Paromomycin Derived from *Streptomyces* sp. AG-P 1441 Induces Resistance against Two Major Pathogens of Chili Pepper

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This is the first report that paromomycin, an antibiotic derived from *Streptomyces* sp. AG-P 1441 (AG-P 1441), controlled *Phytophthora* blight and soft rot diseases caused by *Phytophthora capsici* and *Pectobacterium carotovorum*, respectively, in chili pepper (*Capsicum annum* L.). Chili pepper plants treated with paromomycin by foliar spray or soil drenching 7 days prior to inoculation with *P. capsici* zoospores showed significant (*p* < 0.05) reduction in disease severity (%) when compared with untreated control plants. The disease severity of *Phytophthora* blight was recorded as 8% and 50% for foliar spray and soil drench, respectively, at 1.0 ppm of paromomycin, compared with untreated control, where disease severity was 83% and 100% by foliar spray and soil drench, respectively. A greater reduction of soft rot lesion areas per leaf disk was observed in treated plants using paromomycin (1.0 μg/ml) by infiltration or soil drench in comparison with untreated control plants. Paromomycin treatment did not negatively affect the growth of chili pepper. Furthermore, the treatment slightly promoted growth; this growth was supported by increased chlorophyll content in paromomycin-treated chili pepper plants. Additionally, paromomycin likely induced resistance as confirmed by the expression of pathogenesis-related (PR) genes: PR-1, β-1,3-glucanase, chitinase, PR-4, peroxidase, and PR-10, which enhanced plant defense against *P. capsici* in chili pepper. This finding indicates that AG-P 1441 plays a role in pathogen resistance upon the activation of defense genes, by secretion of the plant resistance elicitor, paromomycin.

Keywords: Chili pepper, induced resistance, paromomycin, *Phytophthora* blight, soft rot, *Streptomyces* sp.

Introduction

*Phytophthora capsici* is a soil-borne pathogen that causes *Phytophthora* blight in nearly all cultivars of chili pepper (*Capsicum annum* L.). *Phytophthora* blight is one of the most economically destructive diseases in chili pepper production, causing annual losses in regions growing this species worldwide [9, 12]. In general, it is difficult to manage diseases caused by *Phytophthora* spp. because of their aggressiveness and increasing resistance to chemical compounds [8]. *Pectobacterium carotovorum* subsp. *carotovorum*, formerly known as *Erwinia carotovora* subsp. *carotovorum*, mainly affects crops in subtropical and temperate regions [13]. It is the causal agent of bacterial soft rot, a severe disease of many economically important food crops such as potato, tomato, chili pepper, eggplant, and Chinese cabbage [5, 7]. Various chemical fungicides have been used to control root diseases, and the extensive use of chemicals has led to the development of resistant strains of pathogens [24, 34]. The need to reduce pesticide application on food crops and
the concern for environmental pollution require alternative methods for disease control, such as biocontrol agents. The aminoglycoside paromomycin (Fig. 1) was first isolated from the actinobacterium Streptomyces rimosus subsp. paromomycinus and some of its biological properties have been documented [29]. The aminoglycosides, commonly known as a group of bactericidal antibiotics derived from Streptomyces spp., are the most promising biocontrol agents of plant diseases. They are effective owing to secondary metabolite production, and they are also ubiquitous in the rhizosphere. Their ability to exude a variety of fungal cell-wall- and insect exoskeleton-degrading enzymes has been well documented [20, 33]. The antibiotics produced by Streptomyces spp. may protect the host plants against phytopathogens [28]. In support of this finding, Xiao et al. [34] reported that Streptomyces isolates substantially reduced the root rot severity in alfalfa and soybean caused by Phytophthora spp. The tissue-cultured seedlings of rhododendron treated with non-antagonistic Streptomyces spp. showed minor wilting due to Pestalotiopsis sydowiaviana. The seedlings accumulated anthocyanin(s) and activated defense responses through the phenylpropanoid pathway rather than through antibiosis [25]. To date, most of the studies concerning the biological properties of aminoglycoside antibiotics have focused on the activities against bacteria, yeast, and protozoa [3, 11].

Induced resistance in plants can be developed by the application of a variety of biotic and abiotic agents [32]. Chemical activators, including acibenzolar-S-methyl (ASM) and β-aminobutyric acid, are widely reported to induce resistance against a broad spectrum of pathogens in many plant species [32]. The resistance may be systemic-acquired resistance (SAR) or induced systemic resistance (ISR) based on the signaling pathway. For example, ASM was reported to induce SAR against rust in faba beans [26], and resistance to P. infestans in squash [14]. Some aminoglycoside antibiotics from Actinomycetes spp. were found to be selectively active against oomycetes such as Phytophthora and Pythium species, and paromomycin exhibited the highest activity both in vitro and in vivo [18]. The ability of the aminoglycoside antibiotics to induce systemic resistance against pathogens in chili pepper has not yet been reported. This research gap encouraged us to investigate the efficacy of Streptomyces sp. AG-P 1441-derived paromomycin for the disease control of P. capsici and P. carotovorum in chili pepper through induced resistance.

Materials and Methods

Aminoglycoside Paromomycin from Streptomyces sp. AG-P 1441

The aminoglycoside paromomycin, derived from Streptomyces sp. AG-P 1441 (AG-P 1441), was kindly provided by Dr. Chang-Jin Kim of the Korean Research Institute of Bioscience and Biotechnology (KRIBB, Daejeon, South Korea). Isolation and purification of paromomycin from AG-P 1441 were performed at KRIBB. The purified paromomycin was stored at room temperature. Paromomycin was dissolved in 1.0 N NaOH before dilution with distilled water at various concentrations (μg/ml).

Isolation and Purification of Paromomycin Compound from Streptomyces sp. AMG-P1

Purification of paromomycin from Streptomyces sp. AMG-P1 is shown in Fig. S1. The extract of Streptomyces sp. was obtained from TSA with 80% methanol using a rotary vacuum evaporator. The residue was subjected to column chromatography (Amberite IRC50, Sigma-Aldrich Co., USA) to obtain 0.5 N NH₄OH and 1 N NH₄OH gradient fractions. The fraction from 1 N NH₄OH was subjected to Amberite CG50 column chromatography (IRC50) to obtain three different fractions on the basis of gradient, of which the active fraction was subjected to Sephadex LH20 column chromatography, eluted with methanol and 1 N NH₄OH at 1:1 ratio to obtain different fractions. On the basis of antibiosis assay, LH41 was found to be an active fraction, and thus the obtained fraction was subjected to TLC for purification. The 1H NMR spectra of authentic (Sigma Co., USA) and isolated samples of paromomycin were recorded using a Bruker 500 MHz NMR instrument in D₂O (Fig. S2).

Preparation of Spore Suspensions of P. capsici and Bacterial Pathogen P. carotovorum Inocula

The fungal pathogen P. capsici and the bacterial pathogen P. carotovorum were obtained from the Korean Agriculture Cultural Collection, National Academy of Agricultural Sciences, Suwon, South Korea. P. capsici inoculum was prepared as described by Ploetz et al. [21]. In brief, a 5-mm diameter mycelial plug of the isolate was transferred to a V8 agar plate. After 1 week incubation at 25°C, V8 agar plugs with mycelia were placed onto a Petri dish
containing V8 broth and allowed to grow for another week under the same conditions. The V8 broth was then drained, and each plate was washed twice with sterile distilled water (SDW). SDW was added to cover the mycelia on each plate; afterwards, the plates were placed under a wide-spectrum light at room temperature for 24–48 h to induce sporangial development. The sporangia were chilled at 4°C for 45 min to induce the release of zoospores. The zoospore suspensions were adjusted to a final concentration of $1 \times 10^8$ zoospores/ml using a hemocytometer before the challenge inoculation. For the preparation of *P. carotovorum* inoculum, the bacterial cell suspensions were prepared from 24-h-old culture at 28°C. Ten milliliters of SDW was poured on a tryptic soy agar culture plate and scraped with a sterile plastic loop and adjusted to a final concentration of $1 \times 10^6$ CFU/ml (OD$_{600}$ = 0.8) before application.

**Evaluation of Paromomycin for Induced Resistance against *P. capsici* in Chili Pepper under Greenhouse and Field Conditions**

Chili pepper cv. Hanbyul seedlings at the first-branch stage were used in this study. The seeds were sown in a plastic tray (35 cm × 35 cm × 15 cm) containing a soilless potting mix (TKS2, Flora Gard Ltd., Germany). Seedlings at the two-leaf stage were transplanted to plastic pots (5 cm × 15 cm × 10 cm) containing the same soil mix. Complex fertilizer was applied to the plants once a week after transplanting. Chili pepper plants were raised in a growth room under light conditions of 16 h/day at 27°C ± 2°C. The plants were transplanted under field conditions in naturally contaminated soil. Plants were treated with 30 ml of paromomycin at different concentrations (0.1, 1.0, 10, 100, and 1,000 μg/ml) under greenhouse conditions. Each experiment used a randomized complete block design with six replications per treatment. Dimethomorph, a common systemic fungicide at 1.0 μg/ml, and SDW were used as positive and negative controls, respectively. A week later, the third or fourth bottom leaves (untreated leaves) from the treated plants were harvested for disease assessment, and leaf disks (8 mm in diameter) were made using a sterile cork borer. The disks (12) were placed in sterile 24-well culture plates containing the *P. carotovorum* pathogen suspensions ($1 \times 10^6$ CFU/ml). The percent disease lesion area per leaf disk was recorded 24 h after incubation of the plates at 28°C by visual observation. The experiment was performed twice, and each treatment (concentration) consisted of 12 replicates (plants). Twelve discs from 12 plants were used per treatment.

**Effect of Paromomycin on Plant Growth Promotion and Chlorophyll Content**

The effect of paromomycin treatment on growth promotion of chili pepper plants was evaluated. The purified compound was tested for its plant growth-promoting activity by the soil drench method at various concentrations (1.0, 10, and 100 μg/ml). Three-week-old seedlings were soil drenched with 30 ml of paromomycin per pot. BTH (0.1 mM) and SDW were used as positive and negative controls, respectively. The height of the plants was recorded 40 days after the treatments during two experiments. The estimation of chlorophyll content was determined by the method described by Grøn and Ort [10]. The chlorophyll content was determined from the leaves of paromomycin-treated chili pepper plants under greenhouse conditions after the extraction of the pigment with 80% acetone. Fresh chili pepper leaves (1 g) obtained from the field conditions were ground in a small volume of acetone solution. The extract was diluted to a final volume of 4 cm$^3$. The absorbance was measured at 600 nm by spectrophotometer. Twelve plants were used in each treatment and the experiment was repeated twice.

**Molecular Analysis for the Expression of Pathogenesis-Related (PR) Defense Genes**

To analyze the expression of PR defense genes, the third leaves from treated plants were sampled at 12 and 24 h after the challenge inoculation with *P. capsici*, and the leaf tissues were frozen in liquid nitrogen until use. Leaf samples collected from BTH- and SDW-treated plants were used as positive and negative controls, respectively. Defense-related PR-1, β-1,3-glucanase, chitinase, PR-4, peroxidase, and PR-10 genes were assessed for expression using the reverse transcriptase (RT)-PCR method. Total RNA was isolated using the easy-spin II Total RNA Extraction Kit (iNTRON Biotechnology, South Korea). RT-PCR was performed according to Kishimoto et al. [16] with Ex Taq polymerase (Takara Biomedicals, J. Microbiol. Biotechnol.)
Paromomycin Induces Resistance in Chili Pepper

Table 1. Sequences of gene-specific primers used for RT-PCR analysis.

<table>
<thead>
<tr>
<th>Gene family</th>
<th>Specific class</th>
<th>Accession number*</th>
<th>5' Primer</th>
<th>3' Primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>PR-1</td>
<td>PR protein 1</td>
<td>AF053343</td>
<td>5'-TGCAACACTCTGGTGCCCT-3'</td>
<td>5'-AAGGCCGGTGTTGCTCGAG-3'</td>
</tr>
<tr>
<td>GLU</td>
<td>β-1,3-Glucanase</td>
<td>AF227953</td>
<td>5'-GCTGCCACCCCTCTAATGCAA-3'</td>
<td>5'-TGTCACGCGGATTACCAAGCA-3'</td>
</tr>
<tr>
<td>Chi</td>
<td>Class II chitinase</td>
<td>AF091235</td>
<td>5'-CATTTACTGACAAGTCCACAGC-3'</td>
<td>5'-GCAGAAGCTTCAAATTTGCC-3'</td>
</tr>
<tr>
<td>PR-4</td>
<td>PR protein 4</td>
<td>AF244122</td>
<td>5'-GGCGCAGAGTGCTACGAAC-3'</td>
<td>5'-AGTGTCCAATTTGTTAACACG-3'</td>
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<tr>
<td>PO 1</td>
<td>Peroxidase</td>
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<td>5'-CTATGGTATTAGGCCAAGGG-3'</td>
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<tr>
<td>PR-10</td>
<td>PR protein 10</td>
<td>AF244121</td>
<td>5'-CGTCCGCCCTATGGTGACCCAGCT-3'</td>
<td>5'-GAGCCGGTGTTGCTCGAG-3'</td>
</tr>
<tr>
<td>18s rRNA</td>
<td>18s rRNA</td>
<td>EF564281</td>
<td>5'-CGTCCGCCCTATGGTGACCCAGCT-3'</td>
<td>5'-GTCATCCAAAGCTTCTCGAG-3'</td>
</tr>
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</table>


Table 2. Suppression of Phytophthora blight disease caused by Phytophthora capsici in paromomycin-treated red-pepper plants by foliar spray and soil drench under greenhouse conditions.

<table>
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<tr>
<th>Treatment</th>
<th>% of disease severitya</th>
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<tbody>
<tr>
<td></td>
<td>Foliar spray</td>
</tr>
<tr>
<td>Control (water)</td>
<td>83.3b</td>
</tr>
<tr>
<td>Dimethomorph 1.0 μg/ml</td>
<td>0.00</td>
</tr>
<tr>
<td>Paromomycin 0.1 μg/ml</td>
<td>33.3b</td>
</tr>
<tr>
<td>Paromomycin 1.0 μg/ml</td>
<td>8.3b</td>
</tr>
<tr>
<td>Paromomycin 10.0 μg/ml</td>
<td>38.3b</td>
</tr>
<tr>
<td>Paromomycin 100.0 μg/ml</td>
<td>33.3b</td>
</tr>
<tr>
<td>Paromomycin 1000.0 μg/ml</td>
<td>33.3b</td>
</tr>
<tr>
<td>LSD (p = 0.05)</td>
<td>26.4</td>
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Three-week-old plants treated with various concentrations of paromomycin or sterile distilled water (negative control) or 0.1 ppm dimethomorph (positive control) by foliar spray and soil drench were challenge inoculated with zoospore suspensions of P. capsici after 7 days of treatment by soil drench. Percent disease severity was recorded 7 days after inoculation. The experiment was repeated at least two times, with 12 replicates per treatment. The values presented in the column followed by the same letter(s) are not significantly different from each other according to least significant difference (p < 0.05).

Results

Effect of Paromomycin Treatment on Chili Pepper Infection by P. capsici

The paromomycin treatments reduced Phytophthora leaf blight infection by P. capsici substantially, under greenhouse conditions, when compared with the water-treated control upon pathogen challenge infection. Among the different modes of applications, foliar spraying was found to be more effective than soil drenching. The greatest reduction of Phytophthora blight infection in chili pepper was obtained using foliar spray with paromomycin at 1.0 μg/ml, which resulted in a disease severity of 8% in comparison with water-treated control (83%), whereas the paromomycin treatment at higher dosages (100 and 1,000 μg/ml) resulted in a disease severity of 33% (Table 2 and Fig. 2). The foliar spray treatment with dimethomorph (1.0 μg/ml) suppressed the P. capsici infection completely. There were still 33% and 100% disease severities for soil drenching in the positive (dimethomorph) and negative (SDW) controls, respectively (Table 2). The highest reduction of P. capsici infection for soil drenching was found to be 50% at 1.0 μg/ml of paromomycin when compared with other concentrations, where there was a minimum level of disease suppression. However, the disease severity (83%) was at the same level at concentrations of 10 and 1,000 μg/ml. Under field conditions, the disease severity was significantly (p < 0.05) reduced to 25% compared with the water-treated control (66%) for the soil drench method, whereas the chemical control (BTH) at 0.1 mM also showed a greater disease

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reduction (33%) when compared with the water-treated control, but was less effective than the paromomycin treatment (Fig. 3). Thus, our results demonstrate that the paromomycin treatment is beneficial in protecting chili peppers from *Phytophthora* leaf blight.

**Effect of Paromomycin on Disease Suppression of* P. carotovorum* in Chili Pepper**

Treatments with paromomycin and BTH (positive control) by leaf infiltration significantly (*p* < 0.05) reduced the soft rot incidence of chili pepper plants treated with paromomycin when compared with the water-treated control in a 24-well-plate assay (Fig. 4). There was a greater reduction of lesion area per leaf disk (3.3%) at 1.0 μg/ml of paromomycin when compared with the water-treated control (71.7%) and the chemical inducer BTH (16.6%). However, paromomycin at higher concentrations did not show any significant effect in the reduction of lesion area percentage per leaf disk. Paromomycin treatment by soil drench had a greater effect on the reduction of the lesion area percentage of soft rot per leaf disk (8%) when compared with the water-treated control (75%), whereas BTH (0.1 mM) treatment also caused a considerable reduction in lesion area per leaf disk (16%), and had a similar effect as the *P. capsici* reduction mentioned above (Fig. 5).

**Effect of Paromomycin on Plant Growth and Chlorophyll Content**

No considerable effect was noted for the growth of chili pepper plants treated with paromomycin when compared with the water-treated control (Fig. 6). However, the SAR inducer, BTH at 0.1 mM, reduced the plant height. Increased chlorophyll content was observed in paromomycin-treated chili peppers when compared with the BTH- and water-treated controls grown under field conditions for two experiments (Fig. 7).

**Effect of Paromomycin on Defense-Related Gene Expression in Chili Pepper Leaves through RT-PCR**

To ascertain the changes in SAR-related genes by the...
paromomycin derived from AG-P 1441, the expression of defense genes in chili pepper plants challenged with *P. capsici* was measured using RT-PCR analysis (Fig. 8). Compared with the treatments without the pathogen, treatments after pathogen challenge generally enhanced the expression of the PR genes studied. After 12 h of pathogen challenge, the BTH treatment increased the expression of chitinase, peroxidase, and PR-10 genes compared with the water-treated control, whereas paromomycin treatment also enhanced the PR-1, β-1,3-glucanase, and PR4 genes. In some cases for paromomycin, the defense gene expression began upon pathogen challenge; for BTH, gene expression was mostly enhanced 24 h following pathogen challenge.

**Discussion**

This study investigated the potential for paromomycin, an aminoglycoside derived from *Streptomyces* sp. AG-P 1441, in controlling *P. capsici* and *P. carotovorum* infections in chili pepper through induced resistance. The results demonstrated that the AG-P 1441-derived paromomycin tested in this study had effectively suppressed the development of both pathogens on the leaves of chili pepper plants. In support of our study, aminoglycoside
antibiotic compounds have been reported to reduce diseases caused by oomycetes, including *Phytophthora infestans* [18, 34]. Commercial aminoglycoside antibiotics such as neomycins, ribostamycin, streptomycin, and paromomycin have been tested against *P. infestans*. It has been reported that paromomycin at 10 µg/ml was the most active against the pathogen under in vitro conditions to reduce mycelial growth [18]. However, in our study, we demonstrated that the antibiotic paromomycin could reduce *P. capsici* and *P. carotovorum* disease symptoms in chili pepper plants under greenhouse conditions. Paromomycin at 125 µg/ml was found to be effective in controlling tomato late blight;
however, at concentrations above 250 μg/ml, there were minor levels of phytotoxicity [18]. In our study, no phytotoxicity was found in paromomycin-treated chili pepper plants. Disease severity was reduced using the lowest concentration of paromomycin, and increased at high concentrations. Foliar spray of paromomycin at 1.0 μg/ml was the most effective in reducing disease incidence. Furthermore, paromomycin is reported to inhibit in vitro growth of oomycete plant pathogens of the genera Phytophthora and Pythium, and to exhibit potent in vivo activity against chili pepper and tomato late blight [17]. Moreover, it was reported that the minimal inhibitory concentration of paromomycin against P. capsici was 500–1,000 μg/ml. On the other hand, our study identified the application of paromomycin at lower concentration (1.0 μg/ml) is an ideal dosage for disease suppression through induced resistance than higher concentrations, suggesting that a dosage-dependency manner is followed.

This study also clearly demonstrated that paromomycin treatment significantly controlled P. carotovorum, and there was increased disease suppression in paromomycin-treated chili pepper plants upon pathogen challenge. Paromomycin treatment of the plants resulted in greater suppression of soft rot disease than BTH (positive control) and SDW (negative control). The antibiosis activity of paromomycin against a major group of plant pathogens has been documented in earlier reports [18, 34]. In addition, paromomycin had no effect on plant growth. Instead, the increased chlorophyll content suggests a change in plant metabolism.

Molecular evidence of PR gene expression demonstrated that paromomycin soil drenching induced resistance in chili pepper leaves. Specifically, paromomycin at 1.0 μg/ml enhanced the expression of the PR genes, PR-1, β-1,3-glucanase, peroxidase, and PR-10 at 12 h after pathogen inoculation, which resulted in induced resistance against P. capsici infection in chili pepper plants. Previously, Lee and Hwang [19] demonstrated that the induction of defense-related genes such as PR-1 was essential for establishing local and systemic acquired resistance in chili pepper plants. Expression of the PR-1 gene is known to be triggered through a SA-dependent signaling pathway and to be related to SAR [4]. It has been well documented that disease-suppressing rhizobacteria enhance a plant’s defense capacity by inducing defense genes against invading pathogens [1, 15]. Conrath et al. [6] described the priming mechanism in plant-microbe interactions in vitro that can help plants overcome biotic or abiotic stresses. Priming in beneficial plant-microbe associations has been studied through the interaction of plants with ISR. In most cases, plant-microbe associations induce the defense capacity of the plant against a broad spectrum of pathogens [22]. The elevated levels of PR gene expression were related to increased activities of β-1,3-glucanase, chitinase, and peroxidase, which are key enzymes in plant defense. These defensive responses of pepper plants may be more rapid and substantially induced compared with water-treated controls because of the induction of PR gene expression in leaves infected by P. capsici, as observed in Arabidopsis inoculated with Pseudomonas [31]. Previous studies [2, 30] have reported that the signaling pathways can be activated by various inducers, such as callose deposition, synthesis of defense enzymes, phytoalexins, accumulation of PR proteins, volatile organic compounds, and antimicrobial compounds.

In conclusion, our results suggested that paromomycin treatment enhanced systemic resistance against P. capsici by activating defense genes. The aminoglycoside paromomycin derived from Streptomyces sp. AG-P 1441 may play a role in protecting chili pepper plants against invading pathogens through induced resistance, and thus might serve as an alternative approach to chemical fungicides.

**Acknowledgments**

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**References**

9. Goldberg NP. 1995. Chile Pepper Diseases, Circular 549. New Mexico Agricultural Experiment Station, College of Agriculture and Home Economics.