Characterization of extracellular chitinase produced from *Streptomyces rubiginosus* isolated from rhizosphere of *Gossypium* sp.

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Characterization of extracellular chitinase produced from *Streptomyces rubiginosus* isolated from rhizosphere of *Gossypium* sp.

Sneha Jha¹, Hasmukh A. Modi¹ and Chaitanya Kumar Jha²*

Abstract: In the present study, 28 chitinase producing Actinomycetes were isolated from rhizospheric soil samples of *Gossypium* sp. (Cotton) growing in Dastan farm, Naroda, Gujarat. Amongst them, eleven of these isolates produced significantly higher levels of chitinase and were further studied quantitatively. Results indicated that SP 24 was found to be the most prominent chitinase producer, giving 2.790 U/ml chitinase activity by using 1% colloidal chitin as substrate concentration, amongst the isolates under study. In case of isolate SP 24, maximum chitinase production was obtained after 72 h of incubation period and in culture medium having pH 7 at temperature 30°C. Amendment of peptone as a sole nitrogen source to colloidal chitin broth (CCB) enhanced chitinase activity. Furthermore, xylose as a sole carbon source added to CCB medium also increased chitinase activity. Effective production method of chitinase by *Actinomycetes* has captured the attention due to its varied applications in industries as well as in medicines. Chitinase enzyme has wide applications such as for control of phytopathogenic fungi, in food industry by increasing shelf-life of fruits, in bioconversion process.

Subjects: Bioscience; Engineering & Technology; Environment & Agriculture

Keywords: enzymes; chitin; *Actinomycetes*; N-acetylglucosamine; cultural conditions
1. Introduction

The annual worldwide capitate of chitin is implicit to be 1–100 billion metric tons, which makes chitin the second most abundant polysaccharide. Chitinase (EC 3.2.1.14), industrially important enzyme belongs to glycosyl hydrolase degrades chitin, an insoluble linear β-1, 4-linked polymer of N-acetylglucosamine into its end products i.e. N-acetylglucosamine, glucosamine and chitobiose. These end products are also responsible to induce the synthesis of chitinase (Montgomery & Kirchman, 1994). Amongst all micro-organisms, about 90–99% of chitinolytic organisms are Actinomycetes. Actinomycetes and particularly Streptomyces species are important saprophytic soil microorganisms and good sources of novel antibiotics, enzymes, enzyme inhibitors, immunomodifiers, and vitamins (Kumar & Gupta, 2006). Several chitinolytic enzymes have been identified in various Streptomyces sp., including, Streptomyces plicatus, S. lividans, S. virdificans, S. halstedii, S. aureofaciens, S. diasitapiticus, S. thermoviolaceus and S. griceus. For the partial degradation of old exoskeletons in invertebrates, chitinases are essential (Ruiz-Herrera & Martinez-Espinoza, 1999). In plants, they act in defense mechanism against phytopathogenic fungi (Honee & Visser, 1993).

Screening and isolation of microorganisms capable of producing chitinase is usually done on a medium containing chitin. Resistances to drought condition as well as towards wet and dry heat were found by aerial spores of most Actinomycetal genera compare to that of the vegetative hyphae. Accordingly, if soil is pretreated by either drying or heating enhanced the growth of rare Actinomycetes (Kim, Lee, Shimazu, Kwon, & Park, 1995). As an alternative for isolation of Actinomycetes, use of chemicals such as phenol was made (Hayakawa, Tamura, Iino, & Nonomura, 1991). Production of chitinase as well as growth of Actinomycetes varied with culture conditions such as carbon and nitrogen sources, pH, Temperature and metal ions. It is essential to increase enzymatic activity of microorganisms so that a symbiotic relationship exists between plants as well as microbes. This also depends on nutritional supply, physical parameters and genetic structure of that strain. To optimize cultural conditions for the economical production of chitinase, ideal selection of carbon and nitrogen sources as well as cultural conditions like pH, temperature should be studied.

The aim of this research was to screen potential chitin degraders which produce significant amount of chitinase that can further be utilized for various applications.

2. Methodology

2.1. Chemicals

Chitin, p-dimethylaminobenzaldehyde, N-acetylglucosamine, glacial acetic acid, potassium tetraborate those were procured from (Hi-media) which is commercially available.

2.2. Colloidal chitin preparation

Chitin flakes (10 g) were suspended in concentrated HCl (60 mL) and incubated on rotary shaker for 1–2 h at room temperature until chitin flakes were completely dissolved. The resulting solution was then poured into doubly deionized water (400 mL) with rapid stirring to form the precipitates of colloidal chitin, which were subsequently collected by centrifugation at 7,000 × g for 10 min at 4°C. The precipitates were washed with sterile distilled water (10 mL each) several times to bring the pH value of the colloidal chitin suspension to 2.0–3.0. After neutralization with sodium hydroxide (1 M), the low-salt colloidal chitin was obtained on repeated centrifugation (7,000 × g, 10 min) and washing with sterile water 2–3 times. The colloidal chitin was kept at 4°C as a pellet for further applications (Wu, Cheng, & Li, 2009).

2.3. Isolation of Actinomycetes

Soil samples were collected in sterile polypropeline bags from the rhizosphere of Gossypium sp. (Cotton) grown in Dastan farm, Naroda, Gujarat. For isolation, Bennet’s agar medium was used. First, little amount of the soil from different fields were taken in petriplates and on them calcium carbonate was sprinkled, these was kept for drying for a period of 4–5 days to allow Actinomycetes to
sporulate. Dilutions ($10^{-3}$ to $10^{-6}$) of enriched soil samples were plated which were incubated at 30°C. Desired colonies culturally appeared as *Actinomycetes* were picked up and restreaked for obtaining pure colonies. *Actinomycetes* were isolated by the serial dilution and spread plate techniques.

2.4. **Primary screening of chitin degrading Actinomycetes**

Actinomycetal isolates obtained from Bennet’s agar media were then screened for chitin hydrolysis by spotting on the centre of 1% colloidal chitin agar (CCA) (colloidal chitin-1%, NaNO$_3$–0.2 g, K$_2$HPO$_4$–0.1 g, MgSO$_4$–0.1 g, CaCO$_3$–0.1 g, FeSO$_4$·7H$_2$O–0.001 g, KCL–0.05 g) media at neutral pH and were incubated at 30°C. The zone of clearance due to chitin hydrolysis was recorded up to 8 days. The isolates those formed clear zone of hydrolysis above 15 mm were selected. Selection ratio (S/R) for each isolates was determined on the basis of zone of hydrolysis and colony diameter. Isolate showing highest S/R was considered for further optimization process.

Selection ratio = Zone of clearance (mm)/Colony diameter (mm)

2.5. **Nucleotide sequence accession number**

Sequence data of 16s rRNA gene of isolate SP 24 giving highest activity in this study was deposited in the GenBank nucleotide sequence databases under accession number KT198721.

2.6. **Optimization of cultural conditions for chitinase production**

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

2.6.1. **Effect of substrate concentration on chitinase production**

Fifty milliliters of sterile production medium prepared with different concentrations of colloidal chitin (0.5, 1.0, 1.5, 2.0, and 2.5%) were inoculated with 3 ml inoculum in 250 ml Erlenmeyer flasks and incubated under shaking conditions (100 rpm) at 30°C. Then culture filtrate was harvested every day and the enzyme assay as well as the protein content were estimated till 96 h of incubation period.

2.6.2. **Effect of pH on chitinase production**

Fifty milliliters of four different buffer medium were prepared for studying effect of pH (Glycine-HCl pH 2.2–3.4, Acetate pH 3.6–5.6, phosphate pH 5.8–8.0 and glycine-NaOH pH 8.6–11.0) and inoculated with 3 ml of culture in 250 ml Erlenmeyer flasks and incubated under shaking conditions (100 rpm) at 30°C. Then culture filtrate was harvested every day after 24 h and the enzyme assay as well as the protein content were estimated till 96 hrs of incubation period.

2.6.3. **Effect of temperature on chitinase production**

Fifty milliliters of sterile production medium were prepared and 3 ml inoculum was added, incubated at different temperatures at 25, 30, 40, 50, and 60°C. The culture filtrate was harvested for every 24 h. The enzyme activity and protein content were studied till 96 h of incubation period.

2.6.4. **Effect of incubation time**

To determine the optimum incubation period for maximum chitinase production, inoculated flasks were incubated in a rotary shaker in 100 rpm at 30°C for 96 h. Every 24 h the culture filtrate was harvested and the enzyme activity as well as the total protein content were measured.

2.6.5. **Effect of supplemental carbon sources on chitinase production**

Effect of different carbon sources on chitinase production were studied by inoculating 3 ml of inoculum in 50 ml of production medium with different carbon sources (2%) such as sucrose, dextrose, lactose, xylose and mannitol and incubated up to 96 h under shaking condition at 100 rpm. The culture filtrate was harvested after every 24 h and the enzyme activity as well as the protein content were measured.
2.6.6. Effect of supplemental nitrogen sources on chitinase production
Effect of different nitrogen sources on chitinase production were studied by inoculating 3 ml of inoculum in 50 ml of sterile production medium with different nitrogen sources (2%) such as yeast extract, beef extract, peptone, ammonium sulphate and ammonium nitrate, incubated up to 96 h under shaking conditions at 100 rpm. The culture filtrate was harvested after every 24 h and the enzyme activity as well as the protein content were estimated.

2.6.7. Chitinase assay
The reaction mixture contained 1 ml of 0.1% colloidal chitin in phosphate buffer (0.05 M, pH 7.0) and 1 ml culture filtrate was incubated at 37°C for 1 h. Suitable substrate and enzyme blanks were included. Chitinase activity was assayed by following the spectrophotometric method of Reissig, Strominger, and Leloir (1955). The reaction was terminated by adding 0.1 ml of 0.08 M potassium tetraborate, (pH 9.2) to 0.5 ml of reaction mixture and then was boiled in a water bath for 3 min. Then 3 ml of diluted p-dimethylaminobenzaldehyde [10 g of DMAB dissolved in 100 ml of glacial acetic acid containing 12.5% 10 N (v/v) hydrochloric acid] reagent was added and again incubated at 37°C for 15 min. The released product in the reaction mixture was read at 585 nm in a visible spectrophotometer. Chitinase activity was determined using N-acetylglucosamine (Hi-media) as the standard. One unit of chitinase activity is defined as the amount of enzyme, which produces 1 μ mole of N-acetylglucosamine in 1 ml of reaction mixture under standard assay condition (Mathivanan, Kabilan, & Murugesan, 1998).

2.6.8. Estimation of protein
Protein content of all enzyme preparations was estimated by Folin-Lowry method (Lowry, Rosenbrough, Fan, & Randall, 1951).

3. Results

3.1. Isolation of Actinomycetes from Gossypium rhizosphere
There were number of colonies that were obtained on Bennet’s agar plates. The major pigmentation observed amongst rhizosphere of Gossypium sp. were white (50%), light pink (30%), yellow (25%), greyish white (10%), golden yellow (10%), and off white (5%).

3.2. Screening of chitin degrading Actinomycetes
In the present study, CCA media amended with colloidal chitin-1%, NaNO₃–0.2 g, K₂HPO₄–0.1 g, MgSO₄–0.1 g, CaCO₃–0.1 g, FeSO₄·7H₂O–0.001 g, KCl–0.05 g with pH 7 was found to be suitable for the growth of chitin degrading Actinomycetes at 37°C. Isolated organisms on Bennet’s agar media were then screened and from those about 28 different organisms showed chitin degradation. The zone size of the isolates was measured against creamy background and it was found on the basis of S/R that SP 24 showed maximum chitin degradation (Table 1).

| Isolate no. | Zone of clearance (mm) | Colony diameter (mm) | Selection ratio |
|-------------|------------------------|----------------------|----------------|
| SP 2        | 25                     | 12                   | 2.083          |
| SP 5        | 30                     | 15                   | 2              |
| SP 9        | 28                     | 17                   | 1.64           |
| SP 10       | 20                     | 12                   | 1.66           |
| SP 11       | 27                     | 15                   | 1.8            |
| SP 12       | 30                     | 20                   | 1.5            |
| SP 14       | 25                     | 15                   | 1.66           |
| SP 22       | 28                     | 15                   | 1.86           |
| SP 24       | 32                     | 15                   | 2.13           |
| SP 25       | 18                     | 11                   | 1.636          |
| SP 26       | 29                     | 18                   | 1.61           |
3.3. Molecular identification of strain SP 24

The 16s rRNA sequence obtained after sequencing is shown in Figure 1. The sequence is rich in its G + C content as known for Streptomyces spp. Phylogenetic analysis based on 16s rRNA gene sequences available from the European Molecular Biology Laboratory data library constructed after multiple alignments of data by ClustalX. Distances and clustering with the neighbor-joining method was performed by using the software packages Mega version 4.0. Bootstrap values based on 500 replications are listed as percentages at the branching points.

The strain SP 24 formed a separate branch in neighbor-joining (Figure 1) and was identified as *Streptomyces rubiginosus* after 16s rRNA sequencing, grouped most closely to a cluster containing to *Streptomyces vinaceus*. This work is novel in terms of chitinase production by *S. rubiginosus* and we are the first reporting it.

3.4. Optimization of cultural conditions

The “altering one factor at a time” method was used to determine the impact of various culture conditions, such as substrate concentration, pH, temperature, incubation time, additional carbon and nitrogen sources, on the production of chitinases by SP 24 cultured and incubated for 96 h. Figure 2 shows Colloidal chitin broth (CCB) medium after inoculation of isolate SP 24.

3.4.1. Effect of substrate concentration

Amongst five different concentrations tested, colloidal chitin at 1.0% considerably enhanced the chitinase activity for isolate SP 24 by 2.76 U/ml followed by 1.5% which gave 1.65 U/ml chitinase activity (Figure 3). Protein content was about 371.30 μg/ml. Beyond 1.5% substrate concentration enzyme activity decreased. Lower the substrate concentration (0.5%) lower is the chitinase activity (1.42 U/ml).

Figure 1. Phylogenetic analysis based on 16S rRNA gene sequences of SP 24.

Notes: Phylogenetic analysis based on 16S rRNA gene sequences available from the European Molecular Biology Laboratory data library constructed after multiple alignments of data by ClustalX. Distances and clustering with the neighbor-joining method was performed by using the software packages Mega version 4.0. Bootstrap values based on 500 replications are listed as percentages at the branching points.
3.4.2. Effect of pH
The effect of pH on the chitinase activity was studied with glycine-HCl (pH 2.2–3.4), acetate (pH 3.6–5.6), phosphate (pH 5.8–8.0) and glycine-NaOH (pH 8.6–11.0) under the standard conditions. The pH-activity profile was bell shaped for SP 24 with maximum value at pH 7.0 (Figure 4). This enzyme showed its activity over broad pH range (5–7 pH). At pH 7 maximum chitinase activity was found about 2.45 U/ml. Chitinase activity gradually decreased as pH increased above 7–9 about 1.67 U/ml and it also decreased if pH lowered from 7 to 5 about 1.35 U/ml.

3.4.3. Effect of temperature
The activity of the enzyme increased as the temperature was increased. The enzyme activity dramatically fell upon reaching 60°C and activity was lost at 70°C and beyond, a temperature optimum was displayed at approximately 30°C for isolate SP 24 and its activity was found to be about 2.96 U/ml while its protein content was found to be about 397 μg/ml. Increasing the temperature from 30 to 40°C gradually lowered the chitinase activity (Figure 5) and it was found to be about 2.64 U/ml but protein content increased to about 432 μg/ml. At higher temperature 50 and 60°C, chitinase activity dropped down (1.64 and 0.56 U/ml) and protein content also reduced to 312 and 263 μg/ml.
3.4.4. Effect of incubation time
Present study showed that there was no degradation on first day of incubation and hence this suggests that there was no N-acetylglucosamine production and so, activity could not be found. There was very less chitinase activity in the first 48 h (Figure 6) but it increased rapidly in the next 72 h and after that gradually declined.

3.4.5. Effect of supplemental carbon source
Effect of different carbon source (sucrose, dextrose, xylose, lactose, mannitol) and the protein content was studied and it was observed that 2% xylose showed maximum chitinase activity (2.5 U/ml) after 72 h of incubation period followed by sucrose (1.9 U/ml), dextrose (1.5 U/ml), lactose (0.92 U/ml) and mannitol (0.3 U/ml). The protein content obtained by xylose was 150.02 μg/ml. Chitinase activity increased after every 24 h, in medium containing xylose, but it decreased after 96 h of incubation period (1.2 U/ml). Protein content was found utmost in 2% sucrose about 176.09 μg/ml (Figure 7).

3.4.6. Effect of supplemental nitrogen sources
Effect of different nitrogen source (ammonium sulphate, peptone, yeast extract, beef extract, ammonium nitrate) on chitinase production and total protein content was studied and it was observed that the production medium containing peptone showed maximum activity (2.0 U/ml) after 72 h of incubation period and total protein content was about 240.09 μg/ml. Peptone showed maximum chitinase activity followed by beef extract (1.75 U/ml) and yeast extract (1.5 U/ml). Ammonium sulphate and ammonium nitrate showed less chitinase activity (0.9 U/ml) as described in Figure 8.
4. Discussion
A group of enzymes which define a chitinolytic system is involved in degradation of chitin to yield free monomers of N-acetylglucosamine units which is present in a variety of organisms such as bacteria, fungi, Actinomycetes, yeast, plants, insects and also in humans (Bhattacharya, Nagpure, & Gupta, 2007; Mellor, Nicholas, & Adams, 1994; Royer, Fraichard, & Bouhin, 2002; Tjoelker et al., 2000). Amongst Actinomycetes, the best known producer of chitinase is Streptomyces spp. (Ohno et al., 1996; Okazaki, Takahashi, Kizuka, & Enokita, 1995). Chitinase is also produced by several other Actinomycetes such as Nocardia, Actinoplanes, and Micromonospora spp. (Iverson, Bromel, Anderson, & Freeeman, 1984). It was observed during screening experiments that one percent (1%) CCA media...
amended with other salts increased chitinase production. In plates containing CCA media each isolate showed a good zone of clearance. The ratio of zone of clearance and colony diameter was measured and amongst them highest zone of clearance was observed by SP 24 (32 mm).

Chitinase from isolate SP 24 hydrolyzed 1% colloidal chitin to produce N-acetylglucosamine. Hydrolysis of β-1, 4 linkages in chitin produced GlcNAc and N,N-diacyctethylchitobiose (CHBdiNAc) as single reaction product (Ohno et al., 1996). Amendment of different concentrations of colloidal chitin to production medium either increased or decreased chitinase activity. Flasks were inoculated with spore suspension containing $1 \times 10^6$ cfu and incubated at 30°C at 100 rpm in a rotary shaker. For chitinase assay, crude enzyme extract was obtained by centrifugation of culture filtrate after every 24 h in cooling centrifuge at 4°C. Maximum chitinase production was obtained in 1% colloidal chitin concentration (Substrate) about 2.76 U/ml. As reported by Gupta, Saxena, Chaturvedi, and Virdi (1995) S. viridificans showed maximum chitinase activity in 1.5% chitin. For enhancing the production of chitinase of Aeromonas hydrophilia, Yabuki et al. (1986) used 1.0% chitin as an optimal carbon source. Maximum chitinase yield was obtained using colloidal chitin as a substrate for B. alvei about 1.34 U/ml, for B. sphaericus about 1.29 U/ml, for B. cereus about 1.34 U/ml (Wang & Hwang, 2001).

Optimum chitinase activity (2.45 U/ml) was observed at pH 7 because of which graph appears bell shaped curve. This is due to ionization of proton gradient at catalytic site of enzyme. As reported by Brurberg, Nes, and Eijsink (1996) chitinase enzyme from Serratia marcescens and Serratia liquificaciens showed its activity between pH 5.0–6.0. Chitinase from Streptomyces erthraceus has pH optima of 5.0 as reported by Hara, Yamamura, Fyjii, Mega, and Ikenaka (1989). Nawani and Kapadnis (2002) reported that chitinase enzyme from Microbispora are active at around pH 7.0.

Conformational changes occurring in structure of enzyme due to temperature differences is a common mechanism because of which enzyme activity is affected. The optimum temperature at which chitinase activity (2.96 U/ml) was found maximum is 30°C. The highest activity of S. lividans was found at temperature 50°C as reported by Neugebauer, Gamoche, Dery, and Brzezinski (1991) while S. erythraceus showed its activity at temperature ranging between 60 and 70°C. Above 70°C temperature this organism was not found to be active as reported by Hara et al. (1989). Narayana and Vijayalakshmi (2009) reported that Streptomyces Anu 6277 showed its optimum activity at temperature 35°C.

In our study, maximum chitinase activity were obtained after 72 h of incubation period after which chitinase activity decreased gradually. Young and Bell (1985) reported that the chitinase activity was present till 288 h in S. marcescens. B. licheniformis, B. cereus, B. chitinolyticus, B. ehimensis and S. griseus showed the presence of chitinase enzyme after 72–96 h (Yuli, Suharton, Rukayadi, Hwang, & Pyunb, 2004).

Of different carbon source, maximum chitinase production was observed by 2% xylose enhancing enzyme activity by acting as an inducerin CCB medium. Chandrasekar et al. (2012) who observed that in Streptomyces sp. Two percentage of sucrose gave maximum chitinase activity (118 U/ml) and protein content was about 210 μg/ml. Narayana and Vijayalakshmi (2009) reported that the maximum chitinase activity was observed when the medium was supplemented with starch as a carbon source. According to Elad, Chet, and Henis (1982) in Trichoderma harzianum there is a slight increase in chitinase activity when the medium supplemented with N-acetylglucosamine and glucose. Enhanced chitinase production (7.22 U/ml) was found by strain ANU 6277 in CYS medium amended with 0.2% starch.

There was a considerable increase in chitinase production by strain ANU 6277 in CYS medium amended with organic nitrogen sources such as 0.6% soybean meal giving chitinase activity of about 8.05 U/ml and 0.4% yeast extract giving chitinase activity of about 8.89 U/ml. Chitinase production by Alcaligenes xylosoxydans enhanced on addition of organic nitrogen sources such as yeast extract
and peptone (Vaidya, Shah, Vyas, & Chhatpar, 2001). Priya, Jagannathan, and Kalaiichelvan (2011) studied various nitrogen source like ammonium sulphate, peptone, yeast extract, colloidal chitin and malt extract and observed that colloidal chitin gave maximum chitinase activity (7.1 U/ml) followed by peptone.

5. Conclusion
In the present investigation, eleven isolates were found significant chitin degraders showing maximum zone of clearance. Amongst them, strain SP 24 showed maximum chitinase activity on 1% CCA media amended with xylose and peptone act as inducers enhancing chitinase activity in its presence. Optimal conditions for increased production of chitinase was obtained when the medium having pH 7, temperature 30°C and incubated for a period of 72 h.

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Competing Interests
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References
Bhattacharya, D., Nagpure, A., & Gupta, R. K. (2007). Bacterial chitinases: Properties and potential. Critical Reviews in Biotechnology, 27, 21–28. http://dx.doi.org/10.1080/07388550601168223

Brurberg, M. B., Nes, I. F., & Eijsink, V. G. H. (1996). Comparative studies of chitinases A and B from Serratia marcescens. Microbiology, 142, 1581–1589. http://dx.doi.org/10.1099/13500872-142-7-1581

Chandrasekar, U., Balokrishnan, S., Duraisamy, G., Manokaran, K., Ganesan, R., & Chinthamani, A. (2012). Production and purification of chitinase by Streptomyces sp. from Soil. Journal of Advanced Scientific Research, 3, 25–29.

Chandrasekar, U., Balokrishnan, S., Duraisamy, G., Manokaran, K., Ganesan, R., & Chinthamani, A. (2012). Production and purification of chitinase by Streptomyces sp. from Soil. Journal of Advanced Scientific Research, 3, 25–29.

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References
Bhattacharya, D., Nagpure, A., & Gupta, R. K. (2007). Bacterial chitinases: Properties and potential. Critical Reviews in Biotechnology, 27, 21–28. http://dx.doi.org/10.1080/07388550601168223

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Chandrasekar, U., Balokrishnan, S., Duraisamy, G., Manokaran, K., Ganesan, R., & Chinthamani, A. (2012). Production and purification of chitinase by Streptomyces sp. from Soil. Journal of Advanced Scientific Research, 3, 25–29.

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Chandrasekar, U., Balokrishnan, S., Duraisamy, G., Manokaran, K., Ganesan, R., & Chinthamani, A. (2012). Production and purification of chitinase by Streptomyces sp. from Soil. Journal of Advanced Scientific Research, 3, 25–29.

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Chandrasekar, U., Balokrishnan, S., Duraisamy, G., Manokaran, K., Ganesan, R., & Chinthamani, A. (2012). Production and purification of chitinase by Streptomyces sp. from Soil. Journal of Advanced Scientific Research, 3, 25–29.

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Chandrasekar, U., Balokrishnan, S., Duraisamy, G., Manokaran, K., Ganesan, R., & Chinthamani, A. (2012). Production and purification of chitinase by Streptomyces sp. from Soil. Journal of Advanced Scientific Research, 3, 25–29.
Priya, C. S., Jagannathan, N., & Kalaichelvan, P. T. (2011). Production of chitinase by Streptomyces hygroscopicus VMCH2 by optimization of cultural conditions. International Journal of Pharma and Bio Sciences, 2, 210–219.

Reissig, J. L., Strominger, J. L., & Leloir, L. F. (1955). A modification colorimetric method for the estimation of N-acetylamino sugars. The Journal of Biological Chemistry, 27, 959–966.

Royer, V., Fraichard, S., & Bouhin, H. (2002). A novel putative insect chitinase with multiple catalytic domains: Hormonal regulation during metamorphosis. Biochemical Journal, 366, 921–928.

Ruiz-Herrera, J., & Martinez-Espinoza, A. D. (1999). Chitin biosynthesis and structural organization in vivo. In: P. Julles & R. A. A. Muzzarelli (Eds.), Chitin and chitinases (pp. 39–53). Basel: Birkhäuser.

Tjoelker, L. W., Gosting, L., Frey, S., Hunter, C. L., Le Trong, H. L., Steiner, B. ... Gray, P. W. (2000). Structural and functional definition of the human chitin chitin-binding domain. Journal of Biological Chemistry, 275, 514–520.

Vaidya, R. J., Shah, I. M., Vyas, P. R., & Chhatpar, H. S. (2001). Production of chitinase and its optimization from a novel isolate Alcaligenes xylosoxydans: Potential in antifungal biocatalysis. World Journal of Microbiology and Biotechnology, 17, 691–696.

Wang, S. L., & Hwang, J. R. (2001). Microbial reclamation of shellfish wastes for the production of chitinases. Enzyme and Microbial Technology, 28, 376–382.

Wu, Y. J., Cheng, C. Y., & Li, Y. K. (2009). Cloning and expression of chitinase A from Serratia marcescens for large-scale preparation of N,N-diacyetylchitobiose. Journal of the Chinese Chemical Society, 56, 688–695.

Yabuki, M., Mizushima, K., Amatatsu, T., Ando, A., Fujii, T., Shimada, M., & Yamashita, M. (1986). Purification and characterization of chitinase and chitobiase produced by Aeromonas hydrophila subsp. Anaerogenes A52. The Journal of General and Applied Microbiology, 32, 25–38.

Yuli, P. E., Suharto, M. T., Rukayadi, Y., Hwang, J. K., & Pyunb, Y. R. (2004). Characteristics of thermostable chitinase enzymes from the Indonesian Bacillus sp. 13.26. Enzyme and Microbial Technology, 35, 147–153.