological control tools since they grow even at 4°C (EF-22, EF-34, EF-76 and DVD 4) (Table 2).

Table 1. Phenotypic characterization of 11 actinomycete strains antagonistic to *Phytophthora fragariae* var. *rubi*

Strain	Reverse colony color on YME	Spore mass color on YME	Chain spore morphology
EF-14	yellow	dark gray	*Retinaculiaperti*
EF-22	yellow	gray	*Rectiflexibiles*
EF-25	light brown	gray	*Rectiflexibiles*
EF-27	light brown	gray	*Rectiflexibiles*
EF-34	dark brown	gray	*Retinaculiaperti*
EF-43	dark brown	gray	*Retinaculiaperti*
EF-72	yellow	white	*Rectiflexibiles*
EF-76	light brown	gray to black	*Spirales*
EF-97	dark brown	gray	*Retinaculiaperti*
DVD 3	yellow	white	*Rectiflexibiles*
DVD 4	light brown	gray	*Rectiflexibiles*

Table 2. Radial growth (mm) of the antagonistic streptomycetes on V8 agar under different temperatures and pH conditions

	4°C			15°C			30°C		
Strain	pH 5.0	pH 7.0	pH 9.0	pH 5.0	pH 7.0	pH 9.0	pH 5.0	pH 7.0	pH 9.0
EF-14	0 (0)[a]	0 (0)	0 (0)	10.2 (3.4)	11.9 (1.7)	10.2 (3.4)	23.8 (6.8)	20.4 (5.1)	18.7 (1.7)
EF-22	0.3 (0.3)	3.4 (1.7)	1.7 (0.0)	5.1 (3.4)	5.1 (1.7)	6.8 (3.4)	10.2 (3.4)	10.2 (3.4)	17.0 (1.7)
EF-25	0 (0)	0 (0)	0 (0)	3.4 (1.7)	6.8 (3.4)	6.8 (1.7)	11.9 (5.1)	11.9 (1.7)	3.4 (0)
EF-27	0 (0)	0 (0)	0 (0)	5.1 (3.4)	5.1 (3.4)	5.1 (3.4)	11.9 (5.1)	11.9 (3.4)	10.2 (1.7)
EF-34	2.7 (1.0)	5.1 (1.7)	5.1 (3.4)	5.1 (3.4)	6.8 (1.7)	6.8 (5.1)	5.1 (3.4)	5.1 (3.4)	10.2 (3.4)
EF-43	0 (0)	0 (0)	0 (0)	5.1 (3.4)	5.1 (1.7)	6.8 (3.4)	10.2 (3.4)	13.6 (3.4)	17.0 (3.4)
EF-72	0 (0)	0 (0)	0 (0)	0.3 (0)	0.3 (0.2)	0 (0)	10.2 (5.1)	11.9 (1.7)	8.5 (1.7)
EF-76	0.3 (0.3)	0.5 (0.3)	0.3 (0.2)	6.8 (5.1)	6.8 (6.8)	6.8 (3.4)	15.3 (5.1)	13.6 (5.1)	13.6 (3.4)
EF-97	0 (0)	0 (0)	0 (0)	5.1 (1.7)	6.8 (1.7)	5.1 (5.1)	11.9 (5.1)	13.6 (3.4)	8.5 (3.7)
DVD 3	0 (0)	0 (0)	0 (0)	5.1 (3.4)	10.2 (5.1)	10.2 (5.1)	6.8 (3.4)	6.8 (1.7)	23.8 (5.1)
DVD 4	0 (0)	1.7 (0.9)	3.4 (1.7)	11.9 (3.4)	10.2 (3.4)	10.2 (3.4)	5.1 (5.1)	8.5 (3.4)	20.4 (3.4)

[a] Mean of three repetitions, standard deviation in parentheses.

Table 3. Growth of the antagonistic actinomycetes in the presence of chemicals used in Canada to protect raspberry crops

	Radial growth (mm)							
Strain	no pesticide	metalaxyl	benomyl	simazine	captan	ferbam	napropamide	dichlobenil
EF-14	8.5 (1.7)[a]	10.2 (3.4)	8.5 (1.7)	10.2 (3.4)	11.9 (3.4)	13.6 (3.4)	8.5 (1.7)	8.5 (1.7)
EF-22	8.5 (1.7)	8.5 (1.7)	8.5 (3.4)	11.9 (3.4)	11.9 (5.1)	13.6 (5.1)	8.5 (5.1)	11.9 (3.4)
EF-25	8.5 (5.1)	8.5 (1.7)	8.5 (5.1)	11.9 (3.4)	0 (0)	0 (0)	10.2 (5.1)	10.2 (3.4)
EF-27	8.5 (5.1)	8.5 (5.1)	11.9 (5.1)	8.5 (3.4)	10.2 (1.7)	0 (0)	8.5 (5.1)	8.5 (5.1)
EF-34	8.5 (5.1)	11.9 (5.1)	8.5 (3.4)	11.9 (3.4)	10.2 (3.4)	0 (0)	8.5 (5.1)	11.9 (5.1)
EF-43	11.9 (3.4)	10.2 (3.4)	11.9 (1.7)	11.9 (3.4)	11.9 (1.7)	8.5 (1.7)	11.9 (5.1)	11.9 (3.4)
EF-72	1.2 (0.3)	0.7 (0.3)	0.5 (0.5)	0.9 (0.5)	0 (0)	0.9 (0.2)	0.9 (0.2)	0.7 (0.2)
EF-76	8.5 (5.1)	10.2 (3.4)	10.2 (5.1)	6.8 (1.7)	0 (0)	0 (0)	8.5 (3.4)	5.1 (1.7)
EF-97	8.5 (5.1)	8.5 (3.4)	8.5 (3.4)	8.5 (3.4)	0 (0)	0 (0)	8.5 (5.1)	8.5 (1.7)
DVD 3	11.9 (1.7)	11.9 (5.1)	15.3 (5.1)	13.6 (1.7)	0 (0)	0 (0)	5.1 (3.4)	6.8 (1.7)
DVD 4	13.6 (1.7)	11.9 (5.1)	13.6 (5.1)	11.9 (1.7)	0 (0)	0 (0)	0 (0)	0 (0)

[a] Mean of three repetitions, standard deviation in parenthesis.

Since fungicides and herbicides are commonly used in Canada to protect raspberry crops, the antagonistic actinomycetes were tested for their ability to grow in the presence of various commercial pesticides. The antagonistic actinomycetes differed in regard to their resistance against the pesticides. Three strains (EF-14, EF-22 and EF-43) could grow in the presence of all pesticides tested while the growth of the other strains was inhibited by at least one product. The use of some pesticides, especially captan and ferbam, should thus be avoided during field trials since they interfere with the growth of most antagonistic strains (EF-25, EF-27, EF-34, EF-72, EF-76, EF-97, DVD 3 and DVD 4). The growth of the antagonistic strains was not affected by metalaxyl (Table 3). Therefore, an integrated approach using the biocontrol agents and metalaxyl can be considered.

Antagonistic properties of actinomycetes

Previously, we had shown that the antagonistic strains had both the ability to hydrolyze *Phytophthora* cell walls and to inhibit *Phytophthora* growth at 15°C at pH 7 (Valois *et al.* 1996). We confirmed these results and we demonstrated that seven antagonistic strains (EF-14, EF-22, EF-25, EF-27, EF-34, EF-76 and EF-92) retained these properties at 15°C at pHs 5 and 9. Some strains lost their ability to hydrolyze *Phytophthora* cell walls or to produce antifungal metabolites at pH 5 or 9 (Table 4). Amongst the low temperature-growing strains (EF-22, EF-34, EF-76 and DVD 4), two (EF-34 and EF-76) kept their properties between pH 5 and pH 9 (Tables 2 and 4).

Metabolites produced by the actinomycetes on solid media exhibited various spectra of fungal and bacterial growth inhibition. All actinomycetes inhibited the growth of *P. fragariae* var. *rubi* and of *Pythium ultimum*. However, the growth of the nine other fungal species tested was inhibited only in the presence of three strains (EF-14, EF-72 and EF-76). The actinomycetes had no effect on growth of the Gram-negative bacteria but four strains (DVD 4, EF-14, EF-72 and EF-76) inhibited growth of at least one Gram-positive species (Table 5).

Identification of the antibiotic produced by EF-76

Strain EF-76 appears as a promising biocontrol candidate in regard to both its ability to grow at low temperature and under a wide range of pH and its ability to efficiently reduce raspberry root rot under controlled conditions (Valois *et al.* 1996). Consequently, EF-76 was further characterized. The antibiotic produced by EF-76 was obtained in a pure form from eluting a spot from the silica gel (Rf 0.47). From the eluted material, spectra obtained by HRMS and ^{1}NMR revealed the nature of the antibiotic produced by strain EF-76. The antibiotic produced by EF-76 was a yellow product that has been identified as geldanamycin. This antibiotic has been first isolated from a culture of *Streptomyces hygroscopicus* var. *geldanus* (De Boer *et al.* 1970). Identities between mass spectra, peak matching (HRMS) and ^{1}NMR of the antibiotic produced by strain EF-76 and *S. hygroscopicus* var. *geldanus* strain ATCC 55256 allowed the identification of this antibiotic as geldanamycin (De Boer and Dietz 1976; De Boer *et al.* 1970).

Geldanamycin was previously shown to exert an inhibitory effect on the growth of a wide spectrum of microorganisms including Gram-negative bacteria (De Boer *et al.* 1970). Similarly to the geldanamycin-producing strain ATCC 55256, strain EF-76 inhibited the growth of several fungi and Gram-positive bacteria. However, EF-76 did not seem to affect the growth of Gram-negative bacteria (Table 5). Possibly, the quantity of geldanamycin produced by EF-76 in vitro was not sufficient to detect the inhibitory effect. Accordingly, it has been previously demonstrated that the concentration of geldanamycin necessary to inhibit the growth of Gram-negative bacteria was four times higher than that necessary to inhibit Gram-positive bacteria (De Boer *et al.* 1970).

Table 4. Effect of pH on the ability of antagonistic actinomycetes to inhibit *Phytophthora* growth and to hydrolyze *Phytophthora* cell walls at 15°C

	Inhibition of *Phytophthora* growth			Hydrolysis of *Phytophthora* cell walls		
Strain	pH 5.0	pH 7.0	pH 9.0	pH 5.0	pH 7.0	pH 9.0
EF-14	+[a]	+	+	+	+	+
EF-22	+	+	+	+	+	+
EF-25	+	+	+	+	+	+
EF-27	+	+	+	+	+	-
EF-34	+	+	+	+	+	+
EF-43	-	+	+	+	+	+
EF-72	-	+	n.g.[b]	+	+	+
EF-76	+	+	+	+	+	+
EF-97	+	+	+	+	+	-
DVD 3	-	+	-	-	+	+
DVD 4	-	+	-	+	+	+

[a] + : growth inhibition or hydrolysis was recorded; - : growth inhibition or hydrolysis was not recorded.

[b] n.g. : strain EF-72 did not grow on V8 agar under the culture conditions used.

Table 5. Inhibition of 11 antagonistic actinomycetes against fungi and bacteria

	Growth inhibition caused by										
	EF-14	EF-72	EF-76	EF-22	EF-25	EF-27	EF-34	EF-43	EF-97	DVD 3	DVD 4
Fungal species											
Rhizopus stolonifer	+[a]	+	+	-	-	-	-	-	-	-	-
Phycomyces blackesleeanus	+	+	+	+	-	+	+	+	+	+	+
Phytophthora fragariae var. *rubi*	+	+	+	+	+	+	+	+	+	+	+
Pythium ultimum	+	+	+	+	+	+	+	+	+	+	+
Aspergillus niger	+	+	+	+	-	+	+	-	+	-	-
Penicillium chrysogenum	+	+	+	+	-	+	+	+	-	-	+
Penicillium italicum	+	+	+	+	-	+	+	-	+	-	+
Rhizoctonia solani	+	+	+	-	-	-	-	-	+	-	-
Sclerotinia sp.	+	+	+	+	-	+	-	+	+	-	-
Saccharomyces cerevisiae	+	+	+	-	-	-	-	+	-	-	-
Schizosaccharomyces octosporus	+	+	+	-	-	+	+	-	-	-	-
Bacterial species											
Bacillus cereus	-	+	+	-	-	-	-	-	-	-	+
Staphylococcus aureus	+	+	+	-	-	-	-	-	-	-	+
Streptococcus faecalis	-	+	+	-	-	-	-	-	-	-	-
Acinetobacter calcoaceticus	-	-	-	-	-	-	-	-	-	-	-
Citrobacter freundii	-	-	-	-	-	-	-	-	-	-	-
Escherichia coli	-	-	-	-	-	-	-	-	-	-	-
Klebsiella oxytoca	-	-	-	-	-	-	-	-	-	-	-
Proteus vulgaris	-	-	-	-	-	-	-	-	-	-	-
Pseudomonas fluorescens	-	-	-	-	-	-	-	-	-	-	-

[a] + : growth inhibition was recorded; - : no growth inhibition was recorded.

Taxonomic identification of EF-76

Phenotypic properties of strain EF-76 were similar to those exhibited by *Streptomyces hygroscopicus* var. *geldanus* (De Boer and Dietz 1976). Both strains produced a brownish vegetative mycelium and gray spores borne in spiral chains and at maturity, their spores became black. DNA of *Streptomyces hygroscopicus* var. *geldanus* ATCC 55256 was hybridized to DNA from EF-76, which was suspected to belong to the same subspecies as strain ATCC 55256 and to DNA of *Streptomyces scabies* strain EF-46, which was used as negative control. A high level of relatedness (99.5 ± 8.0%) was found between DNAs of strains ATCC 55256 and EF-76 while strains ATCC 55256 and EF-46 shared a low level of DNA relatedness (19.5 ± 6.5%). Since strains EF-76 and ATCC 55256 exhibited a high level a DNA relatedness, one can conclude that EF-76 belongs to the subspecies *Streptomyces hygroscopicus* var. *geldanus* (Scheifer and Stackebrandt 1981).

Rothrock and Gottlieb (1981, 1984) characterized a geldanamycin-producing strain that reduced infections caused by *P. megasperma* var. *sojae* Drechsler and *Rhizoctonia solani*. This suggests that strain EF-76 could be efficient not only to control diseases caused by oomycetes but also to control other fungal or bacterial plant diseases. Also, this indicates that EF-76 could exert an influence on saprophytic organisms especially fungi and Gram-positive bacteria. The further characterization of new actinomycete strains antagonistic to plant pathogens will hopefully contribute to develop new biocontrol tools.

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