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Reduction of *Rhizoctonia* damping-off in Chinese cabbage seedlings by fungal protein

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ABSTRACT

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Total proteins were purified from 12 *Alternaria* species and tested for their ability to promote growth and disease reduction in Chinese cabbage. Our results indicated that proteins purified from some but not all *Alternaria* spp. were capable of promoting plant growth, stimulating root elongation and the formation of lateral roots, and reducing damping-off incidence caused by *Rhizoctonia solani*. Proteins purified from a cherry isolate designated APR01 exhibited most promising results in suppressing damping-off by 30%. APR01 isolate was identified as *A. tenuissima* based on cultural and morphological characteristics and sequence analysis of rDNA internal transcribed spacer (ITS). *In vitro* assays revealed no fungistatic activity of APR01 protein against *R. solani*. APR01 proteins with M.W. greater than 10 kDa displayed ability to reduce *R. solani* damping-off, whereas proteins M.W. less than 10 kDa had no effects. APR01 proteins heated at temperatures higher than 50°C completely or nearly abolished the ability to reduce disease, indicating that the major components contributing to disease reduction could be enzymes or heat-labile proteins. Nonetheless, our studies have demonstrated the possibility of fungal proteins in the reduction of a soil-borne disease, likely via the induction of plant defense mechanisms.

Keywords: *Alternaria*, elicitor, defense, disease resistance, fungal pathogens

INTRODUCTION

Many pathogens including fungi, bacteria, viruses, phytoplasmas, and nematodes can attack plants in natural conditions. Natural selection and adaptation during evolution also render plants develop a wide range of defense mechanisms, both constitutive and inducible, in order to ward off pathogens [12,19,22,31,37]. Since Ross and colleagues [39] studied induced resistance in tobacco in 1961, the subject concerning plant defense mechanisms has been widely pursued [2,3,9,12,15,17,27,31,41,43].

Studies have found that plants are capable of modifying and intensifying physical structures as well as changing physiological and biochemical reactions in response to pathogen attacks [19-22, 37]. For example: plants, in response to pathogen invasion, would produce callose and papilla structures to deter pathogen penetration and colonization [10,19,20,22]. In addition, plants can turn on or up-regulate the genes encoding pathogenesis-related proteins (PR-proteins), which have diverse functions in plant-microbial interactions [32,45]. PR-2 and PR-3 have been demonstrated to have 1,3-glucanase and chitinase activities, respectively and both play a pivotal role in defense by degrading fungal cell walls and thereby, interfering pathogen colonization [42]. Constitutive expression of a PR-3 gene in tobacco and rape renders plants resistant to damping-off diseases caused by *Rhizoctonia solani* [8]. PR-1 has also been shown to inhibit zoospore germination of *Phytophthora infestans* with unknown mechanisms [33]. The structure of PR-5 protein is similar to thaumatin-like family. PR-5 has been shown to inhibit fungal growth and conidial germination due to its ability to disrupt membrane permeability resulting in imbalance of osmotic status in fungal cells [1].

Of all elicitors studied, proteinaceous elicitors are the largest group and their biological activities have been documented in a wide range of plants [1-4,11,13,23,33,34,38,42,47,50,51,52]. One of the best known examples is the harpin purified from the pear fire blight bacterium *Erwinia amylovora* [47]. Harpin is an acidic protein of M.W. 44 kDa that can trigger HR in tobacco. Harpin has been found widely in many Gram negative bacteria and has been shown to induce systemic acquired resistance (SAR) in *Arabidopsis thaliana* [11,32]. Harpin was commercialized under the brand name "Messenger." Harpin interacts with plant receptors, subsequently triggers the onset of signaling transduction pathways associated with defense reactions, and reduce plant diseases. "Messenger" has been demonstrated in field conditions to reduce various diseases in many economically important crops including wheat, rice, cotton, citrus, tomato, and cucumber [21].

Elicitin (Ppn 46e) was first purified from cultural filtrates of

P. parasitica var. *nicotianae* Race 0 and was detected in tobacco after pathogen infection [14]. Elicitin is an acidic protein (pI=4.67) mainly composed of aspartic acid, asparagine, alanine, glycine, glutamic acid, glutamine and serine. Tobacco calli treated with elicitin accumulated high amounts of sesquiterpenoid and capsidiol. An oligopeptide (13 amino acids) derived from a glycoprotein of *P. megasperma* f. sp. *glycinea* also exhibits elicitor activity [34]. This elicitor was found to specifically interact with a cellular receptor of celery, trigger influx/efflux of ions through membranes, induce oxidative burst, up-regulate the expression of defense-associated genes, and promote the accumulation of phytoalexins.

Rhizoctonia solani can survive as mycelium and sclerotia in the soil and in plants. This pathogen can cause damping-off, stem rot and head rot on vegetables, resulting in serious yield loss. Studies conducted in China have demonstrated that proteins purified from several filamentous fungi were capable of promoting seed germination and growth of various plants [28, 29,46,49-52]. In the present study, we purified proteins from 12 different *Alternaria* spp. and have found that proteins from some but not all *Alternaria* spp. were capable of promoting plant growth, stimulating root elongation and the formation of lateral roots, and reducing damping-off incidence caused by *R. solani* on Chinese cabbage. One of the *Alternaria* isolates designated APR01 was identified as *A. tenuissima* whose proteins were investigated in detail. The purified proteins (molecular weight greater than 10 kDa) had no fungistatic activities and were demonstrated to reduce damping-off incidence by 30%. This study provides insight into the possible role of fungal proteins in triggering defense reactions in plants.

MATERIALS AND METHODS

Biological materials and their maintenance

Plant pathogenic fungi used in this study and their origins are listed in Table 1. Fungal cultures were single-spore isolated from surface-sterilized plant materials using a semi-selective medium [20 g glucose, 3 g $(NH_4)_2SO_4$, 3 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 15 g peptone, 2% agar, 20 ppm neomycin, 50 ppm chloramphicol, 50 ppm ridomial, and 50 ppm benomyl] and were subsequently streaked three times for single colonies. Antibiotics and fungicide were added into medium after autoclaving. Fungi were routinely maintained on potato dextrose agar (PDA) at 25 to 28°C. For protein extraction, fungi were cultured in 500 ml potato dextrose broth (PDB) for 7 to 9 days. Chinese cabbage (*Brassica campestris* L. chinensis group cv. Speedy) was used throughout the study. Seeds were purchased from Known-You Seed Co. (Kaohsiung, Taiwan). Plants were grown in a peat moss (BVB no. 4, Bas Van Buuren, Maasland, New Zealand) and maintained in a greenhouse.

Extraction of fungal proteins

Fungal proteins were purified according to protocols reported by Bridge and colleagues [6,7] with modifications. Unless otherwise stated, all steps were performed at 4°C. Fungal hyphae (3 g) grown in PDB were harvested by passing through two layers of cheesecloth, frozen at -20°C and blended in a stainless cup using a MM400 model of Oscillating mill (Retsch GmbH, Germany) for 30s or ground in liquid nitrogen and sea sand using a mortar and a pestle. Fungal mycelium was suspended completely in a 10-ml extraction buffer containing 0.7 M sucrose, 0.5 M Tris-base, 50 mM EDTA-$Na_2 \cdot H_2O$, 0.1 M KCl, 30 ml mM HCl, 2% β-mercapto ethanol (2-ME), 2.8% polyvinyl pyrrolidone, and 2 mM phenylmethylsulfonyl fluoride (PMSF). After centrifugation at 10,000 x g for 20 min, supernatant was filtered through a 0.22 μm membrane (Millipore, Billerica, MA), mixed with equal volume of water-saturated phenol for 5 min, and centrifuged again at 20,000 x g for 30 min. Supernatant was mixed with equal volume of extraction buffer and centrifuged again for 30 min. The last step was repeated once. Supernatant was mixed with 3x (v/v) cold (-20°C) 0.1 M ammonia acetate/ methanol (HPLC grade), incubated at -20°C for 16 h, and centrifuged (20,000 x g) at 4°C for 20 min. Protein pellets were dissolved in 1.8 ml of 0.1 M ammonia acetate/ methanol and 0.7 M 2-ME, centrifuged at 10,000 x g for 5 min, and the step was repeated once. Supernatants collected from last two steps were combined, mixed with 1 ml acetone and 4 μl of 0.7 M 2-ME, incubated at -20°C for 1h, and centrifuged at 10,000 x g for 5 min. Proteins were air-dried for 1 min, dissolved in a solution containing 9.5 M urea, 65 mM CHAPS (Bio-Rad, Hercules, CA), 39 mM Tris-base, 4% Triton x-100, 4% NP-40, and filtered through a 0.22 μm membrane. After adding 5 μl of PMSF, crude protein extracts were sonicated in a Branson 3210 Ultrasonic (Danbury, CT).

Fungal proteins were mixed with 3x cold 0.1 M ammonia acetate/ methanol (v/v) for 16 h and precipitated by centrifugation at 20,000 x g for 20 min. Proteins were re-dissolved in PBS lysis buffer, each liter containing 8 g NaCl, 0.2 g KCl, 1.44 g $Na_2H_2PO_4$, 0.24 g KH_2PO_4, 0.1% NaN_3, and 0.1% glycerol (pH 7.4), dialysed against PBS buffer for 24 h, and quantified based on the Bradford dye-binding method [8]. Proteins were separated by sodium dodecyl sulfate-polyacrylamide (SDS-PAGE) gel electrophoresis and stained with Coomassie brilliant blue. Proteins were placed into an Amicon Ultra-15 centrifugal filter device with cut-off at 10 KDa (Millipore) and separated into two parts by centrifugation at 4,000 x g for 2 h.

Assays for antagonistic activity against *R. solani*

Assays for antagonistic activity against *R. solani* were carried out on water agar spread with fungal protein extracts (2 μg/ml). An 8.4 mm agar plug bearing *R. solani* mycelium was placed on a sterilized cover glass overlaid on the center of plate and fungal growth was monitored daily. Control plates were spread with sterile water or PBS lysis buffer (100x) only.

Assays for seed germination and plant growth

Seed germination was assessed in two different ways. Protein extracts dissolved in PBS lysis buffer were prepared in a series of 2-fold dilution. Chinese cabbage seeds (30 for each treatment) were soaked in protein extract solution for 12 h and planted in potting mix in a 3-inch plastic box. Germination of the seeds was recorded 5 days after planting. Seeds treated with water or buffer only were used as controls. Alternatively, Chinese cabbage seeds were soaked in distilled sterile water for 24 h and subsequently in fungal protein extracts (2 μg/ml) for an additional 6 h. The treated seeds were

transferred onto a glass Petri dish (9 x1 cm) with 4.5 ml sterile water and incubated in a growth chamber set on a daily 12/12-h light/dark cycle at 24°C for 3 days. The length of roots and the number of lateral roots were determined. Each treatment contained four replicates and each replicate had 10 seeds.

The effect of protein extracts on plant growth was assessed on plants grown in peat moss in a 128-well tray (dia. 2.5 cm). Plants bearing the true second leaves (5 to 9 days) were sprayed to run off weekly with protein crude extracts (2 μg/ml) and plant height and fresh weight were measured 30 days after treatment. Each treatment contained four replicates and each replicate had 16 seedlings.

Preparation of *Rhizoctonia solani* inoculum

Rhizoctonia solani AG-4 inoculum was prepared in two different ways. The pathogen was grown on sterilized shred potato (100 g) for 7 days, mixed with sterilized peat moss (1:10, w/v) and 400 ml water in a 500-ml flask, and incubated at room temperature (25 ~28°C) with stirring every 2 to 3 days for 2 weeks. The resultant culture was mixed further with unsterilized peat moss (3:100, w/w) to become 3% infested soils. Each batch of *R. solani* inoculum was performed in a 10-fold serial dilution and tested for infectivity by growing Chinese seedlings in the infested soils with three to four replicates. Alternatively, *R. solani* inoculum was prepared by culturing on 3% water agar for 3 days. Agar medium carrying fungal mycelium was cut into small plugs (5 mm^2) and mixed with peat moss (150 ml per plate) to make infested substrate. Chinese seedlings (10 to 24 seedlings for each replicate) sprayed to run-off with or without protein extracts were grown in *R. solani*-infested peat moss. Disease incidence was assessed 7 to 14 days post inoculation (dpi). In some experiments, Chinese cabbage (28 days) was sprayed with protein extracts and inoculated with *R. solani* by placing a 3.75 mm agar plugs carrying fungal mycelium grown on PDA (four replicates with two plugs for each replicate) on the third and fourth true leaves or on basal areas of stems. Inoculated plants were bagged for 16 to 24 h and moved to greenhouses after bag removal.

Molecular identification of *Alternaria* spp.

Alternaria spp. isolates were cultured on clarified V8 juice agar (175 ml V8 juice, 3 g $CaCO_3$, 20 g agar per liter) for conidial formation. Morphological identification was performed based on the distinct characteristics of conidia and colony morphologies formed on V8 as described by Rotem [40]. *Alternaria* sp. isolate APR01 was further characterized by sequencing the internal transcribed spacer (ITS) DNA fragment consisted of the entire 5.8S and partial 18S ribosomal DNA (rDNA). ITS region was amplified by PCR with primers ITS1-F (5'-cttggtcatttagaggaagtac-3') and ITS4 (5'-tcctccgcttattgatatgc-3') [16,48]. For DNA isolation, fungal mycelium was boiled in a microwave for 4.5 min, suspended in 30 l 10 mM Tris-EDTA buffer (pH 7.0), and centrifuged at 13,800 x g at 4°C for 1 min. PCR was set up in a 25 μl reaction and carried out in a TC-96-G thermal cycler (Infinigen Biotech, CA, USA). The cycling profile for amplification started with an initial cycle of 95°C for 2 min, immediately followed by 35 cycles of 95°C for 30 s, 55°C for 45 s, 72°C for 10 min and completed by incubating at 72°C for 4 min. The amplified ITS fragment was sequenced at Tri-I Biotech (Taipei, Taiwan). Similarity searching was performed at the National Center for Biotechnology Information (NCBI) using the BLASTX program.

Statistical analysis

Data of damping-off incidence on Chinese cabbage were analyzed by one-way analysis of variance (ANOVA) using SAS/STAT software. Significance of treatments was determined based on Duncan's multiple range test (P=0.05).

RESULTS

Identification of *Alternaria* spp.

Ten fungal isolates were collected from diseased plants (Table 1) and were identified as *Alternaria* spp. according to morphological characteristics of conidia and colonies grown on V8 juice agar. The APR01 isolate collected from cherry plant was identified further as *A. tenuissima* (Nees & T. Nees:Fr.) based on ITS rDNA sequence. A 550-bp DNA fragment was amplified with primers ITS1-F and ITS4 from genomic DNA of APR01 and sequenced. The amplified DNA displayed strong similarity (99%) to ITS sequence of *A. tenuissima* EGS34-015 (accession no. AY751455.1) and *A. alternata* (accession no. FJ809940.1). APR01 produced dark greenish colony with 2 to 3 concentric rings and brownish conidia on V8 agar. Conidia (12.5-187.5 x 5-15 μm) often arranged in chains were multicellular and ovoid, obclavate, obpyriform, or oblong in shape with 1 to 7 septa (Fig. 1). Conidia produced by APR01 often had long beaks and thus, were distinguishable from short beak conidia produced by *A. alternata*.

TABLE 1. Fungal pathogens and their origins used in this study

Fungal pathogen	Isolates	Original host	Geographic location
Alternaria brassicicola	ABA31	Cabbage	Hsinshe, Taichung
Rhizoctonia solani AG-4	RST-04	Cabbage	Dali, Taichung
Alternaria alternata	AAL01	Leek	Wufeng, Taichung
Alternaria tenuissima	APR-01	Cherry	Wufeng, Taichung
Alternaria spp.	ABR03	Cabbage	Known Seed Co.
Alternaria spp.	ACO01	Taro	Wufeng, Taichung
Alternaria spp.	ALY02	Tomato	Known Seed Co.
Alternaria spp.	AOR01	Rice	Minhsiung, Chiayi
Alternaria spp.	AOR02	Rice	Minhsiung, Chiayi
Alternaria spp.	AOR05	Rice	Minhsiung, Chiayi
Alternaria spp.	AOR06	Rice	Minhsiung, Chiayi
Alternaria spp.	ATA01	African marigold	Wufeng, Taichung

Proteins purified from *Alternaria* spp. promote root development

Fungal cellular proteins were purified from 12 *Alternaria* spp. and tested for Chinese cabbage root development. Seeds were soaked in protein extracts (2 μg/ml) for 12 h, resulting in more than 90% germination rates. Proteins purified from 7 isolates (ATA01,

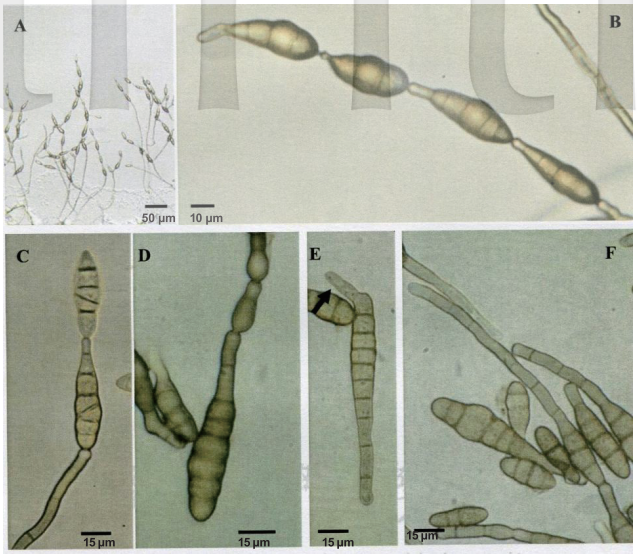


Fig. 1. Morphological characteristics of conidia produced by the APR01 isolate of *Alternaria* sp. collected from cherry. APR01 was identified as *A. tenuissima* based on cultural and morphological characteristics and sequence analysis of ITS rDNA. (A－F) Conidia having multiple septa are often formed in chains showing brownish color, slightly verruculose, and ovoid to obclavate in shape with or without long conical or cylindrical beaks.

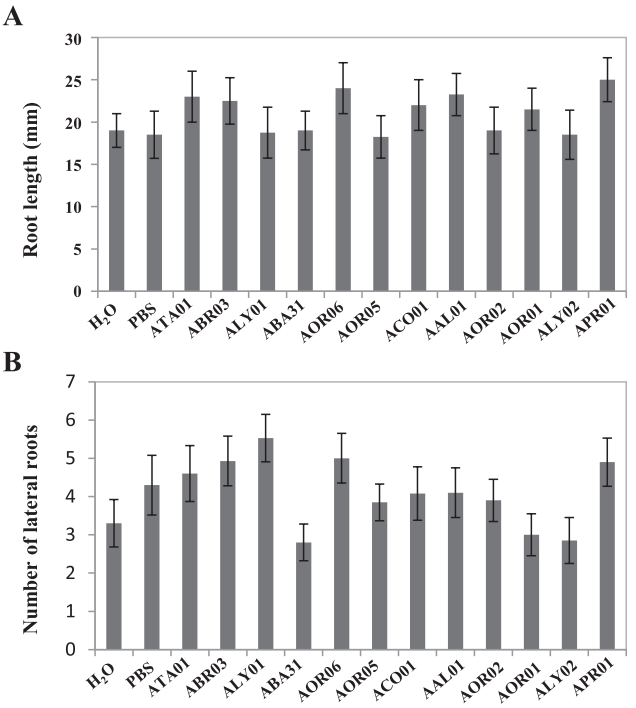


Fig. 2. Assays for fungal proteins purified from 12 *Alternaria* spp. on root growth of Chinese cabbage. **(A)** Root length of Chinese cabbage seeds treated with or without fungal proteins (2 μg/ml) for 6 h and incubated at 24°C for 3 days. **(B)** Number of lateral roots in Chinese cabbage after treatments. Seeds soaked in water or PBS buffer (100x) were used as controls. Each column represents the mean of root length ± the standard error from four replicates (each with 10 seeds).

ABR03, AOR06, ACO01, AAL01, AOR01, and APR01) promoted root elongation, increasing root length by an average of 33 to 37 % compared to those of controls (Fig. 2A). It was observed that fungal proteins could stimulate the development of lateral roots, exemplified by those prepared from ATA01, ABR03, ALY01, AOR06, and APR01 (Fig. 2B).

Reduction of *Rhizoctonia* damping-off

Chinese cabbage seedlings were sprayed with protein extracts prepared from eight *Alternaria* spp. and inoculated with *R. solani* AG4 by placing agar plugs on stem base. Fungal proteins were nontoxic to plants causing no visible lesions at all (data not shown). Disease incidence measured at 7 dpi revealed that only proteins purified from APR01 and ACO01 were able to reduce damping-off by an average of 33.3% compared to those treated with water or buffer (Fig. 3). Protein concentrations adjusted to 2 μg per ml had the best effect on the reduction of damping-off. However, *in vitro* assays indicated that APR01 proteins had no inhibitory effects on the growth of *R. solani* (data not shown).

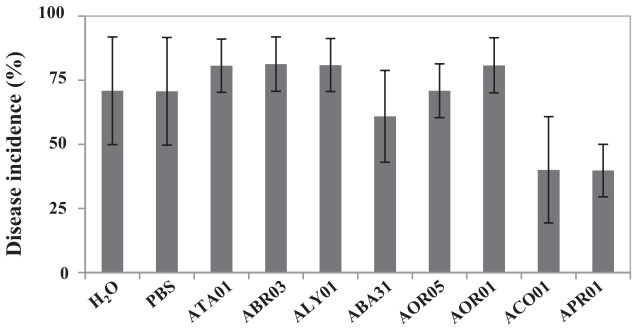
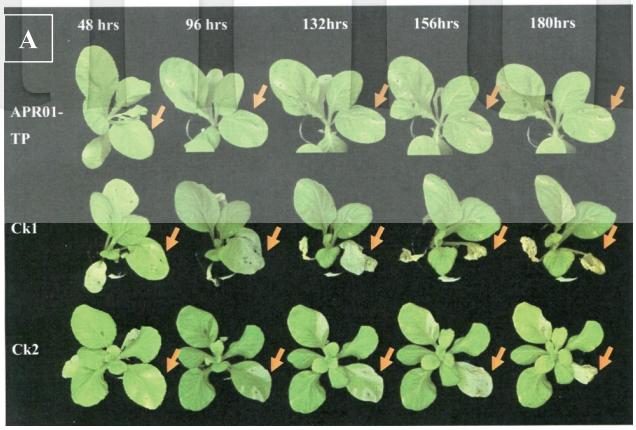


Fig. 3. Assays for *Alternaria* protein extracts for controlling *Rhizoctonia solani* damping-off disease in Chinese cabbage seedlings. Seedlings were sprayed to run-off with protein extracts (2 μg/ml) prepared from eight *Alternaria* spp. and inoculated with *R. solani* AG4 by placing agar plugs on stem base. Seedlings treated with water or PBS buffer (100x) were used as controls. Disease incidence was determined 7 dpi. Experiments were carried out in three replicates, each containing three seedlings. Each column represents the mean of disease incidence (%) ± the standard error.

APR01 proteins reduce disease progression on Chinese cabbage leaves

Experiments were set up to evaluate the effects of APR01 protein extracts on the development of *R. solani*-induced damping-off on Chinese cabbage leaves. Agar plugs bearing *R. solani* mycelium were placed onto the third and fourth true leaves 12 h after seedlings were sprayed with APR01 proteins. The inoculated leaves developed water-soaking and/or necrotic lesions surrounded by yellow halos 24 dpi. Lesions induced by *R. solani* progressed at rates much slower on leaves treated with APR01 proteins (Fig. 4A) compared to controls treated with water or buffer. Quantitative assays revealed that lesion sizes appearing on the leaves treated with APR01 proteins were significantly smaller than those of controls (Fig. 4B). When assayed in *R. solani* infested substrates, APR01 proteins reduced damping-off incidence by 45 to 53% compared to controls.



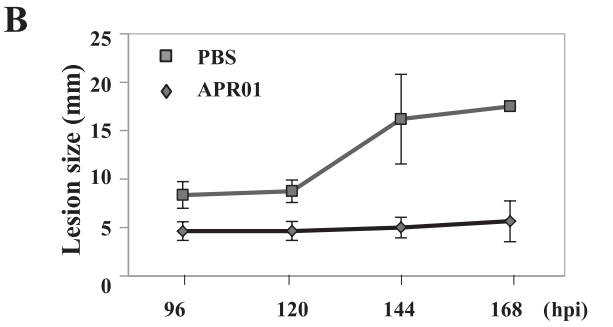
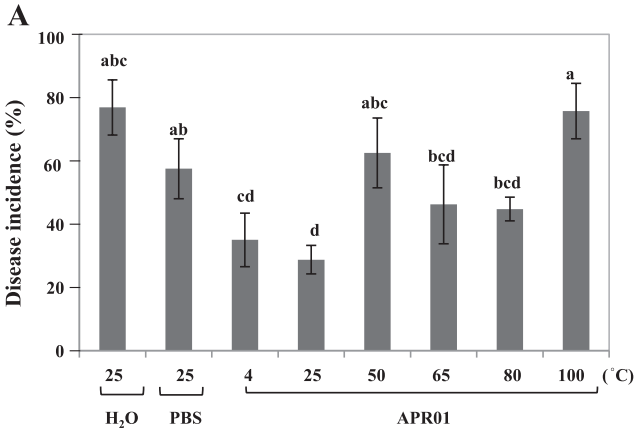


Fig. 4. Fungal protein activators reduce *Rhizoctonia solani* – induced necrotic lesions on Chinese cabbage leaves over time. (A) Progression of water-soaking and chlorotic lesions on seedling leaves sprayed with protein extracts (2 μg/ml) of APR01 and inoculated with *R. solani* by placing agar plugs on the third and fourth true leaves. Seedlings treated with water (CK1) or PBS buffer (100x, CK2) were used as controls. (B) Quantitative analysis of lesion size on seedling leaves induced by *R. solani* after protein treatment. Experiments were performed in four replicates with two spots for each leaf. Only representatives are shown.



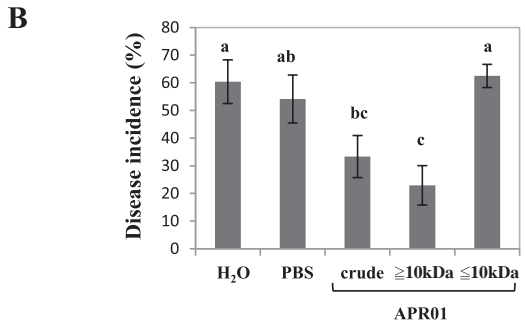


Fig. 5. Physical properties of protein activators purified from the APR01 isolate of *Alternaria tenuissima*. (A) APR01 proteins (2 μg/ml) heated at temperatures higher than 50˚C failed to reduce damping-off incidence induced by *Rhizoctonia solani* in Chinese cabbage seedlings (four replicates, each containing 12 seedlings). (B) Proteins M.W. greater than 10 kDa reduced damping-off incidence at levels resemble those of crude extracts. Seedlings were inoculated with *R. solani* by placing agar plugs with fungal mycelium on basal areas of stem 12 h after treatments. Seedlings treated with water or PBS buffer (100x) were used as controls. Disease incidence was determined 7 dpi. Each column represents the mean of disease incidence (%) ± the standard error. Treatments with the same letter are not significantly different (P=0.05) based on Duncan's multiple range test.

The overall fresh weight of seedlings treated with APR10 proteins was also greater than those treated with water only (data not shown).

Physical properties of APR01 protein activators

APR01 proteins were treated at 4, 25, 50, 65, 80, or 100˚C for 10 min and sprayed onto 7-day-old Chinese cabbage seedlings. The treated seedlings were inoculated with *R. solani* by placing agar plugs with fungal mycelium on basal areas of stem 12 h after treatment. The results indicated that APR01 proteins treated with heat (≥50˚C) failed to reduce damping-off incidence (Fig. 5A). APR01 proteins that were unable to pass through the filter (M.W. ≥10 kDa) displayed disease reduction effects against *R. solani* damping-off (Fig. 5B). In contrast, proteins passed through a centrifugal filter (10 kDa cut off) had no inhibitory effects.

DISCUSSION

In the present study, we purified proteins from 12 *Alternaria* spp. and have demonstrated that proteins purified from some but all *Alternaria* spp. were able to promote plant growth and reduce damping-off disease induced by *R. solani* in Chinese cabbage seedlings. Proteins purified from an APR01 isolate had no anti-fungal activity.

APR01 collected from cherry was identified as *A. tenuissima* based on cultural and morphological characteristics and sequence analysis of rDNA ITS. Infectivity assays on peach fruit and Cherry Hill leaves (*Cerasus serrulata*) revealed that APR01 failed to induce any visible lesions (data not shown). APR01 pathogenicity and host range remain largely unknown. Crude proteins purified from *A. brassicicola*, *A. alternata*, and other *Alternaria* spp. had less effects on the reduction of damping-off. Assays for disease reduction also revealed that proteins purified from *Alternaria* spp. had moderate effects on diseases induced by *Pythium aphanidermatum*, *Colletotrichum higginsianum*, and *A. brassicicola* (data not shown).

Studies conducted in China revealed that proteins purified from several filamentous fungi including *Botrytis* spp. *Alternaria* spp.

Aspergillus spp. *Pyricularia* spp. *Penicillium* spp. *Rhizoctonia* spp., *Trichoderma* spp. and *Fusarium* spp. were able to promote seed germination and growth of cotton, tomato, loofah, and Chinese cabbage [46,49-52]. However, of proteins purified from 12 *Alternaria* spp., none was able to promote seed germination of Chinese cabbage even though some were found to enhance root elongation and branching. Quantitative analysis revealed that APR01 proteins enhanced root growth by 32.5% and the formation of lateral roots by 36.5% compared to water controls. Unlike PB90 elicitor purified from *Phytophthora boehmeriae* [28], APR01 proteins did not induce any hypersensitivity on Chinese cabbage leaves. Elicitins have also been shown to cause wilting or senescence on radish plants [25]. In the present study, we found that Chinese cabbage seedlings treated with APR01 proteins reduced damping-off incidence and increased overall fresh weight. *In vitro* assays revealed no fungistatic activity of APR01 protein, suggesting the changes of host physiological and biochemical metabolisms and induction of plant defense mechanisms by APR01 proteins after spraying onto plants. These aspects deserve further investigation.

Ample studies have shown that proteins of plant pathogens were able to induce plant defense reactions [35,36]. Elicitins of *Phytophthora* spp. were able to induce defense mechanisms in tobacco and rendered plants resistant to fungal, bacterial, and viral diseases [2,23,38]. It has also been speculated that elicitins serving as sterol carriers interact with host cell membrane proteins triggering defense reactions and limiting the availability of sterols [4]. Sterols are absolutely required for *Phytophthora* growth. Proteins purified from *Pythium oligandrum* cell wall also rendered sugar beet resistant to *R. solani* [44]. APR01 proteins could reduce *R. solani* incidence by 30% on Chinese cabbage even lacking antifungal activity. Heat treatment experiments indicated that the major components contributing to disease reduction could be enzymes or heat-labile proteins as APR01 proteins heated at temperatures higher than 50°C completely or nearly abolished the capacity to reduce disease. The results are inconsistent with finding of crude proteins purified from *A. alternata* and *Curvularia lunata*, in which heating fungal proteins at 100°C for 7 min enhanced rust resistance on corn [46]. This discrepancy could be due to the divergence of fungal pathogens and/or the presence of different active components in crude proteins. Whether or not APR01 proteins contain more than one active components and whether or not they interact synergistically remain uncertain.

In conclusion, our studies have demonstrated the possibility of fungal proteins in the reduction of a soil-borne disease, likely via the induction of plant defense mechanisms. The active components are likely heat-labile proteins. Future studies shall focus on how to improve the efficacy of disease control and on the physiological and biochemical changes of plants after treating with *Alternaria* proteins. We have recently demonstrated that application of a nonionic detergent Nonidet P-40 alone activates disease resistance against cucumber anthracnose [30]. It will be of great interest to determine the synergistic effects of both Nonidet P-40 and *Alternaria* proteins in controlling plant diseases.

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摘　要

謝子揚、林宗俊、林秋琍、鍾光仁、黃振文。2016。利用真菌蛋白激活子防治白菜立枯絲核病。植物醫學 58(1):1-8。

將12菌株的鏈格孢屬 (Genus *Alternaria*) 真菌，逐一純化它們的總量蛋白後，分別測試各總蛋白促進白菜生長及減少病害發生的效果，結果發現部分菌株的總量蛋白質具有促進植物生長、刺激根系伸長及側根形成的功效外，尚可減少立枯絲核菌 (*Rhizoctonia solani*) 引起苗立枯病的罹病率。由山櫻花分離的APR01 菌株真菌蛋白可顯著降低白菜苗立枯病的發生率達30%以上。依據產 孢方式、孢子形態及 rDNA ITS的序列分析，我們將APR01 菌株鑑定為*Alternaria tenuissima*。分析APR01 菌株之總量蛋白的抑菌活性，發現其無法有效抑制立枯絲核菌的菌絲生長。研究結果顯示 APR01的總量蛋白分子量大於10 kDa者，具有減少苗立枯病發生的效果; 然而其分子量小於10 kDa者，卻不具有 抑病的功效。將APR01 菌株之總量蛋白加熱處理，達50℃以上時，其抑制苗立枯病發生的能力幾乎完全喪失，顯示其 抑病的主要成分可能是酵素或為熱敏感蛋白質。歸言之，本研究的結果顯示真菌蛋白有可能經由誘導植物防禦機制的啟動，進而有效降低作物土壤媒病害的發生。

關鍵詞：鏈格孢菌、激活子、防禦反應、抗病性、真菌性病原菌