Asian Journal of Biochemistry, Genetics and Molecular Biology

6(2): 46-56, 2020; Article no.AJBGMB.60739
ISSN: 2582-3698

Purification and Characterization of a Thermostable Chitinase Produced by a Fungus Isolated from Fruit Tree Rhizosphere

I. A. Afolayan[1*], J. F. Oyun[1], E. A. Ekundayo[2] and F. O. Ekundayo[1]

[1]Department of Microbiology, Federal University of Technology, Akure, P.M.B. 704, Akure, Ondo State, Nigeria.
[2]Department of Biological Sciences, University of Medical Sciences, University of Medical Sciences, Ondo, Ondo State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Authors IAA and JFO designed the study, managed the literature searches, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript and managed the analyses of the study. Authors EAE and FOE supervised the study. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJBGMB/2020/v6i230149
Editor(s):
(1) Dr. Arulselvan Palanisamy, Muthayammal College of Arts and Science, India.
Reviewers:
(1) Seint San Aye, Yezin Agricultural University, Myanmar.
(2) Mônica Vieira de Souza, Oswaldo Cruz Foundation-FIOCRUZ, Brazil.
(3) Jane Melita Keliat, University of Sumatera Utara, Indonesia.
Complete Peer review History: http://www.sdiarticle4.com/review-history/60739

Original Research Article

Received 12 September 2020
Accepted 24 November 2020
Published 11 December 2020

ABSTRACT

This study reveals the chitinase producing ability of some fungi isolates cultured from the rhizosphere of different fruit trees such as mango, cassava, guava, and banana inside FUTA farm. A total of 16 isolates identified as *Aspergillus nidulans, A. niger, A.flavus, A. fumigatus, A. ripens, Trichoderma viride, Mucor mucedo, Penicillium frequentans, Rhizopus stolonifer, Paecilomyces fumosoroseus, Gibbelula suffulfa and Geotrichum albidum* were obtained and screened for chitinolytic activity. The effect of cultural conditions such as pH, temperature, metal ion, nitrogen source and carbon source was determined on *Aspergillus nidulans,* being the best chitinase producer. Further, the chitinase produced by *Aspergillus nidulans* was concentrated by ammonium sulphate precipitation and purified consecutively by gel filtration and ion-exchange chromatography. The optimum pH and temperature for chitinase activity and stability were examined as well as the effect of metal ions on the enzyme activity. The enzyme was most active at pH 7.0 and it was

**Corresponding author: E-mail: oyunjustina@gmail.com;*

relatively stable at pH 4.0 - 9.0 retaining over 60% of initial activity after 120 min of incubation. The enzyme was most active at 50°C, possessing high thermal stability at high temperature of 70°C. The purified chitinase was significantly inactivated at 80°C and almost completely at 90°C when it was pre-incubated at these temperatures for 60 min. The enzyme was strongly inhibited by $FeSO_4$, $ZnCl_2$ and $MnCl_2$ and was less sensitive to $CaCl_2$ and KCl. This purified *Aspergillus nidulans* chitinase can be used as a catalyst for the degradation of chitin-containing compounds.

Keywords: Chitinase; rhizosphere; fungi; chitin; thermostable.

1. INTRODUCTION

The rhizosphere is the zone of soil surrounding a plant roots in which complex relations exist among the plant, the soil microorganisms and the soil itself, it is also where the biology and chemistry of the soil are influenced by the root [1]. The rhizosphere saprotrophic fungal community appears to consist of both yeasts and filamentous fungi with representatives of all major terrestrial phyla (Ascomycota and Basidiomycota) and sub-phyla [2]. Chitin, a linear polymer of β-1, 4-N-acetylglucosamine (GlcNAc), is the second most abundant biopolymer on the planet. Chitinases are a group of enzymes which hydrolyse the -1, 4-linkages in chitin to low-molecular-weight products and have been shown to be produced by a number of microorganisms. They have the ability to degrade chitin directly to low molecular weight chitooligomers, which serve a broad range of industrial, agricultural, and medical functions such as elicitor action and anti-tumor activity [3]. A number of soil-borne fungi have been reported to exhibit a chitinolytic activity that surpasses that of bacteria. *Aspergillus and Trichoderma* are among the most studied chitinolytic species and they are present in the soil [4]. In bacteria, chitinases play roles in nutrition and parasitism whereas in fungi they are also involved in morphogenesis. The destruction of crop plants by fungal pathogens is a serious problem worldwide that annually leads to losses of about 15%, even with intensive fungicide use [5]. Hence, any development aimed to diminish this problem will be useful. Biological control of some soil-borne fungal diseases has been correlated with chitinase production [6]. Thus, the aim of this research is to study the chitinase producing ability of some fungal isolates from rhizosphere of some fruit trees.

2. MATERIALS AND METHODS

2.1 Collection of Soil Samples and Sterilization and Preparation of Media

Soil samples were collected with soil auger at depth of 15 cm from root of banana, cassava, guava, mango and fish pond sediment. These samples were placed in sterile polythene bags and immediately transported to the Microbiology Laboratory, FUTA for microbiological analysis. Chitin powder was obtained from Sigma Aldrich Co, Germany.

2.2 Isolation and Identification of Soil Fungi

Five-fold serial dilution was performed on the soil samples (rhizosphere) mentioned above and 1 ml of the diluent was introduced into petri-dishes and pourplated with molten potato dextrose agar and saboraud dextrose agar. The plates were then incubated at 25ºC for 72 h for fungal growth. The isolated fungi species were identified based on their micro-morphological features according to the method described by Jankiewicz et al. [7].

2.3 Qualitative and Quantitative Screening for the Chitinolytic Activity of Fungal Isolates

Pure cultures of the isolates were inoculated onto freshly prepared agar plates minimal synthetic medium, containing in gram per litre: $MgSO_4$ 0.2, K_2HPO_4 0.9, KCl 0.2, NH_4NO_3 1.0, $FeSO_4.7H_2O$ 0.002, $ZnSO_4$ 0.002, agar 15g and colloidal chitin 1% (w/v) as carbon source and observed for zones of clearance after 72 h of incubation. The zone of inhibition was calculated by measuring the width of the clear zone and then the distance of the hole which was 1 cm was subtracted from it. The wider the clear zone, the greater the zone of inhibition and hence the greater the chitinolytic activity of the fungi isolate. The fungal isolates selected based on large hydrolysis zones were further screened quantitatively for maximum enzyme production in liquid medium incubated in a rotary shaker at 150 rpm and 25°C. The cultures were sampled at different time intervals within a 3-day incubation period and the aliquots were centrifuged at 4°C for 10 min using a refrigerated centrifuge. The supernatant was used for chitinase assay [8].

2.4 Chitinase Activity Assay

Chitinase activity was assayed using colorimetric method with minor modification. The assay mixture contained 1 ml of 1% pure chitin (sigma suspended in 50 mM acetate buffer pH 5.2) and 1 ml of enzyme solution. The reaction mixture was incubated for 1 h at 37°C with shaking and was stopped by centrifugation at 5000 rpm for 10 min before the addition of dinitrosalicyclic acid (DNSA) reagent to the supernatant to measure the amount of the reducing sugar released at 540 nm. One unit (U) of chitinase activity is defined as the amount of enzyme required to release 1 micromole of glucose under standard assay conditions.

2.5 Protein Concentration Determination

The concentration of protein in the sample was determined by the method of Bradford (1976) using bovine serum albumin (BSA) as the standard protein.

2.6 Optimization of Chitinase Production

2.6.1 Effect of temperature on chitinase production

Production medium of 10 ml was prepared, sterilized and 2 ml inoculum was added then incubated at different temperature at 30 – 90°C. The culture filtrate was studied for up to 3 days and it was harvested for enzyme activity at an interval of 12 h [9].

2.6.2 Effect of pH on chitinase production

Production medium of 10 ml was prepared and pH of the medium was adjusted to different pH (4.0 – 8.0). The sterilized production medium was inoculated with 2 ml spore suspension and incubated under the shaking condition. The enzyme activity and protein content were carried out every 12 h for up to 3 days. Sterile inoculated medium was used as blank for chitinase assay [9].

2.6.3 Effect of metal ions on chitinase production

A production medium of 10 ml was prepared, sterilized and 2 ml inoculum was added then incubated with different metal ions. The culture filtrate was harvested and analysed for chitinolytic activity [9].

2.6.4 Effect of carbon source on chitinase production

The effect of using additional carbon source in media at a concentration of (1%) such as glucose, fructose, galactose, sucrose and maltose for maximum enzyme production was also investigated. The supplemented media were inoculated with 2% inoculum and fermented at an optimized chitin source [10].

2.6.5 Effect of nitrogen source on chitinase production

To investigate the influence of using various nitrogen sources on the production of chitinase, 10ml of production medium with different nitrogen sources such as NH_4NO_2, NH_4Cl, $NH_4(NO_3)_2$,NH_4 $(SO_4)_2$, $(NH_4)_2CO_3$ and $(NH_4)PO_4$ was prepared, sterilized, inoculated and incubated at 30°C for optimum incubation period under the shaking conditions. The culture filtrate was harvested for the enzyme activity [10].

2.6.6 Purification of chitinase

The crude protein obtained from the production of enzyme at optimized conditions was first concentrated with solid ammonium sulphate on ice and allowed to precipitate overnight. The precipitate was obtained by centrifugation at 10,000 rpm for 10 min and dissolved in 100 mM sodium acetate buffer of pH 5. The solution was dialyzed for 24 h at 4°C in the same buffer before it was loaded into a DEAE-Cellulose ion-exchange column (2.5 x 20 cm) equilibrated with 100 mM acetate buffer (pH 5) at a flow rate of 60 mL/h while the bound protein fractions were eluted with buffer containing sodium chloride (0-1 M). The protein in the eluted fractions was measured at 280 nm and the chitinase activity determined (Shimadzu, UV 1800). The tubes having chitinase activity were pooled together and concentrated using 4 M sucrose solution. The concentrate was thus loaded onto a Sephadex G-100 gel filtration column (2.5 x 75 cm, flow rate of 20 mL/h) previously equilibrated with 100 mM acetate buffer (pH 5.0). The proteins were eluted from the column in fractions of 5 ml using the same buffer and assayed for chitinase activity. The purified enzyme was used for biochemical studies Han et al. [11], Joo [12] and Sharma and Shanmugam [13].

2.7 Biochemical Characterization of the Purified Chitinase

2.7.1 Effect of temperature on the activity and stability of purified chitinase

The effect of temperature on the activity of purified chitinase was determined by carrying out the enzyme assay at varying temperature from 30 to 90°C. Thermal stability profile of the purified chitinase was studied by pre-incubation at different temperatures for 120 min. Aliquots of the enzyme were withdrawn at 20 min interval and the residual activity of the enzyme was measured.

2.7.2 Effect of pH on the activity and stability of purified chitinase

The effect of temperature on the activity of purified chitinase was determined by carrying out the enzyme assay at different solution pH 3-10. The buffers used include sodium acetate buffer (100 mM, pH 4-5), sodium phosphate buffer (100 mM, pH 6-7), Tris-HCl buffer (100 mM, pH 8-10). Enzyme stability at different pH was studied by incubating the purified enzyme at various solution pH for 120 min and withdrawing aliquot of enzyme every 20 min to measure the residual activity.

2.7.3 Effect of metal ions on purified chitinase activity

The activity of the purified chitinase was determined in the presence of Zn^{2+}, Mn^{2+}, K^{+}, Ca^{2+} and Fe^{2+} at 5 mM salt concentration. The control has no metal ion. The assay was carried out as earlier described.

3. RESULTS

3.1 Identification of Fungal Isolates Obtained From Selected Soil Samples of Different Sources

The fungal isolates from the selected rhizosphere *Aspergillus nidulans, A.niger, A.flavus, A.fumigatus, A.ripens, Trichoderma viride, Mucor mucedo, Penicillium frequentans, Rhizopus stolonifer, Emericella rugulosa, Fusarium merismoides, Chaetospina fulva, Streptomyces* sp, *Paecilomyces fumosoroseus, Gibbelula suffulfa and Geotrichum albidum* as shown in Table 1.

3.2 Screening for Chitinase Activity of Fungal Isolates

All the fungal isolates were screened and found to possess chitinolytic activity in varying degrees. *Aspergillu nidulans* had the highest chitinase activity followed by *Aspergillus niger*, and then followed by *Aspergillus flavus* as shown in Table 2.

3.3 Optimization of Cultural Condition for Chitinase Production by *Aspergillus nidulans*

The rate of Chitinase production by *Aspergillus nidulans* decreased with increase in temperature and the production was favoured most by 40°C and 50°C with activity of 67 U/ml and 73 U/ml, respectively as shown in Fig. 1. After 72 h of incubation, chitinase production by *Aspergillus nidulans* was maximum at neutral pH 7 and slightly basic pH 8.0 but no activity was observed at pH 9 (Fig. 2). As shown in Fig. 3, chitinase production by *Aspergillus nidulans* was repressed by all metal ions indicating that they are not necessary inducers for the production of chitinase. $NH_4(NO_3)_2$, NH_4Cl and $NH_4(SO_4)_2$ enhanced chitinase production while NH_4NO_2, $(NH_4)_2CO_3$ and $(NH_4)PO_4$ repressed the production chitinase by *Aspergillus nidulans* (Fig. 4). Sucrose, maltose and fructose increased chitinolytic activity but sucrose has the highest chitinase activity of *Aspergillus nidulans*. Conversely, glucose and galactose decreased chitinase activity as shown in Fig. 5.

3.4 Enzyme Purification

The summary of purification steps were presented in Table 3. Purification of chitinase with ammonium sulphate (60%) concentration followed by DEAE-cellulose column and Sephadex-100 chromatography yielded 19.20, 16.04 and 9.74% recovery with 1.90, 3.70 and 8.86- fold respectively.

3.5 Characterization of Chitinase Produced by *A. nidulans*

3.5.1 Effect of temperature on enzyme activity and stability

The enzyme was active at temperatures between 30 and 70°C and optimum being at 50°C (Fig. 6). The enzyme was stable at high temperature of 70°C, retaining over 60% of initial activity after

120 min of incubation. At 80°C, the chitinase only retained about 55% of initial activity after 60 min of incubation. At 90°C, the activity reduced by over 90% after 1 h of pre-incubation (Fig. 7).

3.5.2 Effect of pH on enzyme activity and stability

The chitinase from *Aspergillus nidulans* was active between wide ranges of pH between 6 and 9 and optimum being at pH 7.0 (Fig. 8). Regarding stability, the enzyme was stable at all pH tested. The chitinase activity was highly maintained at pH 6 to 8 for the 2-hour incubation period (Fig. 9). But the activity of the purified chitinase reduced at pH 9, retaining over 60% of initial activity after 120 min of incubation.

3.5.3 Effect of metal ions on enzyme activity

The effect of various metal ions on the activity of the purified enzyme is shown in Table 4. The enzyme was strongly inhibited by $FeSO_4$, $ZnCl_2$ and $MnCl_2$ and was less sensitive to $CaCl_2$ and KCl, these two salts showed a slight inhibitory effect on enzyme activity.

Table 1. Isolation of fungal species from rhizosphere of selected fruit trees and pond sediment

Fungi	***Sources***				
	Guava	***Cassava***	***Mango***	***Banana***	***Fish pond sediments***
Aspergillus flavus	+	+	+	-	+
Aspergillus nidulans	+	+	-	+	+
Penicillium frequentans	+	+	+	+	-
Fusarium merismoides	+	+	-	-	+
Aspergillus niger	+	+	-	-	-
Rhizopus stolonifera	-	-	+	-	-
Mucor mucedo	-	+	-	-	-
Geotrichum candidum	-	+	-	-	-
Streptomyces spp	-	+	-	-	-
Emericella rugulosa	-	+	-	-	-
Aspergillus fumigatus	+	-	+	-	-
Chaetospina fulva	-	-	-	-	+
Trichoderma viride	-	-	-	+	-
Aspergillus repens	-	-	-	+	+
Paecilomyces fumosoroseus	-	+	-	-	-
Gibbelula suffulfa	-	-	-	-	+

Table 2. Quantitative screening of fungal isolates for chitinolytic activity

Fungal isolates	***Chitinase activity(U/ml)***
Trichoderma viride	33.12
Aspergillus fumigatus	30.30
Mucor mucedo	29.60
Penicillium frequentans	24.67
Aspergillus flavus	33.83
Chaetopsina fulva	31.01
Rhizopus stolonifer	28.89
Emericella rugulosa	31.71
Fusarium merismoides	31.71
Streptomyces spp	0.01
Paecilomyces spp	28.89
Gibbelulla suffulfa	25.37
Aspergillus nidulans	35.94
Geotrichum candidum	31.71
Aspergillus niger	34.55
Aspergillus repens	26.8

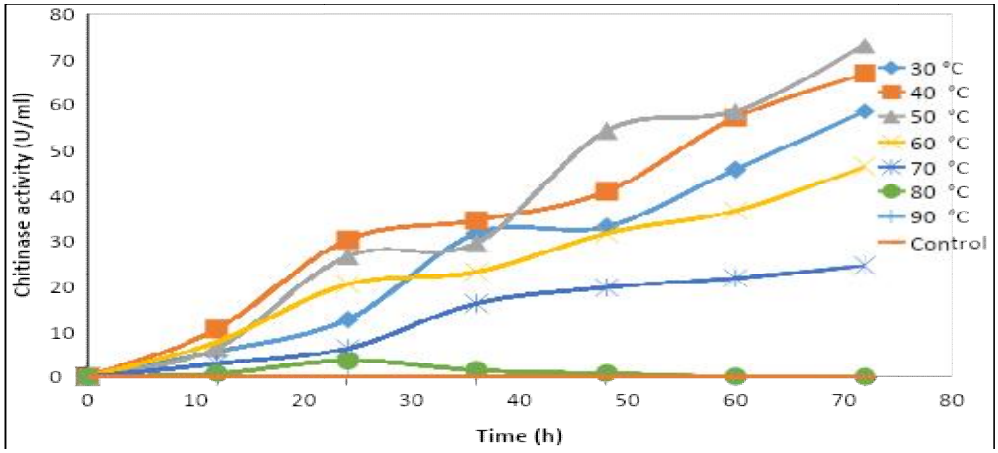

Fig. 1. Effect of temperature on the production of chitinase by *Aspergillus nidulans*

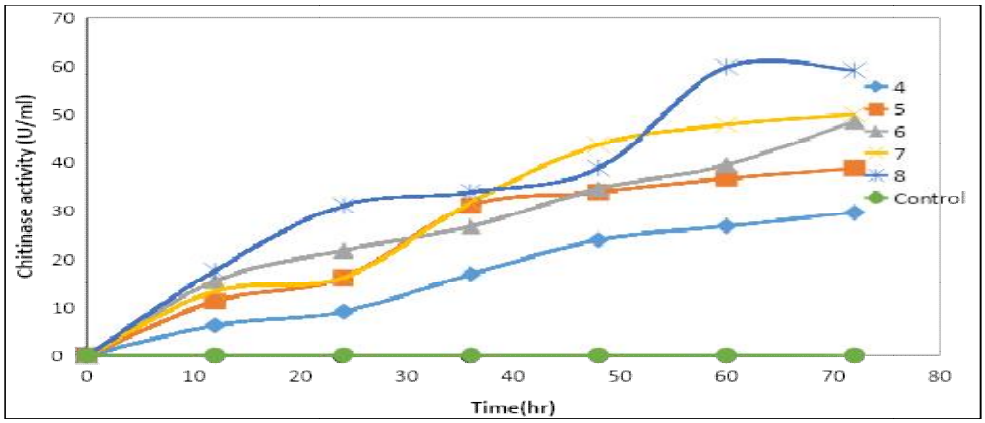

Fig. 2. Effect of pH on the production of chitinase by *Aspergillus nidulans*

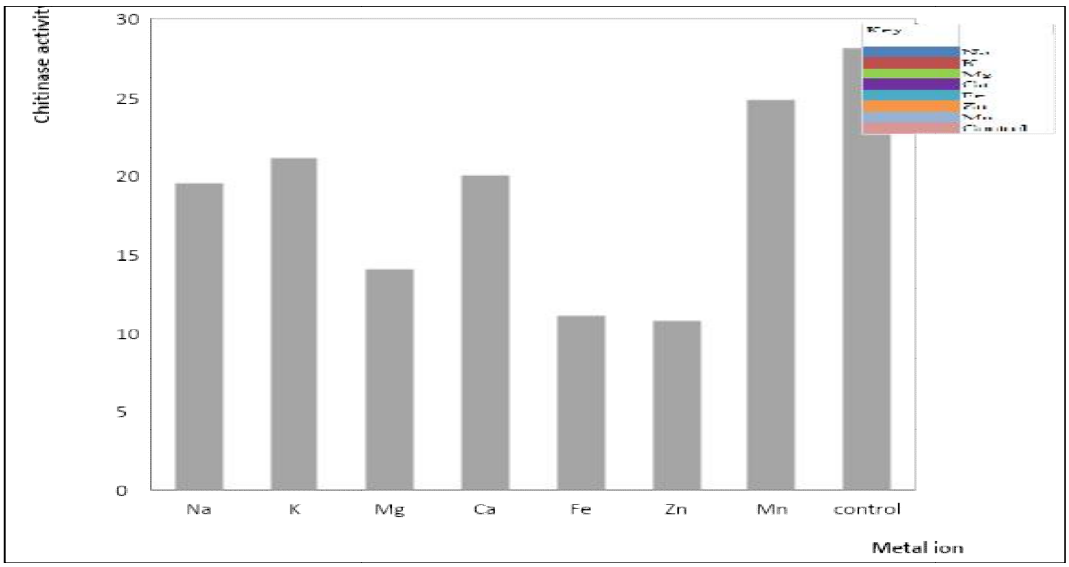

Fig. 3. Effect of metal ions on the chitinolytic activity of *Aspergillus nidulans*

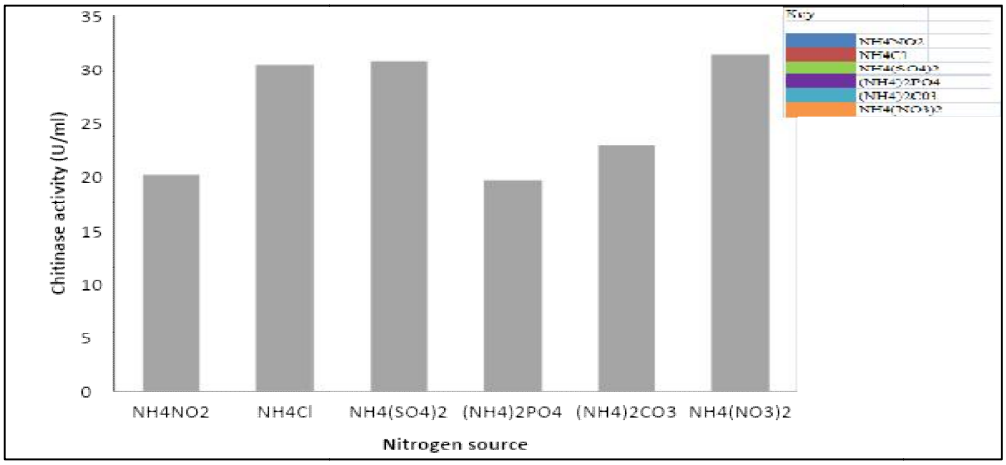

Fig. 4. Effect of nitrogen source on the production of chitinase by *Aspergillus nidulans*

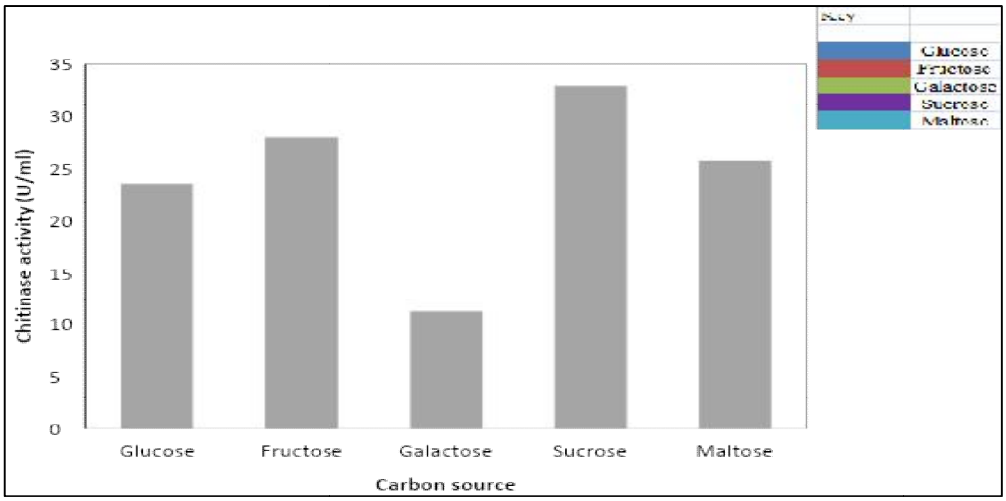

Fig. 5. Effect of carbon source on the production of chitinase by *Aspergillus nidulans*

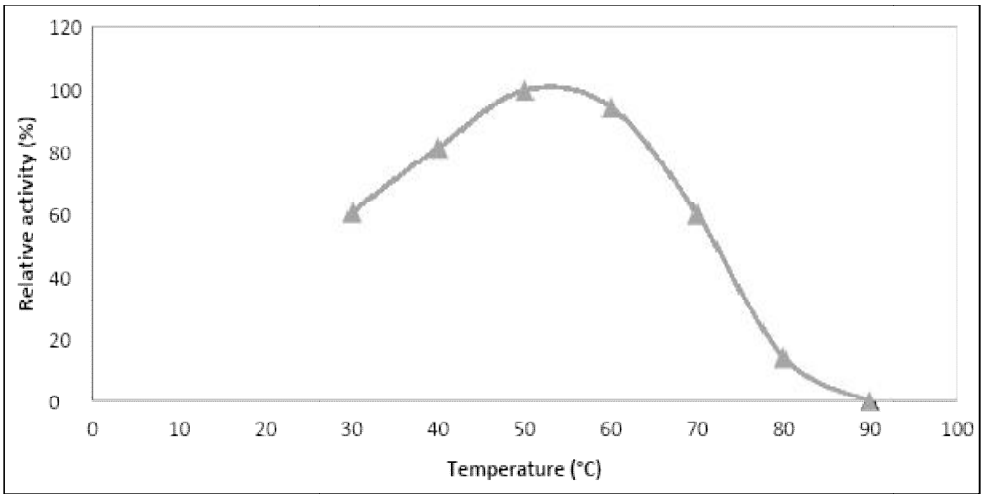

Fig. 6. Effect of temperature on the activity of purified chitinase from *Aspergillus nidulans*

Table 3. Purification steps of chitinase from *Aspergillus nidulans*

Step	Volume (ml)	Chitinase activity (U/ml)	Protein concentration (mg/ml)	Total activity (U)	Total protein (mg)	Specific activity	Yield (%)	Fold
Crude enzyme	300	27.13	16.25	8139.54	4875	1.67	100	1
Ammonium precipitate	54.1	28.89	9.13	1563.50	493.66	3.17	19.20	1.90
Ion exchange chromatography	32.5	40.17	6.5	1305.50	211.25	6.18	16.04	3.70
Gel filtration chromatography	15.3	51.80	3.5	792.49	53.55	14.80	9.74	8.86

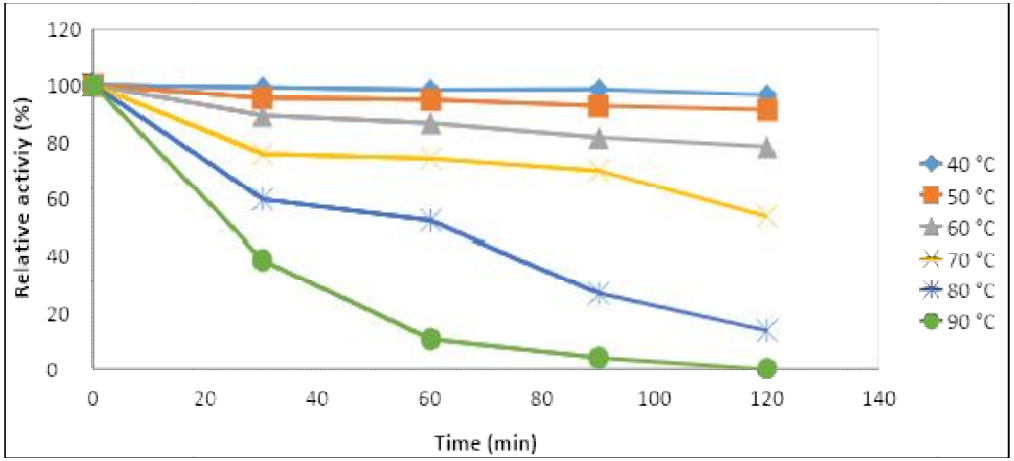

Fig. 7. Temperature stability of purified chitinase from *Aspergillus nidulans*

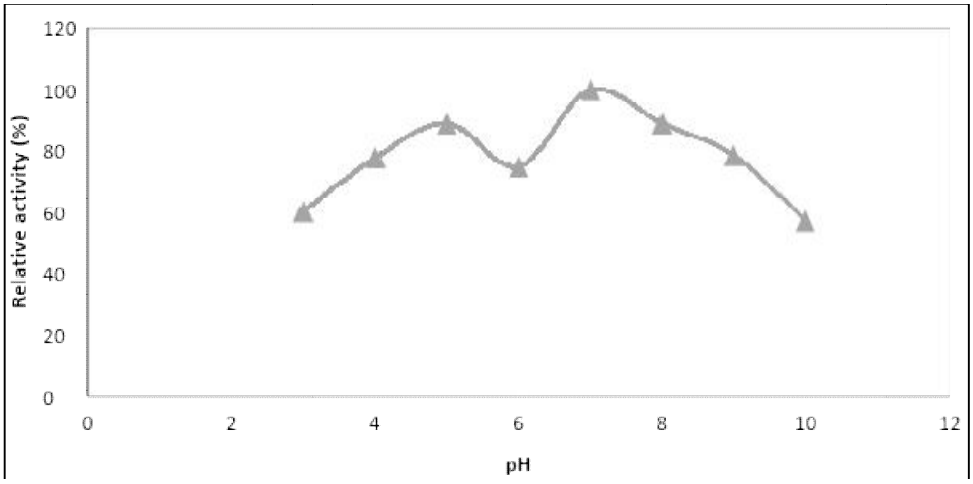

Fig. 8. Effect of pH on the activity of purified chitinase from *Aspergillus nidulans*

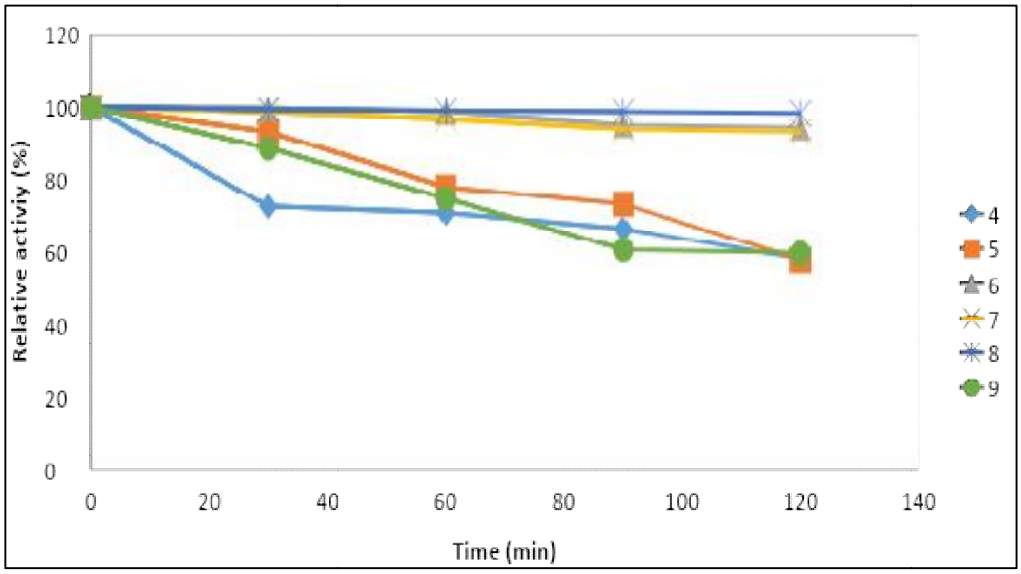

Fig. 9. pH stability of purified chitinase from *Aspergillus nidulans*

Table 4. Effect of metal ions on chitinase activity produced

Metal ions (Salts)	Relative activity (%)
Control	100
$ZnCl_2$	43.48
$MnCl_2$	54.35
$CaCl_2$	87.68
$FeSO_4$	34.78
KCl	92.75

4. DISCUSSION

Fungal species such as *Aspergillus nidulans, A. repens, A. niger, Penicillium frequentans, Mucor mucedo, Trichoderma viride* and many other fungi were isolated from rhizosphere of cassava, banana, fish pond sediment, guava and mango. Some microorganisms inhabit the soil and these

make the soil their microbial habitat. Rhizosphere fungi are found in many environments (soil and marine) [14]. A wide range of eukaryotic and prokaryotic organism has the ability to produce cell wall degrading enzymes when chitin is present in the growth medium [15]. Chitinolytic enzymes are able to lyse the cell wall of many fungi. The microorganisms that produce these enzymes are able to destroy the cell wall of many harmful fungi and capable of eradicating fungal diseases that are a problem for global agricultural production [16]. All the fungal isolates showed varying degrees of chitinolytic activity with *Aspergillus nidulans* having the maximum value while *Streptomyces* sp. had the least activity. The production of chitinase was influenced by the culture conditions of pH, temperature and presence of inducers. The production of chitinase was influenced by the nitrogen source when incorporated into the medium. The highest chitinase activity was recorded in $NH_4(NO_3)_2$ at a concentration of 1%. These results are a bit similar with those reported by Rattanakit et al. [17] in the production of chitinase from *Aspergillus* spp.

Using the 3-stage purification of ammonium sulphate precipitation, ion-exchange chromatography and gel filtration, the enzyme was purified 8.6-fold with a recovery of 9.7% and specific activity of 14.8 U/mg of protein. Exochitinase has been purified from *Aspergillus* sp. S1-13 approximately 22-fold with 1% yield. The optimal pH for chitinase activity and stability were examined. The enzyme was most active at pH 7.0. It was relatively stable at pH between 4.0 and 6.0. However, beyond these pH ranges, it rapidly lost its activity. Compared with other *Aspergillus* chitinases, similar optimal pH were obtained; pH 4 for *Aspergillus niger* and pH 5.0 for *Trichoderma atroviride* PTCC 5220 [18]. The chitinase activity was most active at 50°C, similar to most of the other fungal chitinases [18]. Above 50°C, the activity decreased and was lost completely at 70°C. When the enzyme was kept at various temperatures for 60 min in an acetate buffer (pH 5.0), it was significantly inactivated above 60°C and completely at 80°C. Similar thermostability was obtained between 40°C and 50°C for other *Aspergillus* chitinase activity. The high temperature inactivation may be due to incorrect confirmation due to hydrolysis of the peptide chain, destruction of amino acid, or aggregation [19]. The enzyme was strongly inhibited by $FeSO_4$, $ZnCl_2$ and $MnCl_2$ and was less sensitive to $CaCl_2$ and KCl, these two salts showed a slight inhibitory effect on enzyme activity. The inhibitory effect of $FeSO_4$ has also been reported on chitinase produced by *Trichoderma harzianum* [18].

5. CONCLUSION

This research confirms the rhizosphere of fruit trees as good source for soil fungal species which are beneficial. The *Aspergillus nidulans* thus isolated had high chitinase activity with enviable physicochemical properties. The purified enzyme from this fungus can be used as a catalyst in biotechnological industries for the degradation of chitin.

COMPETING INTERESTS

Authors have declared that no competing interests exist

REFERENCES

1. Sharma N, Sharma K, Gaur R, Gupta V. Role of chitinase in plant defense. Asian Journal of Biochemistry. 20116:29–37.
2. Marc B, Wietse B, Francis MM, Leonard VO, Jurkevitch E. The rhizosphere zoo: An overview of plant-associated communities of microorganisms, including phages, bacteria, archaea, and fungi, and some of their structuring factors. Plant and Soil. 2009;321(1):189-212. DOI:10.1007/s11104-009-9991-3
3. Hamid R, Khan M, Ahmad M, Ahmad M, Abdin M, Musarrat J, et al. Chitinases: An update. Journal pf Pharmacy and Bio Allied Sciences. 2013;5(1):21.
4. Veliz EA, Martinez-Hidalgo P, Hirsch AM. Chitinase-producing bacteria and their role in biocontrol. AIMS Microbiology. 2017;3(3):689-705.
5. Chang W, Chen M, Wang S.An antifungal chitinase produced by *Bacillus subtilis* using chitin waste as a carbon source. World Journal of Microbiology and Biotechnology. 2010;26:945–950.
6. Swiontek Brzezinska M. Jankiewicz, U, Burkowska A, Walczak, M. Chitinolytic microorganisms and their possible application in environmental protection. Current microbiology. 201468(1):71-81
7. Jankiewicz U, Swiontek Brzezinska, M, Saks E.. Identification and characterization of a chitinase of Stenotrophomonas malt philia, a bacterium that is antagonistic towards fungal phytopathogens. Journal of Bioscience and Bioengineering. 2012: 113(1):30–35.

8. Thirumurugan D, Sankari D, Vijayakumar, R.. Screening of chitinase production and antifungal activity of Streptomyces sp. Act7 from east coast region, south india. International Journal of Pharmacy and Pharmaceutical Sciences. 2015;7(5):38-41.
9. Nampoothiri K, Baiju T, Sandhya C, Sabu A, Szakacs G, Pandey A.Process optimization for antifungal chitinase production by *Trichoderma harzianum*. Process Biochemistry. 2004;39:1583–1590.
10. Donderskin W, Trzebiatowska M. Influence of physical and chemical factors on the activity of chitinases produced by planktonic bacteria isolated from Jeziorak Lake. Journal of Environmental Studies. 2000;9(2):77–82.
11. Han Y, Yang B, Zhang F, Miao X, Li Z.Characterization of antifungal chitinase from marine *Streptomyces* sp. DA11 associated with South China Sea Sponge Craniella Australiensis. Journal of Biotechnology. 2009;11:132–140.
12. Joo GJ. Purification and characterization of an extracellular chitinase from the antifungal biocontrol agent. Streptomyces halstedii. Biotechnology Letter. 2005;27: 1483–1486.
13. Sharma V, Shanmugam V. Purification and characterization of an extracellular 24 kDa chitobiosidase from the mycoparasitic fungus *Trichoderma saturnisporum*. Journal of Basic Microbiology. 2011;51:1–8.
14. Prahbat N, Garma G, Pramella J, Rajesh M. Microbial enrichment to enhancement, the diseases suppressive of compost. European Journal of soil Biology. 2013; 39:157-163.
15. Schlegel HG.General microbiology. Warsaw, Poland: Polish Scientific Publishers. 2006;237-242.
16. Brzezinska MS, Jankiewicz U. Production of antifungal chitinase by *Aspergillus niger* LOCK 62 and its potential role in the biological control. Current Microbiology. 2012;65(6):666-672.
17. Rattanakit N, Yano SH, Plikomol A, Wakayama M, Tachiki T. Purification of Aspergillus sp. S1-13 chitinases and their role in saccharification of chitin in mash of solid-state culture with shellfish waste. Journal of Bioscience and Bioengineering. 2007;103(6):535–541.
18. Harighi M, Motallebi M, Zamani MR. Purification of chitinase from *Trichoderma atroviride* PTCC5220. Iran Journal of Biology 2006;19:203–214.
19. Yuli, P, Suhartono M, Rukayadi Y, Hwang J, Pyunb YR. Characteristics of thermostable chitinase enzymes from the Indonesian Bacillus sp .13.26. Enzyme Microbiology and Technology 2004;35: 147–53.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/60739