JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

pubs.acs.org/JAFC

Protein from Red Cabbage (*Brassica oleracea*) Seeds with Antifungal, Antibacterial, and Anticancer Activities

Xiu-Juan Ye,[*,†,§,#] Tzi-Bun Ng,[*,§] Zu-Jian Wu,[†] Lian-Hui Xie,[†] Evandro- Fei Fang,[§] Jack-Ho Wong,[§] Wen-Liang Pan,[§] Sze-Stephen-Cho Wing,[#] and Yan-Bo Zhang[*,#]

[†]Institute of Plant Virology, Fujian Province Key Laboratory of Plant Virology, Fujian Agriculture and Forestry University, Fuzhou, Fujian 350002, People's Republic of China

[§]School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Shatin New Territories, Hong Kong, China

[#]School of Chinese Medicine, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Sassoon Road, Hong Kong, China

ABSTRACT: A 30 kDa antifungal protein was purified from red cabbage (*Brassica oleracea*) seeds. It exhibited a molecular mass and N-terminal amino acid sequence disinct from those of previously isolated *Brassica* antifungal proteins. The protocol used entailed ion exchange chromatography on Q-Sepharose and SP-Sepharose followed by fast protein liquid chromatography on Mono S. The protein hindered mycelial growth in *Mycosphaerella arachidicola* (with an IC_{50} = 5 μM), *Setospaeria turcica*, and *Bipolaris maydis*. It also inhibited the yeast *Candida albicans* with an IC_{50} = 96 μM. It exerted its antifungal action by permeabilizing the fungal membrane as evidenced by staining with Sytox green. The antifungal activity was stable from pH 3 to 11 and from 0 to 65 °C. It manifested antibacterial activity against *Pseudomonas aeruginosa* (IC_{50} = 53 μM). Furthermore, after 48 h of culture, it suppressed proliferation of nasopharyngeal cancer and hepatoma cells with IC_{50} = 50 and 90 μM, respectively.

KEYWORDS: *Brassica oleracea*, seeds, purification, antifungal protein

INTRODUCTION

Pathogenic fungi may elicit devastating damage to crops and produce adverse health consequences in farm animals and humans. Existing antifungal drugs may not be able to effectively combat emerging resistant fungal strains. They may also produce untoward side effects.[1] Hence, there are continuing efforts to search for new antifungal reagents that may circumvent the aforementioned problems.

Antifungal proteins and peptides are produced by a diversity of organisms ranging from bacteria[2] to flowering plants[3] to mammals.[4] The antifungal proteins and peptides reported to date exhibit a variety of amino acid sequences and molecular sizes.[3,5−26]

Biological activities other than antifungal activity, including antiproliferative activity toward tumor cells and inhibitory activity toward HIV-1 reverse transcriptase, have been reported for these proteins.[3−10,14,17−19,22,23]

Previously we have isolated, from plants of the genus *Brassica*, antifungal peptides with different N-terminal amino acid sequences.[3,5−9] We undertook the present investigation to isolate an antifungal peptide from the seeds of the red cabbage (*Brassica oleracea* L. var. *capitata* var. *rubra*). The characteristics of the isolated peptide were then compared with those of its previously reported counterparts from other *Brassica* species.[3,5−9]

MATERIALS AND METHODS

Materials. Seeds of the red cabbage *Brassica oleracea* were obtained from a seed vendor in Beijing, China. The fungi used were provided by the Department of Microbiology, China Agricultural University, China. SP-Sepharose, Q-Sepharose, and Mono S columns were from GE Healthcare (Hong Kong).

Isolation of Antifungal Protein. The crude extract of red cabbage (*B. oleracea*) seeds (82 g) was prepared by homogenization in 20 mM Tris-HCl buffer (pH 7.4), followed by centrifugation (10000*g*, 4 °C, 30 min) and collection of the supernatant. The supernatant (650 mL) was chromatographed on a column (5 × 20 cm) of Q-Sepharose Fast Flow in 20 mM Tris-HCl buffer (pH 7.4). The column had been equilibrated with the same buffer. Absorbed proteins were desorbed with 20 mM Tris-HCl buffer (pH 7.8) containing 1 M NaCl. Chromatographic fractions containing unabsorbed proteins were pooled and subjected to ion exchange chromatograpy with a flow rate of 60 mL/h on a column (2.5 × 30 cm) of SP-Sepharose Fast Flow that had previously been equilibrated with 20 mM Tris-HCl buffer (pH 7.4). After unadsorbed proteins had come off the column, the column was eluted with a linear gradient of 0−0.5 M NaCl in 20 mM Tris-HCl buffer (pH 7.4) to yield peak SP1 (pool of fractions 9−41), peak SP2 (pool of fractions 47−62), and peak SP3 (pool of fractions 84−100). Peak SP3 was subjected to a final purification on an FPLC-Mono S HR 5/5 column with a linear gradient of 0−0.7 M NaCl in 20 mM NH_4OAc buffer (pH 4.5). The second peak S2 (pool of fractions 22−26) constituted purified antifungal protein.

Protein Determination. Protein concentration was determined according to the dye-binding method (Bio-Rad) using bovine serum albumin as standard.

Sodium Dodecyl Sulfate−Polyacrylamide Gel Electrophoresis (SDS-PAGE). SDS-PAGE was conducted according to the method of Laemmli and Favre.[27] After electrophoresis, the gel was stained with Coomassie Brilliant Blue. The molecular mass of the

Received: February 19, 2011
Revised: August 7, 2011
Accepted: August 10, 2011
Published: August 10, 2011

ACS Publications © 2011 American Chemical Society

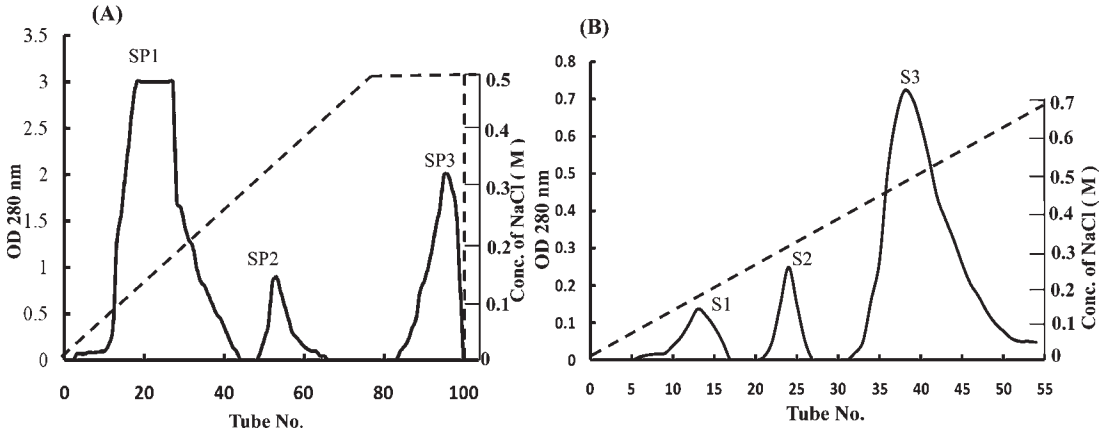

Figure 1. Profile with absorbance (OD 280 nm) following (A) cation exchange chromatography of the proteins (300 mL sample) extracted from red cabbage seeds and unabsorbed on Q-Sepharose (conditions: column, SP-Sepharose (2.5 × 30 cm); equilibration and elution buffer, 20 mM NH_4OAc (pH 4.5); fraction size, 5.5 mL; flow rate, 0.5 mL/min; antifungal activity was detected in peak SP3 (fractions 82−100) and (B) FPLC cation exchange chromatography of peak SP3 (conditions: sample, 99 mL; column, Mono S HR 5/5 (1 mL volume); equilibration and elution buffer, 20 mM NH_4OAc (pH 4.5); fraction size, 0.8 mL; flow rate, 0.4 mL/min; antifungal, antibacterial, and anti-*Candida albicans* activities resided in peak S2 (fractions 22−26). The dotted line represents the concentration gradient of NaCl in the elution buffer, which was linear for the Mono S run (from 0 to 0.7 M) and for the SP-Sepharose run (from 0 to 0.5 M) from fraction 0 to fraction 75 followed by a steady state elution (0.5 M) from fraction 76 to fraction 100.

isolated antifungal protein was determined by comparison of its electrophoretic mobility with those of molecular mass marker proteins from GE Healthcare including phosphorylase *b* (94 kDa), bovine serum albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20 kDa), and α-lactalbumin (14.4 kDa).

N-Terminal Amino Acid Sequence Analysis. The N-terminal amino acid sequence of the purified protein was performed by Edman degradation using a Hewlett-Packard amino acid sequencer. [17]

Assay of Antifungal Activity. The assay for antifungal activity against *Mycosphaerella arachidicola* was performed in triplicate using 100 × 15 mm Petri plates containing 10 mL of potato dextrose agar. After the mycelial colony had developed, sterile blank paper disks (0.625 cm in diameter) were placed around and at a distance of 1 cm away from the rim of the mycelial colony. An aliquot (8 μL containing 0.5 or 1 μg) of the purified protein in 20 mM phosphate-buffered saline (pH 6.0) was introduced to a disk. The plates were incubated at 23 °C for 72 h until mycelial growth had enveloped peripheral disks containing the control (buffer) and had produced crescents of inhibition around disks containing samples with antifungal activity.[18]

To determine the IC_{50} value for the antifungal activity of the isolated antifungal protein, five doses (2.5, 5, 10, 20, and 40 μM) of the protein were added separately to three aliquots each containing 4 mL of potato dextrose agar at 45 °C, mixed rapidly, and poured into three separate small Petri dishes. After the agar had cooled, a small amount of mycelia, the same amount to each dish, was added. Buffer without antifungal protein served as a control. After incubation at 23 °C for 72 h, the area of the mycelial colony was measured and the inhibition of fungal growth determined from the following equation: % inhibition of growth = % reduction in area of mycelial colony = (area of mycelial colony in the absence of antifungal protein − area in the presence of antifungal protein) ÷ area in the absence of antifungal protein × 100%. The concentration of the isolated antifungal protein that brought about 50% reduction in the area of mycelial colony is the IC_{50}.[17]

The tests for themostability and pH stability were performed as previously described.[6,7]

Assay of Permeabilization of Hyphal Membrane. This assay was performed by observing the uptake of Sytox Green (Invitrogen),

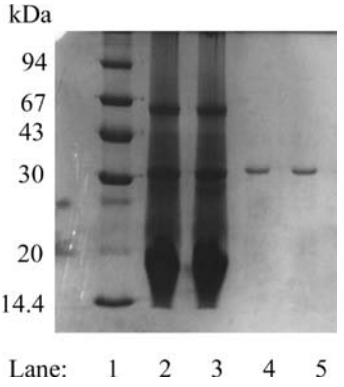

Figure 2. SDS-PAGE pattern. Molecular markers obtained from GE Healthcare (lane 1), including, from the highest to the lowest MW, phosphorylase *b*, bovine serum albumin, ovoalbumin, carbonic anhydrase, soybean trypsin inhibitor, and α-lactalbumin, respectively. Lanes 2 and 3 show the peak SP3 with antifungal activity (pool of fractions 84−100) obtained from red cabbage seed extract, which was most strongly absorbed on SP-Sepharose; lanes 4 and 5 were loaded with 10 μg of protein from peak S2 with antifungal activity (pool of fractions 22−26) absorbed on Mono S.

a high-affinity nuclear stain that penetrates cells with compromised membranes, as described by Thevissen et al.[24] Briefly, yeast (*Candida albicans*) cultures were grown in the presence or in the absence of antifungal protein. Sytox Green was added to the yeast cultures (at various final concentrations up to 0.5 μM). After incubation for 10 min, yeast cells were observed under a fluorescence microscope (Nikon TE2000). Purple pole bean defensin was used as a positive control.

Assay of Chitin Deposition at Hyphal Tips. This assay was conducted as described by Moreno et al.[25] to observe chitin deposition at hyphal tips of *M. arachidicola*. Pregermination and incubation of fungal cultures were performed in 96-well microplates. Following incubation with the antifungal protein for 12 h, Congo Red was added until a final concentration of 1 mM was reached. Staining with Congo Red was allowed to proceed for 5 min with agitation in the dark.

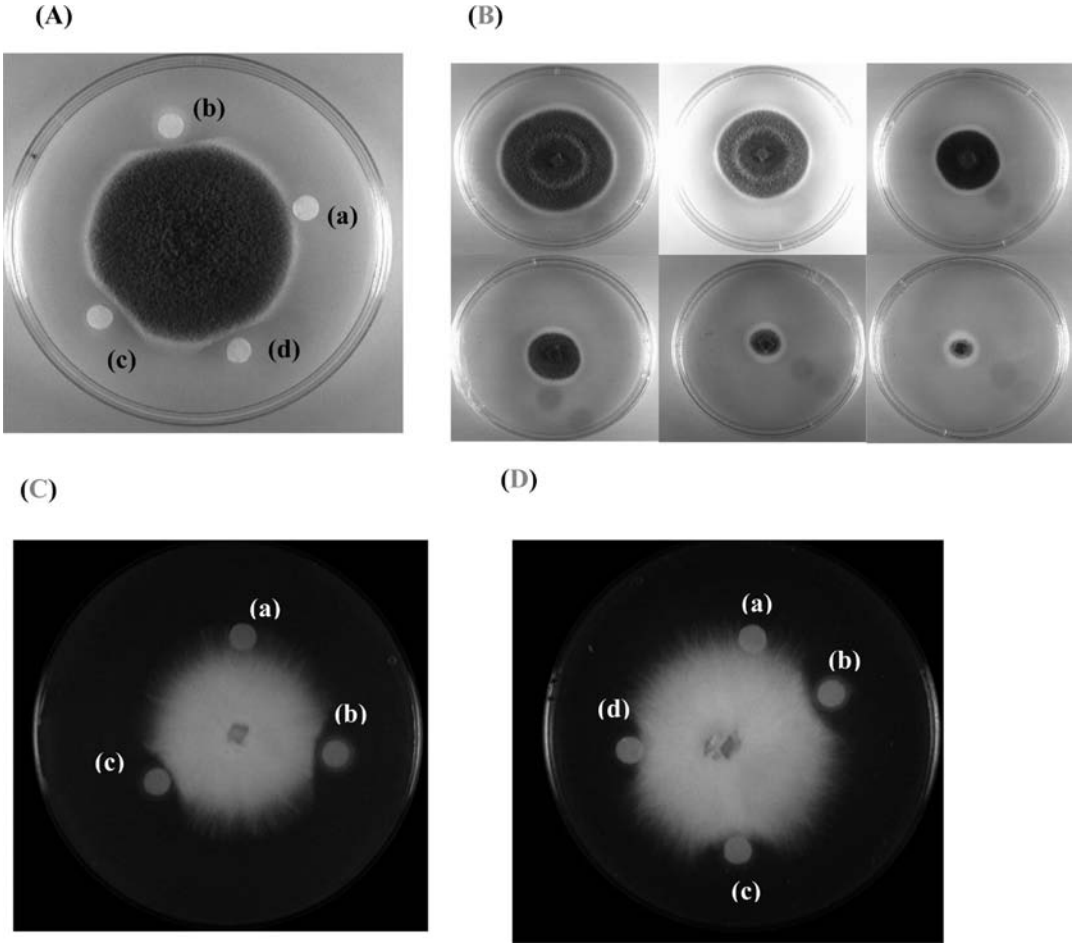

Figure 3. (A) Inhibitory activity of isolated antifungal protein (fraction S2) toward *Mycosphaerella arachidicola*: (a) 0 μg, (b) 0.5 μg, (c) 2.5 μg, or (d) 1 μg antifungal protein was dissolved in 10 μL of 20 mM Tris-HCl buffer (pH 7.2) and applied on a paper disk. (B) Determination of IC_{50} value of antifungal activity of isolated antifungal protein toward *M. arachidicola*: 0 (control), 2.5, or 5 μM and 10, 20, or 40 μM (from upper left to right and from lower left to right, respectively) IC_{50} value was determined to be 5 μM (upper right). (C) Inhibitory activity of isolated antifungal protein (fraction S2) toward *Setospaeria turcica*. (D) Inhibitory activity of isolated antifungal protein (fraction S2) toward *Bipolaris maydis*.

Fluorescence was examined 10 min later by confocal microscopy using a Nikonte 2000 microscope with an excitation wavelength of 543 nm and an emission wavelength of 560−635 nm. The tips of growing hyphae are not stained by Congo Red, whereas hyphal tips with inhibited growth are stained. Purple pole bean defensin[26] was used as a positive control.

Assay of Antifungal Activity on *C. albicans*. *C. albicans* was incubated in 10 mL of potato dextrose agar in a thermal shaker for 6 h at 37 °C, and then 5 mL of this yeast suspension was transferred to 50 mL of potato dextrose agar and incubated for another 6 h to shift yeast growth to the midlogarithmic phase. The yeast suspension was then centrifuged at 2000*g* for 10 min, and the yeast pellet was washed and resuspended in normal saline. A total of 10^7 yeasts/mL was obtained by dilution guided by the optical density at 595 nm. The experiment was conducted in triplicate. The samples were then incubated in a shaker for 6 h, and aliquots were obtained, serially diluted with potato dextrose agar, and spread on agar plates. After 24 h of incubation at 37 °C, the colonies were counted. The number of yeasts for each dilution was determined from the average colony counts for three plates.

Assay of Antibacterial Activity. The assay was performed using various bacteria, including *Bacillus cereus*, *Bacillus megaterium*, *Bacillus subtilis*, *Proteus vulgaris*, *Mycobacterium phlei*, and *Pseudomonas aeruginosa*. Each of the bacterial species was inoculated into a 100 mL flask containing 25 mL of the LB medium and cultured at 37 °C with shaking at 200 rpm for 24 h. Bacterial growth was measured from the linear relationship between bacterial concentration and absorbance at 610 nm (bacterial suspension dilluted to $OD_{610} < 0.4$) measured using a spectrophotometer. IC_{50} is the concentration of isolated antifungal protein required to bring about 50% inhibition of bacterial growth. Precent of inhibition is calculated from the following equation: (OD_{610} in the absence of isolated antifungal protein − OD_{610} in the presence of isolated antifungal protein) ÷ OD_{610} in the absence of isolated antifungal proteint × 100%).

Assay of Antiproliferative Activity on Tumor Cell Lines. The assay was carried out in view of previous reports that some antifungal proteins have this activity.[9,14] Nasoharyngeal cancer NE-1 cell line and hepatoma HepG2 cell line were suspended in RPMI medium (GIVCO) and the cell density adjusted to 2×10^4 cells/mL. A 100 μL aliquot of this cell suspension was seeded to a well of a 96-well plate, followed by incubation for 24 h. Different concentrations of the antifungal protein in 100 μL complete RPMI medium were then added to the wells and incubated for 48 h. After 48 h, 20 μL of a 5 mg/mL solution of [3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide] (MTT) in phosphate buffered saline was spiked into each well and the plates were incubated for 4 h. The plates were then centrifuged at 324*g* for 5 min. The supernatant was carefully removed and 150 μL of dimethyl sulfoxide was added in each well to dissolve the

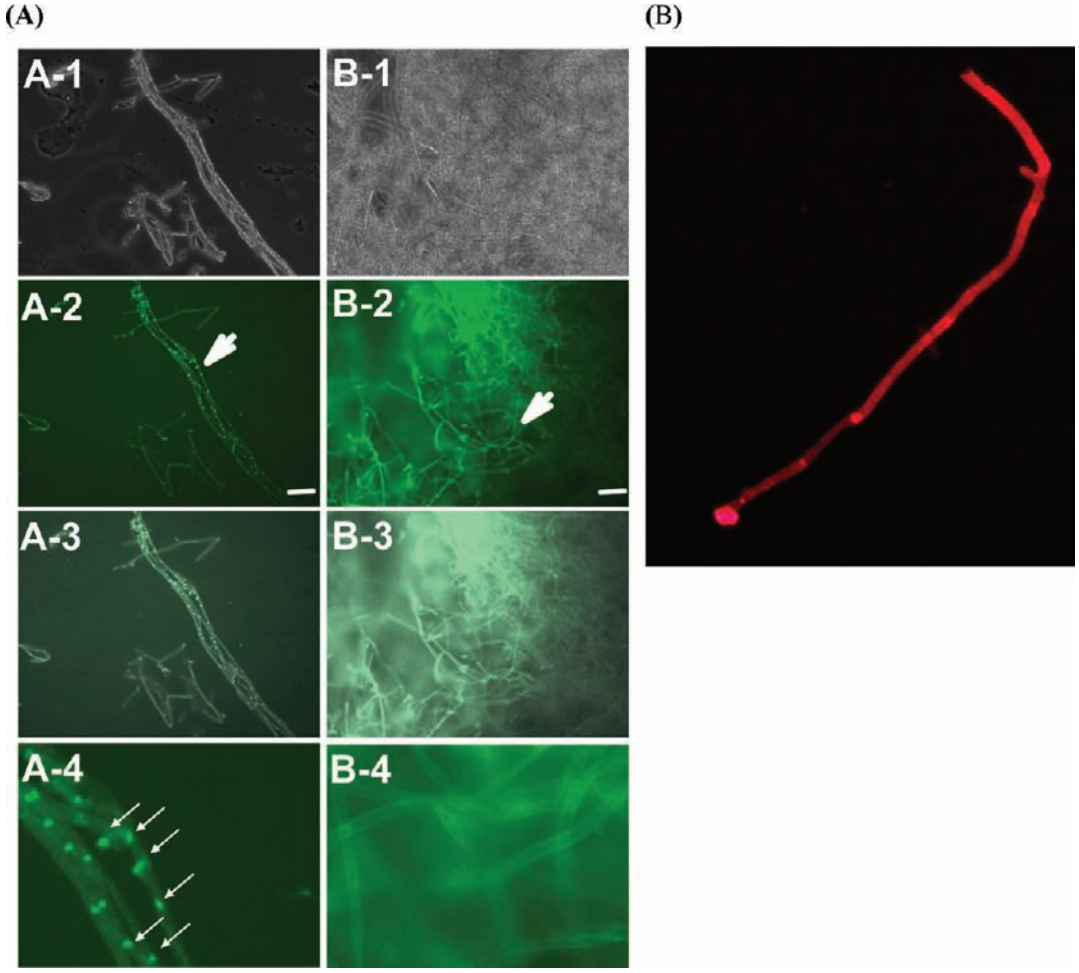

Figure 4. (A) Permeabilization of *Mycosphaerella arachidicola* hyphal membrane caused by the purified antifungal protein (Sytox Green uptake assay): A, treatment; B, negative control; 1, bright field; 2, green fluorescence; 3, merged; 4, 5-fold amplification of a random location indicated by bold arrows (method in an analogous manner, stained with 1 μM Sytox Green. (B) Inhibitory activity of purified antifungal protein (20 μM) on *M. arachidicola* by inducing chitin deposition (Congo Red uptake assay).

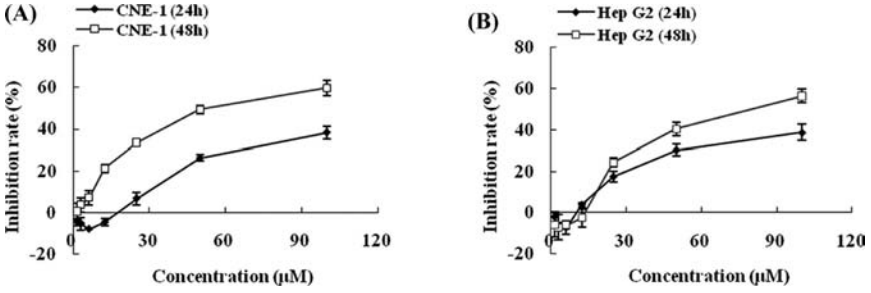

Figure 5. Antiproliferative activity on (A) nasopharyngeal cancer NE-1 cells and on (B) hepatoma HepG2 cells after incubation for 24 and 48 h with 0, 30, 60, 90, and 120 μM of the antifungal protein extracted from red cabbage seeds. Results are the mean ± SD (n = 3).

MTT-formazan at the bottom of the wells. After 10 min, the absorbance at 590 nm was measured by using a microplate reader.

RESULTS AND DISCUSSION

The aqueous extract of red cabbage seeds was first fractionated on Q-Sepharose. Antifungal activity resided in the peak unadsorbed on Q-Sepharose. The adsorbed peak was devoid of antifungal activity. The unadsorbed peak was then resolved by chromatography on SP-Sepharose to yield an unadsorbed peak and three adsorbed peaks (corresponding to fractions 9−41, 47−62, and 84−100, respectively) (Figure 1A). Antifungal activity was detected only in the most strongly adsorbed peak, SP3 (pool of fractions 84−100). Peak SP3 was subjected to final purification on an FPLC-Mono S column to yield peaks S1, S2, and S3 (pools of fractions 9−16, 22−26, and 31−51, respectively) (Figure 1B). The second peak, S2, from the Mono S column displayed a single 30 kDa band in SDS-PAGE (Figure 2). It inhibited mycelial growth in the fungus *M. arachidicola*

(Figure 3A). The IC_{50} value of its antifungal activity toward *M. arachidicola* was 5 μM (Figure 3B). It also manifested antifungal activity toward *Setospaeria turcica* (Figure 3C) and *Bipolaris maydis* (Figure 3D). In the assay of Sytox Green uptake, 20 μM isolated antifungal protein could induce membrane permeabilization when viewed under a fluorescence microscope (Figure 4A). The hyphal tips of *M. arachidicola* were stained by Congo Red after treatment with 20 μM isolated antifungal protein, indicating inhibition of growth (Figure 4B). The antifungal activity of the protein was fully preserved within the temperature range 0−65 °C and within the pH range from 3 to 11. The activity was reduced to half at 80 °C and completely destroyed at 100 °C. At pH 2 and 12 the activity was halved, and at pH 1 and 14 the activity was undetectable.

The antifungal protein inhibited proliferation of NE-1 cells (nasopharyngeal cancer) (Figure 5A) and Hep G2 (hepatoma) cells (Figure 5B) with IC_{50} values of 50 and 90 μM, respectively. It also displayed an inhibitory action on the yeast *Candida albicans* with IC_{50} of 96 μM and manifested antibacterial activity against *Pseudomonas aeruginosa* with an IC_{50} of 53 μM (data not shown). There was no antibacterial activity toward *B. cereus*, *B. megaterium*, *B. subtilis*, *Proteus vulgaris*, and *Mycobacterium phlei* when the *Brassica* antifungal protein was tested up to 170 μM. The yield of the antifungal protein was 2.1 mg from 82 g of seeds (Table 1). The protein did not resemble other antifungul proteins in N-terminal sequence (Table 2).

A comparison of antifungal proteins from various *Brassica* species is presented in Table.3. It can be seen that they display a variety of N-terminal sequences (Table 2) and molecular masses (Table 3), although they exhibit similar chromatographic behaviors and antifungal potencies.

The isolated antifungal protein has a molecular mass of 30 kDa, much larger than those of antifungal proteins from *B. juncea* (18.9 kDa), *B. campestris* (9.4 kDa), *B. alboglabra* (5.9 kDa), and *B. parachinensis* (5.7 kDa). Its N-terminal amino acid sequence is also distinctly different from those of its other *Brassica* counterparts.[3,5−7,9] These results suggest that the isolated antifungal protein is structurally different from the other *Brassica* antifungal proteins isolated to date. Its N-terminal sequence also differs from other non-*Brassica* antifungal proteins reported in the literature.[10,14,17−19] Its molecular mass, however, is within the range (from a few kilodaltons to >60 kDa) observed for other antifungal proteins. It can be isolated by using a protocol composed of ion exchange chromatography and gel filtration, which has been utilized for purifying other antifungal proteins. It is unadsorbed on the anion exchanger Q-Sepharose and adsorbed on the cation exchangers SP-Sepharose and Mono S. Hence, it resembles other antifungal proteins in chromatographic behavior.[3,5−7,9,17,19]

The potency of the antifungal activity of the isolated protein toward *M. arachidicola* (IC_{50} = 5 μM) is similar to the potencies (IC_{50} = 2.4−10 μM) of other *Brassica* antifungal proteins. However, its antiproliferative potency toward HepG2 cells (IC_{50} = 90 μM) is weaker than those of other *Brassica* antifungal proteins (IC_{50} = 2.7−9.2 μM).[3,5−9] It resembles antifungal peptides from *B. aboglabra*[7] and *B. parachinensis*[6] in pH stabbility, albeit slightly attenuated in thermostability.

The isoalted protein exhibits antibacterial activity toward *Pseudomonas* sp. However, lipid transfer protein from *B. campestris*[8,9] has no inhibitory activity toward *B. subtilis*. The other *Brassica* antifungal proteins[5−7] have not been tested for antibacterial activity. The isolated protein manifests anti-*Candida* activity, which has not been shown for other *Brassica* antifungal proteins. The mechanism of antifungal activity of the isolated protein has not previously been demonstrated for other *Brassica* antifungal proteins.[3,5−9] When taken together, the findings indicate that seeds of different *Brassica* species produce different antifungal proteins. This is reminiscent of the observation that different mushroom species may produce structurally different antifungal proteins, ribosome-inactivating proteins and lectins.[16,17]

Some types of antifungal proteins such as defensins[26] and thaumatin-like proteins[22,23] are highly homologous in amino acid sequence, even when proteins from different genera are compared. On the contrary, antifungal proteins from different *Brassica* species display (for example, refs 1−6 and as concluded in this study) dissimilar N-terminal sequences and molecular masses, indicating that they are distinct proteins. Despite these differences in characteristics, they demonstrate antifungal potencies. The different characteristics of these proteins may lead to different modes of action or interference at different levels of pathogenesis.

In summary, the isolated antifungal protein from seeds of red cabbage is distinct from previously reported *Brassica* antifungal proteins and peptides. It is effective against *C. albicans* and *P. aeruginosa*.

Table 1. Yields and Activities of Chromatographic Fractions Obtained during Purification of Antifungal Protein from *Brassica oleracea* Seeds

chromatographic fraction	total protein yield from 82 g of seeds (mg)	IC_{50} of antifungal activity toward *Mycosphaerella arachidicola* (mg/mL)
extract	2630	93
unadsorbed on Q-Sepharose	720	not tested
SP3	23	2.1
S2	2.1	0.2 (=5 μM)

Table 2. N-Terminal Sequence of *Brassica oleracea* (Red Cabbage) Antifungal Protein in Comparison with Antifungal Proteins from Other *Brassica* Species (Results of BLAST Search)

	amino acid residue no.	N-terminal sequence	amino acid residue no.	ref
B. oleracea antifungal protein	1	RQGPTTGPTRKATGL	15	this study
B. campestris antifungal peptide	1	ALSCGTVSGNLAACAGYV	18	6
B. juncea antifungal protein	1	GVEVTRELRSERPSGKIVTI	20	2
B. alboglabra antifungal peptide	1	PEGPFQGPKATKPGDLAXQTWGGWXGQTPKY	31	4
B. parachinensis antifungal peptide	1	DQFPQEYPGDVQFSFN	16	3

Table 3. Comparison of Characteristics of Isolated Antifungal Protein with Antifungal Proteins from Other *Brassica* Species

antifungal protein	mol wt (Da)	chromatographic characteristics	antifungal activity (IC_{50})	antiproliferative activity (IC_{50})	ref
B. oleracea antifungel protein	30000	Q-Sepharose, unadsorbed; Affi-gel blue gel, adsorbed; SP-Sepharose, adsorbed	*M. arachidicola* (5 μM), *C. albicans*(96 μM)	Hep G2 cells (90 μM)	this study
B. alboglabra antifungal protein	5907	Affi-gel blue gel, adsorbed; SP-Sepharose, adsorbed; Mono S, adsorbed	*F. oxyspum* (1.3 μM)	Hep G2 cells (2.7 μM)	4
			H. maydis (2.1 μM)	MCF7 cells (3.4 μM)	
			M. arachidicola (2.4 μM)		
			V. mali (0.15 μM), stable at 20−80 °C for 10 min and at pH 2−3 and 10−11 for 30 min		
B. campestris nonspecific lipid transfer protein	9412	Q-Sepharose, unadsorbed; Affi-gel blue gel, adsorbed; Mono S, adsorbed	*F. oxysporum* (8.3 μM)	not tested	5
			M. arachidicola (4.5 μM)		
B. juncea antifungal protein	189000	Q-Sepharose, unadsorbed; Affi-gel blue gel, adsorbed; SP-Sepharose, adsorbed	*F. oxysporum* (13.5 μM)	Hep G2 cells (5.6 μM)	2
			H. maydis (2.7 μM)	MCF7 cells (6.4 μM)	
			M. arachidicola (10 μM)		
Brassica parachinensis antifungal protein	5716	Affi-gel blue gel, adsorbed; SP-Sepharose, adsorbed; Mono S, adsorbed	*F. oxysporum* (3.6 μM)	Hep G2 cells (9.2 μM)	3
			H. maydis (4.7 μM)	MCF7 cells (4.8 μM)	
			M. arachidicola (2.6 μM)		
			V. mali (0.22 μM), stable at 4−100 °C and pH 1−3 and 10−13		

AUTHOR INFORMATION

Corresponding Author

*(X.-J.Y.) E-mail: xiujuanye2004@gmail.com. Fax: +852-2603-5123. Phone: +852-2609-6872. (T.-B.N.) E-mail: b021770@mailserv.cuhk.edu.hk. Fax: +852-2603-5123. Phone: +852-2609-6872. (Y.-BZ.) E-mail: ybzhang@hku.hk. Fax: +852-2872-5476. Phone: +852-2588-0436.

Funding Sources

The award of a grant (Research Fund for the Control of Infections Diseases, Grant 09080432) from the Food and Health Bureau, Government of Hong Kong Special Administrative Region, is gratefully acknowledged.

REFERENCES

(1) Riahi, R. R.; Cohen, P. R. Voriconazole-associated phototoxicity. *Dermatol. Online J.* **2011**, *17*, 15.

(2) Wong, J. H.; Hao, J.; Cao, Z.; Qiao, M.; Xu, H.; Bai, Y.; Ng, T. B. An antifungal protein from *Bacillus amyloliquefaciens*. *J. Appl. Microbiol.* **2008**, *105*, 1888–1898.

(3) Lin, P.; Wong, J. H.; Xia, L.; Ng, T. B. Campesin, a thermostable antifungal peptide with highly potent antipathogenic activities. *J. Biosci. Bioeng.* **2009**, *108*, 259–265.

(4) Kondori, N.; Baltzer, L.; Dolphin, G. T.; Mattsby-Baltzer, I. Fungicidal activity of human lactoferrin-derived peptides based on the antimicrobial $\alpha\beta$ region. *Int. J. Antimicrob. Agents* **2011**, *37*, 51–57.

(5) Ye, X.; Ng, T. B. Isolation and characterization of juncin, an antifungal protein from seeds of Japanese takana (*Brassica juncea* var. integrifolia). *J. Agric. Food Chem.* **2009**, *57*, 4366–4371.

(6) Lin, P.; Ng, T. B. Brassiparin, an antifungal peptide from *Brassica parachinensis* seeds. *J. Appl. Microbiol.* **2009**, *106*, 554–563.

(7) Lin, P.; Ng, T. B. A novel and exploitable antifungal peptide from kale (*Brassica alboglabra*) seeds. *Peptides* **2008**, *29*, 1664–1671.

(8) Lin, P.; Ng, T. B. First isolation of an antifungal lipid transfer peptide from seeds of a *Brassica* species. *Peptides* **2007**, *28*, 1514–1522.

(9) Lin, P.; Xia, L.; Wong, J. H.; Ng, T. B.; Ye, X.; Wang, S.; Shi, X. Lipid transfer proteins from *Brassica campestris* and mung bean surpass mung bean chitinase in exploitability. *J. Pept. Sci.* **2007**, *13*, 642–648.

(10) Wong, J. H.; Ng, T. B. Vulgarinin, a broad-spectrum antifungal peptide from haricot beans (*Phaseolus vulgaris*). *Int. J. Biochem. Cell Biol.* **2005**, *439*, 91–98.

(11) De Lucca, A. J.; Cleveland, T. E.; Wedge, D. E. Plant-derived antifungal proteins and peptides. *Can. J. Microbiol.* **2005**, *51*, 1001–1014.

(12) Joshi, B. N.; Sainani, M. N.; Bastawade, K. B.; Gupta, V. S.; Ranjekar, P. K. Cysteine protease inhibitor from pearl millet: a new class of antifungal protein. *Biochem. Biophys. Res. Commun.* **1998**, *246*, 382–387.

(13) Broekaert, W. F.; Van Parijs, J.; Leyns, F.; Joos, H.; Peumans, W. J. A chitin-binding lectin from stinging nettle rhizomes with antifungal properties. *Science* **1989**, *245*, 1100–1102.

(14) Wong, J. H.; Ng, T. B. Gymnin, a potent defensin-like antifungal peptide from the Yunnan bean *Gymnocladus chinensis* Baill. *Peptides* **2003**, *24*, 963–968.

(15) Huang, X.; Xie, W. J.; Gong, Z. Z. Characterization and antifungal activity of a chitin binding protein from *Ginkgo biloba*. *FEBS Lett.* **2000**, *478*, 123–126.

(16) Grenier, J.; Potvin, C.; Asselin, A. Some fungi express β-1, 3-glucanases similar to thaumatin-like proteins. *Mycologia* **2000**, *92*, 841–848.

(17) Lam, S. K.; Ng, T. B. First simultaneous isolation of a ribosome inactivating protein and an antifungal protein from a mushroom (*Lyophyllum shimeji*) together with evidence for synergism of their antifungal effects. *Arch. Biochem. Biophys.* **2001**, *393*, 271–280.

(18) Wang, H. X.; Ng, T. B. Ginkbilobin, a novel antifungal protein from *Ginkgo biloba* seeds with sequence similarity to embryo-abundant protein. *Biochem. Biophys. Res. Commun.* **2000**, *279*, 407–411.

(19) Ye, X. Y.; Ng, T. B. Isolation of a novel peroxidase from French bean legumes and first demonstration of antifungal activity of a non-milk peroxidase. *Life Sci.* **2002**, *71*, 1667–1680.

(20) Gozia, Q.; Ciopraga, J.; Bentia, T.; Lungu, M.; Zamfirescu, I.; Tuder, R. Antifungal properties of lectin and new chitinase from potato tuber. *FEBS Lett.* **1995**, *370*, 245–249.

(21) Chilosi, G.; Caruso, C.; Caporale, C.; Leonardi, L.; Bertini, L.; Buzi, A.; Nobile, M. Antifungal activity of a Bowman−Birk type trypsin inhibitor from wheat kernel. *J. Phytopathol.* **2000**, *148*, 477–481.

(22) Wang, H.; Ng, T. B. Isolation of an antifungal thaumatinlike protein from kiwi fruits. *Phytochemistry* **2002**, *61*, 1–6.

(23) 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.

(24) Thevissen, K.; Ghazi, A.; De Samblanx, G. W.; Brownlee, C.; Osborn, R. W.; Broekaert, W. F. Fungal membrane responses induced by plant defensins and thionins. *J. Biol. Chem.* **1996**, *271*, 15018–15025.

(25) Moreno, A. B.; Martinez Del Pozo, A.; San Segundo, B. Biotechnologically relevant enzymes and proteins. Antifungal mechanism of the *Aspergillus giganteus* AFP against the rice blast fungus *Magnaporthe grisea*. *Appl. Microbiol. Biotechnol.* **2006**, *72*, 883–895.

(26) Lin, P.; Wong, J. H.; Ng, T. B. A defensin with highly potent antipathogenic activities from the seeds of purple pole bean. *Biosci. Rep.* **2009**, *30*, 101–109.

(27) Laemmli, U. K.; Favre, M. Gel electrophoresis of proteins. *J. Mol. Biol.* **1973**, *80*, 575–599.