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Identification, characterization and functional analysis of a GH-18 chitinase from *Streptomyces roseolus*

Xiayun Jiang[a,1], Daochun Chen[a,1], Shenle Hong[a], Weifen Wang[a], Shunshen Chen[a], Shuming Zou[b,*]

[a] College of Food Science and Technology, Shanghai Ocean University, Shanghai 201306, China
[b] Key Laboratory of Freshwater Aquatic Genetic Resources, Shanghai Ocean University, Ministry of Agriculture, Shanghai 201306, China

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ABSTRACT

A 40 kDa chitinase from *Streptomyces roseolus* DH was purified to homogeneity from culture medium. The N-terminal sequence was TPPPAKAVKLGYFTNWGVYG, which was highly homologous to the glycoside hydrolase (GH) 18 conserved domain of *Streptomyces* chitinases and included the two crucial Trp and Tyr sites. The purified enzyme showed maximal activity at 60 °C, pH 6.0 and exhibited good thermal and pH stabilities. The enzyme displayed strict substrate specificity on colloidal or glycol chitin, but not on chitosan derivatives. It was activated by Mg^{2+}, Ba^{2+} and Ca^{2+}, and inhibited by Cu^{2+}, Co^{2+}, Mn^{2+}, whereas Zn^{2+} and ethylenediamine tetraacetic acid showed little inhibitory effects. Morphological changes observed by scanning electron microscopy revealed the occurrence of regular pores on the surface with the progress of enzymatic chitinolysis. Additionally, this GH-18 chitinase had a marked inhibitory effect on fungal hyphal extensions. In conclusion, this chitinase may have great potential for the enzymatic degradation of chitin.

1. Introduction

Chitin, a linear β-1,4-linked insoluble homopolymer of *N*-acetyl-D-glucosamine (GlcNAc), comprises the major source of amino sugars in soils and is the second most abundant polysaccharide in nature after cellulose. In marine invertebrates, the annual worldwide chitin biomass reaches 3.7×10^4 metric tonnes (Mukherjee & Sen, 2006). Chitinases (EC 3.2.1.14) catalyze the glycosidic bonds in chitin and are distributed widely in diverse organisms that include bacteria, fungi, plants, insects and animals, which perform various functions dependent upon their own origins. Bacterial chitinases are associated primarily with the digestion and degradation of chitin as carbon and nitrogen nutrient in soils, and fungal chitinases are required for morphogenesis during cell wall growth. Human and plant chitinases are involved in the host defensive response against infection by phytopathogenic fungi (Li & Greene, 2010; Kezuka et al., 2006; Seidl, 2008). Thus, prokaryotic chitinases have been of special interest for the preparation and bioconversion of chitinous materials from processing the waste of marine invertebrates (Bhattacharya, Nagpure, & Gupta, 2007; Muzzarelli et al., 2011).

According to the Carbohydrate-Active Enzymes database (http://www.cazy.org/), various chitinases are classified into glycoside hydrolase (GH) families either the GH-18 or GH-19 (Cantarel et al., 2009). The two families differ in terms of their amino acid sequence, three-dimensional structure and molecular mechanisms of catalytic reactions due to their different evolutionary sources (Ohno et al., 1996; van Aalten et al., 2001). Contrary to the original GH-18 chitinases, bacterial GH-19 chitinases are more likely to originate from plants by horizontal gene transfer (Watanabe et al., 1999). The GH-18 chitinases may participate in the assimilation of chitin, while GH-19 chitinases are involved in defensive systems, such as antifungal activity (Kawase et al., 2006). Many chitinolytic bacteria produce only GH-18 chitinases, while some bacteria, such as *Streptomyces* species, produce both GH-18 and -19 chitinases (Watanabe et al., 2003). Therefore, *Streptomyces* microorganisms are proposed to be a good source for chitinase fermentation and the degradation of chitin.

Streptomyces, the largest genus of Actinobacteria, comprises at least 500 species that are known as saprophytic bacteria and is a good source of chitinase suitable for the degradation of chitin (Christodoulou, Duffner, & Vorgias, 2001). *Streptomyces* spp. degrade chitin with several chitinases that act synergistically (Seidl, 2008). The multiple chitinase genes have been cloned in several *Streptomyces* strains (Christodoulou et al., 2001; Saito, Fujii, & Miyashita, 2003; Yano et al., 2008a). For example, *Streptomyces*

* Corresponding author at: Key Laboratory of Freshwater Aquatic Genetic Resources, Shanghai Ocean University, Huchenghuan Road 999, Shanghai 201306, China. Tel.: +86 21 61900435.
E-mail address: smzou@shou.edu.cn (S. Zou).
[1] These authors contributed equally to this work.

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coelicolor A3 possesses 13 chitinase genes that encode 11 GH-18 chitinases and two GH-19 chitinases in its genome (see Kawase et al., 2006). For decades, chitinases have been purified successfully from just a small number of species of *Streptomyces* (Han, Yang, Zhang, Miao, & Li, 2009; Kim, Yang, & Kim, 2003; Mukherjee & Sen, 2006; Ohno et al., 1996; Okazaki et al., 1995; Radwan, Plattner, Menge, & Diekmann, 1994; Yano et al., 2008b). Therefore, more effective chitinases are still available to be explored because of the high multiplicity of chitinases in *Streptomyces*. Furthermore, only a few wild-type GH-18 chitinases, which are considered to be the main contributors in chitin degradation, have been fully investigated in terms of their enzymatic characteristics and functions, as well as family identification. At the present stage, this approach is still one of the best ways to identify chitinases that are produced in large amounts from microorganisms from nature. Here, we purified, identified and characterized a new GH-18 chitinase induced from the culture medium of a chitin-degrading strain of *Streptomyces roseolus*. Also, important functional properties with respect to antifungal activity and hydrolysis behavior toward insoluble substrate were investigated.

2. Materials and methods

2.1. Materials

Chitin powder was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Colloidal chitin was prepared by the method of Rodriguez-Kabana, Godoy, Morgan-Jones, and Shelby (1983). Glycol chitosan was product of Sigma (USA) and glycol chitin was prepared according to that described by Trudel and Asselin (1989). Columns of HiPrep 16/10 Q XL and Superdex™ 75 HR 10/30 were purchased from GE Healthcare. Amicon Ultra-4 (10 kD) was obtained from Millipore (USA). All other reagents used were of the highest grade available.

2.2. Enzyme purification

The strain *S. roseolus* DH was isolated originally and screened from the shrimp shell-rich soils near the East China Sea in Shanghai, China (Wei, Jiang, Chen, Chen, & Dang, 2011). One hundred milliliters of the culture medium (1.0% chitin, 0.05% KH_2PO_4, 0.05% NaC1, 0.05% $MgSO_4{\cdot}7H_2O$, 0.001% $FeSO_4{\cdot}7H_2O$, 0.4% NH_4NO_3, pH 7.0) in a 250-ml Erlenmeyer flask was cultured under aerobic conditions at 28 °C for 3 days on a rotary shaker (135 rpm). The supernatants were spun in a centrifuge at 12,000 × *g* for 20 min (4 °C), and then collected for ammonium sulfate precipitation. The precipitates were formed, kept overnight and collected by centrifugation at 12,000 × g for 20 min (4 °C). They were then dissolved in a small amount of 50 mM Tris–HCl buffer (pH 8.5) and dialyzed against the same buffer. The dialysate was loaded onto a HiPrep Q XL column equilibrated with 50 mM Tris–HCl buffer (pH 8.5). The column was washed with the same buffer and eluted with a linear gradient of buffer that contained 0–1 M NaCl at a flow rate of 1 ml/min. Fractions of 2.5 ml were collected. The fractions with high chitinase activity were pooled and concentrated by ultrafiltration (Amicon Ultra-4). The concentrates were used for a further gel filtration on Superdex™ 75 HR column at a flow rate of 1 ml/min. Fractions of 1.5 ml were collected. The chitinase-active fractions were obtained and combined for subsequent determination.

2.3. Enzyme and protein assay

Chitinase activity was measured with colloidal chitin as substrate. Enzyme solution (0.5 ml) was added to 1.5 ml of substrate solution that contained 0.3% colloidal chitin in sodium acetate buffer (50 mM, pH 6.0). The mixtures were incubated at 50 °C for 60 min and the reaction was terminated by heating in boiling water for 10 min. After centrifugation, the amount of reducing sugar produced in the supernatant was determined by the method of Imoto and Yagishita (1971). One unit (U) of chitinase activity was defined as the amount of enzyme that liberated reducing sugars that corresponded to 1 μmol of *N*-acetyl-glucosamine per minute.

Protein concentration was determined by the method of Bradford (1976) and bovine serum albumin was used as the standard. After column chromatography, the protein content in collected fractions was estimated by measuring the absorbance of the fraction spectrophotometrically at 280 nm.

2.4. Electrophoresis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed in accordance with the method of Laemmli (1970) that used 15% (w/v) polyacrylamide resolving gels and 5% (w/v) polyacrylamide stacking gels.

2.5. Effects of temperature and pH on enzyme activity and stability

The optimum temperature for chitinase activity was examined by incubation of the purified enzymes with the substrates at temperatures that ranged from 30 to 80 °C under standard assay conditions. Thermal stability was determined by preincubation of the enzymes without substrates in sodium acetate buffer (pH 6.0) for 30 min at temperatures from 30 to 80 °C. Then the remaining activity was measured under standard conditions.

The optimum pH of chitinase was investigated as for the enzyme assays except that the pH of reaction buffers ranged from 3.0 to 10.0, and used 50 mM of each buffer: glycine–HCl (pH 3.0), acetate (pH 4.0–6.0), potassium phosphate (pH 7.0–8.0) and glycine–NaOH (pH 9.0–10.0). The enzymes were preincubated at various pH buffers that used the above systems at 4 °C for 30 min without substrates and then subjected to enzyme assays under standard conditions to determine the pH stability. All measurements were repeated at least three times.

2.6. Effect of various chemicals and substrates on chitinase activity

The effects of various chemicals on chitinase activities were investigated by addition of 10 mmol/l of different reagents in reaction mixtures and preincubation of the mixtures for 30 min at 4 °C. The following solutions were used in the assay: Mn^{2+} ($MnCl_2$), Cu^{2+} ($CuSO_4$), Mg^{2+} ($MgCl_2$), Ba^{2+} ($BaCl_2$), Co^{2+} ($CoCl_2$), Ca^{2+} ($CaCl_2$), Zn^{2+} ($ZnSO_4$) and ethylenediamine tetraacetic acid (EDTA). The residual activities were then measured under standard assay conditions. Colloidal chitin, glycol chitin and its analogues (colloidal chitosan, glycol chitosan and carboxylmethyl cellulose) were used as substrates and the corresponding enzyme activities were assayed under the standard conditions.

2.7. N-terminal amino acid sequences of chitinase

Purified chitinase was subjected to 15% SDS-PAGE, after which the protein was electro-blotted using polyvinylidene fluoride (PVDF) membrane (Millipore, USA). The membrane was stained with Coomassie Brilliant Blue R-250 and destained with methanol. N-terminal amino acid sequences were analyzed by stepwise Edman degradation in PROCISE™ 494cLC protein sequencer (Applied Biosystems) in Shanghai Genecore Biotechnologies Co., Ltd. (Shanghai, China).

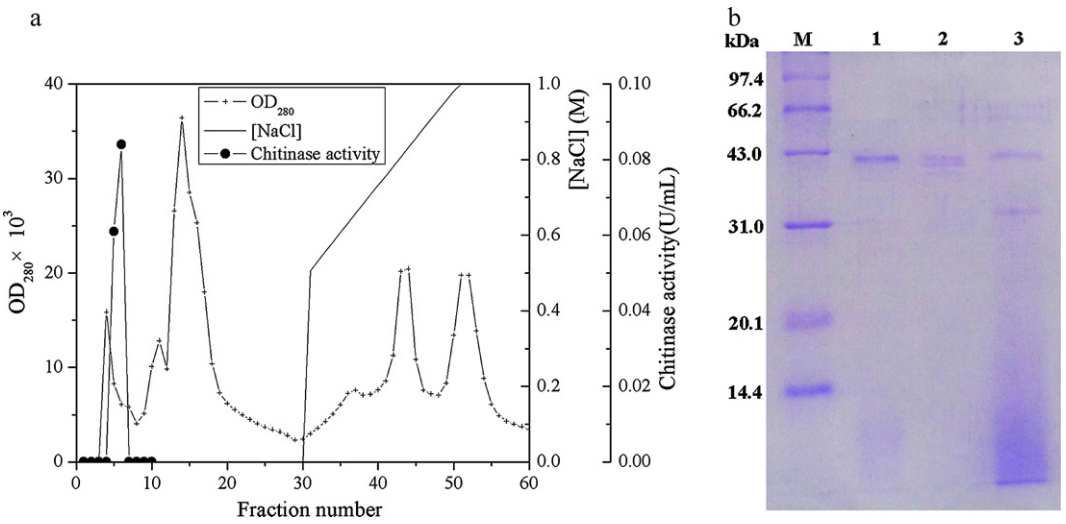

Fig. 1. Purification of chitinase from culture medium of *Streptomyces roseolus*. (a) Elution profile of chitinase on a HiPrep Q XL column. (b) SDS-PAGE analysis in each step of chitinase purification. M, low molecular weight standards. Lane 3, ammonium sulfate precipitation. Lane 2, chitinase-active fractions collected from HiPrep Q XL column. Lane 1, chitinase-active fractions collected from Superdex G-75 HR column.

2.8. Enzymatic hydrolysis of chitinase on insoluble chitin polymer

Scanning electron microscopy (SEM) was used for morphological observation of chitinase hydrolysis of chitin polymer. Chitin powders were incubated with chitinases for 0 h (control), 1 h or 2 h individually. The mixtures were then centrifuged at 10,000 × *g* for 5 min and the precipitates were rinsed in phosphate buffer (pH 7.0), dehydrolyzed by ethanol and then freeze dried. The control and chitinase-treated samples were sputter-coated with a thin layer of gold, then observed and photographed in an analytical field emission SEM JSM-7500 (JEOL, Japan).

2.9. Antifungal activity of chitinase

Cup-Plate assays were applied to test the antifungal activity of chitinase through the inhibition of fungal spore germination. Four fungi (*Aspergillus* spp., *Rhizopus chinensis*, *Penicillium* spp. and *Mucor* spp.) that grew in agar slants were washed with sterile H_2O and then obtained spore suspensions. The spore fluids (100 μl, about 10^7 spores/ml) was mixed with the 100 μl of purified enzymes in 50 mM sodium phosphate buffer (pH 6.0), while the control conditions were the same amount of spore fluids with the same buffers. These sample mixtures were added to the stainless steel cylinders placed on the potato dextrose agar (PDA) plates. The plates then were incubated at 28 °C for 2 days and inhibition of hyphal extension was evaluated by visual inspection.

3. Results and discussion

3.1. Purification and identification of chitinase from S. roseolus

S. roseolus was cultured in a minimal liquid medium with the addition of powdered chitin as the sole carbon source, which was induced to produce both chitinase and chitosanase (data not shown). Crude enzymes from 72 h-cultured medium were concentrated by 100% ammonium sulfate precipitation. The precipitate was then applied onto an anion-exchange chromatograph column. As shown in Fig. 1, chitinase-active fractions did not bind to the column and were eluted directly without NaCl (Fig. 1a). Further fractionation by size-exclusion filtration was carried out and the enzymes were shown by SDS-PAGE to be almost homogeneous. The molecular weight was estimated about 40 kDa and the enzyme was designated as Chi40 (Fig. 1b). A typical purification protocol is summarized in Table 1. The chitinase enzyme was purified by 23-fold with an overall yield of 34% and the specific activity of 30 U/mg. *S. roseolus* is generally considered as a good source for chitinase compared with other fungal strains (Lee, Chung, Wi, Lee, & Bae, 2009).

The N-terminal sequence of Chi40 from *S. roseolus* was determined by Edman degradation to be TPPPAKAVKLGYFTNWGVYG (Fig. 2). The Chi40 sequence was compared with that of other proteins using the BLASTP 2.2.25 program (Altschul et al., 1997) in the non-redundant protein database at NCBI. Results suggested that it was highly homologous at the C-terminal catalytic domain to a series of chitinases from *Streptomyces* species. Thus, Chi40 might be generated by proteolytic removal of N-terminus of the chitin-binding II and/or fibronectin type III domains from chitinase precursor genes. A multiple sequence alignment of N-terminal amino acid residues of Chi40 and corresponding conserved domains was constructed and it was confirmed as a member of the GH-18 family (Fig. 2). In addition, two conserved aromatic residues that corresponded to Trp249 and Tyr 252 in chitinase C from *S. coelicolor* (Kawase et al., 2006) were also found. These residues were considered as essential only for hydrolysis of crystalline chitin (Watanabe et al., 2003). Moreover, it could be speculated that Chi40 belongs to GH-18 subfamily A, as its sequence clustered with chitinase C of *S. lividans*, chitinase C of *S. coelicolor*, chitinase III of *S. griseus* and chitinase from *S. thermoviolaceus* (Fig. 2), which are classified as members of subfamily A by phylogenetic analysis (Saito et al., 2003). These results reasonably led us to predict that Chi40 would have high activity toward crystalline chitin.

3.2. Effect of temperature and pH on chitinase activity

The effect of temperatures on the activity of Chi40 was investigated under standard assay conditions, except that the incubation temperatures ranged from 30 to 80 °C. The purified enzyme maintained more than 60% activity at 30–70 °C and showed its highest activity at 60 °C (Fig. 3a). Its temperature profile was more like a GH-18 chitinase C, a subfamily A chitinase from *S. coelicolor*, than that of a GH-19 chitinase F (Kawase et al., 2006). After preincubation for 30 min, more than 80% residual activities were still retained at 60 °C

Table 1
Summary of purification of chitinase from culture medium of *Streptomyces roseolus* DH.

Step	Total protein (mg)	Total activity (U)	Specific activity (U/mg)	Purification fold	Yield (%)
Culture supernatant	43.5	57.0	1.3	1	100
$(NH_4)_2SO_4$ precipitation	28.1	39.3	1.4	1.1	69
HiPrep Q XL	2.9	32.0	11	8.5	56
Ultrafiltration	2.3	30.4	13	10	53
Superdex G-75	0.65	19.2	30	23	34

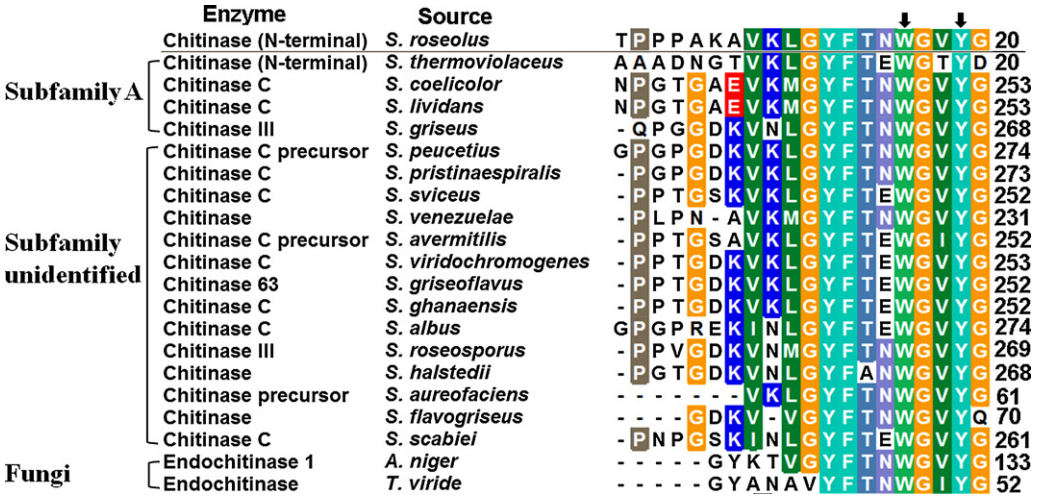

Fig. 2. Multiple sequence alignment of the purified enzyme and other chitinases from *Streptomyces* or fungus species in the N-terminal amino acid residues of GH-18 chitinase. Identical amino acid residues are shaded according to the color table. The dark arrow indicates the conserved aromatic amino acid residues for enzymatic catalyzation. The protein sequences used here for comparison and their GenBank accession numbers were *S. thermoviolaceus* N-terminal chitinase (AAB25383.1), *S. coelicolor* chitinase C (NP_629515.1), *S. lividans* chitinase C (ZP_05523602.1), *S. griseus* chitinase III (BAB86377.1), *S. peucetius* chitinase C precursor (AAF43629.1), *S. pristinaespiralis* chitinase C (ZP_06909435.1), *S. sviceus* chitinase C (ZP_06919803.1), *S. venezuelae* chitinase (CCA58314.1), *S. avermitilis* chitinase C precursor (NP_824054.1), *S. viridochromogenes* chitinase C (ZP_07306522.1), *S. griseoflavus* chitinase 63 (ZP_07310707.1), *S. ghanaensis* chitinase C (ZP_04685832.1), *S. albus* chitinase C (ZP_06593565.1), *S. roseosporus* chitinase III (ZP_04696241.1), *S. halstedii* chitinase (BAF49409.1), *S. aureofaciens* chitinase precursor (BAC66179.1), *S. flavogriseus* chitinase (320011991), *S. scabiei* chitinase C (YP_003488531.1), *Aspergillus niger* endochitinase 1 (XP_001401833.2) and *Trichoderma viride* endochitinase (AAF19619.1).

(Fig. 3a). These characteristics of optimum temperature and thermal stability were superior to those of bacteria and fungus species (usually 20–40 °C) and were equivalent to a few thermostable chitinases (Bhushan, 2000; Christodoulou et al., 2001; Dai, Hu, Huang, & Li, 2011).

The effect of pH on enzyme activity and stability was examined in accordance with the similar methods used to study the effect of temperatures. The optimum pH for Chi40 was 6.0 (Fig. 3b). The enzyme showed almost the same level of activity at a broadly neutral pH from 6.0 to 8.0, these finding were quite similar to the results from 49 kD-chitinase from *S. griseus* (Tanabe, Kawase, Watanabe, Uchida, & Mitsutomi, 2000) and 66 kD-chitinase from *S. venezuelae* P_{10} (Mukherjee & Sen, 2006). In contrast, Chi40 also presented good pH stability, as more than 90% of relative activity was maintained after preincubation at pH 6.0–8.0 for 30 min (Fig. 3b). Taken together, we concluded that the temperature and pH stability of

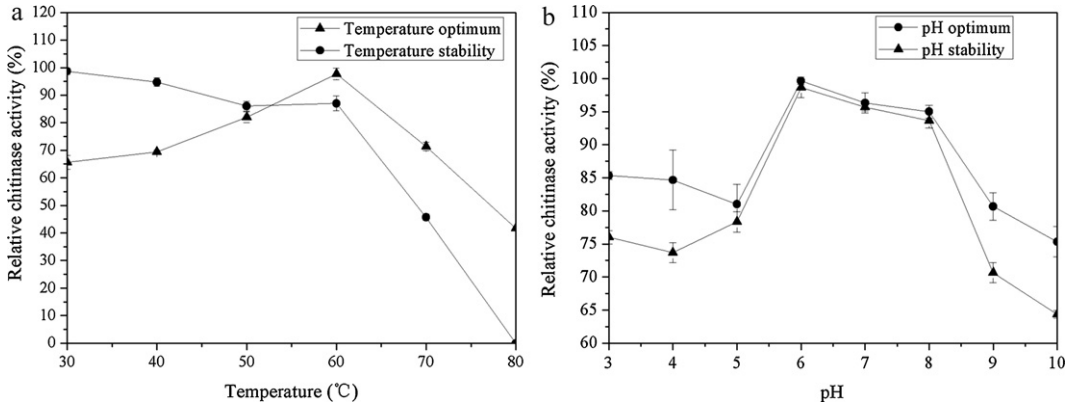

Fig. 3. Effects of temperature and pH on the relative activity and stability of the purified chitinase. (a) The optimum temperature (solid triangles) was measured in sodium acetate (pH 6.0) at 30–80 °C. Thermal stability (dark dots) was determined under the standard assays after preincubation of the enzymes in sodium acetate (pH 6.0) at 30–80 °C for 30 min, and the residual activities were determined. The activities at 60 °C are shown as 100%. (b) The optimum pH (dark dots) was measured under the standard assays at 50 °C in various buffer systems: glycine–HCl (pH 3.0), acetate (pH 4.0–6.0), potassium phosphate (pH 7.0–8.0), glycine–NaOH (pH 9.0–10.0), respectively. The pH stability (solid triangles) was determined after preincubation of the enzymes in various buffers as above, at 4 °C for 30 min, and the residual activities were determined. The activity at pH 6.0 is indicated as 100%.

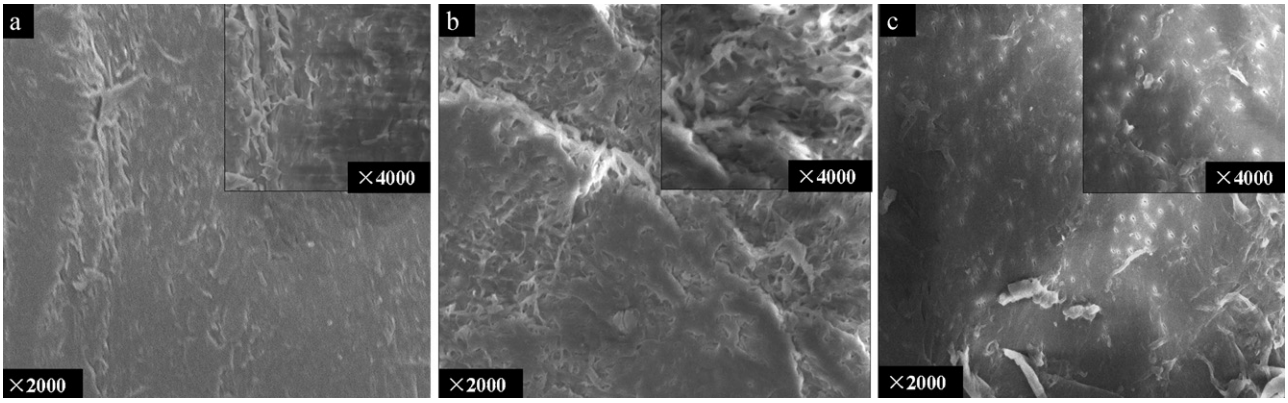

Fig. 4. Scanning electron microscopy photomicrographs of chitin powder treated with the purified chitinases for different times. (a) Control. (b) 1 h. (c) 2 h.

Table 2
Effect of substrate specificity on chitinase activity.

Substrate	Relative chitinase activity[a] (%)
Colloidal chitin	100
Glycol chitin	88
Colloidal chitosan	0
Glycol chitosan	0
CMC	0

[a] The activity with colloidal chitosan as substrate was set as 100%.

Chi40 would make it useful for the future separation of enzyme products.

3.3. Effect of ions and substrates on chitinase activity

The activity of Chi40 against various chitinous derivatives were analyzed to determine substrate specificity further. Chi40 exhibited its highest activity toward colloidal chitin and decomposed glycol chitin at 88% of the relative activity (Table 2). However, it could not hydrolyze colloidal chitosan, glycol chitosan and carboxymethylchitosan (CMC). That is, it could hydrolyze GlcNAc–GlcNAc, but not GlcN–GlcN linkages, which indicated its strict specificity compared with *S. griseus* (Tanabe et al., 2000). Extensive studies are needed to clarify whether or not Chi40 is able to cleave GlcNAc–GlcN/GlcN–GlcNAc bonds. As shown in Table 3, Chi40 purified from *S. roseolus* was activated significantly by Mg^{2+}, Ba^{2+} and Ca^{2+} and inhibited strongly by Cu^{2+}, Co^{2+} and Mn^{2+}. Other chemicals, Zn^{2+} and EDTA, had almost no effect on the enzymatic activity of Chi40.

Table 3
Effects of various chemicals on chitinase activity.

Chemical (10 mM)	Relative chitinase activity[a] (%)
None	100
Cu^{2+}	54
Mn^{2+}	76
Ca^{2+}	119
Ba^{2+}	133
Mg^{2+}	135
Zn^{2+}	92
Co^{2+}	60
EDTA	96

[a] The relative activity was expressed as the percentage ratio of the specific activity of the enzyme after treatment with various chemicals compared to no chemical treatment.

3.4. Enzymatic hydrolysis of chitin polymers

GH-18 chitinases are thought to have extremely high activity toward crystalline substrates, while family GH-19 chitinases are mainly active toward soluble substrates (Kawase et al., 2006). To clarify this property of the enzyme, its morphology was observation by SEM to reveal any changes in surface properties when crystalline chitin powder was treated with chitinase. The surface of untreated chitin powder appeared to be smooth, in which the original pores appeared as irregular in shape (Fig. 4a). During the course of the chitinolytic reaction, the general occurrence of regular pores (almost circular in shape) was observed (Fig. 4b), which may have resulted from the action of enzymatic hydrolysis. That is, the enzyme acted by random attack and formed pores on the surface of chitinous polymer. The number of pores increased markedly after a further 2 h of treatment (Fig. 4c). This phenomenon is consistent with the only report of chitinase from *Penicillium* spp. LYG 0704, except that lack of changes in crackers, as it was hard to distinguish the original and newly formed ones by observational methods during the degradation of the polysaccharide chains (Lee et al., 2009).

3.5. Antifungal activity of chitinase

The antifungal activity of Chi40 was tested for an inhibitory effect on hyphal extension of fungal colonies formed on PDA plates. When the spores were mixed with the purified enzymes, fungal hyphae of *Aspergillus* spp. (Fig. 5a), *R. chinensis* (Fig. 5b) and *Mucor* spp. (Fig. 5c) grew with little extension of hyphae from the centre of steel cylinders on the plates. In contrast, the spores mixed with buffers germinated normally and the hyphae grew around the cylinders. Chi40 can inhibit slightly the growth of the *Penicillium* spp., which may be caused by the slow growth of this *Penicillium* strain (Fig. 5d). Bacterial chitinases, referred to GH-18 family members, are thought to be involved mainly in digestion of chitin for nutritional purposes, and are reported not to be effective with respect to antifungal activity (Kawase et al., 2006; Watanabe et al., 1999). However, our results demonstrated that the GH-18 chitinase from *Streptomyces* spp. had notable antifungal ability. The antifungal feature of Chi40 raised the question if the antifungal ability in GH-18 protein was unique to this stain or was common in the *Streptomyces* genus. More detailed study is necessary to elucidate this point. To date, the antifungal activity of GH-18 has only been reported

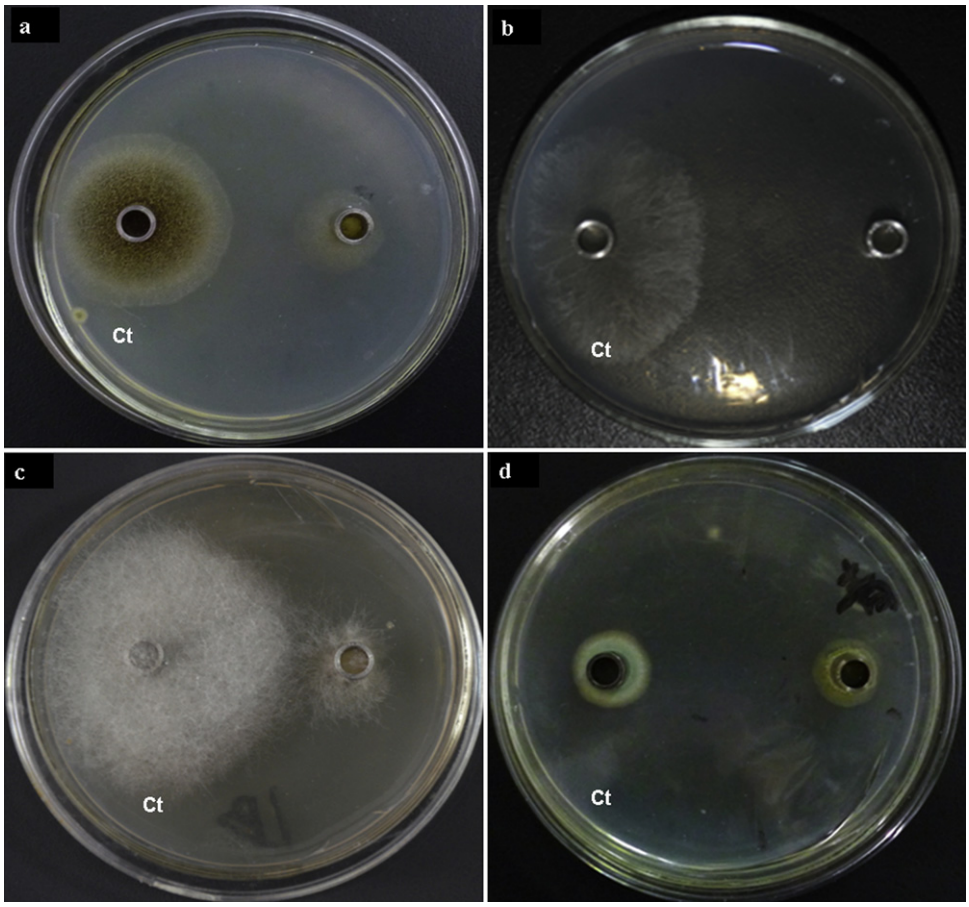

Fig. 5. Antifungal activity of the purified chitinase on the spores of four species of fungi. In each plate, the right side is the test group (chitinases in the buffer mixed with spore suspension) and the left side is the control group (the same buffer mixed with spore suspension). (a) *Aspergillus* spp. (b) *Rhizopus chinensis.* (c) *Mucor* spp. (d) *Penicillium* spp.

previously in a *Bacillus* bacterium (Xiao, Xie, Cai, Lin, & Chen, 2009).

4. Conclusion

A 40 kDa chitinase was purified from culture medium of *S. roseolus.* The enzyme showed good potential for future use with respect to thermal and pH stability, chitinolytic properties and antifungal activity. The chitinase was shown to be a member of the GH-18 family, confirmed by N-terminal Edman sequencing and BLASTP search at the NCBI database. The optimum temperature and pH for chitinase activity was 60 °C and 6.0, respectively. This enzyme displayed good thermal and pH stability compared with other chitinases. The enzyme displayed strict substrate specificity on colloidal or glycol chitin, but not on chitosan derivatives. It was activated by Ba^{2+}, Ca^{2+} and Mg^{2+}, and inhibited by Cu^{2+}, Mn^{2+}, Co^{2+} ions, whereas the addition of Zn^{2+} and EDTA had little inhibitory effects. This GH-18 40 kDa chitinase displayed functions of enzymatic degradation of crystalline chitin, as seen by morphological observations of the polymer surfaces. Furthermore, its novel function of the inhibition of fungal growth was demonstrated.

Acknowledgments

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