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Full Length Research Paper

Chitinase produced by *Serratia marcescens* SMG isolated from decomposed *Volvariella volvacea*

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***Serratia marcescens* strain was isolated at the laboratory from decomposed stalk of paddy straw mushroom (*Volvariella volvacea)* by using swollen chitin as a source of carbon. Morphological and biochemical characteristics of the bacterium were studied. The bacterium showed exponential growth up to 18 h after inoculation in batch culture. The maximum enzyme production by the bacterium was analyzed at 92 h of inoculation at 30°C. Similarly, with respect to different concentration of chitin, the minimal medium supplemented with 1.75% of swollen chitin produced maximum amount of chitinase enzyme. Therefore, the present study showed that isolated bacterium is a good source of chitinase. Moreover, though the bacterium was grown at the cheaper medium, the enzyme can be used for biodegradation of chitinous wastes as well as biological control of fungal pathogen at cheaper cost.**

Key words: Chitin, chitinase, swollen chitin, *Serratia marcescens.*

INTRODUCTION

Chitin, a homopolysaccharide of N-acetyl D-Glucosamine, is the second most abundant organic compound, found naturally in the shells of crustaceans, exoskeleton of insects, fungal cell wall, microfuna and planktons (Kaomek et al., 2003). The commercial source of chitin is shrimp, crab and lobster wastes (Wang et al., 2002). In natural state, chitin is tightly bound with other compounds like protein, lipids, pigments and calcium carbonate. Conversion of these chitinous wastes to useful chitin and its oligomers involves processes like demineralization, deproteinization or hydrolysis, which was earlier carried out with strong acid and bases that involves high cost, low yields and corrosion problem. So, the only alternative to solve this problem is utilization of chitinolytic enzyme, chitinase. Therefore, production of inexpensive chitinase for utilization of chitinous waste that not only solves environmental problem but also reduces the production cost of chito-oligosaccharides derived which are useful in agrochemical and pharmaceutical purposes. Furthermore, chitinase has also many other applications such as enhancement of plant defense mechanism particularly against fungal pathogens (Lorito et al., 1993), as biocontrol agent (Tom and Carroad, 1981), degradation of fish wastes (Ulhoa and Pebero, 1993) and clinical industries (Willey et al., 2008) etc. Due to over exploitation of chitinase enzyme for different purposes, warrants the search of different source of chitinase with respect to both quantity as well as quality of the enzyme. In recent past many microorganisms like *Serratia* sp. and *Streptomyces* sp. reported by several workers for good sources of chitinase (Ueda and Arai, 1992; Bhusan and Hoondal, 1998; Patil et al., 2000; Wen et al., 2002).Therefore, the present investigation is carried out to extract and estimate the chitinase enzyme produced by a bacterium, isolated from decomposed stalk of paddy straw mushroom *V. volvacea*.

MATERIALS AND METHODS

Isolation of the bacterium

The chitinase producing bacterium was isolated form decomposed stalk of paddy straw mushroom *V. volvacea*. The mushroom was collected from a local farm and authenticated at Department of Botany, Berhampur University, Berhampur, Orissa. The mushroom was allowed to decompose naturally inside desiccators at the laboratory. Small pieces of decomposed mushroom are placed on the chitin agar medium in which swollen chitin was used as a source of carbon for microorganisms (Kiyoshi and Okazaki, 1991). The swollen chitin was prepared by using 10 g of flake chitin (Hi media Pvt. Ltd, Mumbai) and phosphoric acid (85%) with little modifications as described by the method of Monreal and Ruse (1969). The microorganism was isolated by appearance of a clean zone around the decomposed mushroom pieces on the culture

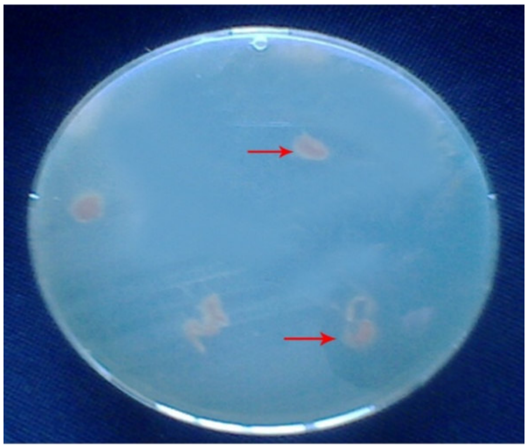

Figure 1. A clear zone of utilization of chitin by the bacterium.

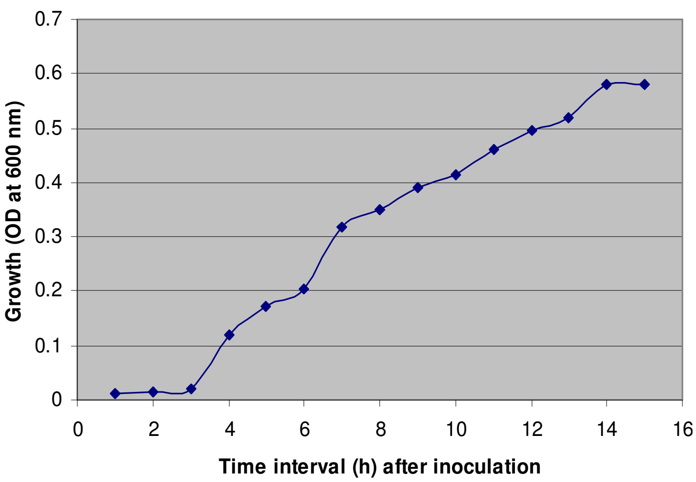

Figure 2. Growth curve of the isolated *Serratia* strain.

medium and was characterized by following Bergey's Manual of Determinative Bacteriology (Holt et al., 1994). Pure culture of the bacterium was maintained at the laboratory by using the same medium for future use.

Determination of growth curve and viable counting

It was evaluated as per standard protocol (Anas et al., 2008). The broth culture of the bacterium having 10^6 cells per ml was used for the study. The culture of bacterium (0.1 ml) was inoculated into the 10 ml freshly prepared chitin liquid medium and incubated at rotary shaker at 37°C. Optical density of the culture was measured at 600 nm at an interval of 1 h against the culture medium as blank for a period of 15 h after inoculation. Similarly, for viable counting samples were removed at 3, 6, 12 and 24 h after inoculation. At each time of counting, the cell strength of the culture was determined by haemocytometer and 0.5 ml of the diluted samples was plated carefully on chitin agar medium by pour plate method. The plates were then incubated for 72 h before the colonies were counted.

Enzyme extraction

The extraction of enzyme was made as per the procedure described by Vishalakshi et al. (2009). Chitin broth culture medium having 0.5% swollen chitin was used for extraction of the enzyme. The freshly prepared medium was inoculated with 1% seed culture (10^8 cell/ml). The inoculum was incubated at temperature controlled rotary shaker under shaking conditions (150 rpm) at 30°C for the period of 96 h. The culture was centrifuged at 10,000 g for 10 min at 4°C. Then the supernatant was again filtrate with Whatman's filter paper to remove the further suspended particles present at the supernatant.

The enzyme assay

Swollen chitin was used as substrate for extraction of the enzyme. The substrate was prepared by 12 mg/ml swollen chitin in acetate buffer, pH 5.5. A solution of 1.0 ml of the substrate and 0.5ml of the enzyme was incubated at 45°C for 30 min. Then the solution was put in boiling water bath for a period of 2 to 3 min to stop the enzyme action. The solution was centrifuged at 5,000 rpm for 10 min. The amount of N-acetyl D-glucosamine present in the supernatant was measured following the method of Reissig et al. (1955). The unit of enzyme represented the 1.0 μmol of carbohydrate released in 1 h at 45°C. Effect of different concentration of chitin (0.5 to 3.0%) in the medium for production chitinase was determined to establish the optimum concentration of chitin which is suitable for extraction of the enzyme.

RESULTS AND DISCUSSION

A clear zone of utilization of chitin by the bacterium was appeared in the chitin agar medium (Figure 1). The bacterium was characterized as the strain of *S. marcescens* SMG.

The growth curve of the isolated *Serratia* strain is represented in Figure 2. The bacterium showed exponential growth up to 18 h of inoculation and then followed the stationary phase. Similarly, the viable cell counting increased from 1×10^6 cells to 2×10^9 cells during the period of culture (Figure 2). Singh et al. (2005) observed that *S. marcescens* GG5 retained the growth in batch culture up to 16 h of inoculation and cell counting increased to 3×10^9 CFU/ml. Every microorganism shows a definite growth pattern in batch culture due to nutrient and oxygen are the limiting factor for the growth of the bacteria (Sharma and Das, 2010).

Chitinase production by *S. marcescens* SMG is given in Table 1. There is no enzyme production was observed at first 14 h of the inoculation. Then the enzyme production increased up to 92 h of inoculation (0.25 U/ml), then found to be declined till the end of the experiment. The least value was observed at 120 h of inoculation i.e. 0.10 U/ml. Singh et al. (2005) reported that *S. marcescens* GG5 showed maximum enzyme production (0.2 U/ml) at 96 h of inoculation and 0.12 U/ml at 120 h of inoculation. Therefore, production of chitinase by the isolated bacterium followed the same trend as reported earlier. The shifting of pH from neutral to alkaline was observed in the culture medium during growth of the bacterium.

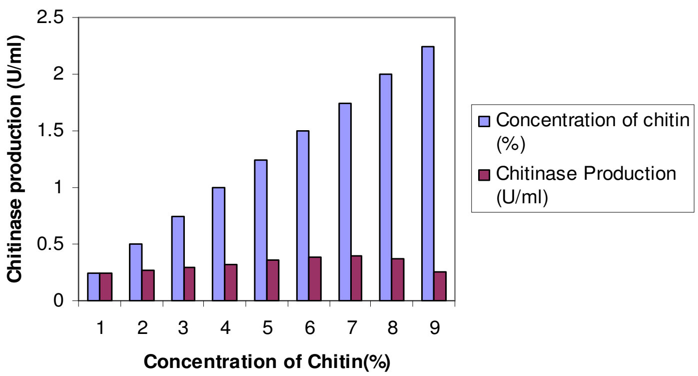

Figure 3. Effect of different concentration of chitin on production of chitinase by *S. marcescens* SMG. Value is mean of three replicates

Table 1. Chitinase production by *Serratia marcescens* SMG. Value is mean of three replicates.

Inoculation time (in hours)	Chitinase production (U/ml)
10	-
20	0.13
30	0.15
40	0.17
50	0.18
60	0.21
70	0.22
80	0.24
90	0.25
100	0.18
110	0.14
120	0.12

The pH was recorded quite alkaline (i.e. pH 9.0) at 120 h of growth of the bacterium.

Effect of different concentration of chitin on production of chitinase by *S. marcescens* SMG is given in Figure 3. Chitinase production by the bacterium was increased with increase of chitin up to 1.75% in the culture medium. Then the production of chitinase decreased with further increase of chitin in the culture medium. The maximum chitinase production was recorded as 0.4 U/ml. It has earlier been reported that minimum culture medium supplemented with different sources of carbon enhance the production of chitinase (Yabuki et al., 1986; Suzuki et al., 1998). But certain carbohydrates like glucose, starch, pectin, rafinose have been identified for their adverse effect on production of chitinase when they are used either directly or as supplement to chitin in the culture medium (Gupta et al., 1995; Bhusan, 2000). The present study showed that *S. marcescens* SMG is a good source of chitinase, however its economics for commercial exploitation has to be worked out.

REFERENCES

Anas IK, Jayasree PR, Vijaykumar T, Manish PRK (2008). *In vitro* antibacterial activity of *Psidium guava* Linn. leaf extract on clinical isolates of multi drug resistant *Staphylococcus aureus*. Indian J. Exp. Biol., 46: 41-46.

Bhusan B (2000). Production and characterization of a thermo stable chitinase from a new alkalophilic *Bacillus* sp. BG11. Appl. Microbilo., 88: 800-808.

Gupta R, Sexens RK, Chaturvedie P, Virdi JS (1995). Chitinase production by *Streptomyces viridificans*: It's potential in fungal cell wall lyses. J. Appl. Bacterial., 87: 378-383.

Holt G, Keong NR, Sneath PMA, Staley JT, Williams ST (1994). Bergey's Manual of Determinative Bacteriology Williams and Wilkins Press, Baltimore, USA.

Kaomek M, Mizuno K, Fujinira T, Sriyotha P, Cairna JRK (2003). Cloning, expression and characterization of an antifungal chitinase from *Leucaena leucocephla* De Wit. Biosci. Biotechnol. Biochem., 67: 667-676.

Kiyoshi T, Okazaki K (1991). Isolation and some cultural conditions of *Streptomyces* species which produce enzymes lysing *Aspergillus niger* cell wall. J. Fermen. Bioeng., 71: 230-236.

Lorito M, Harman GE, Hayes CK, Broadway RM, Tronsmo A, Woo SL, Pietro AD (1993). Chitinolytic enzyme produced by *Trichoderma harzianum*, antifungal activity of purified endo- chitinase and chitobiosidase. Phytopotho., 83: 309-307.

Monreal J, Ruse ET (1969). The chitinase of *Serratia mercescens*. Can. J. Microbiol., 15: 689-696.

Patil RS, Ghormade V, Deshande MV (2000). Chitinolytic enzyme: an exploration. Enz. Micro. Techno., 26: 473-483.

Reissig JL, Strominger JL, Leloir LF (1955). A modified colorimetric method for the estimation of N- acetyl amino sugar. J. Biol. Chem., 217: 959-966.

Sharma D, Das M (2010). Efficacy of antimicrobial metabolites of *Pseudomonas fluorescens* PFG against phytopathogenic fungi. Proc. Nat. Acad. Sci. India, 80: 68-71.

Single G, Dahiya N, Hoondal GS (2005). Optimization of chitinase production by *Serratia marcescens* GG5. Asian. J. Micro. Bio. Env. Sc., 11(3): 587-591.

Suzuki K, Suzuki M, Taiyoku M, Nikaidou N, Watanabe T (1998). Chitin Binding Protein (CBP21) in the culture supernatant of *Serratia marcesens* 2170. Biosci. Biotechnol. Biochem., 62: 128-135.

Tom RA, Carroad PA (1981). Effect of reaction on hydrolysis of chitin by *Serratia marcescens* QMB 1466 chitinase. J. Food Sci., 46: 646-648.

Ueda M, Arai M (1992). Purification and some properties of chitinase from *Aeromonas* sp. No. 105. 24. Biosci. Biotechnol. Biochem., 56: 460-464.

Ulhoa CJ, Peberdy JF (1993). Effect of carbon source on chito-biase production by *Trichoderma harzianum*. Myco. Res., 97: 45-48.

Vishalakshi N, Lingappa K, Amena S, Prabakar M, Dayanad A (2009). Production of alkaline protease from *Streptomyces gulbargensis* and its application in removal of blood strains. Indian J. Biotechn., 8: 280-285.

Wang SL, Shih IL, Wang CH, Tseng KC, Chang WT, Twu YK, Kau JJ, Wang CL (2002). Production of antifungal compounds from chitin by *Bacillus subtilis*. Enz. Microbial. Technol., 31: 321-328.

Wen CM, Tseng CS, Cheng CY, Li YK (2002). Purification, characterization and cloning of a chitinase from *Bacillus* sp NCTU2. Biotechnol. Appl. Biochem., 35: 213-219.

Willey JM, Sherwood LM, Woolverton CJ, Prescott H, Klein L (2008). Microbiology. Mc Graw Hill International Edition New York, USA.

Yabuki M, Mizushina K, Amatatsu T, Akikaju A, Fujii T, Shimada M, Yamashita M (1986). Purification and characterization of chitinase and chitobiase produced by *Aeromonas hyprophila* subsp *anaerogenes* A52. J. Gen. Appl. Microbiol., 32: 25-38.