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FULL LENGTH ARTICLE

Purification, characterization and antimicrobial activity of chitinase from marine-derived *Aspergillus terreus*

Aida M. Farag [a,*], Hanan M. Abd-Elnabey [b], Hassan A.H. Ibrahim [b], Moustafa El-Shenawy [c]

[a] *Marine Biotechnology and Natural Products Laboratory, National Institute of Oceanography and Fisheries, Alexandria, Egypt*
[b] *Microbiology Laboratory, National Institute of Oceanography and Fisheries, Alexandria, Egypt*
[c] *Dept. Food and Environmental Microbiology, National Research Center, Cairo, Egypt*

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KEYWORDS

Chitinase;
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Abstract Chitinase (EC 3.2.1.14) was produced from the culture filtrate of marine-derived *Aspergillus terreus* and purified by 65% ammonium sulphate precipitation, followed by gel filtration on Sephadex G-100 and DEAE-Sephadex A-50 ion exchange chromatography, with 5.16-fold of purification and specific activity of 182.08 U/mg protein. The molecular weight of the purified chitinase was 60 kDa, determined by a sodium dodecyl sulphate polyacrylamide gel electrophoresis. The optimum pH and temperature of purified chitinase were 5.6 and 50 °C, respectively. The chitinase enzyme was stable from pH 5 to 7.5 and stable up to 70 °C. The effect of activators and inhibitors was studied, Hg^{+}, pb, EDTA, ethanol, methanol and acetone strongly inhibited the enzyme activity, while, metal ions such as Ca^{2+}, Mn^{2+} and Na^{2+} highly increased chitinase activity. The purified chitinase produced by *A. terreus* inhibited the growth of *Aspergillus niger*, *Aspergillus oryzae*, *Penicillum oxysporium*, *Rhizocotonia solani*, *Candida albicans* and *Fusarium solani*, while did not inhibit the growth of *Rhizopus oryzae*. Moreover, the purified enzyme had antibacterial effects against some pathogenic bacteria such as; *Staphylococcus aureus*, *Salmonella typhi* and *Pseudomonas aeruginosa*, while, it had not any activity against *Escherichia coli*, *Aeromonas hydrophila* and *Photobacterium damsela*.

Introduction

After cellulose, chitin was the second most abundant polymer occurring in the nature (Stoykov et al., 2014; Wang et al., 2015). Chitin consists of N-acetyl-D-glucosamine residues linked by β 1,4 bonds. It widely exists in shells of crustaceans, constituents of insects' exo-skeleton, and algae and fungal and

* Corresponding author at: National Institute of Oceanography and Fisheries (NIOF), Marine Biotechnology Lab, Kayet Bay, El-Anfushy, Alexandria, Egypt. Tel.: +20 1028011767; fax: +20 03 48801553.
E-mail address: aidafarag92@yahoo.com (A.M. Farag).

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cell walls (Grimont and Grimont, 2006). Some chitins and their derivatives with a specific degree of polymerization appeared antitumor, hypolipidemic, haemostatic and antimicrobial activities (Shen et al., 2009; Xia et al., 2011; Anitha et al., 2014; Ni et al., 2015). Marine wastes are considered the great sources of chitin, Rinaudo (2006) mentioned that over 80,000 tons of chitin was produced from marine wastes every year, which need to be more effectively utilized to avoid the harmful impact on the environment. The accumulation of large amounts of wastes from crustaceans has become a main concern in the industry of seafood processing. Therefore, the production of high value-added products, such as, chitin, chitosan and their derivatives for valuable applications in different fields has vital demand (Shahidi et al., 1999). Chitinases (EC 3.2.1.14) belong to glycoside hydrolases that catalyse the process of chitin degradation. They are produced by many microbes including; fungi (*Aspergillus*, *Trichoderma*), bacteria (*Aeromonas*, *Actinomyces*, *Bacillus*, *Enterobacter*, *Pseudomonas*, *Serratia*), insects, higher plants, crustaceans and animals. Stoykov et al. (2014) and Ni et al. (2015) mentioned that the chitinase enzymes are constituted of not less than three functional domains, namely catalytic domain, chitin-binding domain, and cadherin-like domain or fibronectin type III-like domain. Dahiya et al. (2006) mentioned also that, as there are major advances in biotechnology, mainly in the field of protein engineering, genetics, the availability of sequence data, and bioinformatics, many industrial processes are witnessing a new epoch of enzyme applications. Nevertheless, a large number of biotechnological applications for chitinases is already in use, for example the isolation of protoplasts from fungi and yeasts (Dahiya et al., 2005); the preparation of N-acetyl-D-glucosamine and pharmaceutically important chitooligosaccharides (Kuk et al., 2005; Sorbotten et al., 2005); the treatment of chitinous wastes (Wang and Hwang, 2001); and the preparation of single cell protein (Wang et al., 2015). As well as, chitinase have been implicated in the biocontrol of fungal phytopathogens (Nawani, 2005).

This work reports the purification, characterization and antimicrobial activity of chitinase secreted by an osmoduric strain of marine – derived *Aspergillus terreus*. It may be used for biocontrol of pathogenic fungi, some pathogenic bacteria and, also, in applications in different biotechnological fields

Material and methods

Culture conditions

Aspergillus terreus which produces high amounts of chitinase enzyme, was isolated from Al-Jouf sediment, Saudi Arabia. This isolate was identified as *Aspergillus* by microscopic examination and further identification to species level with the help of Mycological Center, Faculty of Science, Assiut University, Egypt. The strain was cultivated in a minimal salt medium (gl^{-1}): $(NH_4)_2SO_4$, 0.5; KCl, 0.5; 1.0; $MgSO_4{\cdot}7H_2O$, $CaCl_2$, 0.02; $FeSO_4{\cdot}7H_2O$, K_2HPO_4, 1.0; traces, pH 5.0. Shrimp-shell powder was used at a concentration of 20 gl^{-1} (Farag and Al-Nusarie, 2014). Cultivation of *A. terreus* for the production of chitinase, was carried out in 250 ml Erlenmeyer flasks each containing 50 ml of basal medium of the same composition as that used for isolation. Each flask was inoculated with 2 ml of a spore suspension (2×10^6 spore/ml) prepared from 5-day old slants of *A. terreus*. The flasks were incubated at 30 °C in an incubator shaker (120 rpm) for 5 days.

Preparation of cell-free extracts

The fungal growth was separated by centrifugation at 6000 rpm for 15 min in a cooling centrifuge and the supernatant was used as the source of the crude enzyme.

Protein determination

The extracellular protein in crude culture supernatant was measured following the Lowry et al. method (1951).

Chitinase assay

The activity of chitinase was measured colorimetrically by detecting the amount of reducing sugar (N-acetylglucosamine (NAGA)) liberated from the degradation of substrate (colloidal chitin) (Thamthiankul et al., 2001). The reaction mixture containing 1.0 ml 1% (w/v) colloidal chitin (in 0.02 M phosphate buffer, pH 5.2) and 1.0 ml of diluted enzyme solution was incubated for 30 min at 40 °C, followed by the addition of 3,5-dinitrosalicylic acid (DNS) reagent and boiled for 10–15 min, after cooling, the developed colour, as indication to the quantity of releasing (NAGA), was measured at 530 nm (Binod et al., 2005). The amount of NAGA was calculated from the standard curve of NAGA. The yield of the enzyme was also measured by calculating the specific activity and fold purification of the enzyme. IU of chitinase activity is defined as the amount of enzyme that liberates 1 μmol of N-acetylglucosamine under standard assay conditions.

Chitinase production

The production of chitinase was carried out by cultivation of *A. terreus* in basal medium supplemented by shrimp-shell powder as a sole carbon source. After sterilization, each flask (containing 50 ml of the media) was inoculated with two ml of a spore suspension (2×10^6 spore/ml) from slants of the test fungus. The inoculated media was incubated at 30 °C in an incubator shaker (120 rpm). After incubation, the culture medium was filtered and centrifugated at 8000*g* for 20 min.

Chitinase purification

Chitinase was purified by three steps of purification involving precipitation (either by ammonium sulphate, ethanol or acetone), gel filtration and ion exchange.

Precipitation step

Ammonium sulphate fractionation and fractional precipitation by acetone and ethanol were applied for such procedures. Firstly, the supernatant (about 300 ml) was precipitated at 4 °C using different concentrations of ammonium sulphate to obtain different fractions at 25%, 35%, 50%, 65%, 75% and 85% saturation. The protein deposit obtained by centrifugation (8000×*g* for 30 min), dissolved in a defined volume of 50 mM phosphate buffer (pH 7.0) and dialysed against the

same buffer overnight in a refrigerator (Farag and Hassan, 2004). Secondly, cooled acetone or ethanol was added to the supernatant slowly. Enzyme fractions were obtained at concentrations of acetone or ethanol (as in ammonium sulphate). The precipitate was dialysed against 50 mM potassium phosphate buffer for 12 h at 4 °C. After dialysis, chitinase activity and protein content of each fraction were estimated.

Gel filtration chromatography on Sephadex G-100

The concentrated protein was applied to gel filtration which was carried out using the column (2.5, 45.0 cm) pre-equilibrated with 50 mM phosphate buffer (pH 7). Five ml of concentrated enzyme was loaded to the top of the column. The enzyme was eluted using 50 mM phoshate buffer pH 7 at a flow rate of about 30 ml h^{-1} using a peristaltic pump (Masterflex, Cole-Parmer). and fractions of 5.0 ml were collected. The enzyme activity and protein in each fraction were analysed (Farag and Hassan, 2004).

Ion-exchange chromatography on DEAE Sephadex A-50

A column (2.5 × 30.0 cm) was packed with a slurry of the diethylaminoethyl (DEAE) Sephadex A-50 bed was equilibrated with 200 ml of 0.02 M phosphate buffer pH 7.0. The fractions of highest specific activity was obtained from gel filtration on Sephadex G-100 column pooled and applied to DEAE-Sephadex A-50 column. Elution was performed with 0.05 M phosphate buffer, followed by 0.05 M NaCl in 0.05 M phosphate buffer at pH 7.0, at a flow rate of 60 ml h^{-1}. Fractions (about five ml) were collected, chitinase activity and protein content for each fraction were estimated. The fractions having higher specific activity were pooled.

Characterization of purified chitinase enzyme

Polyacrylamide gel electrophoresis

The molecular weight of purified chitinase was estimated using a sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS–PAGE) according to Laemmli (1970).

Amino acid analysis

A pure enzyme sample (1 ml containing 1 mg protein) was hydrolysed by heating on a sealed, evacuated tube for 24 h at 110 °C with 5 ml 6 N HCl and 10 ml mercaptoethanol. The excess of HCl was removed by evaporation to dryness under vacuum. Amino acid analysis was estimated using Beckman Amino Acid Analyzer (Moore et al., 1958).

Effect of pH and temperature on the chitinase activity

Effect of the pH of reaction mixture on the activity of the purified enzyme was determined by incubating the purified chitinase and substrate at different pH levels (3.6–10.7) using the following buffers: 0.05 M acetate buffer (3.6–5.4), 0.05 M phosphate buffer (5.6–8.0) and 0.05 M sodium carbonate buffer (9.2–10.7). The chitinase activity was estimated at various pH levels as described before. The effect of the reaction temperature on the chitinase activity. The reaction was carried out for 60 min at various temperatures ranging from 20 to 80 °C using an enzyme protein and substrate concentration of 0.439 and 7.5 mg, respectively, per 1 ml reaction mixture.

The pH stability and thermal stability of the purified enzyme

The purified enzyme was incubated at different pH levels (4–9) at 50 °C for 60 min. then the residual activity was measured at optimum pH and optimum temperature. The thermal stability of chitinase was carried out by preincubation identical enzyme solutions in phosphate buffer (0.05 M, pH 5.6) preheated separately at various temperatures (40, 50, 60, 70, 80 and 90 °C) at different periods of time (15, 30 and 60 min). The residual activity of chitinase was determined as described before in the enzyme assay.

Effect of some activators and inhibitors on activity of purified chitinase

The purified chitinase was preincubated at room temperature for 1 h with different concentrations of various metals (5 and 10 mM) Ca^{2+} ($CaCl_2$), Na^{+} (NaCl), K^{+} (KCl), Mg^{2+} ($MgSO_4{\cdot}7H_2O$), Mn^{2+} ($MnCl_2$), Zn^{2+} ($ZnCl_2$), Cu^{2+} ($CuSO_4$), Cd^{2+} ($CdCO_3$), Pb^{2+} ($PbCl_2$), Hg^{2+} ($HgCl_3$), ethylenediaminetetraacetic acid (EDTA), acetone, ethanol and methanol. After incubation, the activity was assayed under the above optimal conditions.

Antimicrobial activity of purified chitinase

The antimicrobial activity of chitinase was estimated by well diffusion method (Perez and Anesini, 1993). The wells were punctured in the Mueller-Hinton sterile agar plates (for bacteria) and potato dextrose agar (PDA) medium (for fungi) with sterile borer and the enzyme supernatant (10, 25 and 50 U) was introduced in the wells in each of the bacterial and fungal plates. The plates were kept for 24 and 72 h at 37 °C for bacterial and fungal cultures, respectively. The diameter of inhibition zone observed was recorded. The tested pathogenic fungi were *Aspergillus niger*, *Aspergillus oryzae*, *Candida albicans*, *Fusarium solani*, and *Rhizopus oryzae*. The tested pathogenic bacteria used were *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Aeromonas hydrophila*, *Salmonella typhi* and *Photobacterium damsela*. These pathogens were kindly provided by the staff members of Marine Microbiology Lab, National Institute of Oceanography and Fisheries (NIOF), Egypt.

Results

Partial purification of the crude chitinase

Partial purification of the crude *A. terreus* chitinase enzyme was carried out by fractional precipitation with ammonium sulphate, acetone and ethanol, each used separately. Each fraction was assayed for chitinase activity and its total protein content. A total of 18 fractions were obtained and the total proteins recovered by these precipitants were 75.62%, 69.3% and 58.08%, for ammonium sulphate, acetone and ethanol, respectively. As shown in Table 1, the highest total relative activity was obtained by $(NH_4)_2SO_4$ (76.35%) followed by acetone (51.13%) and ethanol (42.99%). Also, the highest recovered protein was found with fractions precipitated at 65%

Table 1 Precipitation of *A. terreus* chitinase using different agents.

Conc. (%)	Ammonium sulphate				Acetone				Ethanol			
	PC	RP	CA	RA	PC	RP	CA	RA	PC	RP	CA	RA
25	5.65	4.76	2.53	0.34	5.65	4.76	2.42	0.32	7.20	2.33	4.75	0.20
35	9.66	8.13	6.52	1.50	10.23	8.62	6.52	1.59	10.55	7.89	11.22	2.11
50	16.6	13.97	20.5	8.13	13.60	11.45	20.50	8.84	12.55	12.65	25.20	7.61
65	36.5	30.72	65.63	57.21	18.06	15.21	29.37	9.54	36.50	12.50	45.45	24.42
75	15.33	12.90	22.63	8.28	25.21	21.22	48.53	29.22	18.50	18.94	18.90	7.46
85	6.10	5.13	6.11	0.89	9.55	8.04	7.11	1.62	3.50	3.77	12.46	1.12
Total	89.84	75.62		76.35	82.30	69.30		51.13	65.68	58.08		42.90

PC: protein content (mg); RP: recovered protein (%); CA: chitinase activity (U/mg protein); RA: relative activity (%).

ammonium sulphate (30.72%), 75% acetone (21.22%) and 75% ethanol saturation (18.94%). Among all the fractions, the 65% ammonium sulphate fraction showed the protein recovery and highest chitinase recovered activity (57.21%), about 1.85-fold purification.

Purification of chitinase by gel filtration on Sephadex G-100

The semipurified chitinase (precipitated by 65% ammonium sulphate saturation) was concentrated by ultra-filtration to about 5-ml and subjected to a column of Sephadex G-100. The elution profiles for chitinase and protein from Sephadex G-100 column are shown in Fig. 1. The total protein and chitinase activity recovered from the Sephadex G-100 reached about 64.22% and 86.43%, respectively, of the applied sample. Three peaks were obtained, one as the major and the other two were minor. The highest chitinase activity (114.47 U mg protein) was found in the first peak contains (Fig. 1).

Purification by ion-exchange chromatography on DEAE Sephadex A-50

The most active fractions (number 12 to 17) from the gel filtration on Sephadex G-100 column were pooled and purified by re-chromatographed on anion-exchange DEAE-Sephadex A-50 column (Fig. 2). A total of about 99.8% of the applied enzyme protein was recovered by the eluting solutions. The recovered protein by the first eluting agent (fraction No. 1 to 25) was about 85.24%, while that obtained by the second agent (Fraction No. 26 to 40) was only 13.39%. The protein recovered comprised 9.86%, 75.38% and 13.55% respectively of the total. These indicate that the second protein component was a major one, while the other ones' components were relatively minor ones. A total chitinase recovered activity reached 91.55% of the total activity of the sample. All the recovered chitinase activity was present in the major protein component (second protein peak). So, the column yielded a major active protein peak which showed specific activity of 182.08 U/mg protein and a purification of 5.16-fold of the crude chitinase produced by *A. terreus*. The purification steps of chitinase from the culture of *A. terreus* were summarized and are represented in Table 2.

Characterization of purified chitinase

Some properties of the purified *A. terreus* chitinase were studied. The molecular weight of the pure enzyme was 60 kDa (Fig. 3). The amino acid composition of the purified chitinase

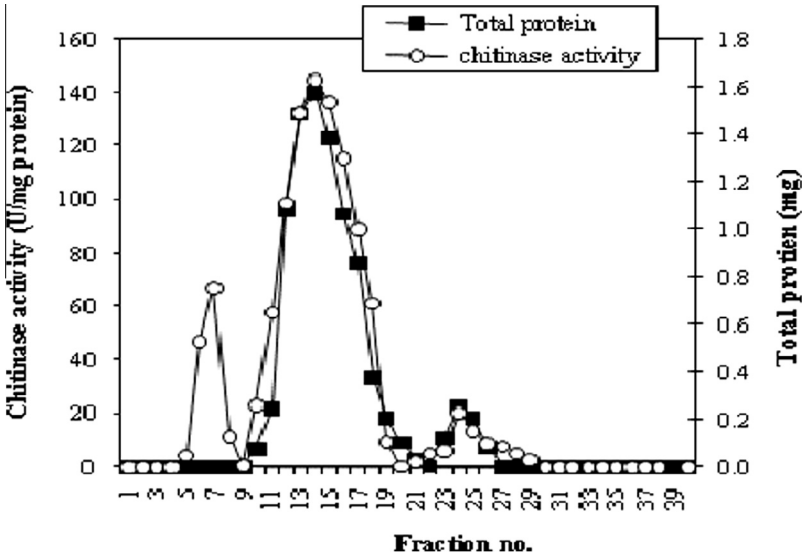

Figure 1 The first gel filtration chromatography partially purified chitinase preparation. The 65% ammonium sulphate fraction was chromatographed on Sephadex G-100.

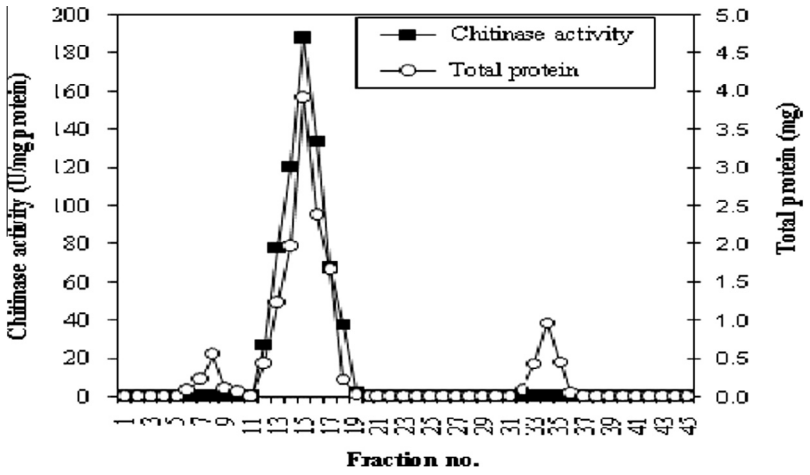

Figure 2 Second DEAE-Sephadex A-50 chromatography of the major active chitinase peak obtained by gel filtration.

Table 2 Summery of purification steps of chitinase from *A. terreus* culture.

Purification step	Total protein (mg)	Total activity (U)	Chitinase activity (U/mg protein)	Yield (%)	Purification (fold)
Crude culture filtrate	118.80	4187.50	35.25	100.00	1.00
Ammonium sulphate*	36.50	2385.64	65.36	85	1.85
Sephadex-G-100	15.00	1717.02	114.47	22	3.23
DEAE-Sephadex A-50	8.79	1607.15	182.08	12	5.16

* Ammonium sulphate was in 65% saturation.

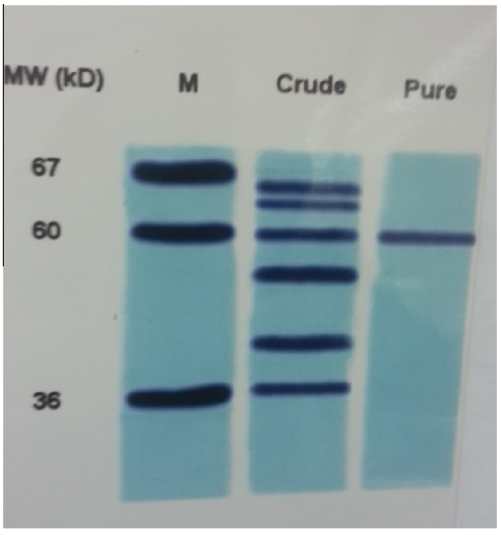

Figure 3 SDS-PAGE electrophoretic pattern of chitinase enzyme from *A. terreus*. Line M: standard protein, Line 2: crude chitinase, Line 3: purified chitinase from DEAE-Sephadex A-50.

showed a relatively high content of aspartic acid (12.10%) followed by Glutamic acid (10.93%), while the remaining fifteen amino acids showed lower percentages (Table 3).

Effect of pH and temperature on the purified chitinase

A high chitinase activity was observed at pH values between 5 and 7 with a maximum activity reaching 208.21 U/mg protein at pH 5.6 (Fig. 4). The optimum temperature of the pure chitinase enzyme was 50 °C showing a maximum activity (265.89 U/mg protein), the enzyme was also active in a temperature range of 40–60° (Fig. 5). Lower or higher incubation temperatures decreased the activity to 39.06%, 47.34% and 20.82% of its maximum value, at 20, 30 and 80 °C, respectively.

Table 3 Amino acid composition of purified chitinase produced by *A. terreus*.

Amino acid	Concentration (%)
Aspartic acid	12.10
Glutamic acid	10.93
Serine	8.90
Glycine	7.90
Leucine	7.40
Alanine	6.60
Proline	6.15
Threonine	5.77
Isoleucine	5.11
Arginine	4.15
Lysine	3.98
Phenylalanine	3.98
Valine	3.20
Tyrosine	2.85
Histidine	2.66
Methionine	1.36
Cystine	0.00

pH and thermal stability of the purified chitinase

It was observed that the purified chitinase retained more than 80% of its activity in a pH range from 5.0 to 8.0 (Fig. 6). The

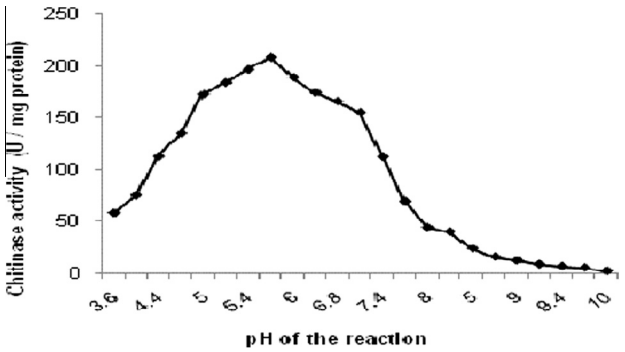

Figure 4 Effect of different pH on the chitinase enzyme activity.

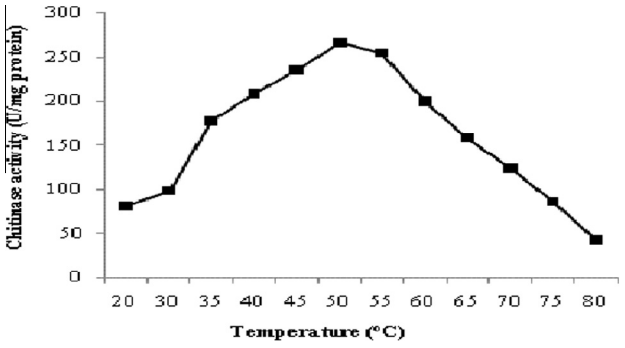

Figure 5 Effect of different temperatures on the chitinase enzyme activity.

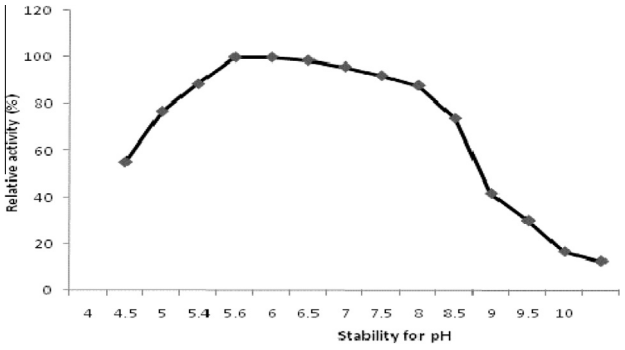

Figure 6 The pH stability of chitinase enzyme.

results in Fig. 7 show that the pure enzyme retained 65.97% and 42.42% of its activity when treated in the absence of its substrate at 60 and 70 °C for 60 min, respectively. However, the enzyme lost most of its original activity (90.77%) by heating at 90 °C for 60 min.

Effect of some ions and chemicals on chitinase activity

Treatment of the purified chitinase of *A. terreus* (Table 4) with Cd^{2+}, Zn^{2+}, pb^{2+} and Hg^{2+} (10 mM), partially inhibited its activity to 54.09%, 46.81%, 28.45% and 19.32%, respectively. A high inhibition was obtained, by treatment with EDTA, ethanol, acetone and methanol, reducing chitinase activity to

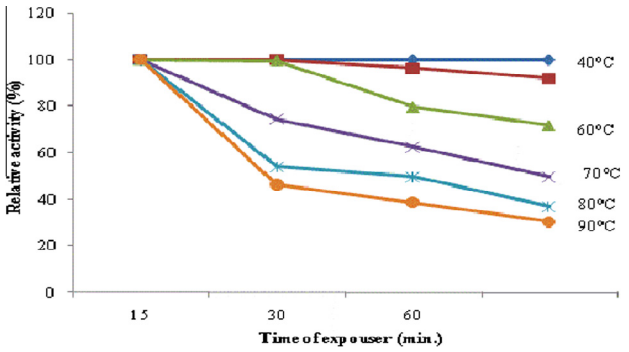

Figure 7 The effect of different temperatures on the stability of chitinase enzyme.

Table 4 Effect of activators and inhibitors chemicals on the purified chitinase produced by *A. terreus*.

Metal ion	Relative chitinase activity (%)	
	5 mM	10 mM
Control	100	100
Ca^{2+}	160.16	130.30
Mn^{2+}	144.85	125.62
Na^{+}	136.02	120.98
K^{+}	125.83	116.09
Mg^{2+}	122.56	111.64
Cu^{2+}	115.42	96.55
Cd^{2+}	84.10	54.09
Zn^{2+}	72.66	46.81
pb^{2+}	45.66	28.45
Hg^{2+}	30.91	19.32
EDTA	24.98	12.11
Ethanol	23.20	11.74
Acetone	19.64	9.03
Methanol	15.43	6.11

12.11%, 11.74%, 9.03% and 6.11%, respectively. On the other hand, enzyme activation was recorded with Ca^{2+}, Mn^{2+} and Na^{2+} (at a concentration 10 mM), increasing chitinase activity to 130.30%, 125.62% and 120.98%, respectively.

Antimicrobial effect of purified chitinase

Inhibition of the growth of the tested fungi was observed on agar plates containing different concentrations of the purified chitinase enzyme (Table 5). The diameter of inhibition zone obtained with 50 U enzyme concentration was higher than that of the 10 U for all tested pathogenic fungi, while the denaturated (boiled) enzyme had no inhibitory activity. The purified chitinase showed much stronger inhibitory activity against *A. niger*, *A. oryzae* and *Penicillum oxysporium* where the diameter of inhibition zone reached to 28, 22 and 18 mm, respectively, compared to *Rhizocotonia solani*, *F. solani* and *C. albicans* (12, 8 and 7 mm, respectively). Among the tested bacterial cultures, only the 50 U purified chitinase showed inhibition against *S. aureus* (inhibition zone diameter: 17 mm) followed by *S. typhi* (inhibition zone diameter of 15 mm) and *P. aeruginosa*, (inhibition zone diameter of 14 mm). Moreover, there was no activity against *E. coli*, *A. hydrophila* and *P. damsela*.

Table 5 Antimicrobial activity of purified chitinase.

Pathogens	Inhibition zone (mm)/purified chitinase	
	10 U	50 U
Fungi		
A. niger	23 ± 1	28 ± 0.8
A. oryzae	18 ± 0.6	22 ± 0.5
P. oxysporium	15 ± 0.5	18 ± 0.4
R. solani	10 ± 0.8	12 ± 0.6
F. oxysporium	6 ± 0.8	8 ± 0.4
C. albicans	4 ± 0.3	7 ± 0.4
R. oryzae	ND*	ND
Bacteria		
A. hydrophila	ND	ND
S. aureus	ND	17 ± 0.6
Salmonella typhi	ND	15 ± 0.4
P. aeruginosa	ND	14 ± 0.7
E. coli	ND	ND
P. damsela	ND	ND

ND*: not detected.

Discussion

The production of microbial chitinases attracted great attention in the last few decades and microorganisms which produce a complex of mycolytic enzymes, are considered to be possible biological control agents (references). Many microbes showed the ability to produced chitinases, among them *Aspergillus* species are the most famous (Ni et al., 2015; Stoykov et al., 2015). Indeed, the fungal strain of *A. terreus,* used throughout this work, was proved to possess a relatively high chitinase activity (Farag and Al-Nusarie, 2014).

Chitinase enzyme precipitation from the culture filtrate of *A. terreus* at 65% ammonium sulphate saturation exhibited the highest recovered activity, protein recovery and about 1.85-fold purification. Similarly, in preliminary precipitation attempts, many investigators have used ammonium sulphate for the precipitation of chitinase enzyme. The percent saturation of ammonium sulphate used for isolation of chitinase from different isolates was not the same. The saturation level of precipitation ranged from 30% to 85% (Rabeeth et al., 2011; Nagpure and Gupta, 2012).

The anion-exchange DEAE-Sephadex A-50 column yields a major active protein peak with a specific chitinase activity of 182.08 U/mg protein and 5.16-fold of purification of the crude chitinase produced by *A. terreus*, therefore it was considered as the pure chitinase preparation. Binod et al. (2005) found that *Penicillium aculeatum* NRRL 2129 yield of chitinase from solid state fermentation was 60.3% and the purification factor was 2.9, while, Rattanakit et al. (2007) purified an exochitinase from *Aspergillus* sp. S1-13 approximately 22-fold with 1% yield.

The molecular mass of the *A. terreus* purified chitinase was found to be 60 kDa. This value laid in the range in which different molecular masses (35–74 kDa) have been reported for other fungal chitinases (Nagpure and Gupta, 2012; Saraswathi and Ravuri, 2013).

The purified chitinase was produced by *A. terreus* had 17 amino acids. The highest percentage was that of Aspartic acid, followed by Glutamic acid. The rest of the detected amino acids showed a relatively low value. A partially similar amino acid profile was obtained also by Wiwat et al. (1999) who found that the amino acid composition of the purified chitinase produced by *Bacillus circulans* was Alanine, Proline, Tryptophan, Asparagine, Serine, Lysine, Glycine, Tyrosine, Leucine, Arginine, and Valine.

The purified chitinase exhibited maximum activity between pHs 5 and 7 with an optimum activity (208.21 U/mg protein) at pH 5.6, while the maximum temperature was in the range of 40–60 °C with optimal activity (265.89 U/mg protein) at 50 °C. These results are in agreement with other chitinases from many microbes (Zeki and Muslim, 2010; Anuradha and Revathi, 2013). Also, these results were in agreement with those other chitinase preparations obtained from *Aspergillus fumigatus* CS-01 (Xia et al., 2011).

In the present work the purified chitinase from *A. terreus* showed a fairly good pH and thermal stability since it retained about 80% of its activity in a pH range from 5.0 to 8.0 and about 42.42% of its activity when treated at 70 °C for 60 min. However it was sensitive to high temperatures of 80 and 90 °C. Similarly, the purified chitinase from *Monascus purpureus* CCRC31499 had a pH stability from 6.0 to 8.0 (Wang et al., 2002). The high temperature inactivation of the studied chitinase may be due to hydrolysis of the peptide chain, incorrect confirmation, aggregation or destruction of amino acids (Schokker and van Boekel, 1999). On the other hand, the chitinase produced from *Penicillium oxalium* was fairly stable below 45 °C (Rodriguez et al. (1995).

The current study detected that some metal ions (Ca^{2+}, Mn^{2+}, Na^{2+}, K^{+}, Mg^{2+} and Cu^{2+}) can be evaluated as activator for the chitinase produced by *A. terreus*. On the contrary, other metals inhibited the chitinase activity (Cd^{2+}, Zn^{2+}, pb^{2+} and Hg^{2+}). These results may prove that studied chitinase is a metaloenzyme. Chitinase from *Enterobacter* sp. was activated by Ca^{2+}, K^{2+} and Mg^{2+}, while it was strongly inhibited by Hg^{2+}, Cu^{2+} and Co^{2+} (Dahiya et al., 2005). Bhushan and Hoondal (1998) found that Ca^{2+}, Ni^{2+} and Triton x-100 stimulated the activity up to 20% whereas, Ag^{2+} and Hg^{2+} inhibited the activity up to 50%. Additionally, Wang et al. (2015) found that metal ions such as Co^{2+}, Fe^{3+}, Zn^{2+}, Cd^{2+} and Cu^{2+} had an obvious promoting effect upon the activity of chitinase.

The antimicrobial activity of purified chitinase was detected to evaluate the usage of *A. terreus* chitinase as a biocontrol agent against some pathogenic microbes. El-Katatny et al. (2005) observed that the purified endochitinase of *Trichoderma* showed antifungal activity against *Sclerotium rolfsii*, *Aspergillus flavus* and *Fusarium moniliforme*. Other fungal chitinases were studied to exhibit antifungal activities like inhibition of, germ tube elongation, spore germination, hyphal tip and bursting of spores (Lin et al., 2009). Our results are in good agreement with other chitinases produced from different microorganisms (Fadhil et al., 2014). The purified *A. terreus* chitinase showed a wide spectrum of antimicrobial activities against bacterial and fungal pathogens.

Conclusion

The present study confirmed the potency of the marine-derived *A. terreus* to produce a highly active chitinase which can be used as a catalyst for the degradation of chitin. In addition this

enzyme can be used commercially for the preparation of N-acetylglucosamine which has a pharmacological activity. Purification and characterization of the enzyme showed good stability of the microbial chitinase preparation and its relatively wide range of pH and temperature of the reaction. This study revealed also that the isolated chitinase has potent antibacterial and antifungal activity. These properties collectively indicate that the chitinase enzyme obtained from *A. terreus* may be a good candidate for biocontrol of pathogenic fungi, some pathogenic bacteria and, also, in applications in different biotechnological fields.

Conflicts of interest

The authors declared that they have no conflicts of interest to this work.

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