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Journal of Advanced Scientific Research

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ISSN
0976-9595
Research Article

Production and Purification of Chitinase by *Streptomyces* sp. from Soil

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ABSTRACT

Microbial production of chitinase has captured the worldwide attention of both industrial and scientific environments, not only because of its wide spectrum of applications but also for the lacuna of an effective production method. Chitinase production by a terrestrial *Streptomyces* species was studied under sub-merged fermentation. Chitinase production started after 24 h of incubation and reached maximum levels during the sixth day of cultivation. A high level of chitinase activity was observed in the culture medium with pH 7 at 30°C. Culture medium amended with 0.4% colloidal chitin was found to be suitable source and the influence of additional carbon on chitinase production was also studied and the results revealed that the sucrose served as a good carbon source to enhance chitinase yield. Chitinase was purified from crude enzyme extract by two steps (ammonium sulphate precipitation and dialysis) and the molecular weight was determined by SDS-PAGE which exhibits a distinct protein band near to 34 kDa. The present study provides a suitable medium for improved chitinase production by *Streptomyces* species. Moreover, the study reflects the potential of *Streptomyces* species to produce chitinase for biotechnological applications.

Keywords: Chitinase, *Streptomyces,* Actinomycetes, Microbial enzymes

1. INTRODUCTION

Chitinase is an important chitin degrading enzyme which is involved in physiological reactions, bioconversion processes of wastes from crustacean chitin, in chitin mineralization from marine and soil environments & in plant protection by preserving them from chitin, containing parasites such as fungi which is used by insects to degrade the structural polysaccharide "chitin" during the molting process [1].

Chitinase enzyme is very important in the biological control of pests [2]. Among the microorganisms, approximately 90-99% of the chitinolytic populations are actinomycetes [3]. Actinomycetes play an important role in the biological control of insects through the production of insecticidally active compounds against the house fly *Musca domestica* [4] and it is also considered to be significant degraders of organic matter [5]. Actinomycetes, particularly *Streptomyces sp*. have been a widely exploited group of microorganisms in the production of secondary metabolites and enzymes of commercial importance in medical and agricultural applications [6].

2. MATERIAL AND METHODS

2.1. Screening of chitin utilizers

Different types of bacteria were isolated from the soil by serial dilution method [7] and they were screened for the production of chitinase by plate assay method. Sterile production media with 0.1% colloidal chitin and 1.5% agar was inoculated with the organism. After incubation period, 0.1% Congo red solution was fed over the plates and observed for clearance zone. Formation of clear utilization zone around the organism was considered as positive (chitin utilizers).

2.2. Selection

The selection of working strain was made on the basis of their chitinase activity determined qualitatively (Measuring clearance zone) and quantitatively (chitinase assay). The selected strains were allowed to grow on chitin mineral salt medium where chitin was the sole source of carbon. The organism showed good visible growth on incubation for 7 days. They showed clear zone around the colonies which indicates extra cellular chitinase production. The organisms (*Streptomyces sp.,)* were identified and maintained on sterile starch casein agar slants at 4°C.

2.3. Preparation of spore suspension

50 ml of sterile distilled water was added to 100 ml flask containing the culture. The flask was shaken well to harvest the spores. From this 1ml was taken and inoculated into 50 ml of liquid medium (production Medium). This method of preparation of inoculum was followed throughout the study.

2.4. Optimization of chitinase production

The optimization study of the following parameters was done for better growth and production of the enzyme.

2.5. Effect of incubation time

To determine the optimum incubation period for chitinase production, inoculated flasks were incubated in a rotary shaker in 100 rpm at room temperature for about eight days. Every 24 hours the culture filtrate was harvested and checked for the enzyme activity as well as the total protein content.

2.6. Effect of substrate concentration on chitinase production

50 ml of sterile production medium prepared with different substrate (chitin) concentration of 0.4 %, 0.45%, 0.5%, 0.55% and 0.6% then all conical flasks inoculated with 2ml spore suspension of the culture and incubated under the shaking condition (120 rpm). Then culture filtrate was harvested for every day and the enzyme assay as well as the protein content was studied for 8 days.

2.7. Effect of pH on chitinase production

50 ml of production medium was prepared and pH of the medium was adjusted to 5.5, 6.0, 6.5, 7.0 and 7.5. The sterilized production medium were inoculated with 2ml spore suspension and incubated under the shaking condition. The enzyme activity and protein content study was done for every day up to 8 days. Sterile uninoculated production medium was used as blank for chitinase assay.

2.8. Effect of temperature on chitinase production

50 ml of production medium was prepared, sterilized and 2ml inoculum was added then incubated at different temperatures at 25°C, 30°C, 35°C and 40°C. The culture filtrate was harvested for every 24 hours. The enzyme activity and protein content was studied for up to 8 days.

2.9. Effect of carbon sources on chitinase production

Effect of different carbon sources on chitinase production 50 ml of production medium with different carbon sources (2%) such as Sucrose, Dextrose, Lactose, Xylose and Mannitol was prepared, sterilized, inoculated and incubated up to 8 days under the shaking condition. The culture filtrate was harvested for every 24 hours and the enzyme activity as well as the protein content was measured.

2.10. Purification of chitinase enzyme

2.10.1. Ammonium Sulphate precipitation

1000 ml of culture filtrate solution was treated with 70% of saturated ammonium sulphate solution. The crude enzyme was continuously stirred using magnetic stirrer and kept at 4°C for overnight, the enzyme was centrifuged at 10,000 rpm for 15 minutes, then supernatant was discarded and the pellets were collected using 0.02 µl (Tris buffer 0.02 M pH 8.8).

2.10.2. Dialysis

Dialysis membrane was treated in water bath at 60^0C for 20 minutes. Then it was washed thoroughly with glass distilled water. A knot was made at one end the membrane and is used for filling the sample. To this dialysis bag, the sample was added and it was dialyzed against 0.02 M Tris buffer of pH 8.8 for overnight at 4°C by placing on a magnetic stirrer. The buffer used for dialysis should be removed twice for the complete removal of salts.

2.10.3. Lyophilization

The dialyzed sample was collected in a sterile round bottom flask. It was rotated on ice at 45° angles for the formation of uniform thin layer coating of sample on the flask and lyophilized using the Virtis lypophilizer.

2.10.4. Polyacrylamide gel electrophoresis

SDS-PAGE electrophoresis was carried out and molecular weight was determined. The protein content was estimated [8].

2.10.5. Chitinase Assay

Chitinase assay was performed using modified colorimetric method for the estimation of N acetyl amino sugar [9]. The unit (U) of chitinase activity is defined as the amount of enzyme required to produce 1.0 VM of N-Acetyl glucosamine per minute.

3. RESULTS AND DISCUSSION

Bacteria produce chitinase to digest chitin primarily to utilize it as a carbon and energy source. *Streptomyces* strains are regarded as the major producer of chitinases in soil. Figure 1 shows clear zone formation around the colonies (plate assay) where 0.1% colloidal chitin was used.

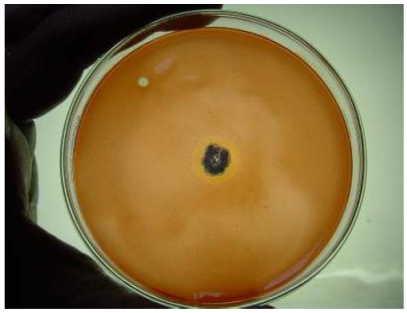

Figure 1: Screening of chitinase production from Streptomyces sp using (0.1%) colloidal chitin as substrate

Figure 2 shows Streptomyces sp crude enzyme, hydrolyzed the 0.1% colloidal chitin. Effect of incubation days on chitinase production and total protein content Time course of enzyme production plays a very critical role in enzyme synthesis. The *Streptomyces sp* was incubated for 1-8 days; the production of chitinase was increased with increase in the incubation period and found maximum on 6th day after incubation (Fig. 3). Our results were supported by Sudhakar P. and Nagarajan P. [10] who reported the Production of Chitinase by Solid State Fermentation from *Serratia marcescens*.

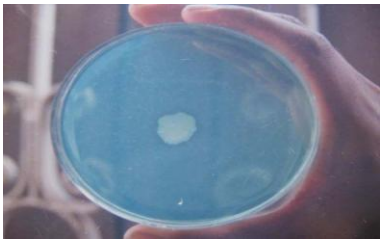

Figure 2: Chitinase activity of Streptomyces isolate in Production medium with chitin

Effect of different concentration of substrate on chitinase production and total protein content was studied. The production medium containing different substrate concentration 0.4%, 0.45%, 0.5%, 0.55%, and 0.6 % were tested for chitinase production at different days of incubation. Figure 4 shows that maximum chitinase production was observed in 0.4% substrate concentration on 6th day (156U/ml) followed by, in 0.5% of substrate concentration (102U/ml) of chitinase production was observed on 6th day. After 6th day of incubation the enzyme production was gradually decreased. Maximum amount of protein content (150µg/ml) was observed in 0.4% substrate concentration at the 6th day. Our results were in line Natarajan and Murty [11] who studied that chitin alone act as inducer and best carbon source to improve the chitinase production when compared to other substrates. Beyer and Diekmann [12] observed the cell wall degradation of *Penicillium chrysogenum* by chitinase system of *Streptomyces* sp. ATCC 11238. An increase in chitinase production was observed from *S. aureofaciens* and *S. halstedii* when cultured in a medium containing colloidal chitin supplemented with fungal cell wall preparations [13, 14].

Chitinases are fairly stable over broad pH range. The pH stability of chitinase varies from organism to organism. The effect of different pH on chitinase production and total protein content is summarized in figure 5. Among the different pH checked, the maximum enzyme production (94U/ml) was observed on 6th day at pH 7. Similar results were observed by Brurberg et al., [15] who invented that the chitinase from *Streptomyces* are found to be stable over a pH range of 4.0 to 10.0 and the optimum pH for *S. marcescens* and *Serratia liquifaciens* was found to be 5.0 to 6.0. In contrast Hara et al. [16] has suggested that the *Streptomyces erythraceus* chitinases has an optimum pH 5.0.

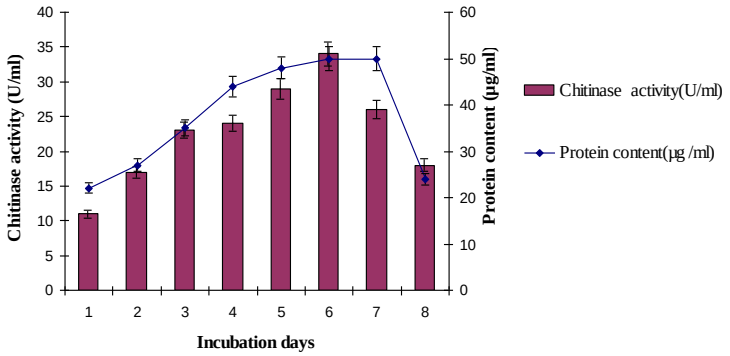

Figure 3: Effect of incubation days on chitinase production

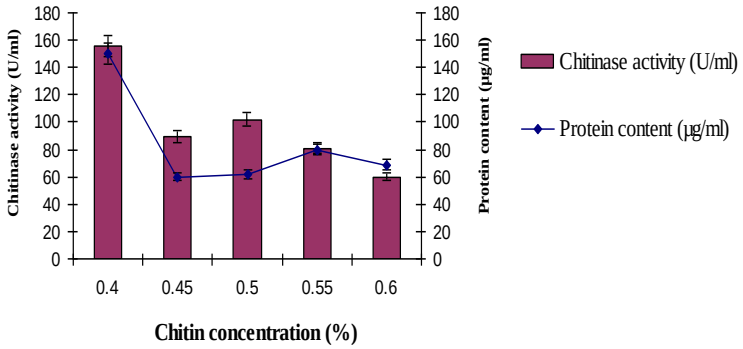

Figure 4: Effect of substrate concentration on chitinase production and protein content in Streptomycec sp.

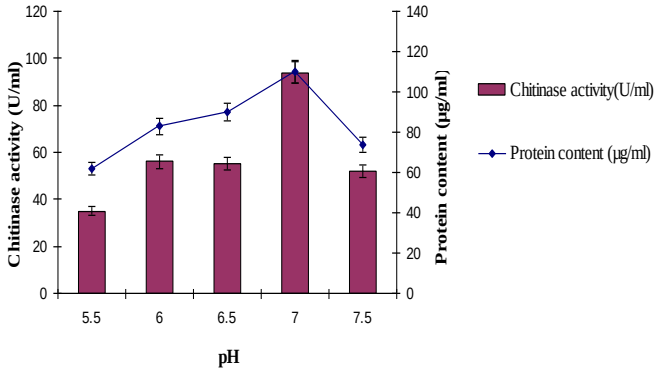

Figure 5: Effect of pH on chitinase production and protein content in Streptomyces sp.

Effect of different carbon sources on chitinase production and protein content is shown in figure 6. The production medium containing different carbon sources (2%) i.e, sucrose, galactose, maltose, and mannitol was incubated up to 8 days under the shaking condition. Results presented in figure 6 showed that the maximum enzyme production (118 U/ml) occur on 6th day when (2%) sucrose is used as carbon sources, followed by dextrose (60 U/ml) and Mannitol (38U/ml). According to Elad et al., [17] in *Trichoderma harzianum* there is a slight increase in enzyme activity when the medium is

supplemented with N-acetyl glucosamine and glucose. Maximum production of chitinase obtained in *M. verrucaria* was observed with chitin as a carbon source and no detectable activity was seen in the culture grown with lactose, maltose, sucrose, chitosan, starch and cellulose [18]. In contrast, Kolla et al., [19] reported that the maximum enzyme activity was observed when the medium was supplemented with starch as a carbon source.

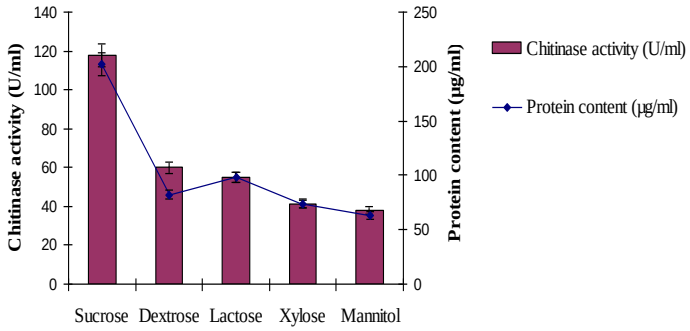

Figure 6: Effect of carbon source on chitinase production and protein content in Streptomyces sp.

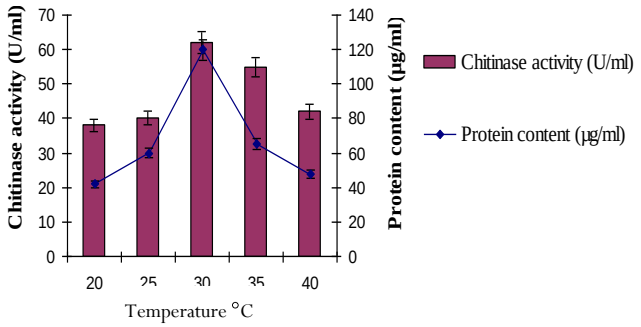

Figure 7: Effect of temperature on chitinase production and protein content in Streptomyces sp.

The production medium inoculated with spores' suspension under the shaking condition at 20°C, 25°C, 30°C, 35°C and 40°C up to 8 days. The results are presented in figure 7.

The maximum enzyme production was observed at 30^0C on 6^{th} day (62U/ml). According to Elad et al., [17] the optimum temperature for chitinase production *Trichoderma harzianum* was 28°C and *Serratia marcescence* produced high amount of chitinase in controlled temperature at 30^0C in continuous culture. The optimum temperature for chitinase production *Mucor rouxii* was 28°C isolated from soil has its optimum temperature at 30°C for produce maximum amount of chitinase. Our results are in line with the results of Priya et al., [20] who reported that the maximum chinitase enzyme production was occurred at pH 7.0, temperature 35°C and 0.2% colloidal chitin was used as substrate.

3.1. Purification of chitinase enzyme

After the production, the enzyme was purified using 70% ammonium sulphate precipitation and dialyzed against 0.02 M Tris buffer of pH 8.8 for overnight at 4°C. The results of purification steps of chitinase of *Streptomyces sp* are presented in Table 1.

After the two steps purification the purification fold was 1.08 with yield 58.3% of the original activity. Similar results were obtained by Ikeda et al., [21] who reported that the chinitase activity was increased about 1.5- to 3-fold with the presence of NaCl.

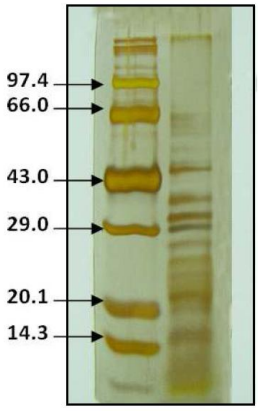

Figure 8: Extracellular protein profile of Streptomyces sp. on SDS-PAGE; *Lane 1: Standard protein marker; Lane 2: Crude chitinase of Streptomyces sp*.

Table 1: Purification and recovery of chinitase from Streptomyces sp

Steps	Chitinase activity (U)	Total protein (mg)	Specific activity (U/mg)	Purification (fold)	Recovery (%)
Crude extract	155	34	4.55	1	100
70% Ammonium sulphate precipitation	120	26	4.61	0.98	77.4
Dialysis	90.5	18	5.02	1.08	58.3

The purified enzyme was subjected to SDS-PAGE for determination of molecular weight of the protein. Purified enzyme preparation showed only one band corresponding to molecular weight of approximately 34kDa (Fig. 8). Kono et al., [22] and Matsumiya et al., [23] reported the molecular mass for this enzyme lay between of 42 kDa and 46 kDa for red sea bream and the 38 kDa for common mackerel.

Chitinases from various *Streptomyces* were shown to possess molecular weights as 20 kDa from *Streptomyces* sp. M-20 [24], 28 kDa, 35 kDa and 45 kDa from *Streptomyces* sp. NK 1057 [25], 43 kDa and 45 kDa from *S. albovinaceus* S-22 [26] and 49 kDa from *S. griseus* HUT 6037 [27] by SDS-PAGE analysis.

4. CONCLUSION

Streptomyces species chitinase is active over a wide range of operating and environmental conditions and hence it is designated as one of the best organism to study the production as well biochemical aspects of chitinase.

5. ACKNOWLEDGEMENT

We, the authors are thankful to our Chancellor, Advisor, Vice Chancellor and Registrar of Karpagam University for providing facilities and encouragement for this study.

6. REFERENCES

1. Zhang H, Huang X, Fukamizo T, Muthukrishnan S, et.al. *Insect Biochem Molec*, 2002; **32:** 1477–1488.
2. El–Tarabily KA. *Australian J Botany*, 2003; **51:** 257–266.
3. Mukherjee G, Sen SK. *Ind J Exp Biol*, 2004; 541-544.
4. Hussain AA, Mostafa SA, Ghazal SA, Ibrahim SY. *African J Mycol Biotechnol*, 2002; **10:** 63–80.
5. Basilio AI, Gonzalez MF, Vicente J, Gorrochategui A, et.al. *J Appl Microbiol*, 2003; **95:** 814–823.
6. Kumar D, Gupta RK. *Ind J Biotechnol*, 2006; **5:** 20-25.
7. Waksman SA, Fred EB. *Soil Sci*, 1922; **14:** 27-28.
8. Bradford MM. *Analyt Biochem*, 1976; **72:** 248-254.
9. Reissig JL, Strominger JL, Leloir LF. *J Biol Chem*, 1955; **217:** 959–966.
10. Sudhakar P, Nagarajan P. *Int J Chem Tech Res*, 2011; **3(2):** 590-598.
11. Natarajan K, Murty VR. *Biological Segment*, 2010; **1(1):** BS/1510.
12. Beyer M, Diekmann H. *Appl Microbiol Biotechnol*, 1985; **23:** 140-146.
13. Joo JG. *Biotechnol Lett*, 2005; **27:** 1483-1486.
14. Taechowisan T, Peberdy JF, Lumyong S. *Anals Microbiol*, 2003; **53:** 447-461.
15. Brurberg MB, Nes IF, Eijsink VGH. *Microbiology*, 1996; **142:** 1581-1589.
16. Hara S, Yamamura Y, Fyjii Y, Mega T, et.al. *J Biochem*, 1989; **105:** 484-489.
17. Elad Y, Chet HK, Henis Y. *Can J Microbiol*, 1982; **28:** 719-725.
18. Vyas P, Deshpande M. *J Gen Appl Microbiol*, 1991; **37(3):** 267-275.
19. Kolla JP, Narayana, Vijayalakshmi M. *Braz J Microbiol*, 2009; **40:** 725-733.
20. Priya CS, Jagannathan N, Kalaichelvan PT. *Int J Pharma Bio Sci*, 2011; **2(2):** 210-219.
21. Ikeda M, Miyauchi K, Mochizuki A, Matsumiya M. *Protein Expr Purif*, 2009; **65:** 214-222.
22. Kono M, Matsui T, Shimizu C. *Nippon Suisan Gakkaishi*, 1987; **53:** 131–136.
23. Matsumiya M, Mochizuki A. *Fish Sci*, 1996; **62:** 150-151.
24. Kim KJ, Yang YJ, Kim JG. *J Biochem Molecular Biol*, 2003**; 36:** 185-189.
25. Nawani NN, Kapadnis BP. *World J Microbiol Biotechnol*, 2004; **20:** 487- 494.
26. El-Sayed ESA, Ezzat SM, Ghaly MF, Mansour M, et.al. *World J Microbiol Biotechnol*, 2000; **16:** 87-89.
27. Tanabe T, Kawase T, Watanabe T, Uchida Y, et.al, *J Biosci Bioeng*, 2000; **89:** 7-32.