Journal of Applied Microbiology 2005, **99,** 836–843 doi:10.1111/j.1365-2672.2005.02684.x

Activity of some aminoglycoside antibiotics against true fungi, *Phytophthora* and *Pythium* species

H.B. Lee[1]*, Y. Kim[2]*, J.C. Kim[3], G.J. Choi[3], S.-H. Park[4], C.-J. Kim[4] and H.S. Jung[1]

[1]Department of Biological Sciences, College of Natural Sciences, Seoul National University, Seoul, [2]Division of Biotechnology, The Catholic University of Korea, Puchon, [3]Biological Function Research Team, Korea Research Institute of Chemical Technology, Daejeon, and [4]Laboratory of Antioxidants, Korea Research Institute of Bioscience and Biotechnology, Daejeon, Korea

2004/1338: received 22 November 2004, revised 11 April 2005 and accepted 16 April 2005

ABSTRACT

H.B. LEE, Y. KIM, J.C. KIM, G.J. CHOI, S.-H. PARK, C.-J. KIM AND H.S. JUNG. 2005.

Aims: To investigate the *in vitro* antifungal and antioomycete activities of some aminoglycosides against true fungi and *Phytophthora* and *Pythium* species and to evaluate the potential of the antibiotics against *Phytophthora* late blight on plants.

Methods and Results: Antifungal and antioomycete activities of aminoglycoside antibiotics (neomycin, paromomycin, ribostamycin and streptomycin) and a paromomycin-producing strain (*Streptomyces* sp. AMG-P1) against *Phytophthora* and *Pythium* species and 10 common fungi were measured in potato dextrose broth (PDB) and on seedlings in pots. Paromomycin was the most active against *Phytophthora* and *Pythium* species with a minimal inhibitory concentration of 1–10 μg ml^{-1} in PDB, but displayed low to moderate activities towards other common fungi at the same concentration. Paromomycin also showed potent *in vivo* activity against red pepper and tomato late blight diseases with 80 and 99% control value, respectively, at 100 μg ml^{-1}. In addition, culture broth of *Streptomyces* sp. AMG-P1 as a paromomycin producer exhibited high *in vivo* activity against late blight at 500 μg freeze-dried weight per millilitre.

Conclusions: Among tested aminoglycoside antibiotics, paromomycin was the most active against oomycetes both *in vitro* and *in vivo*.

Significance and Impact of the Study: Data from this study show that aminoglycoside antibiotics have *in vitro* and *in vivo* activities against oomycetes, suggesting that *Streptomyces* sp. AMG-P1 may be used as a biocontrol agent against oomycete diseases.

Keywords: aminoglycoside antibiotics, antifungal activity, antioomycete activity, biocontrol agent, oomycetes, *Streptomyces* sp. AMG-P1.

INTRODUCTION

Phytophthora blights and rots caused by oomycete plant pathogens with wide host range, such as *Phytophthora capsici*, *Phytophthora infestans* and *Phytophthora medicaginis*, are significant diseases of many important crops such as red peppers, potatoes, tomatoes and alfalfas, and cause worldwide economic loss. *Pythium* blights, rots and damping-off are also caused by oomycete pathogens such as *Pythium aphanidermatum* and *Pythium ultimum*. *Phytophthora* and *Pythium* species are pseudofungi belonging to the phylum Heterokonta of the kingdom Chromista (Gunderson *et al.* 1987; Chesnick *et al.* 1996; Khulbe 2001) and as many as 95 *Phytophthora* and 100 *Pythium* species have been reported (Erwin and Ribeiro 1996; Kamoun *et al.* 1999).

Generally, soil-borne diseases are difficult to control. Late blight, caused by *Phytophthora*, is difficult to control by

*These authors contributed equally to this study.

Correspondence to: Hyang Burm Lee/Hack Sung Jung, Department of Biological Sciences, College of Natural Sciences, Seoul National University, Seoul 151-747, Korea (e-mails: fungallee@yahoo.com/minervas@snu.ac.kr).

chemical means, because of their high aggressiveness and increasing resistance to metalaxyl, a previously effective systemic compound against oomycetes (Gisi and Cohen 1996; Fry and Goodwin 1997; Gavino *et al.* 2000). Identifying targets for new fungicides has a high priority in current *Ph. infestans* research (Cvitanich and Judelson 2003).

Various fungicides like EMET [5-ethoxy-3-(trichloromethyl)-1,2,4-thiazole], metalaxyl [methyl *N*-(2,6-dimethyl phenyl)-*N*-(methylacetyl)-DL-alaninate] and pyroxyfur [2-chloro-6-(2-furanylmethoxy)-4-(trichloromethyl) pyridine] have been used to control root diseases (Xiao *et al.* 2002). It has been shown that a phenylamide fungicide, metalaxyl, provides major systemic protection against oomycete pathogens (Davidse *et al.* 1981; Cohen and Reuven 1983; Schwinn and Staub 1995; Parra and Ristaino 2001; Xiao *et al.* 2002). When metalaxyl was introduced in 1977, it provided excellent control of oomycete diseases caused by *Phytophthora* and *Pythium* species, *Peronospora tabacina* and *Bremia lactucae* (Morton and Urech 1988; Schwinn and Staub 1995). However, the extensive use of metalaxyl has led to the rapid development and widespread distribution of metalaxyl-resistant strains of *Phytophthora* species throughout the world (Davidse *et al.* 1981; Dawley and O'Sullivan 1981; Deahl *et al.* 1993; Goodwin *et al.* 1996). The reported occurrence of *Phytophthora* isolates resistant to metalaxyls and oxadixyls in field crops now pose new challenges to the management of these significant diseases (Davidse *et al.* 1981; Morton and Urech 1988; Parra and Ristaino 2001). Especially, the occurrence of metalaxyl-resistant *Phytophthora* species in potato fields has resulted in devastating late blight problems due to the failure of disease control in most production areas (Deahl *et al.* 1993; Hwang and Kim 1995; Ristaino and Johnston 1999; Parra and Ristaino 2001). Once fungicide-insensitive strains emerge, they are persistent, and reapplication of fungicides can exacerbate the problem. The need to reduce pesticide application to food crops and the concern for environmental pollution call for alternative methods for disease control. One such method is the use of biocontrol agents (BCAs) in oomycete diseases.

Biocontrol is regarded as an attractive, environmentally sound alternative to chemical pesticides for protection against crop diseases (Chet and Inbar 1994; Cartwright and Spurr 1998; Whipps 2001; Chatterton *et al.* 2004; Folman *et al.* 2004). Numerous surveys of soil bacteria have identified strains of *Streptomyces* and *Bacillus* as potential BCAs (Rothrock and Gottlieb 1981; Crawford *et al.* 1993; Mari *et al.* 1996; Georgakopoulos *et al.* 2002). Many biocontrol organisms employ antibiotic production as a mechanism of disease control (Emmert and Handelsman 1999; Whipps 2001). Jones and Samac (1996) showed that a combination of fungicide and *Streptomyces* resulted in high alfalfa seedling survival with no or slight symptoms of *Phytophthora* root rot. Xiao *et al.* (2002) reported that *Streptomyces* isolates significantly reduced root rot severity in alfalfa and soybean caused by *Ph. medicaginis* and *Phytophthora sojae* respectively.

In the course of our initial screening of antioomycete compounds originating from micro-organisms, some aminoglycoside antibiotics from actinomycetes were found to be selectively active against *Phytophthora* and *Pythium*. To date, most studies of the biological properties of aminoglycoside antibiotics such as amikacin, gentamicin, hygromycin B, kanamycin, neomycin, netilmicin, paromomycin, ribostamycin, streptomycin and tobramycin have focused on activities against bacteria, yeast and protozoan (Chambers *et al.* 1998; Gracenea *et al.* 1998; Lerner *et al.* 1998; Vakulenko and Mobashery 2003; Beers and Berkow 2004). So far, only limited information on the activity of aminoglycosides against oomycetes and the sensitivity of oomycetes to them is available.

The objectives of this study were (i) to investigate *in vitro* antifungal and antioomycete activities of four aminoglycoside antibiotics (neomycin, paromomycin, ribostamycin and streptomycin) against *Phytophthora* and *Pythium* species and further 10 common fungi, and (ii) to evaluate the antioomycete potentials of aminoglycoside antibiotics and of a paromomycin-producing strain on plants against red pepper and tomato late blights caused by *Ph. capsici* and *Ph. infestans* respectively.

MATERIALS AND METHODS

Chemicals and micro-organisms

Neomycin, paromomycin, ribostamycin, streptomycin, and related reagents used in this study were obtained from Sigma Chemical Co. (St Louis, MO, USA). The bacterial, fungal and oomycete strains used in the study are listed in Table 1. The strains were identified and deposited at Microbial Resources Data Base, KRIBB, Daejeon, Korea, and SNU Fungus Culture Collection, Department of Biological Sciences, Seoul National University, Korea, under lyophilized condition. The strains were also maintained under refrigeration (2–3°C) until used for cultivation.

Media used for cultivation and bioassay of the strains were potato dextrose agar (PDA) and potato dextrose broth (PDB) consisting of (l^{-1}) PDB 24 g (Difco, Detroit, MI, USA), agar 15 g (or without agar); malt extract agar, consisting of (l^{-1}) yeast malt extract 15 g, agar 15 g; V8 vegetable juice agar (VA), consisting of (l^{-1}) V8 vegetable juice 200 g, $CaCO_3$ 3 g, agar 15 g (adjusted to pH 7·2 before sterilization) respectively. Bennett's agar and Bennett's broth for preservation and liquid culture of the actinomycete strain contained (l^{-1}) glucose 10 g, yeast extract 1 g, Bactopeptone 2 g, beef extract 1 g, agar 20 g (or without agar), 0·5 g each of thiamine-HCl, riboflavin, niacin, pyridoxine-

Table 1 List of strains used in this study

Species	Strain no.*
Aspergillus flavus	KCTC 6633
Aspergillus fumigatus	KCTC 6145
Absidia coerulea	KCTC 6936
Botrytis cinerea	SFCC-BCI01
Colletotrichum gloeosporioides	KCTC 6169
Fusarium culmorum	KCTC 6157
Mucor ambiguous	KCTC 6142
Penicillium oxalicum	KCTC 16912
Penicillium expansum	KCTC 6434
Phytophthora capsici	KRICT-pc101
Phytophthora infestans	ATCC 36609
Phytophthora infestans	KRICT-pi101
Phytophthora megasperma	ATCC 28004
Phytophthora nicotianae	ATCC 15409
Phytophthora palmivora	ATCC 26483
Phytophthora porri	ATCC 52729
Pythium aphanidermatum	ATCC 26081
Pythium dissotocum	ATCC 60519
Pythium graminicola	ATCC 28458
Pythium ultimum	SFCC-PUL01
Rhizopus stolonifer	SFCC-RST01
Streptomyces sp.†	SFCC-AMG-P1

*KCTC, Korea Collection for Type Culture, Daejeon, Korea; SFCC, Seoul National University Fungus Culture Collection, Department of Biological Sciences, College of Natural Sciences, Seoul National University, Korea; KRICT, Korea Research Institute of Chemical Technology, Daejeon, Korea; ATCC, American Type Culture Collection, Manassas, VA, USA.
†Paromomycin-producing actinomycete strain.

HCl, inositol, Ca-pantothenate and aminobenzoic acid, biotin 0·25 mg, cycloheximide 50 mg and nalidixic acid 10 mg (adjusted to pH 7·2 before sterilization).

Screening of aminoglycoside producer and culture

As a preliminary study to identify a potential BCA, the paromomycin-producing actinomycete strain, *Streptomyces* sp. AMG-P1, was screened. The analysis of aminoglycoside antibiotics was performed using liquid chromatography and mass spectrometry according to previously described methods (Umezawa and Kondo 1975; Lu *et al.* 1997; Olson *et al.* 1997). After chemical verification of the antibiotic, the quantity of paromomycin produced was indirectly assayed by microbial paper disc agar diffusion method (Parenti *et al.* 1978), using *Bacillus subtilis* ATCC 1914 as a test organism. For the preparation of culture broth, *Streptomyces* sp. AMG-P1 was first inoculated in 200 ml Erlenmeyer flasks containing 60 ml of the screening medium consisting of (l^{-1}) soluble starch 10 g, glucose 20 g, soybean meal 25 g, beef extract 1 g, yeast extract 4 g, NaCl 2 g, K_2HPO_4 0·25 g and $CaCO_3$ 2 g (adjusted to pH 7·2 before sterilization) and cultured on a rotary shaker (150 rev min^{-1}) at 28°C for 5 days. In addition, for scale-up fermentation, the strain was precultured in a 500-ml Erlenmeyer flask for 3 days, and a stock solution (3%) was used to re-inoculate a 5-l jar fermentor (Korea Fermentor Co., Daejeon, Korea).

Assays of *in vitro* antifungal and antioomycete activities

The activities of aminoglycoside antibiotics against four *Phytophthora* species (*Ph. infestans*, *Phytophthora nicotianae*, *Phytophthora megasperma* and *Phytophthora palmivora*) and two *Pythium* species (*Pythium dissotocum* and *Py. ultimum*) and 10 common fungal species were measured in PDB and PDA augmented with different concentrations (0·1–100 $\mu g\ ml^{-1}$) of antibiotics. PDA was used as the basal medium for all test species, except for *Ph. infestans* (ATCC 36609) and *Ph. capsici* for which VA medium was used. For viability test, submerged growth of the isolates was tested in PDB. Agar discs (5 mm in diameter) from actively growing mycelia were placed into 5-ml test tubes of broth containing antibiotics at different concentrations. After 3 days, discs were removed, rinsed thoroughly with sterile distilled water, and placed back onto fresh PDA. The plates were incubated at 25°C for further 5 days, after which mycelial regrowth was observed, and the minimal inhibitory concentrations (MICs) were evaluated in duplicate.

Assays of *in vivo* antioomycete activity

The four aminoglycoside antibiotics were tested for antioomycete activity on plants against red pepper late blight caused by *Ph. capsici* and tomato late blight caused by *Ph. infestans*. In order to investigate further *in vivo* antifungal activities, paromomycin, which showed the most antioomycete activities against oomycete diseases, was also tested for antifungal activity on plants against the following diseases: rice blast caused by *Pyricularia oryzae*, rice sheath blight caused by *Rhizoctonia solani* and wheat leaf rust caused by *Puccinia recondita*. Red pepper (*Capsicum annuum*), tomato (*Lycopersicon esculentum*), rice (*Oryza sativa*) and wheat (*Triticum aestivum*) plants were grown in vinyl pots (4·5 cm in diameter) in a glasshouse at 25 ± 5°C for 1–4 weeks. Stock solutions of antibiotics were prepared in sterile distilled water containing 0·2% (v/v) Tween-20, and the concentrations adjusted to 100–500 $\mu g\ ml^{-1}$. In addition, *in vivo* antioomycete activity of liquid culture of the strain was investigated against *Ph. infestans* on tomato plants. After incubation at 28°C for 5 days in Bennett's broth medium, liquid culture of *Streptomyces* sp. AMG-P1 was filtered through Whatman No. 2 filter paper, centrifuged at 10 000 *g* for 20 min, and the supernatant was freeze-dried.

The freeze-dried culture extract was dissolved in 0·2% (v/v) Tween-20 solution and then serially diluted to different concentrations [500 (equivalent to 1/50 dilution of culture broth), 250, 125, 60, 30 μg fdw (freeze-dried weight) ml^{-1}]. The aminoglycoside antibiotics and liquid culture extract of *Streptomyces* sp. AMG-P1 as a paromomycin producer were sprayed on plant seedlings in pots with a hand sprayer and allowed to stand for 23 h for safer anchoring of antibiotics on leaves. Control plants were treated with Tween-20 solution alone. The treated seedlings were inoculated with spores or mycelial suspensions of the test organisms. The experiment was conducted twice, and the mean of the six estimates made for each treatment was converted into a percentage disease control. The percentage of disease control was determined using the following equation: % control = $100[(A - B)/A]$, where A = the area of infection (%) on leaves or sheaths sprayed with Tween-20 solution alone and B = the area of infection (%) on treated leaves or sheaths. Treatment with broth-only inoculation was used as a negative control. Values were expressed as a percentage control (±SD). The detailed procedures for the *in vivo* antifungal and antioomycete assays have been described previously (Kim *et al.* 2001, 2004).

RESULTS

In vitro antifungal and antioomycete activities of aminoglycoside antibiotics

Inhibitory effects of four aminoglycoside antibiotics (neomycin, paromomycin, ribostamycin and streptomycin) on mycelial growth of four *Phytophthora* (*Ph. infestans*, *Ph. nicotianae*, *Ph. megasperma* and *Ph. palmivora*) and two *Pythium* (*Py. dissotocum* and *Py. ultimum*) species in PDB varied with tested antibiotics and micro-organisms (Tables 2 and 3). The four *Phytophthora* species were very sensitive to aminoglycoside antibiotics at MICs of less than 10 μg ml^{-1}.

Table 3 *In vitro* antimicrobial activity of paromomycin against various fungal and oomycete isolates*

Species	Paromomycin (μg ml^{-1})				
	10	20	40	80	100
Aspergillus flavus KCTC 6633	+†	+	+	+	+
Aspergillus fumigatus KCTC 6145	+	+	+	+	+
Absidia coerulea KCTC 6936	+	+	+	+	+
Botrytis cinerea SFCC-BCI01	+	+	–	–	–
Colletotrichum gloeosporioides KCTC 6169	+	+	+	+	ND
Fusarium culmorum KCTC 6157	+	+	+	–	–
Mucor ambiguous KCTC 6142	+	+	+	+	ND
Penicillium oxalicum KCTC 16912	+	+	+	+	ND
Penicillium expansum KCTC 6434	+	+	+	+	ND
Phytophthora capsici KRICT-pc101	–	–	–	ND	ND
Phytophthora infestans ATCC 36609	–	–	–	ND	ND
Phytophthora infestans KRICT-pi101	–	–	–	ND	ND
Phytophthora megasperma ATCC 28004	–	–	–	ND	ND
Phytophthora nicotianae ATCC 15409	–	–	–	ND	ND
Phytophthora palmivora ATCC 26483	–	–	–	ND	ND
Phytophthora porri ATCC 52729	+	+	+	+	–
Pythium aphanidermatum ATCC 26081	–	–	–	ND	ND
Pythium dissotocum ATCC 60519	–	–	–	ND	ND
Pythium graminicola ATCC 28458	–	–	–	ND	ND
Pythium ultimum SFCC-PUL01	–	–	–	ND	ND
Rhizopus stolonifer SFCC-RST01	+	+	+	+	ND

*Each isolate was grown in PDB at 25°C for 7–10 days in the dark.
†The antioomycete activity was determined by a viability test (+, mycelial regrowth observed; –, no mycelial regrowth observed; ND, not determined).

Of the aminoglycoside antibiotics, paromomycin was the most active against *Phytophthora* and *Pythium* species. Ribostamycin showed lower activity than paromomycin. Interestingly, *Phytophthora porri* was less sensitive to paromomycin than the other *Phytophthora* and *Pythium* species at an MIC of 100 μg ml^{-1} (Table 3). The *in vitro* activities of aminoglycosides against *Ph. infestans* on VA

Table 2 *In vitro* antioomycete activities of four aminoglycoside antibiotics against four *Phytophthora* and two *Pythium* species

Species*	Neomycin†				Paromomycin				Ribostamycin				Streptomycin			
	0	0·1	1	10	0	0·1	1	10	0	0·1	1	10	0	0·1	1	10
Ph. infestans	+‡	+	+	–	+	+	–	–	+	+	–	–	+	+	+	–
Ph. megasperma	+	+	+	–	+	+	–	–	+	+	+	–	+	+	+	–
Ph. nicotianae	+	+	+	+	+	+	–	–	+	+	+	+	+	+	+	–
Ph. palmivora	+	+	+	+	+	+	–	–	+	+	+	–	+	+	+	–
Py. aphanidermatum	+	+	+	+	+	+	–	–	+	+	+	–	+	+	+	ND
Py. ultimum	+	+	+	+	+	+	–	–	+	+	+	–	+	+	+	ND

*Each isolate was grown in PDB at 25°C for 7–10 days in the dark.
†The concentration of each antibiotic ranged from 0 to 10 μg ml^{-1}.
‡The antioomycete activity was determined by a viability test (+, mycelial regrowth observed; –, no mycelial regrowth observed; ND, not determined).

solid medium (Fig. 1) were approximately five times lower than in liquid medium (PDB) (data partly shown). Most of the aminoglycoside antibiotics were active against *Phytophthora* and *Pythium* species at 10 μg ml^{-1} and moderately active against *Botrytis cinerea* and *Fusarium culmorum* at 20 and 40 μg ml^{-1}, respectively, but little activity was shown against 10 eufungi species of *Absidia*, *Aspergillus*, *Botrytis*, *Colletotrichum*, *Fusarium*, *Mucor*, *Penicillium* and *Rhizopus*, even at 80 μg ml^{-1} (Table 3). The results showed that the oomycetes *Phytophthora* and *Pythium* were sensitive to aminoglycoside antibiotics, and further that 2-deoxystreptamine structure-containing antibiotics might be potent inhibitors of both *Phytophthora* and *Pythium*.

In vivo antifungal and antioomycete activities

Antioomycete activity of aminoglycoside antibiotics against red pepper and tomato late blights caused by *Ph. capsici* and *Ph. infestans*, respectively, varied with tested antibiotics and pathogens (Tables 4 and 5, Fig. 2). Paromomycin showed potent *in vivo* activities against red pepper and tomato late blight diseases with 80 and 99% control at 100 μg ml^{-1} respectively. Paromomycin was slightly less active against *Ph. capsici* than *Ph. infestans*. Tomato late blight was completely inhibited by paromomycin with 99% control at 100 μg ml^{-1}, and moderately controlled by streptomycin and neomycin with 76–80% control at 500 μg ml^{-1} (Table 5). Ribostamycin was apparently less active against *Ph. capsici* and *Ph. infestans* than the other antibiotics, displaying (at 500 μg ml^{-1}) 37–40% control of red pepper late blight achieved by paromomycin and streptomycin (Tables 4 and 5). In contrast, when the antibiotics were applied against three other plant diseases (rice blast, rice sheath blight and leaf rust), paromomycin did not inhibit the development of these diseases (Table 6).

The actinomycete strain, *Streptomyces* sp. AMG-P1, was found to produce paromomycin at the rate of approx. 25 mg l^{-1}. When the freeze-dried culture extract was applied against tomato late blight, the actinomycete strain displayed a strong inhibitory effect at 125 μg fdw ml^{-1}

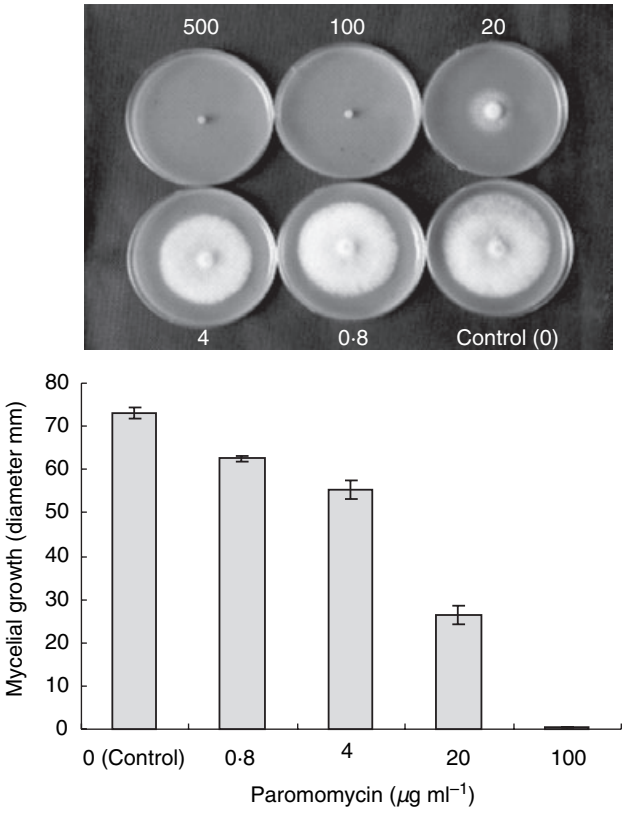

Fig. 1 *In vitro* antioomycete activities of paromomycin against *Phytophthora infestans* on a solid medium. Upper panel: Growth of *Ph. infestans* 10 days after inoculation onto V8 juice agar containing 0·8–500 μg ml^{-1} paromomycin. Lower panel: Average diameter (±SD) of mycelia after 10 days of growth on different concentrations of paromomycin. Each diameter represents a mean value of two replicate plates

Table 4 Antioomycete activity of four aminoglycoside antibiotics against red pepper late blight caused by *Phytophthora capsici* (KRICT-pc101)*

Concentration (μg ml^{-1})	% Disease control ± SD†			
	Neomycin	Paromomycin	Ribostamycin	Streptomycin
100	7 ± 11	80 ± 9·5	7 ± 9·2	47 ± 11
500	40 ± 6·1	93 ± 4·5	37 ± 5·2	80 ± 5·6

*The red pepper seedlings were inoculated with zoospore suspensions of *Ph. capsici* 23 h after treatment with antibiotics.
†% Disease control = 100[(A − B)/A], where A = the area of infection (%) on leaves or sheaths sprayed with Tween-20 solution (5 ± 10^4 zoospores ml^{-1} inoculum concentration) alone and B = the area of infection (%) on treated leaves or sheaths of two replicates.

Table 5 Antioomycete activity of four aminoglycoside antibiotics against tomato late blight caused by *Phytophthora infestans* (KRICT-pi101)*

Concentration (μg ml^{-1})	% Disease control ± SD†			
	Neomycin	Paromomycin	Ribostamycin	Streptomycin
100	20 ± 11	99 ± 0·9	28 ± 6·0	48 ± 7·5
500	80 ± 7·5	99 ± 0·5	28 ± 5·7	76 ± 6·4

*The tomato seedlings were inoculated with zoospore suspensions of *Ph. infestans* 23 h after treatment with antibiotics.
†% Control = 100[(A − B)/A], where A = the area of infection (%) on leaves or sheaths sprayed with Tween-20 solution (5 ± 10^4 zoospores ml^{-1} inoculum concentration) alone and B = the area of infection (%) on treated leaves or sheaths of two replicates.

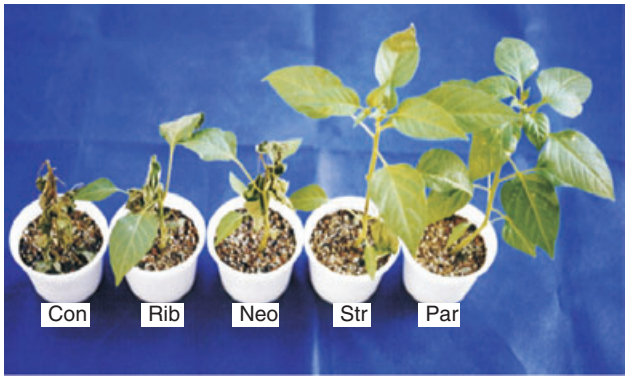

Fig. 2 Antioomycete activities of four aminoglycoside antibiotics against red pepper late blight caused by *Phytophthora capsici* KRICT-pc101 (Con, control; Neo, neomycin; Par, paromomycin; Rib, ribostamycin; Str, streptomycin). Red pepper seedlings were inoculated with zoospore suspensions of *Ph. capsici* 23 h after treatment with antibiotics (500 μg ml^{-1}). Disease severity was evaluated 4 days after inoculation

Table 6 Inhibitory effect of paromomycin (250 μg ml^{-1}) against various plant pathogens*

Plant pathogen	Host	% Disease control ± SD†
Pyricularia oryzae KRICT-po201	Rice	0
Rhizoctonia solani KRICT-rs102	Rice	5 ± 11
Phytophthora infestans KRICT-pi101	Tomato	99 ± 0·5
Phytophthora capsici KRICT-pc101	Red pepper	90 ± 2·7
Puccinia recondita KRICT-pr101	Wheat	16 ± 10

*The plant seedlings were inoculated with spores or mycelial suspensions of the test organisms 23 h after treatment with antibiotics.
†% Control = 100[(A − B)/A], where A = the area of infection (%) on leaves or sheaths sprayed with Tween-20 solution alone and B = the area of infection (%) on treated leaves or sheaths of two replicates.

(equivalent to approx. 1/200 dilution of culture broth), suggesting its potential as a BCA (Fig. 3).

DISCUSSION

Paromomycin was first isolated from an actinomycete species, *Streptomyces rimosus* ssp. *paromomycinus* by Coffey *et al.* (1959). Some of its biological properties have been reported (Vakulenko and Mobashery 2003). Aminoglycoside antibiotics are characterized by the presence of two or more amino sugars linked to an aminocyclitol ring through glycosidic bonds. They bind to bacterial 30S ribosomal subunits, causing misreading by tRNA, and leaving bacteria unable to synthesize proteins essential for growth. These antibiotics have generally proved to be inactive (or at best

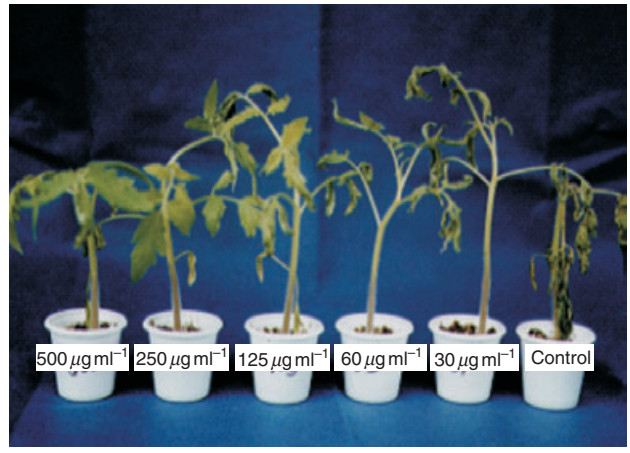

Fig. 3 Antioomycete activities of the culture broth of *Streptomyces* sp. AMG-P1 against tomato late blight caused by *Phytophthora infestans*. Tomato seedlings were inoculated with zoospore suspensions of *Ph. infestans* 23 h after treatment with freeze-dried extract [500 (equivalent to 1/50 dilution of culture broth), 250, 125, 60, 30, 0 (control) μg ml^{-1}] of liquid culture of *Streptomyces* sp. AMG-P1. Disease severity was evaluated 3 days after inoculation

weakly active) against eukaryotic ribosomes. However, certain antibiotics like hygromycin B are active against both prokaryotic and eukaryotic cells (Gonzalez *et al.* 1978; Wilhelm *et al.* 1978; Recht *et al.* 1999; Lynch and Puglisi 2001). Tests of the relative potency of the aminoglycosides have ranked gentamicin > G418 > paromomycin > neomycin > hygromycin B > streptomycin in their ability to cause misreading (Eustice and Wilhelm 1984).

While aminoglycosides are known to interfere with translation in prokaryotic ribosomes, their mode of action against eukaryotes is not well-defined. Some eukaryotic ciliate protozoa (*Tetrahymena thermophila*) and intestinal parasites (*Giardia lamblia*) are sensitive to aminoglycosides with a 6′ hydroxyl group, as paromomycin has (Palmer and Wilhelm 1978; Edlind 1989).

It seems possible that oomycetes represent a rather special group in phylogeny that have prokaryotic sensitivity to the action of aminoglycoside antibiotics. Furthermore, sensitivity may be different within species or genera of oomycetes. Our results showed that the antibiotics were selectively active against *Phytophthora* blight pathogens of red pepper and tomato both *in vitro* and on plants. For example, *Ph. porri* was more resistant to paromomycin than other *Phytophthora* and *Pythium* species (Table 3). Mutants of *Phytophthora parasitica* resistant to streptomycin have been reported (Ann and Ko 1988), and the streptomycin sensitivity of *Pythium splendens* varied in isolates with different colony types (Guo and Ko 1995).

Recently, there has been a rising interest in the development of friendly BCA for disease control leading to a

sustainable agriculture. Various antibiotics active against oomycetes have been isolated from bacteria and actinomycetes (Silo-Suh *et al.* 1994; Hwang *et al.* 2001). Actinomycetes have been recognized as sources for several secondary metabolites and antibiotics, and a number of *Streptomyces* species have been studied as potential BCAs against fungal pathogens (Filonow and Lockwood 1985; Sabaratnam and Traquair 2002; Xiao *et al.* 2002). Our previous work also showed that paromomycin was antibacterial against *Staphylococcus aureus* KCTC 1916 and *Escherichia coli* KCTC 1924, and that neomycin was highly active against some species of potato scab pathogens, *Streptomyces scabiei* (=*Streptomyces scabies*) and *Streptomyces acidiscabies* at 10–50 ppm (Lee *et al.* 2004). The combined results indicate that paromomycin and other similar aminoglycosides are candidates for the control of plant diseases caused by oomycetes and by bacteria such as potato scab pathogens (data partly shown). McGovern *et al.* (2004) reported that Ridomil (metalaxyl) showed 30–50% control of mycelial growth of *Ph. capsici* at 6 μg ml^{-1}. Our work with a paromomycin-producing strain, *Streptomyces* sp. AMG-P1, displayed a strong *in vivo* inhibitory effect on tomato late blight caused by *Ph. infestans* at >125 μg fdw ml^{-1}. Our results suggest that actinomycetous BCAs which produce a high yield of paromomycin or related aminoglycoside antibiotics can also be used for the development of new BCAs against oomycete diseases.

ACKNOWLEDGEMENTS

We are grateful to Dr Bradner of Macquarie University, Australia, for reviewing the manuscript. This research was supported by grants from the Basic Research Program (R01-2002-000-00304-0) of the Korea Science & Engineering Foundation, the 21C Frontier Microbial Genomics and Application Center Program (MG005-0101-1-0) of the Ministry of Science & Technology, the KRIBB-JEJU joint research program funded by Jeju Provincial Government, and by the Brain Korea 21 Research Fellowship from the Ministry of Education & Human Resources Development.

REFERENCES

Ann, P.J. and Ko, W.H. (1988) Hormonal heterothallism in *Phytophthora parasitica*: a novel mode of sexual reproduction? *J Gen Microbiol* **134**, 2895–2992.

Beers, M.H. and Berkow, R. (2004) Aminoglycosides. In *The Merck Manual of Diagnosis and Therapy*, Section 13. Infectious Disorders. Chapter 153. Antibacterial drugs, pp. 1085–1340. Whitehouse Station, NJ: Merck & Co., Inc.

Cartwright, D.K. and Spurr, H.W. Jr (1998) Biological control of *Phytophthora parasitica* var. *nicotianae* on tobacco seedlings with non-pathogenic binucleate *Rhizoctonia* fungi. *Soil Biol Biochem* **30**, 1879–1884.

Chambers, H.F., Hadley, W.K. and Jawetz, E. (1998) Aminoglycosides & spectinomycin. In *Basic and Clinical Pharmacology* ed. Katzung, B.G., pp. 784–792. Stamford, CT: Appleton & Lange.

Chatterton, S., Sutton, J.C. and Boland, G.J. (2004) Timing *Pseudomonas chlororaphis* applications to control *Pythium aphanidermatum*, *Pythium dissotocum*, and root rot in hydroponic peppers. *Biol Control* **30**, 360–373.

Chesnick, J.M., Tuxbury, K., Coleman, A., Burger, G. and Lang, B.F. (1996) Utility of the mitochondrial nad4L gene for algal and protistan phylogenetic analysis. *J Phycol* **32**, 452–456.

Chet, I. and Inbar, J. (1994) Biological control of fungal pathogens. *Appl Biochem Biotechnol* **48**, 37–43.

Coffey, G.L., Anderson, L.E., Fisher, M.W., Galbraith, M.M., Hillegas, A.B., Kohberger, D.L., Thompson, P.E., Weston, P.E. *et al.* (1959) Biological studies of paromomycin. *Antibiot Chemother* **9**, 730–738.

Cohen, Y. and Reuven, M. (1983) Occurrence of metalaxyl-resistant isolates of *Phytophthora infestans* in potato fields in Ireland. *Phytopathology* **73**, 925–927.

Crawford, D.L., Linch, J.M., Whipps, J.M. and Ousley, M.A. (1993) Isolation and characterization of actinomycete antagonists of a fungal root pathogen. *Appl Environ Microbiol* **59**, 3899–3905.

Cvitanich, C. and Judelson, H.S. (2003) Stable transformation of the oomycete, *Phytophthora infestans*, using microprojectile bombardment. *Curr Genet* **42**, 228–235.

Davidse, L.C., Looijen, D., Turkensteen, L.J. and Vander Wal, D. (1981) Occurrence of metalaxyl-resistant strains of *Phytophthora infestans* in Dutch potato fields. *Neth J Plant Pathol* **87**, 65–68.

Dawley, L.J. and O'Sullivan, E. (1981) Metalaxyl-resistance strains of *Phytophthora infestans* (Mont.) de Bary in Ireland. *Potato Res* **24**, 417–421.

Deahl, K.L., DeMuth, S.P., Pelter, G. and Ormrod, D. (1993) First report of resistance of *Phytophthora infestans* to metalaxyl in Eastern Washington and Southwestern British Columbia. *Plant Dis* **77**, 429.

Edlind, T.D. (1989) Susceptibility of *Giardia lamblia* to aminoglycoside protein synthesis inhibitors: correlation with rRNA structure. *Antimicrob Agents Chemother* **33**, 484–488.

Emmert, E.A.B. and Handelsman, J. (1999) Biocontrol of plant disease: a (Gram-) positive perspective. *FEMS Microbiol Lett* **171**, 1–9.

Erwin, D.C. and Ribeiro, O.K. (1996) *Phytophthora* Diseases Worldwide. St Paul, MN: American Phytopathological Society Press.

Eustice, D.C. and Wilhelm, J.M. (1984) Fidelity of the eukaryotic codon-anticodon interaction: interference by aminoglycoside antibiotics. *Biochemistry* **23**, 1462–1467.

Filonow, A.B. and Lockwood, J.L. (1985) Evaluation of several actinomycetes and the fungus *Hyphochytrium catenoides* as biocontrol agents for *Phytophthora* root rot of soybean. *Plant Dis* **69**, 1033–1036.

Folman, L.B., De Klein, M.J.E.M., Postma, J. and van Veen, J.A. (2004) Production of antifungal compounds by *Lysobacter enzymogenes* isolate 3·1T8 under different conditions in relation to its efficacy as a biocontrol agent of *Pythium aphanidermatum* in cucumber. *Biol Control* **31**, 145–154.

Fry, W.E. and Goodwin, S.B. (1997) Resurgence of the Irish potato famine fungus. *Bioscience* **47**, 363–371.

Gavino, P.D., Smart, C.D., Sandrock, R.W., Miller, J.S., Hamm, P.B., Lee, T.Y., Davis, R.M. and Fry, W.E. (2000) Implications of sexual reproduction for *Phytophthora infestans* in the United States: generation of an aggressive lineage. *Plant Dis* **84**, 731–735.

Georgakopoulos, D.G., Fiddaman, P., Leifert, C. and Malathrakis, N.E. (2002) Biological control of cucumber and sugar beet damping-off caused by *Pythium ultimum* with bacterial and fungal antagonists. *J Appl Microbiol* **92**, 1078–1086.

Gisi, U. and Cohen, Y. (1996) Resistance to phenylamide fungicides: a case study with *Phytophthora infestans* involving mating type and race structure. *Ann Rev Phytopathol* **34**, 549–572.

Gonzalez, A., Jimenez, A., Vazquez, D., Davies, J.E. and Schindler, D. (1978) Studies on the mode of action of hygromycin B, an inhibitor of translocation in eukaryotes. *Biochim Biophys Acta* **521**, 459–469.

Goodwin, S.B., Sujkowski, L.S. and Fry, W.E. (1996) Widespread distribution and probable origin of resistance to metalaxyl in clonal genotypes of *Phytophthora infestans* in the United States and Western Canada. *Phytopathology* **86**, 793–800.

Gracenea, M., Gomez, M.S., Fernandez, J. and Feliu, C. (1998) Secnidazole vs. paromomycin: comparative antiprotozoan treatment in captive primates. *J Med Primatol* **27**, 38–43.

Gunderson, J.H., Elwood, H., Ingold, A., Kindle, K. and Sogin, M.L. (1987) Phylogenetic relationships between chlorophytes, chrysophytes, and oomycetes. *Proc Natl Acad Sci U S A* **84**, 5823–5827.

Guo, L.Y. and Ko, W.H. (1995) Continuing variation in successive asexual generation of *Pythium splendens* following sexual reproduction. *Mycol Res* **99**, 1339–1344.

Hwang, B.K. and Kim, C.H. (1995) *Phytophthora* blight of pepper and its control in Korea. *Plant Dis* **79**, 221–227.

Hwang, B.K., Lim, S.W., Kim, B.S., Lee, J.Y. and Moon, S.S. (2001) Isolation and *in vivo* and *in vitro* antifungal activity of phenylacetic acid and sodium phenylacetate from *Streptomyces humidus*. *Appl Environ Microbiol* **67**, 3739–3745.

Jones, C.R. and Samac, D.A. (1996) Biological control of fungi causing alfalfa seedling damping-off with a disease-suppressive strain of *Streptomyces*. *Biol Control* **7**, 196–204.

Kamoun, S., Huitema, E. and Vleeshouwers, V.G.A.A. (1999) Resistance to oomycetes: a general role for the hypersensitive response? *Trends Plant Sci* **4**, 1360–1385.

Khulbe, R.D. (2001) *A Manual of Aquatic Fungi: Chytridiomycetes & Oomycetes*. New Delhi, India: Daya.

Kim, J.-C., Choi, G.J., Park, J.-H., Kim, H.T. and Cho, K.Y. (2001) Activity against plant pathogenic fungi of phomalactone isolated from *Nigrospora sphaerica*. *Pest Manag Sci* **57**, 554–559.

Kim, J.-C., Choi, G.J., Lee, S.-W., Kim, J.-S., Chung, K.Y. and Cho, K.Y. (2004) Screening extracts of *Achyranthes japonica* and *Rumex crispus* for activity against various plant pathogenic fungi and control of powdery mildew. *Pest Manag Sci* **60**, 803–808.

Lee, H.B., Cho, J.W., Park, D.J., Li, C.T., Ko, Y.H., Song, J.H., Koh, J.S., Kim, B.J. *et al.* (2004) *In vivo* screening for biocontrol agents (BCAs) against *Streptomyces scabiei* causing potato common scab. *Plant Pathol J* **20**, 110–114.

Lerner, S.A., Gaynes, R.P. and Nördstrom-Lerner, L. (1998) Aminoglycosides. In *Infectious Diseases* ed. Gorbach, S.L., Bartlett, J.G. and Blacklow, N.R., pp. 775–790. Philadelphia, PA: W.B. Saunders Co.

Lu, J., Cwik, M. and Kanyok, T. (1997) Determination of paromomycin in human plasma and urine by reversed-phase high-performance liquid chromatography using 2,4-dinitrofluorobenzene derivatization. *J Chromatogr B Biomed Sci Appl* **695**, 329–335.

Lynch, S.R. and Puglisi, J.D. (2001) Structural origins of aminoglycoside specificity for prokaryotic ribosomes. *J Mol Biol* **306**, 1037–1058.

Mari, M., Guizzardi, M. and Pratella, G.C. (1996) Biological control of gray mold in pears by antagonistic bacteria. *Biol Control* **7**, 30–37.

McGovern, R.J., Roberts, P.D., Kucharek, T.A. and Gilreath, P.R. (2004) *Phytophthora capsici*: New Problems from an Old Enemy. Gainesville, FL: IFAS, University of Florida.

Morton, H.V. and Urech, P.A. (1988) History of the development of resistance to phenylamide fungicides. In *Fungicide Resistance in North America* ed. Delp, C.J., pp. 59–60. St Paul, MN: American Phytopathological Society Press.

Olson, L.L., Pick, J., Ellis, W.Y. and Lim, P. (1997) A chemical assessment and HPLC assay validation of bulk paromomycin sulfate. *J Pharmacol Biomed Anal* **15**, 783–793.

Palmer, E. and Wilhelm, J.M. (1978) Mistranslation in a eukaryotic organism. *Cell* **13**, 329–334.

Parenti, F., Beretta, G., Berti, M. and Arioli, V. (1978) Teicomycins, new antibiotics from *Actinoplanes teicomyceticus* nov. sp. I. Description of the producer strain, fermentation studies and biological properties. *J Antibiot (Tokyo)* **31**, 276–283.

Parra, G. and Ristaino, J.B. (2001) Resistance to mefenoxam and metalaxyl among field isolates of *Phytophthora capsici* causing *Phytophthora* blight of bell pepper. *Plant Dis* **85**, 1069–1075.

Recht, M.I., Douthwaite, S. and Puglisi, J.D. (1999) Basis for prokaryotic specificity of action of aminoglycoside antibiotics. *The EMBO J* **18**, 3133–3138.

Ristaino, J.B. and Johnston, S.A. (1999) Ecologically based approaches to management of *Phytophthora* blight on bell pepper. *Plant Dis* **83**, 1080–1089.

Rothrock, C.S. and Gottlieb, D. (1981) Importance of antibiotic production in antagonism of *Streptomyces* species to two soil-borne plant pathogens. *J Antibiot (Tokyo)* **34**, 830–835.

Sabaratnam, S. and Traquair, J.A. (2002) Formulation of a *Streptomyces* biocontrol agent for the suppression of *Rhizoctonia* damping-off in tomato transplants. *Biol Control* **23**, 245–253.

Schwinn, F. and Staub, T. (1995) Oomycete fungicides. In *Modern Selective Fungicides, Properties, Applications, Mechanisms of Action* ed. Lyr, H. pp. 323–346. New York: Gustav Fischer Verlag.

Silo-Suh, L.A., Lethbridge, B.J., Raffel, S.J., He, H., Clardy, J. and Handelsman, J. (1994) Biological activities of two fungistatic antibiotics produced by *Bacillus cereus* UW85. *Appl Environ Microbiol* **60**, 2023–2030.

Umezawa, H. and Kondo, S. (1975) Ion-exchange chromatography of aminoglycoside antibiotics. *Methods Enzymol* **43**, 263–278.

Vakulenko, S.B. and Mobashery, S. (2003) Versatility of aminoglycosides and prospects for their future. *Clin Microbiol Rev* **16**, 430–450.

Whipps, J.M. (2001) Microbial interactions and biocontrol in the rhizosphere. *J Exp Bot* **52**, 487–511.

Wilhelm, J.M., Pettitt, S.E. and Jessop, J.J. (1978) Aminoglycoside antibiotics and eukaryotic protein synthesis: structure-function relationships in the stimulation of misreading with a wheat embryo system. *Biochemistry* **17**, 481–489.

Xiao, K., Kinkel, L.L. and Samac, D.A. (2002) Biological control of *Phytophthora* root rots on alfalfa and soybean with *Streptomyces*. *Biol Control* **23**, 285–295.