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Carbohydrate Research 343 (2008) 1171–1179
Carbohydrate RESEARCH

Purification and characterization of chitinases and chitosanases from a new species strain *Pseudomonas* sp. TKU015 using shrimp shells as a substrate

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Received 10 January 2008; received in revised form 4 March 2008; accepted 11 March 2008
Available online 15 March 2008

Abstract—A chitinase (CHT1) and a chitosanase (CHS1) were purified from the culture supernatant of *Pseudomonas* sp. TKU015 with shrimp shell wastes as the sole carbon and nitrogen source. The optimized conditions of this new species strain (Gen Bank Accession Number EU103629) for the production of chitinases were found to be when the culture was shaken at 30 °C for 3 days in 100 mL of medium (pH 8) containing 0.5% shrimp shell powder (SSP) (w/v), 0.1% K_2HPO_4, and 0.05% $MgSO_4{\cdot}7H_2O$. The molecular weights of CHT1 and CHS1 determined by SDS–PAGE were approximately 68 kDa and 30 kDa, respectively. The optimum pH, optimum temperature, pH stability, and the thermal stability of CHT1 and CHS1 were pH 6, 50 °C, pH 5–7, <50 °C and pH 4, 50 °C, pH 3–9, <50 °C, respectively. CHT1 was inhibited completely by Mn^{2+} and Fe^{2+}, and CHS1 was inhibited by Mn^{2+}, Cu^{2+}, and PMSF. CHT1 was only specific to chitin substrates, whereas the relative activity of CHS1 increased when the degree of deacetylation of soluble chitosan increased.

Keywords: *Pseudomonas*; Chitinase; Chitosanase; Shrimp shell wastes

1. Introduction

The production of chitinolytic enzymes in higher plants may be part of their defense mechanism against infection by fungal pathogens.[1] In addition, bacteria may produce chitinolytic enzymes for the assimilation of chitinous materials such as carbon and nitrogen sources.[2] It is suggested that chitinolytic microorganisms or chitinolytic enzymes have potential applications in the biocontrol of plant pathogenic fungi and insects, as a target for biopesticides, and in many other biotechnological areas.[1,2]

The oligosaccharides of chitin/chitosan, prepared by hydrolyzing chitin/chitosan with chitinase/chitosanase, have various potential applications in the fields of food, agricultural, and pharmaceutical industry.[2] Many chitinases/chitosanases derived from different organisms have been purified and their genes analyzed. Almost all of the chitinase/chitosanase-producing strains will use chitin/chitosan (or colloidal chitin/chitosan) as a major carbon source.[2] However, the preparation of chitin involves demineralization and deproteinization of shellfish waste with the use of strong acids or bases.[3,4] The utilization of shellfish waste not only solves environmental problems but also decreases the production costs of microbial chitinases. The production of inexpensive chitinolytic enzymes is an important element in the process.[3,5–7]

The bioconversion of chitinous materials has been proposed as a waste treatment alternative to the disposal of shellfish wastes.[3,4,7] To further enhance the utilization of chitin-containing marine crustacean waste, we have recently investigated the bioconversion of shellfish chitin wastes for the production of chitinases and/or

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doi:10.1016/j.carres.2008.03.018

proteases.[3] In this study, shrimp shell powder, which was prepared by boiling and crushing shellfish processing waste, was used as a substrate for isolating and screening chitinase-producing strains. A strain was isolated and identified as *Pseudomonas* sp. In addition, the chitinase and chitosanase from *Pseudomonas* sp. TKU015 were also purified, characterized, and compared with chitinases and chitosanases isolated from other bacterial sources.

2. Materials and methods

2.1. Materials

The squid pen powder (SPP), shrimp shell powder (SSP), shrimp and crab shell powder (SCSP) used in these experiments were prepared as described earlier.[3] Squid pens, shrimp shells, and crab shells were purchased from Shin-Ma Frozen Food Co. (I-Lan, Taiwan). Katsuobushi from macherel (KM) and bonito (KB) were purchased from Yi-Fu Food Co. (I-Lan, Taiwan).

In the preparation of the SPP, SSP, and SCSP, the squid pens, shrimp shells, and crab shells were washed thoroughly with tap water, and then dried. The dried materials obtained were milled to powders for the use as the carbon source for protease and chitinase production. Soluble chitosan (94% deacetylated) was purchased from Chitosan Biotech Co. (Taipei, Taiwan). Soluble chitosans of 85% deacetylated, 82% deacetylated, and 73% deacetylated were kind gifts from Dr. J.-K. Chen (Taiwanese Petroleum Corp., Chia-Yi, Taiwan). Powdered chitin was purchased from Sigma Chemical Co. (St. Louis, MO). Colloidal chitin was prepared from powdered chitin according to the method of Jeniaux.[7] DEAE-Sepharose CL-6B and Sephacryl S-100 were purchased from Amersham Pharmacia Biotech AB (Uppsala, Sweden). All other reagents used were of the highest grade available.

2.2. Isolation and screening of chitinase-producing strains

Microorganisms isolated from soils collected at different locations in northern Taiwan were screened on agar plates containing 1% SSP, 0.1% K_2HPO_4, 0.05% $MgSO_4{\cdot}7H_2O$, and 1.5% agar powder. The plates were incubated at 30 °C for 2 days. Those organisms obtained from the screening were subcultured in liquid media (containing 1% SSP, 0.1% K_2HPO_4, and 0.05% $MgSO_4{\cdot}7H_2O$) in flasks at 30 °C. After incubation for 2 days, the culture broth was centrifuged (4 °C and 12,000*g* for 20 min) and the supernatants were collected for the measurement of chitinase activity using the procedure described below. The strain TKU015 that showed the highest chitinase and chitosanase activity was isolated, maintained on nutrient agar, and used throughout the study.

2.3. Microorganism and enzyme production

In the investigation of the culture conditions, growth was carried out in a minimal medium containing 0.1% K_2HPO_4 and 0.05% $MgSO_4{\cdot}7H_2O$, and supplemented with 0.5–2% (w/v) of various carbon/nitrogen sources to be investigated. The carbon/nitrogen sources investigated included SPP, SSP, SCSP, KM, and KB. The resultant medium in a 250-mL Erlenmeyer flask was aerobically cultured at 30 °C for 1–4 days on a rotary shaker (150 rpm). After centrifugation (12,000*g*, 4 °C, for 20 min), the supernatants were collected for the measurement of chitinase activity.

2.4. Purification of the enzyme

2.4.1. Production of chitinase. For the production of chitinase, *Pseudomonas* sp. TKU015 was grown in 100 mL of liquid medium in an Erlenmeyer flask (250 mL) containing 0.5% SSP, 0.1% K_2HPO_4, and 0.05% $MgSO_4{\cdot}7H_2O$ (pH 8). The seed culture (1 mL) was transferred into 100 mL of the same medium and grown in an orbital shaking incubator for 3 days at 30 °C and pH 8 (the pH after being autoclaved was 7.5). After incubation, the culture broth was centrifuged (4 °C and 12,000*g* for 20 min), and the supernatant was used for further purification by chromatography.

2.4.2. DEAE-Sepharose CL-6B chromatography. To the culture supernatant (600 mL), ammonium sulfate was added (608 g/L). The resultant mixture was kept at 4 °C overnight and the precipitate formed was collected by centrifugation at 4 °C for 20 min at 12,000*g*. The precipitate was then dissolved in a small amount of 50 mM sodium phosphate buffer (pH 7), and dialyzed against the same buffer. The resultant dialysate (50 mL) was loaded onto a DEAE-Sepharose CL-6B column (5 cm × 16 cm) equilibrated with 50 mM sodium phosphate buffer (pH 7). The chitosanases (appeared in the unadsorbed fractions) were washed from the column with the same eluting buffer, and the chitinases (appeared in the adsorbed fractions) were fractionated with a linear gradient of 0–1 M NaCl in 50 mM phosphate buffer. As shown in Figure 1, the chitinase and chitosanase were effectively separated. The chitinase fractions were combined (250 mL) and concentrated by ammonium sulfate precipitation. The resultant precipitate was collected by centrifugation and dissolved in 50 mM phosphate buffer (pH 7). The obtained concentrated chitinase preparation (4 mL) was used as the sample for further purification by Sephacryl S-100 gel filtration. For the chitosanase, the collected chitosanase fractions (150 mL) were adjusted to contain 1 M ammo-

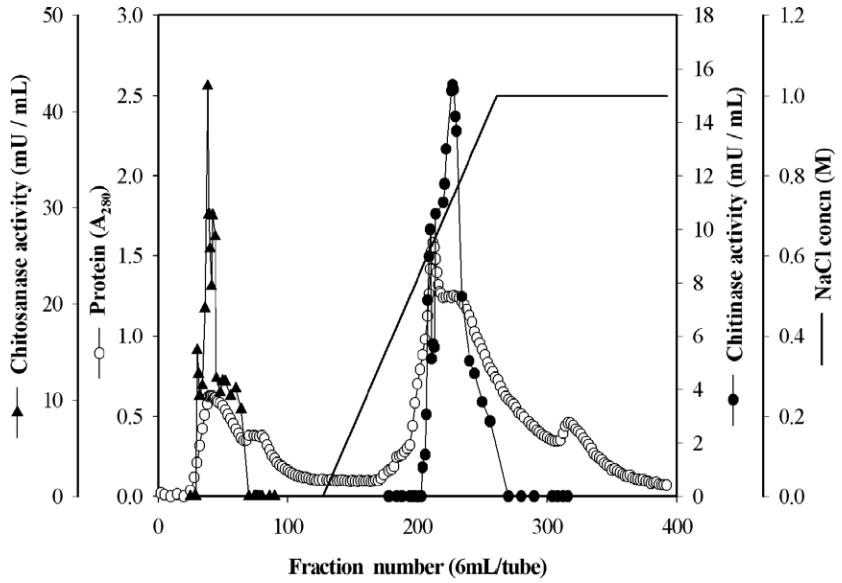

Figure 1. Elution profile of *Pseudomonas* sp. TKU015 chitinase and chitosanase on DEAE-Sepharose CL-6B.

nium sulfate by the addition of ammonium sulfate. The chitosanase preparation containing ammonium sulfate was then used for further purification by Phenyl-Sepharose 6 Fast Flow hydrophobic chromatography.

2.4.3. Phenyl-Sepharose 6 Fast Flow chromatography. The obtained chitosanase solution (100 mL) was then purified by chromatography on a column of Phenyl-Sepharose 6 Fast Flow (1.3 cm × 4.3 cm), which had been equilibrated with phosphate buffer (50 mM, pH 7) containing 1 M ammonium sulfate. The unadsorbed materials were washed from the column with the same ammonium sulfate containing buffer. The adsorbed materials were then fractionated with a linear gradient of 1–0 M ammonium sulfate in 50 mM phosphate buffer (pH 7). As shown in Figure 2, one peak exhibiting chitosanase activity was obtained, combined, and used as purified preparation of chitosanase (CHS1).

2.4.4. Sephacryl S-100 chromatography. The resultant concentrated chitinase preparation (4 mL) as described above was loaded onto a Sephacryl S-100 gel filtration column (1.6 cm × 70 cm), which had been equilibrated with 50 mM sodium phosphate buffer (pH 7), then eluted with the same buffer. One peak exhibiting chitinase activity was obtained, combined, and used as purified preparation of chitinase (CHT1).

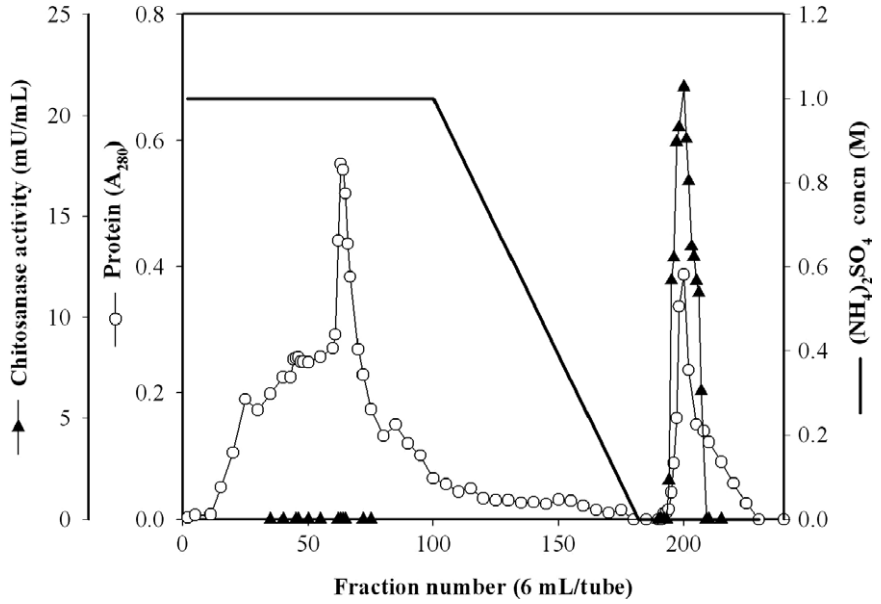

Figure 2. Elution profile of *Pseudomonas* sp. TKU015 chitosanase on Phenyl-Sepharose.

2.5. Protein determination

Protein content was determined by the method of Bradford using Bio-Rad dye reagent concentrate and bovine serum albumin as the standard.[3] After column chromatography, the protein concentration was estimated by measuring the absorbance at 280 nm.

2.6. Measurement of enzyme activity

Colloidal chitin (1.3% in 50 mM phosphate buffer) and soluble chitosan (0.3% in 50 mM phosphate buffer) were used as the substrate for the measurement of chitinase-like and chitosanase-like activities, respectively. The mixture of enzyme solution (0.5 mL) and substrate (1 mL) was incubated at 37 °C for 30 min. After centrifugation, the amount of reducing sugar produced in the supernatant was determined by the method of Imoto and Yagishita with *N*-acetylglucosamine as a reference compound.[7] One unit of enzyme activity was defined as the amount of enzyme that produced 1 μmol of reducing sugars per min.

2.7. Determination of molecular weight

The molecular weight of the purified chitinase and chitosanase were determined by sodium dodecyl sulfate–polyacryamide gel electrophoresis (SDS–PAGE) with a 12.5% polyacrylamide gel at pH 7.0 by the method of Laemmli.[8] The standard proteins used for calibration were phosphorylase b (molecular weight, 97.4 kDa), albumin (66.2 kDa), ovalbumin (45 kDa), carbonic anhydrase (29 kDa), trypsin inhibitor (20.1 kDa), and α-lactabumin (14.4 kDa). Before electrophoresis, equal volume of sample buffer that contained 65 mM Tris–HCl, pH 6.8, 10% (v/v) glycerol, 2% (w/v) SDS, 5% (v/v) 2-mercaptoethanol, and 0.2% (w/v) bromophenol blue was added to the protein sample and exposed overnight prior to loading on the gels. The proteins were separated at constant voltage of 100 V using the running buffer contained 25 mM Tris, 192 mM glycine, and 0.1% (w/v) SDS, pH 8.3. The gels were stained with Coomassie Brilliant Blue R-250 in methanol–acetic acid–water (5:1:5, v/v), and decolorized in 7% acetic acid.

2.8. Effect of pH and temperature on the enzyme activities

The optimum pH of CHT1 and CHS1 was studied by assaying the samples at different pH values. The pH stability of CHT1 and CHS1 was determined by measuring the residual activity at pH 7 as described above after the sample had been dialyzed against a 50 mM buffer solution of various pH values (pH 3–11) in seamless cellulose tubing (Sankyo). The buffer systems used were glycine·HCl (50 mM, pH3), acetate (50 mM, pH 4–5), phosphate (50 mM, pH 6–8), and Na_2CO_3–$NaHCO_3$ (50 mM, pH 9–11). To determine the optimum temperature for CHT1 and CHS1, the activity values of the samples were measured at various temperatures (25–100 °C). The thermal stability of CHT1 and CHS1 was studied by incubating the samples at various temperatures for 30 min. The residual activity was measured as described above.

2.9. Effect of various chemicals

The effect of various chemicals on the enzyme activity was investigated by preincubating the enzyme with chemicals in 50 mM phosphate buffer solution (pH 7) for 10 min at 37 °C followed by measuring the residual chitinase and chitosanase activities.

2.10. Effect of various surfactants

The enzyme solutions (250 μL) were incubated, in the absence or presence of 0.25 mL of surfactant solutions, at 25 °C for 30 min. The residual activities were estimated by the assay procedure described above.

3. Results and discussions

3.1. Identification of the strain TKU015

TKU015 is a gram-negative and nonspore-forming bacillus with catalase, oxidase, and mobility, which grows in both aerobic and anaerobic environments. According to 16S rDNA partial base sequencing, TKU015 is most closely related to the species of *Pseudomonas* with a similarity of more than 97%. On the basis of glucose metabolic patterns, TKU015 is an aerobic organism. Anlaysis of the lipid profile shows that the fatty acids of TKU015 are mainly C16:0 and C18:1ω7c with some hydroxylated fatty acids C10:0 3OH, C12:0 2OH and C12:0 3OH, but without C16:0 2OH or C16:0 3OH. This profile conforms to the description of the *Pseudomonas* species. From these data, we conclude that TKU015 belongs to the *Pseudomonas* species. Further identification by The Bioresource Collection and Research Center (Shin-Chu, Taiwan) found that TKU015 belongs to a new species strain, whose Gen Bank Accession Number is EU487511 (*Pseudomonas* sp. BCRC17752).

3.2. Culture conditions and enzyme production

To study the effect of SSP concentration on the production of chitinase, we found that 0.5% (w/v) of SSP was more suitable for chitinase production than the concentrations of 1%, 1.5%, and 2% (Fig. 3). To investigate the effect of carbon and nitrogen sources on the production

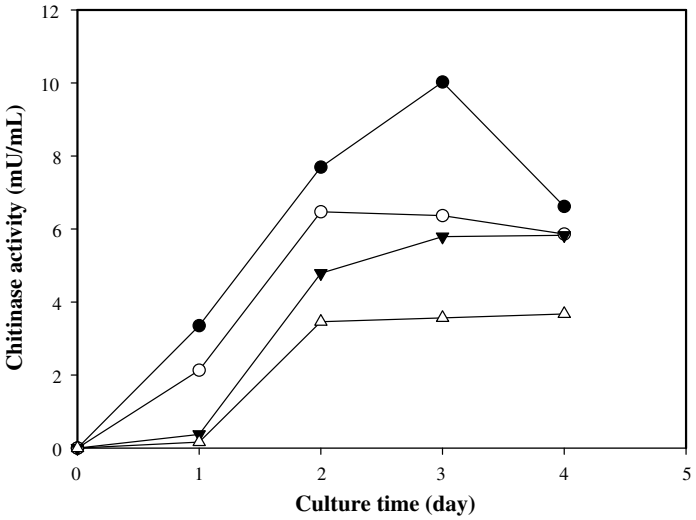

Figure 3. Effect of SSP concentration on the production of TKU015 chitinase. -●-, 0.5%SSP; -○-, 1%SSP; -▼-, 1.5% SSP; -△-, 2% SSP.

of chitinase, growth was carried out in 100 mL of basal medium (0.1% K_2HPO_4 and 0.05% $MgSO_4 \cdot 7H_2O$, pH 7) containing additional carbon/nitrogen sources (0.5%, w/v) of SCSP, SSP, SPP, KM, KB, or chitin, respectively. It was found that SSP (10 mU/mL) was more suitable as an inducer for chitinase production than SPP (7.2 mU/mL), SCSP (2.8 mU/mL), KB (<0.1 mU/mL), KM (<0.1 mU/mL), and chitin (<0.1 mU/mL).

The protein–chitin–mineral salts compositions of the best two inducers (SSP and SPP) were 48:38:14 and 61:38:1, respectively.[2] It is inferred that the carbon and nitrogen sources with the ratio of protein and chitin near 1:1, such as SSP (48:38) and SPP (61:38), were more suitable as the inducers of chitinase production by *Pseudomonas* sp. TKU015 than those with only protein but no chitin (such as KM, KB) or with a high ratio of mineral salts (such as SCSP, 29%/31%/40%).[3] As for SCSP, although their ratio of protein and chitin (29:31) was similar to SSP, the chitinase activity could not be as high as SSP. This might be because the activity was affected by the high mineral salts (40%) in SCSP. Similar results were also found in *Bacillus* sp. TKU004[9] and *B. subtilis* TKU007.[10]

To study the time course of cultivation, 100 mL of the media (0.5% SSP contained basal medium, pH 8) was used, and the relationship between incubation time (1–6 days), chitinase and chitosanase activity was investigated. As shown in Figure 4, maximum activities of chitinase (10 mU/mL) and chitosanase (25 mU/mL) were both found at the third day and then decreased gradually.

3.3. Isolation and purification

The purification of the TKU015 chitinase and chitosanase from the culture supernatant (1000 mL) was described under Section 2. As shown in Table 1, the purification steps were combined to give an overall purification of about 6.0-fold and 6.4-fold for chitinase and chitosanase, respectively. The overall activity yield of the purified chitinase and chitosanase were 11% and 12%, respectively. The purified chitinase (CHT1) and chitosanase (CHS1) were both confirmed to be homogeneous by SDS–PAGE (Fig. 5). The molecular weights of CHT1 and CHS1 were calculated to be 68 kDa and 30 kDa by SDS–PAGE, respectively.

The molecular weights of most microbial chitinases are in the range of 40–65 kDa. The molecular weight of CHT1 (68 kDa) was obviously larger than those of the others. Compared to the other *Pseudomonas* chitinases, it is different from two bifunctional chitinase/lysozymes (30 kDa, 32 kDa) of *P. aeruginosa* K-187[11] and a 58 kDa chitinase of *P. aeruginosa* strain 385.[12] The chitinase of *Pseudomonas* sp. YHS-A2 (67.4 kDa)[13] was the only *Pseudomonas* chitinase that had the similar molecular weight to CHT1 of *Pseudomonas* sp. TKU015. With regard to the chitosanases, there is only one report of a *Pseudomonas* chitosanase, *Pseudomonas* sp. H-14 chitosanase (35 kDa).[14] The molecular weight of CHS1 (30 kDa) was little smaller than the previously reported one.

3.4. Substrate specificity

The activities of CHT1 and CHS1 upon chitin, chitosan, and other polysaccharides are presented in Table 2. The effect of the degree of deacetylation (DD)[15] of chitosan on enzyme activity was studied using chitosan of varying DD as the substrate. CHT1 was specific for colloidal chitin (22.2 mU/mg) and chitin (6.9 mU/mg) but attacked neither soluble chitosan, CM-cellulose, nor xylan. With regard to CHS1, it showed activities

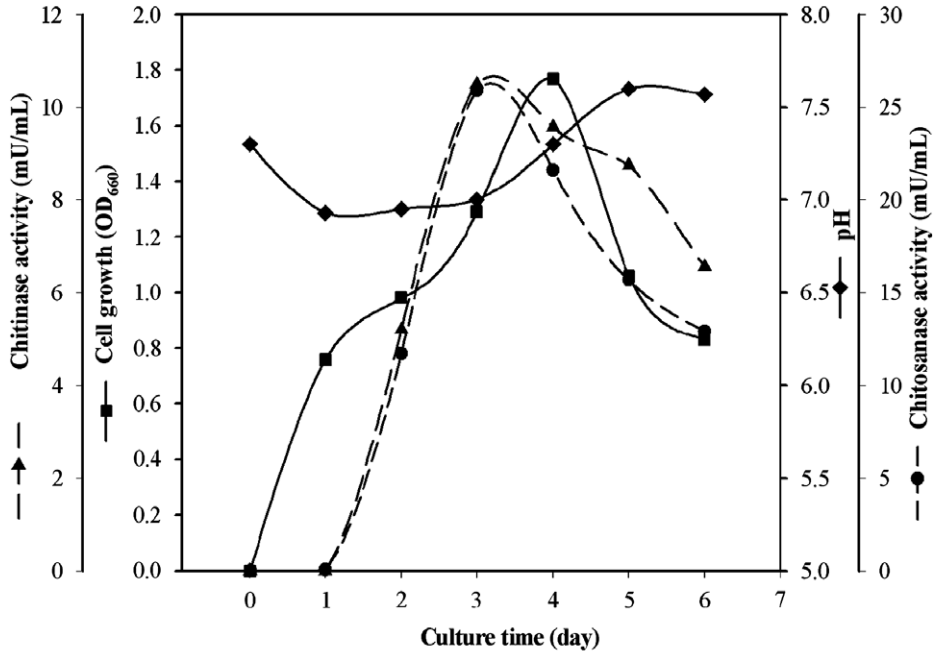

Figure 4. Time courses of chitinase and chitosanase production in a culture of *Pseudomonas* sp. TKU015 on shrimp shell containing media. To reflect the growth of the culture in this medium by the OD660 nm measurement, the residual chitinous materials were allowed to settle out.

Table 1. Purification of CHT1 and CHS1 from *Pseudomonas* sp. TKU015

Step	Total protein (mg)	Total activity (mU)	Spec. activity (mU/mg)	Purif. (fold)	Yield (%)
Chitinase					
Culture sup.	1608	6000	3.7	1	100
$(NH_4)_2SO_4$ ppt.	840	3600	4.3	1.2	60
DEAE-Sepharose	288	2800	9.7	2.6	47
Sephacryl S-100	117	2600	22.2	6.0	43
Chitosanase					
Culture sup.	1608	15,000	9.3	1	100
$(NH_4)_2SO_4$ ppt.	840	8600	10.2	1.1	57
DEAE-Sepharose	130	3000	23.1	2.5	20
Phenyl-Sepharose	32	1890	59.1	6.4	13

toward 94 DD soluble chitosan (59.1 mU/mg), 85 DD soluble chitosan (20.3 mU/mg), and 73 DD soluble chitosan (6.5 mU/mg) but no activities toward 82 DD soluble chitosan, 73 DD soluble chitosan, colloidal chitin, carboxymethyl cellulose, and xylan. Therefore, CHT1 was only specific to colloidal chitin and chitin, whereas for CHS1, its relative activity increased when the DD of soluble chitosan increased. These results indicate that both the physical form and the DD of the substrate affect the rate of hydrolysis.

3.5. Effect of pH and temperature

The pH activity profile of CHT1 and CHS1 showed maximum values at pH 6 and pH 4, respectively. CHT1 and CHS1 were stable at pH 5–7 and pH 3–9, respectively. The optimum temperature for CHT1 and CHS1 were both 50 °C. Both CHT1 and CHS1 maintained their initial activity from 25 to 40 °C and had almost half of their activity at 50 °C but were completely inactivated at 70 °C (Fig. 6).

The optimum pH and pH stability of CHT1 (pH 6, pH 5–7) were different from two bifunctional chitinase/lysozymes (FI, pH 8, pH 6–9; FII, pH 7, pH 5–10) of *P. aeruginosa* K-187[11] and *P. aeruginosa* strain 385 chitinase (pH 6.7, pH 5–10).[12] For CHS1 (pH 4, pH 3–9), which is similar to *Pseudomonas* sp. H-14 chitosanase with optimum pH (pH 4), the pH stability was different (pH 4–7).[14]

CHT1 shows an optimum temperature of 50 °C for catalysis and is stable at <50 °C, comparable

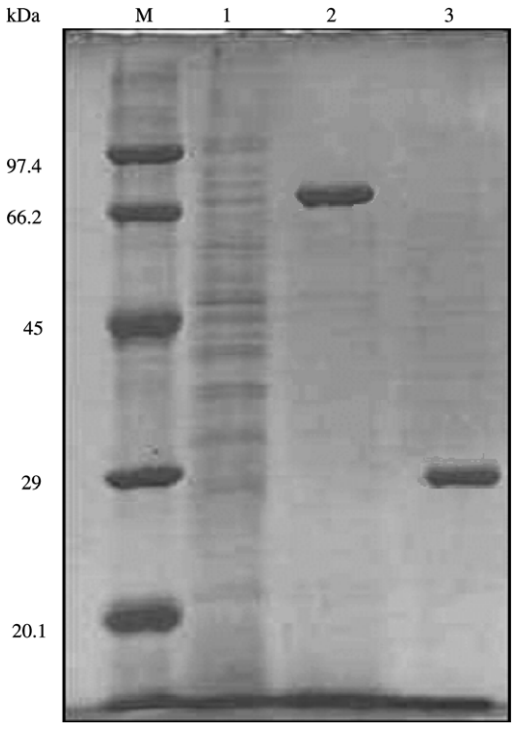

Figure 5. SDS–PAGE of purified chitinase (1 μg, Lane 2) and chitosanase (1 μg, Lane 3): M, standard marker proteins (97.4, 66.2, 45, 29, 20.1, 14.4 kDa); 1, culture supernatant (1 μg).

Table 2. Enzyme activities with various substrates

Substrates[a]	Activity (mean ± SEM [$n = 3$])[b] of	
	CHT1	CHS1
Soluble chitosan (94 DD[c])	0	59.1 ± 0.5
Soluble chitosan (85 DD)	0	20.3 ± 0.2
Soluble chitosan (73 DD)	0	6.5 ± 0.1
Colloidal chitin	22.2 ± 0.3	0
Chitin	6.9 ± 0.1	0
Carboxymethyl cellulose	0	0
Xylan	0	0

[a] CHT1 and CHS1 were assayed at a constant ionic strength (50 mM phosphate buffer) and temperature (37 °C) at pH 7 for 30 min. The amount of enzyme used was less than 100 μg, and it was controlled to a concentration that showed a optical density range of 0.05–0.20. The assay conditions are described in Section 2.

[b] Expressed as units per gram.

[c] Degree of deacetylation was calculated using the first derivative UV-spectrophotometry method.[14]

to the bifunctional chitinase/lysozymes FI (50 °C, <40 °C) and FII (50 °C, <60 °C) from *P. aeruginosa* K-187[11] as well as the chitinase from *P. aeruginosa* strain 385 (50 °C, <50 °C).[12] CHS1 has a same optimal temperature of 50 °C for catalysis as CHT1 yet a lower thermostability of <40 °C, different from the chitosanase (30 °C, <50 °C) from *Pseudomonas* sp. H-14.[14]

3.6. Effects of various chemicals

To further characterize CHT1 and CHS1, we next examined the effect of some known enzyme inhibitors and divalent metals on their activities. The results are summarized in Table 3. The chitinase activity of CHT1 was inhibited completely by Fe^{2+} and Mn^{2+}. The FII chitinase/lysozyme of *P. aeruginosa* K-187 was also found to be inhibited by Mn^{2+}.[11] Differing activation effects were found in both K-187 chitinase/lysozymes (FI, FII), and CHT1 was inhibited by Cu^{2+}. Chitosanase activity of CHS1 was inhibited completely by Cu^{2+}, Mn^{2+}, and PMSF (an inhibitor of serine proteases).

3.7. Effect of various surfactants

Enzymes are usually inactivated by the addition of surfactants to the reaction solution. The effect of different surfactants on the stability of CHT1 and CHS1 was also studied. CHT1 and CHS1 were incubated with surfactants at 25 °C for 30 min and the remaining enzymatic activity was determined under normal assay conditions. The enzyme activity of the sample without any surfactants (control) was taken as 100%. It was found that even at the presence of 2 mM SDS (anionic surfactant), 2% nonionic surfactants of Tween 20, Tween 40, or Triton X-100, the activities of CHT1 retained 87%, 74%, 119%, and 125% of its original activity, respectively. However, at the same conditions, the activity of CHS1 retained 0%, 138%, 106%, and 151% of its original activity, respectively (Table 3). The major difference between CHT1 and CHS1 is that in the presence of 2 mM SDS, CHS1 was completely inactivated but CHT1 still retained its activity. In addition, in the presence of 2% Tween, the activity of CHS1 increased to 138%, but the activity of CHT1 decreased to 74%. These differences between both enzymes might be related to the dissimilarity of the ratio of their hydrophobic and hydrophilic amino acids.

In contract to other reported chitosanase-producing strains,[12–14] this research was aimed at microbial reclamation of shrimp processing wastes. Shrimp shells were used as the sole carbon and nitrogen source to screen for chitinase and chitosanase-producing bacteria. As described above, chitinases or chitosanases have been identified in only four *Pseudomonas* species. Some properties (such as molecular weight) of *Pseudomonas* sp. TKU015 CHT1 and CHS1 were different for these earlier reported enzymes, inlcuding the *P. aeruginosa* K-187 bifunctional chitinase/lysozymes,[11] *P. aeruginosa* strain 385 chitinase,[12] *Pseudomonas* sp. YHS-A2 chitinase,[13] and *Pseudomonas* sp. H-14 chitosanase.[14] Considering the production cost and the reutilization of bioresources, utilizing

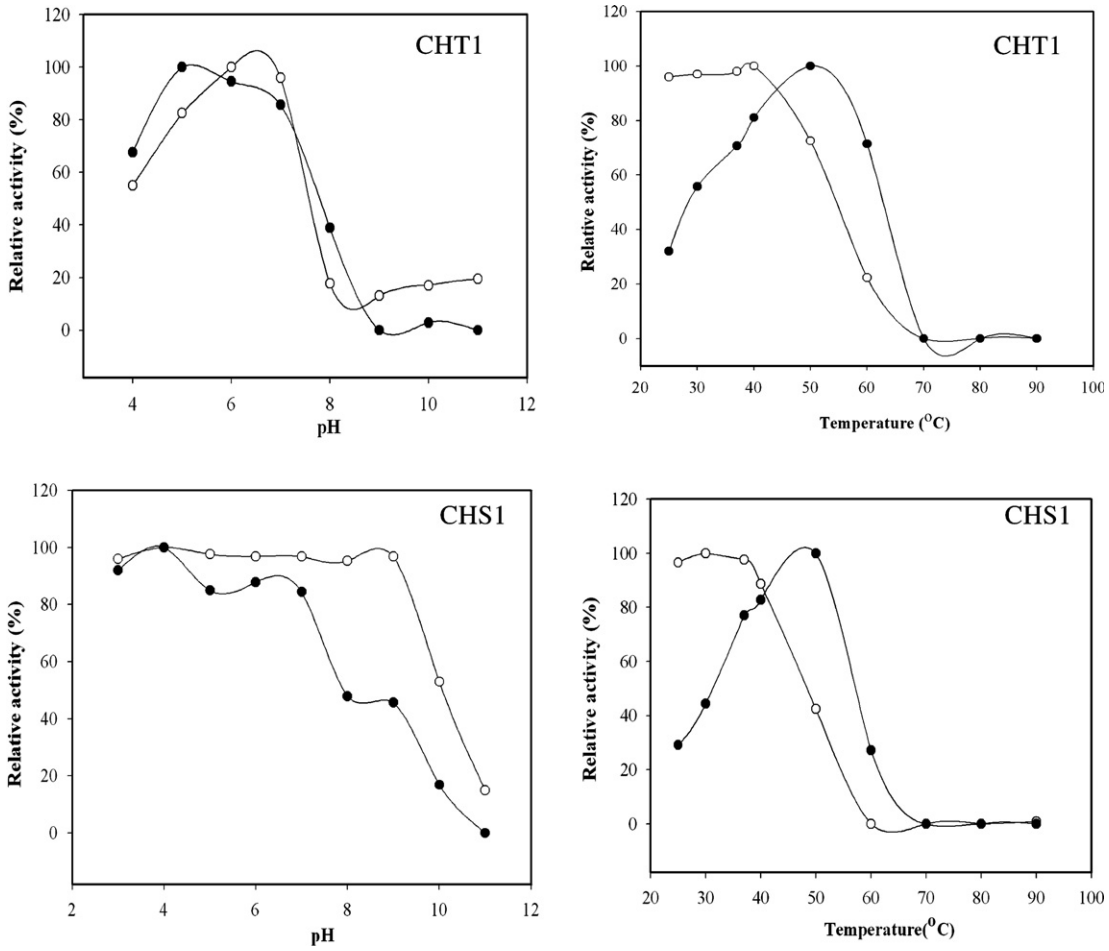

Figure 6. Effect of pH and temperature on the activity and stability of CHT1 and CHS1. -●-, activity; -○-, stability.

Table 3. Effects of various chemicals on enzyme activity

Chemicals	Concentration	Relative activity (%)	
		Chitinase	Chitosanase
None	0	100	100
Mg^{2+}	5 mM	100	98
Ca^{2+}	5 mM	96	111
Mn^{2+}	5 mM	0	0
Zn^{2+}	5 mM	112	79
Ba^{2+}	5 mM	80	105
Cu^{2+}	5 mM	43	0
Fe^{2+}	5 mM	0	33
EDTA	10 mM	25	14
PMSF	10 mM	63	0
SDS	0.5 (2) mM	120 (87)	91 (0)
Tween 20	0.5 (2)%	99 (74)	84 (138)
Tween 40	0.5 (2)%	103 (119)	71 (106)
Triton X-100	0.5 (2)%	118 (125)	44 (151)

Anionic surfactant: SDS.
Nonionic surfactants: Tween 20, Tween 40, Triton X-100.

TKU015 in the microbial reclamation of food processing wastes such as shrimp shell wastes for the production of chitinases and chitosanases seems to be a promising approach.

Acknowledgment

This work was supported in part by a grant of the National Science Council, Taiwan (NSC95-2313-B-032-003).

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