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RESEARCH ARTICLE

Optimization of Chitinase production by soil *Streptomyces* sp. SJKP9

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Abstract

Streptomyces sp. SJKP9 was isolated from the shore soil sample of Dhanushkodi, TN and evaluated for chitinase production. Maximum chitinase production was observed on day 8 of incubation while, pH 6 and temperature 30°C were found to be optimum. Culture medium with colloidal chitin was found to be suitable for chitinase production. Sucrose and yeast extract, when used as additional carbon and nitrogen sources and addition of amino acids to the culture medium influenced chitinase production. Crude chitinase exhibited both antifungal and antibacterial activity against wide range of organisms.

Keywords: *Streptomyces* sp., shore soil sample, chitinase, sucrose, yeast extract, amino acids.

Introduction

The majority of the enzymes used in the industries are of microbial origin since; these microbial enzymes are relatively more stable than the enzymes derived from plants and animals (Mohapatra *et al.,* 2003). Microbial enzymes are economical and can be produced on large scale within the limited space and time. The amount produced depends on size of fermenter, type of microbial strain and growth conditions. It can be easily extracted and purified and they are capable of producing a wide variety of enzymes grown in a wide range of environmental conditions showing genetic flexibility and short generation times (Trevan, 1987).

Chitinases (EC 3.2.1.14) are glycosyl hydrolases which catalyse the degradation of chitin, an insoluble linear β-1, 4-linked polymer of N–acetyl glucosamine (Park *et al*., 2009). It is produced by the organisms having chitin as structural component for their growth processes such as ecdysis in insects and crustaceans and mycelia elongation in fungi (Saito *et al*., 1990). Chitinases are present in a wide range of organisms that do not contain chitin, such as bacteria, viruses, higher plants and animals performing important physiological and ecological roles (Watnabe *et al*., 1999). Chitinolytic enzymes have wide range of applications such as preparation of pharmaceutically important chitooligosaccharides and N-acetyl D-glucosamines, preparation of SCP, isolation of protoplasts from fungi and yeasts, control of pathogenic fungi, treatment of chitinous wastes and control of malaria transmission (Yue Han, 2006). Chitinolytic bacteria are common in nature and are important degraders of chitin. Streptomycetes produce a large number of extracellular enzymes as part of their saprophytic mode of life. Their ability to synthesize enzymes as products of their primary metabolism could lead to the production of many proteins of industrial importance (Gilbert *et al*., 1995).

Streptomycetes are the major chitinivorous microbial groups in soil due to their ability to degrade chitin. This has long been regarded as characteristic feature of a soil strepmycete (Metcalfe *et al*., 2002). Chitinase-producing organisms could be used directly in biological control of fungi, or indirectly by using purified protein, or through gene manipulation (Mahadevan and Crawford, 1997). In this study, optimization of the culture conditions for chitinase production by *Streptomyces* sp. SJKP9 isolated from the shores of Dhanushkodi was analyzed.

Materials and methods

Chemical and reagents: All the chemical and reagents, media used for the study were purchased from Hi-Media Laboratories, Ltd. Mumbai, India and Sigma-Aldrich Chemicals, Co., U.S.A.

Isolation of organism from soil sample: Soil samples were collected from the shores of Dhanushkodi (9°10'N79°28'E) and processed by mixing 1 g of air-dried soil sample to 0.1 g of $CaCO_3$, incubating at room temperature for 7 d. The pre-treated soil sample was plated on 0.1% colloidal chitin agar (Kim *et al*., 2005) amended with cycloheximide at concentration of 25 µg/mL to minimise fungal contamination and incubated at 28°C for 7 to 14 d. Among the 16 isolates of *Streptomyces,* the isolate with clear and large halos and maximum zone size (*Streptomyces* sp. SJKP9) was considered as predominant chitinase producer and selected for further analysis.

Organism Identification: The morphological, physiological and biochemical characterization (Pridham and Gottlieb, 1948) of the *Streptomyces* sp. SJKP9 was identified by Bergey's manual of systematic bacteriology (Crawford *et al*., 1993) and ISP (Shirling and Gottlieb, 1966).

Chitinase production in liquid media: Chitinase production medium (Kim *et al.,* 2003) was inoculated with 4% spore suspension (1.5×10^6 spores mL^{-1}) of *Streptomyces* sp. SJKP9 (30°C, 10 d and 120 rpm. The culture filtrate was harvested for every 2 d and assayed for chitinase activity by the release of N-acetyl glucosamine equivalents from colloidal chitin (Ressig *et al*., 1955) with GLcNAC standards. One chitinase unit is the amount of enzyme which released one micromole of GLcNAC equivalent per mL of reaction mixture per min under the experimental condition.

Optimization of chitinase production: The production medium was incorporated with different substrates like colloidal chitin, glycol chitin, crude chitin and powder chitin and inoculated with 4% of spore suspension, incubated in shaker at 120 rpm for 8 d. An optimum level of the best substrate was determined by amending different concentrations of colloidal chitin in the production medium. The impact of pH and temperature on chitinase production was investigated by cultivating the test isolate in the production medium at various pH (4-9) and temperature (15-45°C) ranges for 8 d. The carbon sources such as dextrose, sucrose, fructose, lactose and mannitol and nitrogen sources such as peptone, beef extract, yeast extract, casein and gelatin were supplemented with the production medium to study their influence on chitinase production. The effect of different amino acids like alanine, valine, methionine, asparagine and histidine was analyzed.

Antifungal activity: Fungal species was cultured on a potato dextrose agar (PDA) medium. Spore suspension was spread onto the plates of PDA. Antifungal activity of the enzyme supernatant against pathogenic fungi was studied by well diffusion method. Control was maintained with heat inactivated enzyme. Inhibition of fungal growth was observed after 3-5 d (Narayana and Vijayalakshmi, 2009).

Antibacterial activity: The Muller Hinton agar plates were prepared and inoculated with bacterial test organism. The antibacterial activity of the enzyme supernatant against pathogenic bacteria was studied by well diffusion method. The plates were incubated at 37^0C for 24 h and the zone of inhibition was measured (Mathur *et al*., 2011).

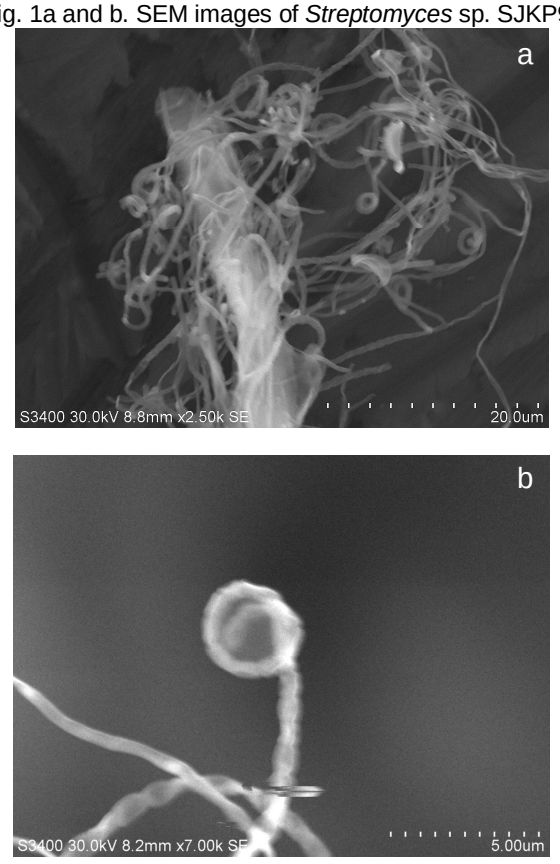
Results

Sixteen isolates of *Streptomyces* spp. were isolated from the sea shore soil samples of Dhanushkodi and among them, 7 isolates were chitinolytic and one isolate, *Streptomyces* sp. SJKP9 which showed maximum zone of clearance on 0.1% colloidal chitin agar was selected for further studies. The morphological and physiological characteristics of the isolate are given in Table 1. Fig. 1a and b shows the scanning electron microscopic image of the isolate.

Table 1. Morphological, biochemical and physiological characteristics of *Streptomyces* sp. SJKP9.

Tests	Results
Grams staining	Gram positive
Motility	Non-motile
Endospore staining	Spore former
Spore chain	Spiral
Spore length	1.15 µm
Spore width	2.25 nm
Spore mass	Dirty white
Mycelial pigment	Negative
Diffusible pigment	Negative
Oxidase	Positive
Catalase	Positive
Melanin production	Negative
Nitrate reduction	Negative
NaCl Tolerance 7% W/V	Positive
Growth in lysozyme	Positive
Gelatin hydrolysis	Clear zone
Cellulose hydrolysis	No zone and growth is present
Pectin hydrolysis	No zone
Carbohydrate fermentation	
Sucrose	Gas negative, Acid positive
Glucose	Gas negative, Acid positive
Fructose	Gas negative, Acid positive
Maltose	Gas negative, Acid positive
Antibiotic resistance	
Rifampicin resistance	Positive
Neomycin resistance	Negative
Ampicillin resistance	Negative
Penicillin G resistance	Positive

Fig. 1a and b. SEM images of *Streptomyces* sp. SJKP9.



a. Mycelium; b. Sporophore.

Fig. 2. Effect of incubation period on chitinase production by *Streptomyces* sp. SJKP9.

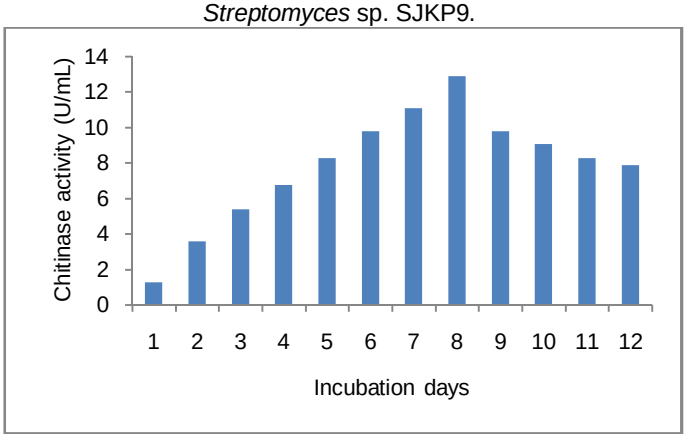


Fig. 3. Effect of different substrates on chitinase production by *Streptomyces* sp. SJKP9.

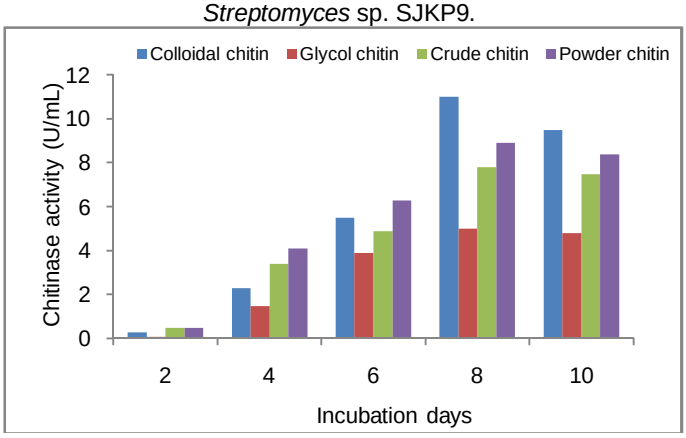


Fig. 4. Effect of different concentrations of colloidal chitin on chitinase production by *Streptomyces* sp. SJKP9.

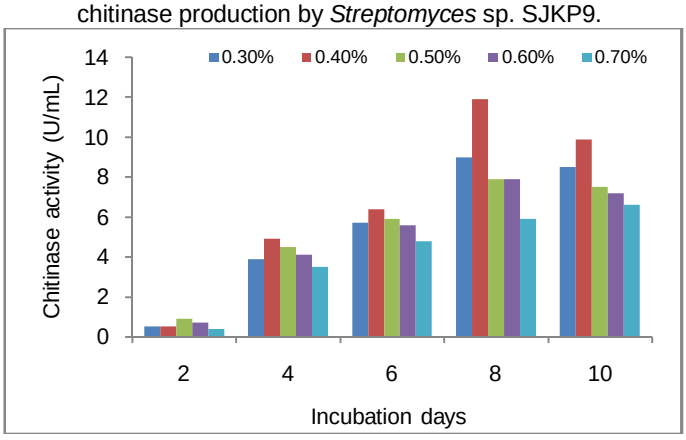


Fig. 5. Effect of pH on chitinase production by *Streptomyces* sp. SJKP9.

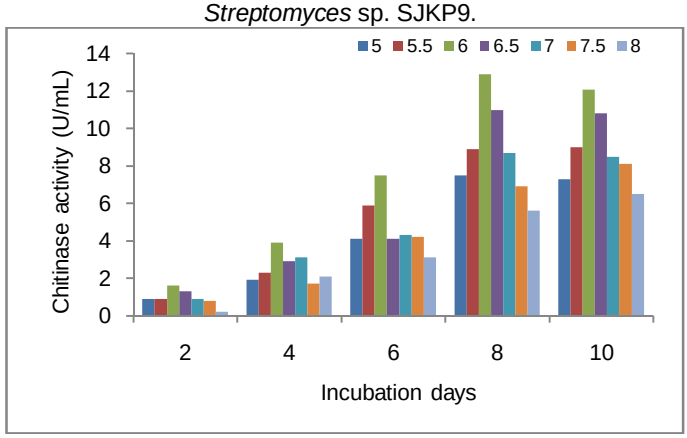


Fig. 6. Effect of temperature on chitinase production by *Streptomyces* sp. SJKP9.

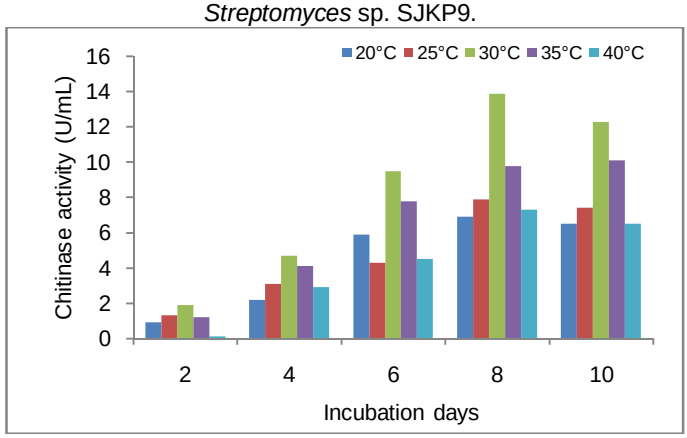
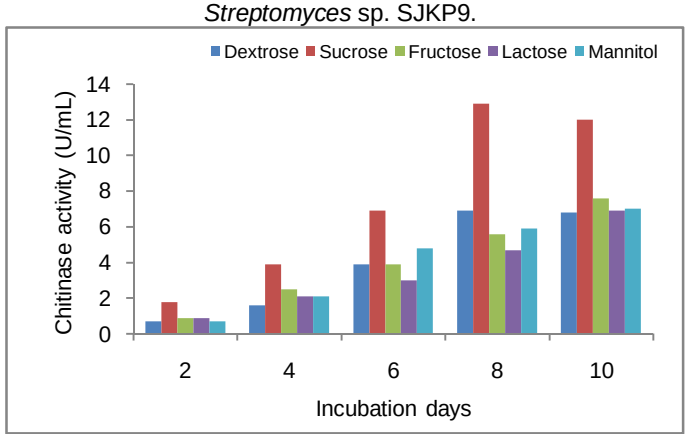


Fig. 7. Effect of carbon source (2%) on chitinase production by *Streptomyces* sp. SJKP9.



The effect of incubation period on chitinase production is presented in Fig. 2. Chitinase activity increased gradually and maximum production was observed on day 8. There was a decrease in enzyme activity after day 8. Among the different substrates (2%) analyzed, colloidal chitin induced a maximum chitinase activity of 11 U/mL on 8th d followed by powdered chitin and crude chitin. Glycol chitin induced least enzyme activity (Fig. 3). Amongst various concentrations of colloidal chitin, maximum chitinase activity of 11.9 U/mL was observed on 8th day with 0.4% of colloidal chitin followed by 0.3 and 0.5% (Fig. 4).

The influence of pH and temperature on chitinase production is shown in Fig. 5 and 6. A high level of chitinase activity was observed in the production medium with pH 6 and temperature 30°C. Additional carbon or nitrogen sources in the production medium influenced chitinase activity (Fig. 7 and 8). Enhanced chitinase production was observed with sucrose (12.9 U/mL) and yeast extract (13.8 U/mL) as carbon and nitrogen sources on 8th d. The production medium when incorporated with 5 different amino acids at 5 mM concentration, alanine induced maximum chitinase activity of 9.3 U/mL on 8th d (Fig. 9).

Fig. 8. Effect of nitrogen source (0.1%) on chitinase production by *Streptomyces* sp. SJKP9.

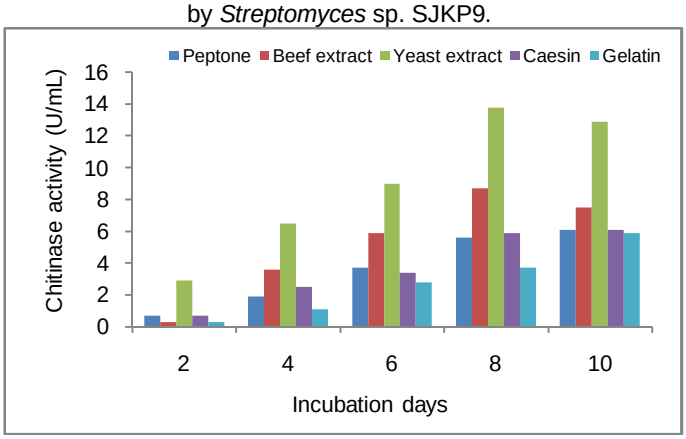


Fig. 9. Effect of different amino acids (5 mM) on chitinase production by *Streptomyces* sp. SJKP9.

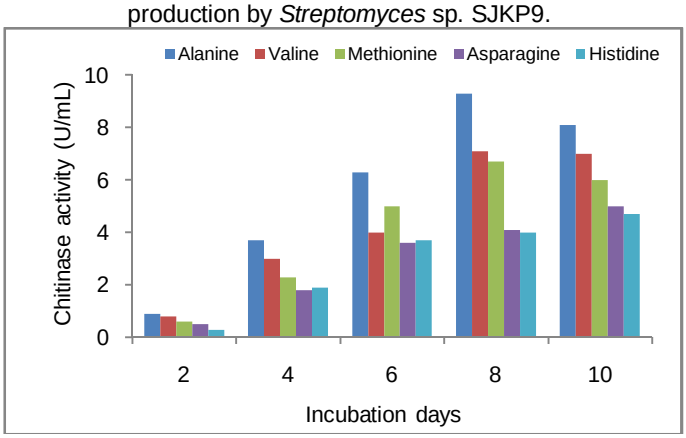


Table 2. Antifungal activity of crude chitinase of *Streptomyces* sp. SJKP9.

Fungi	Dia. of the zone of inhibition (mm)
Alternaria sp.	14
Aspergillus flavus	18
Aspergillus niger	16
Fusarium oxysporum	32
Phythium sp.	26
Sclerotinia sp.	31

Table 3. Antibacterial activity of crude chitinase of *Streptomyces* sp. SJKP9.

Bacteria	Dia. of the zone of inhibition (mm)
Bacillus subtilis ATCC 11774	26
Enterococcus faecalis ATCC 49532	-
Escherichia coli ATCC 10536	27
Pseudomonas aeruginosa ATCC 10145	20
Staphylococcus aureus ATCC 11632	14
S. epidermidis ATCC 12228	23

Crude chitinase obtained from this study exhibited both antifungal and antibacterial ability (Table 2 and 3) against wide range of organisms. Maximum antifungal activity was recorded against *Fusarium oxysporum* and maximum antibacterial activity was recorded against *Escherichia coli* ATCC 10536.

Discussion

Chitinases are a group of enzymes that decompose chitin and are produced by a diverse range of life forms. Among the microorganisms, 90 to 99% of the chitinolytic populations are actinomycetes (Vijay and Shyam, 2006). The chitinase produced by microorganisms is inducible in nature. It is influenced by medium components such as carbon and nitrogen sources, substrates and parameters like pH, temperature and incubation period (Dahiya *et al.*, 2005). Colloidal chitin (0.4%) induced more chitinase production on 8th d when compared to other substrates and other concentrations of colloidal chitin unlike the results of Kavi Karunya *et al*. (2011) who observed maximum production with 0.3% of colloidal chitin. pH has a great impact on chitinase production. In this study, the test isolate produced maximum chitinase on 8th d showing similarity with the efforts of Narayana and Vijayalakshmi (2009). The role of temperature in inducing the production of extracellular chitinase is significant. Here 30°C was optimum for chitinase production on day 8. These results are in line with the observations of Kim *et al*. (2003) who achieved maximum production at 30°C. *Streptomyces* sp. SJKP9 utilized yeast extract for maximum production of chitinase which was observed on day 8 whereas, gelatin was least favourable. Among the carbon sources tested, sucrose induced maximum chitinase production on 8th d. Amendment of different amino acids to the production medium influenced chitinase production as observed in this study. Alanine (5 mM) best suited chitinase production when compared to other amino acids. There are reports stating chitinases as potent biocontrol agents. Production of chitinase and antifungal ability can always be correlated since; the cell wall of fungi is 80% chitin. Thus, they can be considered as effective biocontrol agents of plant pathogens (Kamil *et al*., 2007). Antifungal activity of the test isolate is shown in Table 2. Chitinase from *Streptomyces* sp. SJKP9 also exhibited antibacterial activity (Table 3). The antibacterial and antifungal property of the study is similar to the pattern observed by Mathur *et al*. (2011) in *Bacillus* sp.

Conclusion

A *Streptomyces* sp. SJKP9 isolated from the shore soil sample of Dhanushkodi, TN was evaluated for chitinase production. Maximum chitinase production was observed on day 8 of incubation, pH 6, temperature 30°C, culture medium with colloidal chitin, sucrose, yeast extract and alanine influenced chitinase production. Crude chitinase exhibited both antifungal and antibacterial activity against wide range of organisms. The test isolate can be exploited for large scale production of chitinase, the applications of which is manifold.

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References

1. Crawford, D.L., Lynch, J.M., Whipps, J.M. and Ousley, M.A. 1993. Isolation and characterization of actinomycete antagonists of a fungal root pathogen. *Appl. Envt. Microbiol.* 59(11): 3899-3905.
2. Dahiya, N., Tewari, R. and Hoondal, G.S. 2006. Biotechnological aspects of chitinolytic enzymes: A review. *Appl. Microbiol. Biotechnol.* 71: 773-782.
3. Gilbert, M., Morosoli, R., Shareck, F. and Kluepfel, D. 1995. Production and secretion of proteins by streptomycetes. *Crit. Rev. Biotechnol.*15(1): 13-39.
4. Ha Ju Park, Kim, D., Kim, I.H., Lee, C.E., Ju, CK., Kim, Y., Kim, S.J., Lee, H.K. and Yim, J.H. 2009. Characteristics of cold-adaptive endochitinase from Antarctic bacterium *Sanguibacter antarcticus* KOPRI 21702. *Enz. Microbial Tech.* 45: 391–396.
5. Kamil, Z., Rizk, M., Saleh, M. and Moustaf, S. 2007. Isolation and identification of rhizosphere soil chitinolytic bacteria and their potential in antifungal biocontrol. *Global J. Mol. Sci.* 2(2): 57-66.
6. Kavi Karunya, S., Reetha, D., Saranraj, P. and John Milton, D. 2011. Optimization and purification of chitinase produced by *Bacillus subtilis* and its antifungal activity against plant pathogens. *Int. J. Pharm. Biol. Arch.* 2(6): 1680-1685.
7. Kim, K.J., Yang, Y.J. and Kim, J.G. 2003. Purification and characterization of chitinase from *Streptomyces* sp. M-20. *J. Biochem. Mol. Biol.* 36(2): 185-189.
8. Mahadevan, B. and Crawford, D. L. 1997. Properties of the chitinase of the antifungal biocontrol agent *Streptomyces lydicus* WYEC108. *Enz. Microb. Technol.* 20: 489–493.
9. Mathur, A., Rawat, A., Bhatt, G., Baweja,S., Ahmad, F., Grover, A., Madhav, K., Dhand, M., Mathur, D., Verma, S.K., Singh, S.K. and Dua, V.K. 2011. Isolation of *Bacillus* producing chitinase from soil: production and purification of chito-oligosaccharides from chitin extracted from fresh water crustaceans and antimicrobial activity of chitinase. *Recent Res. Sci. Technol.* 3(11): 1-6.
10. Metcalfe, A.C., Williamson, N., Krsek, M. and Wellington, M.H. 2002. Molecular diversity within chitinolytic actinomycetes determined by *in situ* analysis. *Actinomycetol.* 17(1): 18–22.
11. Mohapatra, B.R., Bapuji, M. and Sree, A. 2003. Production of industrial enzymes (amylase, carboxymethylcellulase and protease) by bacteria isolated from marine sedentary organisms. *Acta Biotechnol*. 23(1): 75-84.
12. Narayana, J.P. and Vijayalakshmi, M. 2009. Chitinase production by *Streptomyces* sp. ANU 6277. *Braz. J. Microbiol.* 40: 725-733.
13. Pridham, T.G. and Gottlieb, D. 1948. The utilization of carbon compounds by some actinomycetales as an aid for species determination. *J. Bacteriol*. 56: 107-114.
14. Reissig, J.L., Strominger, J.L. and Leloir, L.F. 1955. A modified colorimetric method for the estimation of N-acetylamino sugars. *J. Biol. Chem.* 217: 959-966.
15. Saito, A., Fujii, T. and Miyashita, K. 1990. Chitinase system in *Streptomyces. Actinomycetol*. 13: 1-10.
16. Shirling, E.B. and Gottlieb, D. 1966. Methods for characterization of *Streptomyces* species. *Int. J. Syst. Bact.* 16: 313-340.
17. Trevan, M.D. 1987. In Biotechnology, The biological principles. (Indian Ed.), Tata McGraw Hill Publication Co., New Delhi, pp.155- 228.
18. Vijay, B. and Shyam, S. 2006. Characterization of chitinases from microorganisms isolated from Lonar Lake. *Ind. J. Biotech.* 5: 357-363.
19. Watanabe, T., Kanai, R., Kawase, T., Tanabe, T., Mitsutomi, M., Sakuda, S. and Miyashita, K. 1999. Family 19 chitinases of *Streptomyces* species: characterization and distribution. *Microbiol.*145: 3353–3363.
20. Yue Han, Bingjie Yang, Fengli Zhang, Xiaoling Miao and Zhiyong Li. 2009. Characterization of antifungal chitinase from marine *Streptomyces* sp. DA11 associated with South China Sea sponge *Craniella Australiensis*. *Marine Biotechnol*. 11: 132–140.

