JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Isolation and Characterization of an Extracellular Antimicrobial Protein from *Aspergillus oryzae*

SEONG-CHEOL PARK,[†] NAE CHOON YOO,[‡] JIN-YOUNG KIM,[†] HAE KYUN PARK,[†] BYUNG JO CHAE,[§] SONG YUB SHIN,[†,||] HYEONSOOK CHEONG,[⊥] YOONKYUNG PARK,*[,†,⊥] AND KYUNG-SOO HAHM*[,†,||]

Research Center for Proteineous Materials (RCPM), Department of Cellular and Molecular Medicine, College of Medicine, and Biotechnology and BK21 Research Team for Protein Activity Control, Chosun University, Gwangju, 501-759, Korea, Yonsei University College of Medicine, 134 Shinchon-Dong, Seodaemun-Gu, Seoul, 120-752, Korea, and Kangwon National University, Chuncheon, Korea

A 17 kDa antimicrobial protein was isolated from growth medium containing the filamentous fungus *Aspergillus oryzae* by extracting the supernatants from the culture media, ion exchange chromatography on CM-sepharose, and C18 reverse-phase high-performance liquid chromatography. This antimicrobial protein, which we considered to be an extracellular antimicrobial protein from *A. oryzae* (exAP-AO17), possessed antimicrobial activity but lacked hemolytic activity. The exAP-AO17 protein strongly inhibited pathogenic microbial strains, including pathogenic fungi, *Fusarium moniliform* var. *subglutinans* and *Colletotrichum coccodes*, and showed antibacterial activity against bacteria, including *E. coli O157* and *Staphylococcus aureus*. To confirm that the protein acts as a regulation factor for extracellular secretion, we examined growth under varying conditions of N sources, C sources, ions, ambient pH, and stress. Various culture conditions were found to induce characteristic changes in the expression of protein synthesis as analyzed by sodium dodecyl sulfate−polyacrylamide gel electrophoresis. Highly basic polypeptides were regulated by suppressing the ambient pH under acidic conditions and strongly induced under alkaline conditions, thus confirming that pH regulation is physiologically relevant. The expression of exAP-AO17 was upregulated by heat shock upon growth in the presence of NaCl. Automated Edman degradation showed that the N-terminal sequence of exAP-AO17 was NH_2-GLPGPAGAVGFAGKDQNM−. ExAP-AO17 showed partial sequence homology with a collagen belonging to the animal source. These results suggest that exAP-AO17 is an excellent candidate as a lead compound for the development of novel oral or other types of anti-infective agents.

KEYWORDS: Antimicrobial activity; *Aspergillus oryzae*; exAP-AO17; homology with a collagen; anti-infective agents

INTRODUCTION

Organisms use different defense systems, including the production of antimicrobial proteins (*1*−*4*), lectins (*5*), secondary metabolites, membrane-interacting proteins, and antifeedant proteins (*6*), to fight against microbial pathogen infections. Several antimicrobial proteins, which apparently belong to a host defense system against invasive infection, have been isolated from prokaryotes and eukaryotes (*7*−*11*). These proteins show wide variation in length and amino acid composition but are characterized by compact structures, thermal stability, and potent antimicrobial activity. Antimicrobial proteins are a part of the innate immune system widely distributed in nature, and they play the key roles in defending the host against invasive pathogens (*12*).

Filamentous fungi have the unique abilities to economically produce and secrete many different types of products (e.g., antibiotics, commodity chemicals, and enzymes) (*13*) and to grow on a wide variety of inexpensive substrates. An important characteristic of the filamentous fungi used in these industries is their high-efficiency production of various kinds of enzyme production. Furthermore, because filamentous fungi, especially species of the genera *Aspergillus* and *Penicillium*, have a long history of use in the food and beverage industries, they have been granted GRAS (generally regarded as safe) status. *As-*

* To whom correspondence should be addressed. (K.-S.H.) Fax: +82-62-227-8345. E-mail: kshahm@chosun.ac.kr. (Y.P.) Fax: +82-62-230-6854. E-mail: ykpark@chosun.ac.kr.

[†] Research Center for Proteineous Materials (RCPM), Chosun University.

[‡] Yonsei University College of Medicine.

[§] Kangwon National University.

[||] College of Medicine, Chosun University.

[⊥] Biotechnology and BK21 Research Team for Protein Activity Control, Chosun University.

10.1021/jf802373h CCC: $40.75
Published on Web 09/20/2008

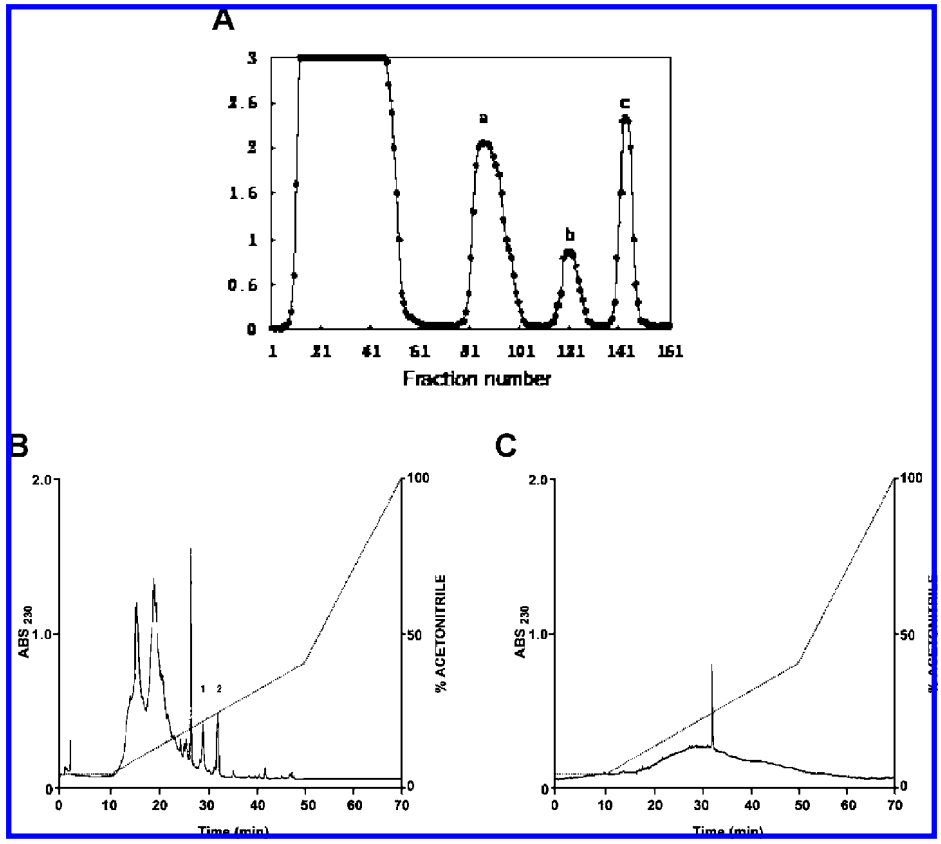

Figure 1. Elution profile of exAP-AO17. (**A**) The fraction containing the three peaks was separated by cationic-exchange chromatography on a CM-sepharose column. The column (1.6 cm × 10 cm) was then eluted with a stepwise pH gradient of 50 mM ammonium acetate (pH 7.0) (*a*), 100 mM ammonium acetate (pH 9.0) (*b*), and 1 M NaCl containing 100 mM ammonium acetate (pH 9.0) (*c*). Panels **B** and **C** show the reverse-phase HPLC profile determined when an Ultrasphere C18 column (4.6 mm × 250 mm) was used to analyze the concentrated fractions eluted from the CM-sepharose column.

pergillus oryzae is one of the most frequently used microorganisms in industries, including the production of soybean paste, alcoholic beverages, and many enzymes (*14, 15*). In the present study, we isolated and characterized a novel antimicrobial protein with a wide range of antimicrobial activity and focused on the production and secretion of inexpensive bulk substrates.

In the present study, we isolated and characterized an antimicrobial protein from *A. oryzae.* In addition, to confirm that the protein acts as a regulation factor for extracellular secretion, we examined growth under a variety of conditions of N sources, C sources, ions, ambient pH, and stress. Various culture conditions were found to induce characteristic changes in the expression of protein synthesis as analyzed by sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS-PAGE). Finally, we analyzed the N-terminal sequence of exAP-AO17 and compared its N-terminal sequence homology with that of animal collagen.

MATERIALS AND METHODS

Preparation of Materials. *A. oryzae* (KCTC 6909) used in this study was obtained from the Korean Collection for Type Cultures (KCTC).

Purification and Characterization of exAP-AO17. To purify exAP-AO17, *A. oryzae* was grown at 28 °C in 1 L of YPD medium (1% yeast extract, 2% peptone, and 2% D-glucose) with 0.5% NaCl. After incubation for 72 h in a shaking incubator (150 rpm), the culture medium was collected by filtering through eight layers of cheesecloth, and the filtrate was centrifuged (12000*g*, 15 min) to remove mycelia debris and spores.

The antifungal protein was isolated and purified using ion exchange chromatography on CM-sepharose and C_{18} RP-HPLC. Briefly, the supernatants were adjusted to pH 5.0 via the addition of 10 mM ammonium acetate buffer and then subjected to CM-sepharose fast flow cation exchange chromatography (Amersham Pharmacia Biotech). Washing to remove unadsorbed compounds was performed with 10 mM ammonium acetate buffer (pH 5.0) until the eluent was free of protein as detected by the absorbance at 280 nm. Fractionation was executed using a stepwise gradient procedure. The first elution was performed with 50 mM ammonium acetate buffer (pH 7.0), the second elution was performed with 100 mM ammonium acetate buffer (pH 9.0), and the column was washed with 1 M NaCl. The fraction was pooled, lyophilized, and tested for antifungal activity.

Fractions showing antifungal activity were further purified using ultrasphere C_{18} reversed-phase high-performance liquid chromatography (RP-HPLC) (4.6 mm × 250 mm), which was previously equilibrated with 0.1% (v/v) trifluoroacetic acid in HPLC-grade water containing 5% acetonitrile. Proteins were eluted with a linear gradient of increasing concentration (2%/min) of acetonitrile containing 0.1% (v/v) trifluoroacetic acid (flow rate, 1 mL/min). Each peak fraction was collected and assayed for antifungal activity.

Mass Spectrometry. MALDI-MS (matrix-assisted laser desorption/ionization mass spectrometry) analysis was performed in the linear mode using a Voyager DE RP instrument (Perseptive Biosystems, Framingham, MA) as described by Pouvreau et al. (*16*).

Bacteria and Fungal Strains for Antimicrobial Assay. *Staphylococcus aureus* (KCTC 1621), *Escherichia coli O157* (ATCC 43895), *Candida albicans* (TIMM 1768), *Saccharomyces cerevisiae* (KCTC 7296), *Fusarium moniliform* (KCTC 6149), and *Colletotrichum coccodes* (KACC 40803) were obtained from the KCTC, American Type Culture Collection (ATCC), Teikyo University Institute of Medical Mycology (TIMM), and Korean Agricultural Culture Collection (KACC).

Antibacterial Activity. Bacteria were grown to the midlogarithmic phase in medium containing (in g/L): bactotryptone, 10; yeast extract,

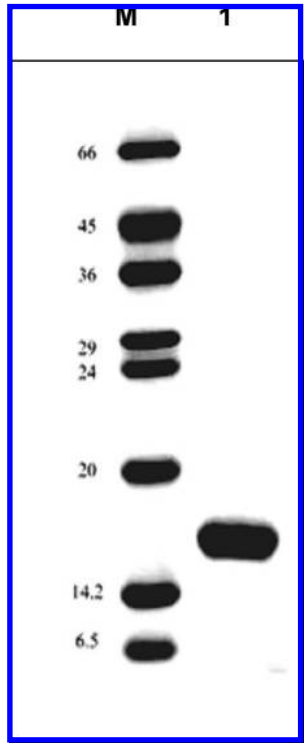

Figure 2. SDS-PAGE of the purified antimicrobial protein. The purified antimicrobial protein was examined by SDS-PAGE. The protein band was identified by staining with Coomassie brilliant blue, after which it was destained with 10% acetic acid and then excised. Lane 1, molecular size marker (66 kDa, bovine serum albumin; 45 kDa, ovalbumin; 36 kDa, glyceraldehyde-3-phosphate dehydrogenase; 29 kDa, carbonic anhydrase; 24 kDa, trypsinogen; 20 kDa, trypsin inhibitor; 14.2 kDa, α-lactalbumin; and 6.5 kDa, aprotinin). Lane 2, purified antimicrobial protein from RP HPLC.

5; and NaCl, 10 (pH 7.0). The protein was diluted stepwise in 1% bactopeptone. The test organism [final bacterial suspension: 5×10^3 colony formation units (CFU)/mL] suspended in growth medium (100 μL) was mixed with the test protein solution in the wells of a microtiter plate such that the final concentration of test protein was 30 μg/mL. Microbial growth was determined based on the increase in OD_{620} after incubation for 10 h at 37 °C. The assay for antibacterial activity against the tested cells was conducted in 100 mm × 15 mm Petri dishes that contained bacteria culture medium. After the colony had developed, sterile blank paper disks (8 mm diameter) were placed 5 mm away from the leading edge of the colony. An aliquot of the test sample in 1% bactopeptone was then added to each disk, after which the plates were incubated at 37 °C for 16 h. The antibacterial activity was indicated by a clear zone of growth inhibition (*17*).

Assay for Antifungal Activity. Microdilution assays were performed to establish minimal inhibition concentration (MIC) values of exAP-AO17. *C. albicans* was grown at 28 °C in YPD (2% dextrose, 1% peptone, and 0.5% yeast extract, pH 5.5) for 3 h. Cell densities were counted with a hemocytometer. The fungal cells (2×10^3/well) were seeded on the wells of a flat-bottom 96-well microtiter plate (Greiner, Nurtingen, Germany) containing YPD (100 μL/well). Serial dilutions of exAP-AO17 solution were added to each well, and the cell suspension was incubated at 28 °C for 24 h. Ten microliters of a 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) solution (5 mg/mL) was added to each well, and the plates were incubated at 37 °C for 4 h (*18*−*20*). The absorbance at 570 nm was measured using an Emax microtiter plate reader (Molecular Devices, CA). All assays were performed in triplicate. To visualize the fungicidal effect, morphological changes were examined by phase contrast light microscopy using an Eclipse TE300 microscope (Nikon, Japan).

The assays for antifungal activity against *F. moniliform* and *C. coccodes* were carried out in 100 mm × 15 mm Petri dishes containing YPD. After the mycelial colony had developed, sterile blank paper disks (8 mm diameter) were placed 5 mm from the leading edge of the mycelial colony. An aliquot of the test sample in MES buffer (20 mM, pH 6.0) was added to each disk, and the plates were incubated at 28 °C for 72 h. The antifungal activity was shown as a clear zone of growth inhibition.

Preparation of Human Red Blood Cells and Assay of Hemolytic Activity. Human red blood cells were centrifuged and washed three times with phosphate-buffered saline (PBS; 35 mM phosphate buffer with 0.15 M NaCl, pH 7.0). The hemolytic activities of potamin-1 and melittin (positive control) were evaluated by measuring the release of hemoglobin from fresh human erythrocytes. Aliquots (100 μL) of an 8% suspension of red blood cells were transferred to 96-well plates, and hemolysis was determined by measuring the absorbance at 414 nm using the Emax plate reader. No hemolysis (0%) and full hemolysis (100%) were determined in the presence of PBS and 0.1% Triton X-100, respectively. The percent hemolysis was calculated using the following equation: % hemolysis = [(A_{414nm} with protein solution − A_{414nm} in PBS)/(A_{414nm} with 0.1% Triton-X 100 − A_{414nm} in PBS)] × 100 (*21*).

Amino Acid Sequencing of the Isolated Protein. Amino acid sequencing of the purified exAP-AO17 protein was performed by the Sequence Centre of the Korea Basic Science Institute (Seoul, Korea).

Physiological Condition for the Production of the exAP-AO17 Protein. *A. oryzae* was cultivated in various media. All liquid cultures were incubated in shaken 500 mL flasks containing 100 mL of medium at 28 °C and 150 rpm. *A. oryzae* was cultivated in media containing different carbon sources, nitrogen sources, and ambient pH. The various media used to cultivate *A. oryzae* were as follows: medium 1 (1% yeast extract), medium 2 (2% peptone), medium 3 (1% yeast extract and 2% D-glucose), medium 4 (2% peptone and 2% D-glucose), medium 5 (1% yeast extract and 2% peptone), medium 6 [1% yeast extract, 2% peptone, 2% D-glucose, and 0.5% NaCl (pH 4.0)], medium 7 (1% yeast extract, 2% peptone, and 2% D-glucose), medium 8 [1% yeast extract, 2% peptone, 2% D-glucose, and 0.5% NaCl (pH 8.0)], medium 9 [1% yeast extract, 2% peptone, 2% D-glucose, and 0.5% NaCl (pH 8.0)], medium 10 (1% yeast extract, 2% peptone, and 2% maltose), medium 11 (1% yeast extract, 2% peptone, and 2% starch), medium 12 (1% yeast extract, 2% peptone, and 2% sucrose), and medium 13 (1% yeast extract, 2% peptone, and 2% glycine). Mycelia were harvested after incubation for 72 h in a shaking incubator (150 rpm), and the filtered supernatants were examined on SDS-PAGE (*22*). The protein band was identified by staining with Coomassie brilliant blue, after which it was destained with 10% acetic acid and then excised.

RESULTS AND DISCUSSION

An antimicrobial protein was purified from the culture supernatants of *A. oryzae*. Protein purification involved the following two steps: CM-sepharose cation-exchange chromatography and C18 RP-HPLC. Each fraction or eluent from the purification step was assayed for growth inhibition activity or cell death against pathogenic yeast strains.

We isolated an exAP-AO17 protein from the culture supernatants of *A. oryzae*. To accomplish this, the supernatants were extracted by filtration and then dialyzed using 1000 MWCO dialysis tubing. Next, the extracts were adjusted to pH 5.0 by the addition of 10 mM ammonium acetate, after which they were directly subjected to cation-exchange chromatography on a CM-sepharose column. The adsorbed proteins were then eluted with 50 mM ammonium acetate (pH 7.0), as shown in **Figure 1A**. This process resulted in the recovery of three components: a−c. The first peak component was the most active; therefore, the first peak fraction was pooled and lyophilized. The active fractions obtained from the CM-sepharose column were then subjected to C_{18} RP-HPLC. The results of this analysis revealed that compound corresponding to peak 2 (**Figure 1B**) showed antimicrobial activity. Therefore, this fraction was collected and

590 600 610 620
Collagen ---GL PGPPGAVGPA GKDGEAGAQG APGPAGPAGE RGEQGPA
---GL PGPAGAVGFA GKD---------- ---------- ----QNM—

Figure 3. Alignment of partial amino acid sequences of exAP-AO17 with that of collagen.

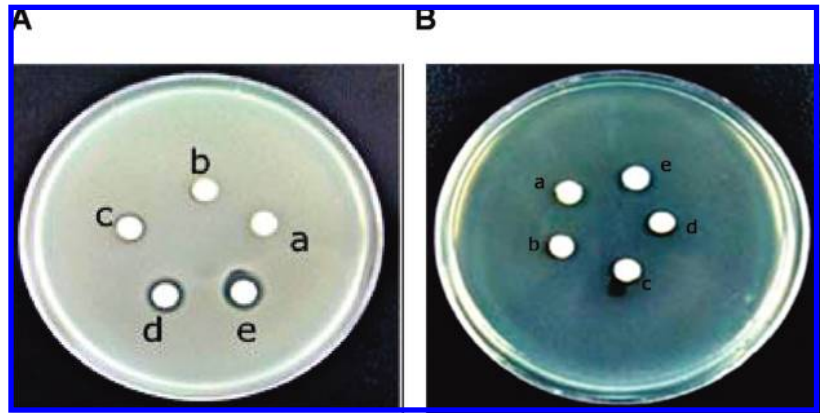

Figure 4. Growth inhibition test with *S. aureus* (**A**) and *E. coli O157* (**B**). Each paper disk was loaded with a 20 μL sample containing various amounts of the purified protein. The amount of applied protein was (a) control (buffer), (b) 0.25 μg, (c) 0.5 μg, (d) 1.0 μg, and (e) 2.0 μg.

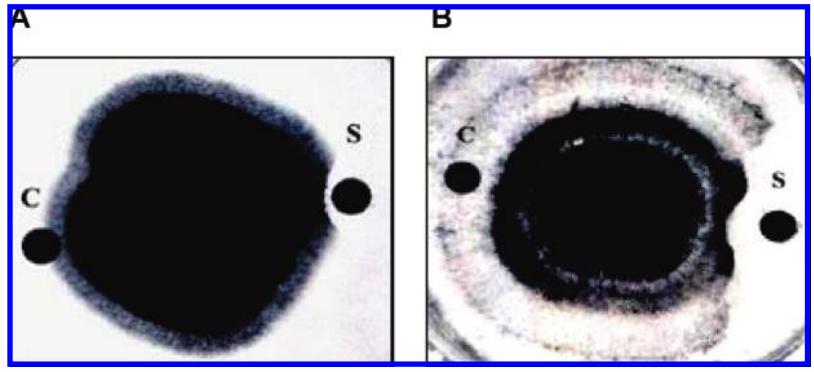

Figure 5. Direct inhibition of fungal growth by the purified protein. *F. moniliform* (**A**) and *C. coccodes* (**B**) were cultured on PDA plates for 48 h. Crescents surrounding the disks indicate the inhibition of hyphal extension. The purified protein was dissolved in 20 μL of 10 mM Mes buffer (pH 5.0). Disc S contained 5 μg of purified protein, and disc C contained only 10 mM Mes buffer and served as the control.

Table 1. Steps in the Purification of exAP-AO17 from *A. oryzae*

fractionation step/fraction	volume (mL)	total protein (mg)	recovery yield (%)
filtered supernatants	900	22.5	100
step 1 (cation-exchange chromatography)	330	7.3	32.44
step 2 (reverse-phase chromatography)			
first run	18	0.42	1.87
second run	2	0.32	1.42

Table 2. Hemolytic Activities of the Antimicrobial Protein exAP-AO17

		% hemolysis							
protein (μg)	200	40	20	10	5	2.5	1.25	0.62	0.31
exAP-AO17	0	0	0	0	0	0	0	0	0
melittin	100	100	100	99	95	78	42	17	0

lyophilized, after which it was subjected to the HPLC procedure described above (**Figure 1C**).

SDS-PAGE revealed that the peak corresponding to exAP-AO17 contained a single protein with an approximate molecular mass of 17 kDa (**Figure 2**). In addition, MALDI-MS revealed that the relative molecular weight of AFP-J was 15690.50 (data not shown).

The purified protein was partially sequenced in N-terminal amino acid. Amino acid sequencing revealed that exAP-AO17 has an N-terminal amino acid sequence of NH_2-Gly-Leu-Pro-Gly-Pro-Ala-Gly-Ala-Val-Gly-Phe-Ala-Gly-Lys-Asp-Gln-Asn-Met−. As shown in **Figure 3**, this sequence was found to be 83% homologous with that of a collagen reported by Valueva et al. (*23*).

The final yield of purified exAP-AO17 from *A. oryzae* was 1.42% (Tale 1). We next examined the antimicrobial activity of the purified exAP-AO17 protein against various human and plant pathogenic microbial cells. As shown in **Figures 4** and **5**, exAP-AO17 exhibited antimicrobial activity against the pathogenic bacteria *S. aureus* (**Figure 4A**), *E. coli O157* (**Figure 4B**), as well as the pathogenic fungi *F. moniliform* (**Figure 5A**) and *C. coccodes* (**Figure 5B**). The microbicidal effect of exAP-AO17 was further visualized by treating cells with exAP-AO17 and spreading them on agar plates. As shown in **Figures 4** and **5**, exAP-AO17 inhibited the growth of both types of microorganisms.

The antifungal activity was observed as a crescent-shaped zone of inhibition at the mycelia. The antifungal activity of exAP-AO17 was tested against both *F. moniliform* (**Figure 5A**) and *C. coccodes* (**Figure 5B**), and the protein showed strong activity below 5 μg. The protein also showed antifungal activity in *C. albicans* (MIC; 150 μg/mL) and *S. cerevisiae* (MIC; 200 μg/mL) but low antibacterial activity against *E. coli O157* (**Figure 4B**) when compared with *S. aureus* (**Figure 4A**).

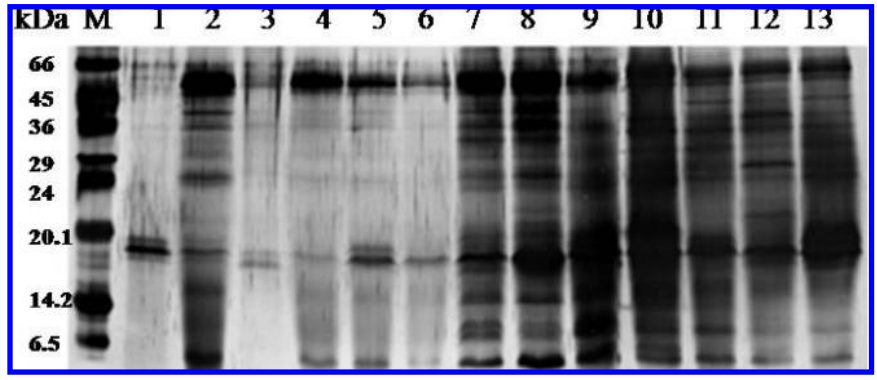

Figure 6. SDS-PAGE analysis of the production of the exAP-AO17 protein under physiological conditions. *A. oryzae* was cultivated in media containing different carbon sources, nitrogen sources, and ambient pH. M, molecular size marker (66 kDa, bovine serum albumin; 45 kDa, ovalbumin; 36 kDa, glyceraldehyde-3-phosphate dehydrogenase; 29 kDa, carbonic anhydrase; 24 kDa, trypsinogen; 20 kDa, trypsin inhibitor; 14.2 kDa, α-lactalbumin; and 6.5 kDa, aprotinin); lane 1, medium 1 (1% yeast extract); lane 2, medium 2 (2% peptone); lane 3, medium 3 (1% yeast extract and 2% D-glucose); lane 4, medium 4 (2% peptone and 2% D-glucose); lane 5, medium 5 (1% yeast extract and 2% peptone); lane 6, medium 6 [1% yeast extract, 2% peptone, 2% D-glucose, and 0.5% NaCl (pH 4.0)]; lane 7, medium 7 (1% yeast extract, 2% peptone, and 2% D-glucose); lane 8, medium 8 [1% yeast extract, 2% peptone, 2% D-glucose, and 0.5% NaCl (pH 8.0)]; lane 9, medium 9 [1% yeast extract, 2% peptone, 2% D-glucose, and 0.5% NaCl (pH 8.0)]; lane 10, medium 10 (1% yeast extract, 2% peptone, and 2% maltose); lane 11, medium 11 (1% yeast extract, 2% peptone, and 2% starch); lane 12, medium 12 (1% yeast extract, 2% peptone, and 2% sucrose); and lane 13, medium 13 (1% yeast extract, 2% peptone, and 2% glycine).

We next examined the cytotoxicity of exAP-AO17 against mammalian cells by measuring the lysis of human erythrocytes. The exAP-AO17 protein had no hemolytic activity, whereas melittin, used as a positive control for lysis, was strongly hemolytic (**Table 2**). These results demonstrated that exAP-AO17 has remarkable antimicrobial activity against various microbial cells but no hemolytic activity.

The regulation of the expression of the purified antimicrobial protein by carbon and nitrogen sources was investigated. Minimal medium was used as standard medium, and different carbon and nitrogen sources were added at a concentration of 1−2% (w/v). Used were the following carbon sources: D-glucose, maltose, starch, sucrose, and glycine. Each carbon source was combined with either yeast extract or peptone as a nitrogen source. To confirm the influence of ambient pH on expression, *A. oryzae* was grown on liquid medium (1% yeast extract, 2% peptone, 2% D-glucose, and 0.5% NaCl) at pH 4.0 and pH 8.0. The pH of the media was adjusted every 12 h with NaOH or HCl to maintain the initial pH value of the culture broth. All filtered supernatants were analyzed by SDS-PAGE (**Figure 6**).

The results of the present study showed that peptone served as a nitrogen source, and dextrose and maltose served as carbon sources. We also found that NaCl treatment increased the level of expression of secreted protein. As shown in **Figure 6** (lanes 6 and 9), the amount of purified antimicrobial protein secretion was strongly dependent on pH. Only small amounts of antimicrobial protein were secreted when the mycelium was grown at pH 4.0, whereas the production of the antimicrobial protein was enhanced by approximately 10-fold at pH 8.0.

Collagen has been found only in the animal kingdom where it is the important component of the animal extracellular matrix, and it is the most abundant animal protein. However, in a previous report, the unexpected finding of collagen in *Microbotryum violaceum* suggested that it might have evolved from a common ancestor that existed before the divergence of fungi and animals (*24*). They reported that the protein might act as a substratum that permits animal cells from adhering, spreading, and proliferating in a manner similar to that of animal collagens. A similar result was also reported in *Aspergillus fumigates* (*25*). However, both sets of data were from studies of collagen identified from fungus. Our results showed that purified exAP-AO17 exhibits homology with collagen as well as potent antifungal activity. Therefore, exAP-AO17, an antimicrobial protein with strong antifungal activity, may have the potential to be a lead compound for the development of agrochemicals or therapeutics.

LITERATURE CITED

(1) Ye, X. Y.; Wang, H. X.; Ng, T. B. First chromatographic isolation of an antifungal thaumatin-like protein from French bean legumes and demonstration of its antifungal activity. *Biochem. Biophys. Res. Commun.* **1999**, *263*, 130–134.

(2) Benhamou, N.; Broglie, K.; Broglie, R.; Chet, I. Antifungal effect of bean endochitinase on *Rhizoctonia solani*: Ultrastructural changes and cytochemical aspects of chitin breakdown. *Can. J. Microbiol.* **1993**, *39*, 318–328.

(3) Vogelsang, R.; Barz, W. Purification, characterization and differential hormonal regulation of a beta-1,3-glucanase and two chitinases from chickpea (*Cicer arietinum* L.). *Planta* **1993**, *189*, 60–69.

(4) Pekkarinen, A. I.; Jones, B. L. Purification and identification of barley (*Hordeum vulgare* L.) proteins that inhibit the alkaline serine proteinases of *Fusarium culmorum*. *J. Agric. Food Chem.* **2003**, *51*, 1710–1717.

(5) Kamemura, K.; Furuichi, Y.; Umekawa, H.; Takahashi, H. C. Purification and characterization of novel lectins from great northern bean, *Phaseolus vulgaris* L. *Biochim. Biophys. Acta* **1993**, *1158*, 181–188.

(6) Scheel, D. Resistance response physiology and signal transduction. *Curr. Opin. Plant Biol.* **1998**, *1*, 305–310.

(7) Hancock, R. E.; Scott, M. G. The role of antimicrobial peptides in animal defense. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 8856–8861.

(8) Broekaert, W. F.; Terras, F. R.; Cammue, B. P.; Osborn, R. W. Plant defensins: Novel antimicrobial peptides as components of the host defense system. *Plant Physiol.* **1995**, *108*, 1353–1358.

(9) Garcia-Olmedo, F.; Molina, A.; Alamillo, J. M.; Rodriguez-Palenzuela, P. Plant defense peptides. *Biopolymers* **1998**, *47* (6), 479–491.

(10) Matsuyama, K.; Natori, S. Purification of three antibacterial proteins from the culture medium of NIH-sape-4, an embryonic cell line of sarcophagi peregrine. *J. Biol. Chem.* **1998**, *263*, 17112–17116.

(11) Boman, H. G. Gene-encoded peptide antibiotics and the concept of innate immunity: An update review. *Scand. J. Immunol.* **1998**, *13*, 61–91.

(12) Risso, A. Leukocyte antmicrobial peptides: Multifunctional effecter molecules of innate immunity. *J. Leukocyte Biol.* **2000**, *68*, 785–792.

(13) Fowler, T.; Berka, R. M. Gene expression system for filamentous fungi. *Curr. Opin. Biotechnol.* **1991**, *2* (5), 691–697.

(14) Jeenes, D. J.; Mackenzie, D. A.; Roberts, I. N.; Archer, D. B. Heterologous protein production by filamentous fungi. *Biotechnol. Genet. Eng. Rev.* **1991**, *9*, 327–367.

(15) van den Hondel, C.; Punt, P. J.; van Gorcom, R. F. M. Production of extracellular proteins by the filamentous fungus *Aspergillus*. *Antonie Van Leeuwenhoek* **1992**, *61*, 153–160.

(16) Pouvreau, L.; Gruppen, H.; Piersma, S. R.; van den Broek, L. A. M.; van Koningsveld, G. A.; Voragen, A. G. J. Relative abundance and inhibitory distribution of protease inhibitors in potato juice from cv. Elkana. *J. Agric. Food Chem.* **2001**, *49*, 2864–2874.

(17) Lee, K. H.; Lee, D. G.; Park, Y.; Kang, D. I.; Shin, S. Y.; Hahm, K. S.; Kim, Y. Interactions between the plasma membrane and the antimicrobial peptide HP (2−20) and its analogues derived from *Helicobacter pylori*. *Biochem. J.* **2006**, *394*, 105–114.

(18) Jahn, B.; Martin, E.; Stueben, A.; Bhakdi, S. Susceptibility testing of *Candida albicans* and *Aspergillus* species by a simple microtiter menadione-augmented 3- (4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide assay. *J. Clin. Microbiol.* **1995**, *33*, 661–667.

(19) Park, Y.; Lee, D. G.; Kim, P. I.; Woo, E. R.; Cheong, G. W.; Choi, C. H.; Hahm, K. S. A Leu-Lys-rich antimicrobial peptide: Activity and mechanism. *Biochim. Biophys. Acta* **2003**, *1645*, 172–182.

(20) Park, Y.; Choi, B. H.; Kwak, J. S.; Kang, C. W.; Lim, H. T.; Haheong, H. T.; Hahm, K. S. Kunitz-typeserine protease inhibitor from potato (*Solanum tuberosum* L. cv. Jopung). *J. Agric. Food Chem.* **2005**, *53* (16), 6491–6496.

(21) Park, Y.; Park, S. N.; Park, S. C.; Park, J. Y.; Park, Y. H.; Hahm, J. S.; Hahm, K. S. Antibiotic activity and synergistic effect of antimicrobial peptide against pathogens from a patient with gallstones. *Biochem. Biophys. Res. Commun.* **2004**, *321*, 631–637.

(22) Laemmli, U. K.; Favre, M. Maturation of the head of bacteriophage T4. *J. Mol. Biol.* **1973**, *80*, 575–579.

(23) Valueva, T. A.; Revina, T. A.; Mosolov, V. V.; Mentele, R. Primary structure of potato Kunitz-type serine proteinase inhibitor. *Biol. Chem. Hoppe-Seyler* **2000**, *381*, 1215–1221.

(24) Celerinl, M.; Ray, M.; Schisler, J.; Day, N. J.; William, A.; Stetler-Stevenson, W. G.; Laudenbach, D. E. Fungal fimbriae are composed of collagen. *EMBO J.* **1996**, *15* (17), 4445–4453.

(25) Fedorova, N. D.; Khaldi, N.; Joardar, V. S.; Maiti, R.; Amedeo, P.; Anderson, M. J.; Crabtree, J.; Silva, J. C.; Badger, J. H.; Albarraq, A.; Angiuoli, S.; Bussey, H.; Bowyer, P.; Cotty, P. J.; Dyer, P. S.; Egan, A.; Galens, K.; Fraser-Liggett, C. M.; Haas, B. J.; Inman, J. M.; Kent, R.; Lemieux, S.; Malavazi, I.; Orvis, J.; Roemer, T.; Ronning, C. M.; Sundaram, J. P.; Sutton, G.; Turner, G.; Venter, J. C.; White, O. R.; Whitty, B. R.; Youngman, P.; Wolfe, K. H.; Goldman, G. H.; Wortman, J. R.; Jiang, B.; Denning, D. W.; Nierman, W. C. Genomic islands in the pathogenic filamentous fungus *Aspergillus fumigatus*. *PLoS Genet.* **2008**, *4* (4), In press.

Received for review April 18, 2008. Revised manuscript received August 27, 2008. Accepted August 28, 2008. This work was supported by a grant from the Ministry of Science and Technology, Korea, and the Korea Science and Engineering Foundation through the Research Center for Proteineous Materials and from the Technology Development Program of the Ministry of Agriculture and Forestry, Republic of Korea.

JF802373H