Extra cellular chitinase production by *Streptomyces* sp. PTK19 in submerged fermentation and its lytic activity on *Fusarium oxysporum* PTK2 cell wall

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**Abstract**

Extracellular chitinase production by a chitinolytic *Streptomyces* sp. PTK19 in submerged fermentation was studied. Different concentration of chitin, pH, temperature, different carbon sources, nitrogen sources and amino acid supplementation were tried in 50 mL of production medium, respectively. Chitin (0.4% w/v) was used as sole carbon source. Maximum chitinase production (16.53 U/mL) was obtained at 30°C under the shaking condition at 120 rpm with sucrose 80 mM as carbon source and (0.1 % w/v) peptone as nitrogen source and pH of the medium maintained at (pH 7). The culture filtrate was analyzed for chitinase activity in every three days of incubation up to 15 days. The fermentation carried out using Stirred-Tank fermenter 5-L containing 2.5-L medium with different aeration 0.5, 0.8, 1.1 vvm at 300 rpm agitation. The temperature maintained at 30°C for eight days of fermentation. Maximum chitinase production was obtained in 300 rpm agitation and 0.8 vvm aeration (31.62 U/mL). The chitinase optimum pH has been found to be 5.5 and optimum temperature at 40°C and it has been stable at 30 to 45°C. The crude chitinase protein bands profile was observed on SDS-PAGE and chitinolytic activity was observed through zymogram analysis. The *Streptomyces* sp. PTK19 crude chitinase efficacy tested against *Fusarium oxysporum* PTK2 and also the crude chitinase has been found to dissolve the phytopathogenic fungal cell wall.

**Key words:** Chitinase, *Streptomyces* sp., chitinolytic activity, submerged fermentation, *Fusarium oxysporum*

**Introduction**

Chitin is the insoluble linear β-1, 4-linked polymer of N-acetylglucosamine. It is an important constituent of the outer layer of many soil organisms, exoskeleton of arthropods and the cell wall of fungi. Chitin is found in the cuticle of insects and the shell of crustaceans and molluscs as well as in the cell walls of most taxonomic groups of fungi (Banicki-Garcia, 1968). Chitin in soil can be degraded by a wide variety of microorganisms including fungal and bacterial species. Chitinolysis, namely hydrolysis of the glycosidic bonds of chitin by chitinases, is probably the most important pathway of degradation of chitin in soil (Gooday, 1990). Streptomycetes are soil-dwelling mycelial bacteria that produce a large number of secreted proteins and many secondary metabolites, including important antibiotics. Chitin is a major nutrient source for many Streptomycetes and these microorganisms have developed complex extracellular systems for chitin utilization (Chater et al., 2009). Chitinases play an important role in the decomposition of chitin and potentially in the utilization of chitin as a renewable resource.

Production of chitinase is widespread in a variety of organisms such as bacteria, fungi, actinomycetes, yeasts, plants, protozoans, coelenterates, nematodes, arthropods and humans (Matsumiya et al., 2002; Gutowska et al., 2004; Matsumiya et al., 2006; Molinari et al., 2007; Wang et al., 2009). Chitinolytic bacteria are common in
nature where they are important degraders of chitin, a natural polymer second in abundance only to cellulose (Knorr, 1984). The sensitivity of the fungal cell wall to lytic enzymes has been exploited by using chitin-producing bacteria to control plant-pathogenic fungi in the rhizosphere (Ordentlich et al., 1988; Inbar and Chet, 1991). Bacteria produce chitinases to digest chitin primarily to utilize it as a carbon and energy source. Streptomyces strains are regarded as the major producers of chitinases in soils. Much research has been carried out to date on the purification and characterization of family 18 chitinases from Streptomyces. Because of their inhibitory abilities, Streptomyces sp. have been actively studied and utilized as biocontrol agents against various plant pathogens (Merriman et al., 1974; El-Abyad et al., 1993; Hiltunen et al., 1995; Jones and Samac, 1996). The goals of this research were to optimize the chitinase production from Streptomyces sp. PTK19. The cultural characters were optimized by amending with different concentration of chitin, various carbon and nitrogen sources, optimum pH, temperature and amino acid supplementation were studied then scale up to the Fermentation in Stirred-Tank fermenter (5L). It contained 2.5-L medium it optimized the fermentation parameters like different aeration. The crude chitinase tested against Fusarium oxysporum PTK2 for cell wall degradation.

Materials and methods

Microorganisms and maintenance

The Streptomyces sp. PTK19 isolated from shrimp cell decomposed soil and maintained on starch casein agar slant. The fungal plant pathogen Fusarium oxysporum PTK2 were used in this study was a laboratory isolates and maintained on Potato Dextrose Agar (PDA) slants and stored at 4°C.

Inoculum preparation

Fifty mL of sterile glass distilled water added to 250 mL conical flask containing the culture and shaken well to harvest the spores. Then 2 mL was inoculated into 50 mL of production medium. The cell concentration was maintained (3 X 10^7 CFU/mL) and adjusted the cell concentration using sterile glass distilled water.

Chitinase screening

The Streptomyces sp. PTK19 chitinolytic activity was tested through the production medium (Mitsutomi et al., 1995) containing 0.1% colloidal chitin and 1.5% agar and incubated for 7 days then agar was stained with 0.2% of congo red.

Cup-plate assays

Chitinase activity was assessed in cup-plate assays as described by (Bertheau et al., 1984). Using 0.1% (w/v) of colloidal chitin dissolved in sodium acetate buffer (0.05 M, pH 5.2) was used as a substrate in 1% of agar medium.

Chitinase production in submerged fermentation by Streptomyces sp. PTK19

The different pH was maintained in production medium at the range of 5.5-8.0, and the concentration of chitin was optimized to 0.1-0.5% w/v. Influence of supplementary carbon sources was studied like 2% (w/v) of dextrose, maltose, sucrose, galactose and mannitol. The effects of different millimolar concentration (20-100 mM) of sucrose were studied. The production medium treated with 0.1% of organic nitrogen sources like beef extract, peptone, casein, yeast extract, gelatin and also supplemented with 5 mM of amino acids such as alanine, valine, aspergine, methionine and histidine. A known quantity of inoculum was transferred to 50 mL of the sterilized production medium containing 80 mM sucrose, 0.1% peptone, 0.4% shrimp shell powder, 0.2% KNO_3, 0.1% K_HPO_4, 0.1% MgSO_4.7H_2O, 0.3% CaCO_3, 0.001% FeSO_4, 0.05% NaCl and pH 7 was maintained as 50 mL production medium in 250 Erlenmeyer flask. Culture was incubated at 30°C for 15 days on a rotary shaker at 120 rpm and was harvested every 3 days interval by centrifugation. The clear supernatant was used for chitinase assay.
Stirred-Tank fermenter (5-L)

Further fermentation studies were carried out using 5-L stirred-tank fermenter (Scigenic Fermenter Pvt. Ltd., Chennai) with 2.5-L culture medium and 4% inoculum. The agitation rates used were 300 rpm with 0.8vvm aeration rate at pH 7. Fermentation was followed for 8 days at 30°C. After completion of fermentation, the culture filtrate was tested for chitinase activity and protein content.

Electrophoresis

The sample was also analyzed by SDS-PAGE (10%) to determine the molecular mass of the proteins as described (Laemmli, 1970). After electrophoresis, proteins in gels were silver stained (Heukeshoven and Dernick, 1985). Medium range molecular markers were purchased from Genei Laboratories.

Preparation of colloidal chitin

Colloidal chitin was prepared by following the method of Skujins et al. (1965). A known amount of dried chitin was suspended in concentrated hydrochloric acid and kept for overnight at 4°C then filtered through glass wool. To this filtrate, 60% ethanol was added to precipitate the chitin and centrifuged at 10000 rpm for 20 minutes at 4°C. The pellet was washed repeatedly with glass distilled water and dialyzed against cold distilled water until the pH of the chitin suspension reached the pH of the distilled water. Finally the substrate was lyophilized and stored at -20°C.

Glycol chitin preparation

Glycol chitin was obtained by acetylation of glycol chitosan (Truel and Asselin, 1989).

Enzyme assay

Chitinase activity was measured by the release of N-acetyl-D-glucosamine equivalents from colloidal chitin by following the method of Reissig et al. (1955). The reaction mixture consisted of 1 mL of enzyme preparation and 1 mL 0.1% (w/v) colloidal chitin in sodium acetate buffer (0.05 M, pH 5.2) and incubated at 37°C for 2 h. The reaction mixture was centrifuged at 3000xg for 3 minutes. To 0.5 mL of the supernatant 0.1 mL of potassium tetra borate buffer (0.08 M, pH 9.2) was added and boiled for 3 minutes at 100°C and cooled. To this 3 mL of diluted dimethyl aminobenzaldehyde reagent was added and incubated at 37°C for 20 minutes for colour development. A heat killed enzyme following the same procedure was kept as control, and read at 585 nm in Milton Roy 601 Spectrophotometer.

Standard graph was prepared with curve for authentic N-acetyl-D-glucosamine to convert the absorbency values to micromoles of N-acetyl-D-glucosamine liberated from colloidal chitin.

Zymogram analysis

The crude chitinase sample was subjected to electrophoresis under Native condition (Davis, 1964). After electrophoresis the gel was equilibrated with sodium acetate buffer (50 mM, pH 5.2) for 5 minutes. This gel was overlay onto a 7.5% (w/v) polyacrylamide gel containing 0.01% (w/v) glycol chitin (Trudel and Asselin, 1989). The entire set up was kept under moist condition for 3 h at 37°C. The glycol chitin containing gel was then stained with freshly prepared 0.01% (w/v) calcoflour white M2R (Sigma Chemical Company, USA) in Tris-HCl buffer (50 mM, pH 6.8). After 5 minutes the brightener solution was removed and the gels were incubated in glass distilled water for 1 h at room temperature. Enzyme activity zone was observed by placing the gels on fotodyne transilluminator (USA) and photographed.

Protein estimation

Protein content was determined by Bradford (1976).

Characterization of crude chitinase

The effect of some factors that influence crude chitinase activity in the reaction mixture as different pH, temperature, substrates and metal ions was also studied.

pH optimum and stability

Optimum pH of the crude chitinase was determined by using 50 mM sodium acetate buffer (pH 3.5-5.5), sodium phosphate buffer (pH 6.0-8.0)
and Tris-HCl buffer (pH 8.0 - 9.5). Ten µg of crude protein was incubated for 1 h at different pH from 3.5-9.5 colloidial chitin as substrate and assayed for chitinase activity. The pH stability was assayed after pre-incubation of enzyme with the pH ranging from 3.5-9.5.

Temperature optimum and thermal stability

The optimum temperature and thermal stability were determined at different temperatures viz., 20, 25, 30, 35, 40, 45, 50, 55, 60, 65 and 70°C. Ten µg of crude chitinase protein was dissolved in 50 mM sodium acetate buffer and incubated at the above temperature for 1 h and the residual enzyme activity was assayed as described earlier. The thermal stability was investigated by incubating the enzyme at different temperature from 25°C-70°C at 120 minutes.

Substrate specificity

The substrates namely colloidial chitin, glycol chitin, fine powder of crab chitin, glycol chitosan, amylose, carboxyl methyl cellulose, maltose, cellobiose and laminarin, 0.1% in sodium acetate buffer (50 mM, pH 5.2) were chosen and incubated with the enzyme 10 µg at 37ºC for 2 h. The enzyme assay was carried out as described earlier.

Effect of different metal ions on crude chitinase

The response of chitinase activity to some metals as ZnSO₄, AlCl₃, CuSO₄, EDTA, BaCl₂, FeCl₃, MgCl₂, MnCl₂, HgNO₃ and HgCl₂ was studied at 1 mM concentration of each metal salt. Each experiment was carried out in triplicate and the results obtained were the arithmetic mean.

Dual plate study

The efficacy of Streptomyces sp. PTK19 was tested against Fusarium oxysporum PTK2 on Potato Dextrose Agar (PDA) medium using dual plate method. The crude chitinase efficacy tested against Fusarium oxysporum PTK2 on the same medium.

Preparation of fungal cell wall

Fusarium oxysporum PTK2 was cultured in 5 Haffkine culture flasks each containing 1L Potato dextrose broth incubated for 5 days at 28°C on orbital shaker at 200 rpm. Cells were harvested on a sheet of tetrion cloth by filtering the culture broth. It was dispersed in water with a homogenizer and filtered through the tetrion cloth. The preparation was washed three times with water, once with ethanol and once with acetone, and then the cell wall was dried in vacuo and pulverized in a mortar.

Lytic activity assay

The reaction mixture consisted of 100 mg of Fusarium oxysporum PTK2 cell wall, 9 mL of 0.05 M Sodium acetate buffer (pH 5.2), 1 mL of enzyme solution and a few drops of toluene aseptically in a L-shaped tube. The reaction proceeded at 40°C for 15 h with vigorous shaking on the reciprocal shaker and then the residual cell wall was collected on a pre-weighed sintered glass funnel. After washing with 20 mL of water, it was dried in vacuo and weighed. A control experiment was run at the same time without enzyme solution. The lytic activity was expressed as percent weight loss of the cell wall under the conditions mentioned above.

Results

The Streptomyces sp. PTK19 produced wider zone of chitin hydrolysis in the plate assay method which indicated the chitinase production (Fig.1). The crude chitinase hydrolyzed the 0.1% colloidal chitin in different concentrations is shown in on (Fig.2).

Effect of substrate concentration

The production medium treated with different concentration of chitin (0.1% to 0.5%) in different days of incubation under the shaking condition (Fig. 3) revealed that 0.4% of substrate concentration induced the maximum level of chitinase production (1.84 U/mL) at 6th day. Followed by 0.5% of chitin induced the (1.40 U/mL) of chitinase on 9th day of incubation. The treated concentrated range (0.1% to 0.5%) of chitin influenced significant level of chitinase production on 6 days of incubation and after that period the chitinase production was gradually decreased.
**Effect of pH of the medium**

The results presented in (Fig-4) indicated a strong influence of chitinase production at pH 7 on sixth day incubation (3.08 U/mL). For this study, enzyme yield was compared in a broad range of pH (5.5 - 8) in shake flask cultures. The pH 6.5 was found to influence (2.46 U/mL) of chitinase production at 6th day incubation. Significant level of chitinase production occurs at pH range 6 to 7.5. Beyond this pH, chitinase production was found to be decreasing.

![Fig. 1. Screening of *Streptomyces* sp. PTK19 for chitinase with 0.1 % colloidal chitin amended medium (a) Chitinase screening plate stained with 0.2% of Congo red (b) Screening of chitinase in *Streptomyces* sp. PTK19 with out congo red staining](image1)

**Table 2. Effect of metal ions and chemical reagents on chitinase of *Streptomyces* sp. PTK19**

<table>
<thead>
<tr>
<th>Metal ions and chemical reagents</th>
<th>Concentration (mM)</th>
<th>Relative activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>ZnSO₄</td>
<td>1</td>
<td>89</td>
</tr>
<tr>
<td>AlCl₃</td>
<td>1</td>
<td>91</td>
</tr>
<tr>
<td>CuSO₄</td>
<td>1</td>
<td>78</td>
</tr>
<tr>
<td>EDTA</td>
<td>1</td>
<td>81</td>
</tr>
<tr>
<td>BaCl₂</td>
<td>1</td>
<td>90</td>
</tr>
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<td>FeCl₃</td>
<td>1</td>
<td>79</td>
</tr>
<tr>
<td>MgCl₂</td>
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<td>87</td>
</tr>
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<td>MnCl₂</td>
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<td>80</td>
</tr>
<tr>
<td>HgNO₃</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>HgCl₂</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

* Each values represent a mean of three experiments

![Fig. 2. Effect of crude chitinase of *Streptomyces* sp. PTK19 on the hydrolysis of 0.1% of colloidal chitin agar medium stained with 0.2% Congo red (a) Crude chitinase 50 µl; (b) Crude chitinase 100 µl](image2)
Optimization of incubation temperature
Submerged fermentation was carried out at different incubation temperatures ranging from 20 to 40°C. The 4% spore suspension was inoculated. Samples were extracted every 3 days of fermentation. The organism exhibited a better growth as well as enzyme production at 30°C and it was (3.170 U/mL) as showed in (Fig-5). The incubation temperature 25°C and 35°C also found to influence the chitinase production and 40°C of incubation is not suitable for chitinase production. The *Streptomyces* sp. PTK19 prefer the favorable temperature range for the better chitinase production from 25°C to 35°C.

Effect of carbon sources
The optimized carbon source concentration for chitinase yield was found to be 2% (Fig -6a) showed sucrose induced maximum level of chitinase production (11.06 U/mL), galactose influenced the (8.38 U/mL) chitinase on six days of incubation. The production medium amended with different millimolar concentration of sucrose (20 mM to 100 mM) concentration. The Fig. 6b showed that 80 mM concentration of sucrose induced the maximum level of chitinase (12.14 U/mL) at sixth day, than100 mM concentration influenced (8.44 U/mL) in the same days.

The 20 mM concentration diminished the chitinase production. Among all the treated carbon sources,
sucrose was found to influence the chitin level as maximum.

**Fig. 6b.** Effect of different concentrations of sucrose on chitinase production in *Streptomyces* sp. PTK19

Submerged fermentation was carried out in 250 mL Erlenmeyer flask containing 50 mL of production medium with (0.1%) of different nitrogen sources like peptone, yeast extract, beef extract, casein and gelatin. On the sixth day the production of chitinase was at maximum level (16.53 U/mL), induced by peptone (Fig-7). Followed by casein induced 9.63 U/mL of chitinase at six days of incubation.

**Effect of nitrogen sources**

Submerged fermentation was carried out in 250 mL Erlenmeyer flask containing 50 mL of production medium with (0.1%) of different nitrogen sources like peptone, yeast extract, beef extract, casein and gelatin. On the sixth day the production of chitinase was at maximum level (16.53 U/mL), induced by peptone the (Fig-7). Followed by casein induced 9.63 U/mL of chitinase at six days of incubation.

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**Fig. 7.** Effect of different nitrogen sources on chitinase production in *Streptomyces* sp. PTK19

**Effect of amino acid supplementation**

Amino acids amended with production medium in 5 mM concentration (Fig-8) showed that valine induced 4.94 U/mL of chitinase on 9 days of incubation under the shaking condition. Among the amino acid supplementation other than valine suppressed the chitinase production.

**Fig. 8.** Effect of different amino acids on chitinase production in *Streptomyces* sp. PTK19

**Fig. 9.** Influence of chitinas enzyme activity of *Streptomyces* sp. PTK19 under different aeration rate at 5 L stirred-tank fermenter, values are means of three replicates of fermentation

**Stirred-Tank fermenter studies**

The production of chitinase was investigated in a 5-L Stirred-Tank fermenter (Scigenic fermentor Pvt LTD, Chennai). The fermentations were carried out at the constant temperature of 30°C and aeration rate 0.5, 0.8, 1.1 vvm at 300 rpm agitation up to eight days. The Fig-9 showed that the highest chitinase (31.62 U/mL) activity was observed at 0.8 vvm aeration of chitinase. Low chitinase activity 12.31 U/mL occur on 0.5 vvm and 1.1 vvm aeration induced 23.39 U/mL of chitinase production. Protein content
of the culture filtrate was estimated to be 65.32 µg/mL.

**Fig. 10.** Extracellular protein profile of *Streptomyces* sp. PTK19 on SDS-PAGE

Lane 1: Standard protein markers
Lane 2 to 6: *Streptomyces* sp. PTK19 crude chitinase

*Electrophoresis*

The protein band profile of concentrated crude chitinase was visualized (Fig-10). The zymogram of crude chitinase reveals that the two isoforms of chitinases present in *Streptomyces* sp. PTK19 (Fig.11).

**Fig. 11.** Zymogram of crude chitinase activity of *Streptomyces* sp. PTK19 on Native-PAGE showed two isoforms of chitinases

**Fig. 12.** Effect of pH on activity and stability of crude chitinase from *Streptomyces* sp. PTK19

**Characterization of crude chitinase**

The optimum pH for the activity of chitinase was found to be pH 5.5 and the enzyme was stable at a range of pH 4.0-7.0. The pH below 4.0 and above 7.0 inactivated the enzyme activity (Fig. 12). The crude chitinase of *Streptomyces* sp. PTK19 showed a maximum enzyme activity at 40°C. To determine its thermal stability, the crude chitinase was pre-incubated from 25 to 70°C for a period of 2 h. The chitinase was stable from 30 to 45°C with more than 60% activity up to 120 minutes of incubation. Whereas, the chitinase at 50°C showed 38% activity up to 40 minutes and after that it decreased. The temperature above 50°C decreased the activity after 60 minutes of incubation (Figs.13a, b).

**Fig. 13a.** Effect of temperature on activity of crude chitinase from *Streptomyces* sp. PTK19
Table 1. Substrate specificity for crude chitinase of *Streptomyces* sp. PTK19

<table>
<thead>
<tr>
<th>Substrate (0.1%)</th>
<th>Relative activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycol chitin</td>
<td>100</td>
</tr>
<tr>
<td>Colloidal chitin</td>
<td>90</td>
</tr>
<tr>
<td>Fine powder of crab chitin</td>
<td>9.5</td>
</tr>
<tr>
<td>Glycol chitosan</td>
<td>0</td>
</tr>
<tr>
<td>Amylose</td>
<td>0</td>
</tr>
<tr>
<td>Carboxyl methyl cellulose</td>
<td>0</td>
</tr>
<tr>
<td>Maltose</td>
<td>0</td>
</tr>
<tr>
<td>Cellobiose</td>
<td>0</td>
</tr>
<tr>
<td>Laminarin</td>
<td>0</td>
</tr>
</tbody>
</table>

* Each values represent a mean of three experiments

Fig. 13b. Effect of temperature on stability of crude chitinase from *Streptomyces* sp. PTK19

The data presented in Table 1 shows that glycol chitin was the suitable substrate followed by colloidal chitin for the crude chitinase of *Streptomyces* sp. PTK19. Fine powders of crab chitin had shown poor relative activity. The chitinase of *Streptomyces* sp. PTK19 did not hydrolyze glycol chitosan, amylase, carboxyl methyl cellulose, maltose cellobiose and laminarin. It was evident from the Table 2 that the activity of crude chitinase was completely inhibited by the metal ions like HgNO₃ and HgCl₂. *Streptomyces* sp. PTK19 and crude chitinase strongly inhibit the growth of *Fusarium Oxysporum* PTK2 in dual plate method (Figs-14a,b) and *Fusarium oxysporum* PTK2 cell wall, and the chitinase, lytic activities were assayed at given times as shown in Fig. 15.

Fig. 14a. *Streptomyces* sp. PTK19 and *Fusarium oxysporum* PTK2 on Potato Dextrose Agar medium in dual plate method

![Fig. 14a](image)

Fig. 14b. Efficacy of crude chitinase of *Streptomyces* sp. PTK19 against *Fusarium oxysporum* PTK2 on Potato Dextrose Agar medium

Discussion

Almost all of the reported chitinase producing strains could use chitin or colloidal chitin as a carbon source (Wang et al., 1995). Actinomycetes express maximum chitinase around the six day of fermentation in most cases and chitin concentration in the range 1 to 1.5%
Aspergillus sp. S1-13, produced two endochitinases and one exochitinase, in a liquid culture with shrimp shellfish waste (Rattanakit et al., 2002). Kim et al. (2011) test the degradation of shrimp shells by Streptomyces sp. TH-11, the bacteria were cultured with degradation media containing 0.1% (w/v) dry and grinded shrimp shells with vigorous agitation at 30°C. pH of the medium plays an important role for the chitinase production. Streptomyces sp. PTK19 was capable of producing high amount of chitinase of 3.08 U/mL at pH 7.0.

Maximum enzyme production of chitinase was observed from 30 to 40°C (Skujins et al., 1965; Gupta et al., 1995; Mahadevan and Crawford, 1997; Gomes et al., 2001). In the present study also temperature showed a significant role in chitinase production on Streptomyces sp. PTK19. It was observed that the organism actively produced the maximum chitinase production at 30°C. There was a gradual decrease in the enzyme production above and below at 30°C and the enzyme production was decreased after six days of incubation. Among the five different carbon sources tested at 2% for chitinase production in the present attempt sucrose induced more amount of chitinase production when compared to the rest of other carbon sources. However amendment of dextrose inhibited the chitinase production. The type and nature of carbon source is one of the most important factors for any type of fermentation process (Pandey et al., 1999). The carbon source represents the energetic source that will be available for the growth of the microorganism. Glucose was found to repress chitinase production in all the strains. In general, chitinase production was prolonged both in the presence of 0.1 and 1.0% glucose (Nawani and Kapadnis, 2005) In the present study amendment of different concentrations of sucrose revealed that sucrose at 80 mM enhanced the chitinase production in Streptomyces sp. PTK19.

Among the different nitrogen sources tested, the organism Streptomyces sp. PTK19 utilized peptone for maximum chitinase production. Seong and Sang-Dal (1994) and Vaidya et al. (2001) where addition of organic nitrogen sources to the culture medium increased chitinase production in Pseudomonas stutzeri YPL-1 and Alcaligenes xylosoxydans IMI 385022. The medium containing malt extract and peptone led to the highest chitinase activity in Serratia marcescens B4A when compared to that of the control (Mandana Zarei et al., 2010). Vaidya et al. (2001)
reported through parametric optimization that inorganic nitrogen sources were not found to be significant whereas organic nitrogen sources such as yeast extract and peptone were found to be significant for chitinase production. Supplementation of five different amino acids in the basal medium on chitinase production in *Streptomyces* sp. PTK19 revealed that the amino acids did not influence the chitinase production.

*Streptomyces* sp. PTK19 produced 2 folds of chitinase in Stirred-Tank fermenter when compared to shake flask method. Under the fermentation maximum activity of chitinase was recorded within 8 days of fermentation. Maximum chitinase production of *Streptomyces* sp. C10 was recorded on 6 days after incubation and then it decreased (Reynolds, 1954). The crude chitinase of *Streptomyces* sp. PTK19 extracellular protein profile showed on SDS-PAGE. Two chitinase isoforms hydrolytic activity in crude chitinase on zymogram analysis were recorded. As shown in the observation all the enzymes had shown optimum activity at the acidic pH region and their molecular weights were rather small, ranging from 19 kDa to 33 kDa as similar observation made from this study in *Streptomyces* sp. PTK19, optimum pH 5.5.

The stability of crude chitinase of *Streptomyces* sp. PTK19 pH 4.0-7.0 as similar observation revealed that the stability of chitinase 49 kDa *Streptomyces griseus* HUT 6037 was at pH 5.5-7.0 (Toshiaki et al., 2000). The temperature optimum of the crude chitinase recorded from *Streptomyces* sp. PTK19 was at 40°C and stability 30-45°C as similar to the observations made on *Streptomyces albolineatus* S-22, and the enzymatic activities significantly increased with temperature within the temperature range of 20 to 60°C. The optimum temperature at 40°C was shown a maximal relative chitinase activity of 99% (Sayed et al., 2000). The substrate specificity of crude chitinase from *Streptomyces* sp. PTK19 had showed more relative activity to glycol chitin and colloidal chitin and it did not hydrolyze glycol chitosan, amylose, carboxyl methyl cellulose, maltose, cellobiose and laminarin. The substrate specificity of *S. aureofaciens* CMUA130 chitinase was investigated. It also capable of hydrolyzed several insoluble chitin substrates, with highest activity on ball milled chitin compared to crude chitin and cell wall material from *Schizosaccharomyces* sp. The enzyme was also active on the chitooligosaccharides, chitotriose and chitotetraose, but was inactive towards chitobiose and CM-cellulose (Taechowisan et al., 2003).

The *Streptomyces* sp. PTK19 chitinase lysis the *Fusarium oxysporum* PTK2 cell wall significantly the similar results obtained Tagawa and Okazaki, (1991) reported that *Streptomyces* spp. isolates showing lytic activity towards *A. niger* cell wall, some preferentially produced chitinase and others α-glucanase, but they did produce simultaneously the other enzyme, even if only in a small quantity. This indicates that the cell wall may be decomposed by the synergistic actions of chitinase and α-glucanase.

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**References**


