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Utilization of Shrimp and Crab Wastes for the Production of N-Acetylglucosamine by Chitinolytic Soil *Streptomyces* sp. SJKP9

Sridharan Jagadeeswari[1*] • Manickam Dakshinamoorthy Balakumaran[2] • Padmanabhan Vidya[1] • Pudupalayam Thangavelu Kalaichelvan[2] • Kanesan Panneer Selvam[3]

[1] Department of Microbiology, D.G. Vaishnav College, Chennai – 600106, India
[2] Centre for Advanced Study in Botany, University of Madras, Chennai – 600025, India
[3] Department of Microbiology, M.R. Government Arts College, Mannargudi – 614001, India

Corresponding author: * **jaga.dgvc@yahoo.in**

ABSTRACT

Shrimp, crab and other crustacean shells are the major wastes of sea food processing industries, possessing a rich amount of protein and oligosaccharides such as chitin and chitosan. In this study, shrimp and crab wastes were utilized to produce the amino sugar compound N-acetylglucosamine using chitinolytic *Streptomyces* sp. SJKP9 isolated from a sea shore soil sample. The chitin flakes obtained from the sources were digested using crude chitinase enzyme derived from *Streptomyces* sp. SJKP9 and N-acetylglucosamine was precipitated from a concentrated solution of hydrozylate by adding ethanol. Crab chitin produced more N-acetylglucosamine than shrimp chitin.

Keywords: chitin, chitosan, crab, FTIR, N-acetylglucosamine, shrimp, *Streptomyces*
Abbreviations: GlcNAc, N-acetylglucosamine; **SEM**, scanning electron microscopy; **FTIR**, Fourier transform infrared spectroscopy

INTRODUCTION

Crustacean wastes are one of the main sources for the production of value-added products such as chitin and chitosan (Knorr 1991; Shahidi 1991). Chitin, a high molecular weight polysaccharide composed primarily of N-acetylglucosamine units (Brine 1991; Chang 1997), is the second most abundant biopolymer on earth next to cellulose. Its annual production in aquatic systems alone is estimated to be 10^6 to 10^8 tons (Cauchi 2002). It is widely distributed in nature as a structural component of the exoskeletons of crustaceans, insects and other arthropods as well as a component of the cell wall of most fungi and some algae (Shahidi *et al.* 1999). Chitin can be easily extracted from these sources and hence it is a readily renewable resource. The main commercial process for chitin extraction from crustacean wastes is based upon demineralization and deproteinization by acid and alkali treatments (Prashanth *et al.* 2007).

Chitin is extremely insoluble and has yet to find large-scale industrial uses (Kao *et al.* 2009). Chitin can be converted to chitosan without depolymerization by deacetylation. Chitin is broken down by chitinases, a group of complex hydrolytic enzymes produced by bacteria, fungi and actinomycetes that catalyze the depolymerization of chitin (Jami *et al.* 2008). Chitin degradation starts generally as random cleavage within the polymer by chitinase to release an oligomer and subsequently the monomer N-acetylglucosamine (GlcNAc). This hydrolytic product of chitin has gained special interest in agriculture, food and pharmaceutical industries (Nawani and Kapadnis 2005) and also paved the way for the production of ethanol by yeasts (Cody *et al.* 1990). Members of the genus *Streptomyces* from soil are widely employed in the production of various enzymes and antibiotics whose rate of production and recovery is economically viable (Rabah *et al.* 2006). A study was designed to evaluate the potential of chitin flakes obtained from the shells of shrimp and crab in the production of GlcNAc using partially purified chitinase obtained from *Streptomyces* sp. SJKP9.

MATERIALS AND METHODS

All chemical and reagents were purchased from Hi-Media Laboratories Ltd. (Mumbai, India) and (Sigma-Aldrich Chemicals Co. (St. Louis, USA). Shrimp and crab shell wastes were obtained from the local market, cleaned, washed with distilled water and processed to get chitin and chitosan (Toan 2009). Soil samples collected from Dhanushkodi shore (9° 10′ N 79° 28′ E) were used and were processed using 1% colloidal chitin agar (Kim *et al.* 2003) so as to isolate *Streptomyces*. The colonies of *Streptomyces* showing clear and large halos around them were considered for predominant chitinase activity and were selected for further analyses. The isolate SJKP9 which showed predominant activity was subjected to various morphological and biochemical analysis. For SEM analysis the isolate on agar blocks was fixed with 1.0% glutaraldehyde in phosphate buffer (0.1M, pH 7.2) for 24 h, post fixed with 1% osmium tetroxide in phosphate buffer for 24 h and dehydrated in acetone series (10-100%) and air dried. The specimens were sputtered with gold and viewed under scanning electron microscope and photographed (Leica, Cambridge, UK). Initially they were grown in chitinase production medium that contained the following per litre; 1 g colloidal chitin, 0.7 g KH_2PO_4, 0.3 g K_2HPO_4, 4 g NaCl, 0.5 g $MgSO_4{\cdot}7H_2O$, 1 mg $FeSO_4{\cdot}7H_2O$, 0.1 mg $ZnSO_4$, 0.1 mg $MnSO_4{\cdot}7H_2O$ (Kim *et al.* 2003) for 8 days at 30°C and were subsequently harvested, filtered and centrifuged. The supernatant was assayed for chitinase activity by the release of N acetyl D glucosamine equivalents from colloidal chitin following the method of Reissig *et al.* (1955). The reaction mixture consisted of 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 3000 × *g* for 3 min. To 0.5 ml of the supernatant 0.1 ml of potassium tetraborate buffer (0.08 M, pH 9.2) was added and boiled for 3 min at 100°C then cooled. To this, 3 ml of diluted dimethyl aminobenzaldehyde reagent was added, incubated at 37°C for 20 min for colour development; absorbance was read at 585 nm (Milton Roy 601 Spectrophotometer). A standard graph was prepared with a curve for authentic GlcNAc to convert the absorbance values to micromoles of GlcNAc liberated from colloidal chitin. One chitinase unit was the amount of en-

Received: 16 September, 2011. Accepted: 28 December, 2011.

Research Note

Table 1 Morphological and physiological characteristics of potent chitinolytic *Streptomyces* sp. SJKP9.

Test	Results
Gram staining	Gram-positive
Motility	Non-motile
Endospore staining	Spore former
Spore structure	Spiral
Spore size	
Length	1.15 µm
Width	2.25 nm
Spore mass	Dirty white
Pigment production	No pigment
Oxidase	Positive
Catalase	Positive
Nitrate reduction	Negative
NaCl Tolerance	4%
Growth in lysozyme	Present
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 sensitivity	
Chloromphenicol	Sensitive
Ampicillin	Resistant
Streptomycin	Sensitive
Rifampicin	Sensitive

zyme which released one micromole of GlcNAc equivalent per ml of reaction mixture per minute under the experimental conditions.

The crude chitinase enzyme thus obtained was used in the production of GlcNAc by utilizing the chitin substrates obtained from shrimp and crab wastes (Setthakaset *et al.* 2008) and compared. The dried final product, GlcNAc, was subjected to Fourier transform infrared (FTIR) spectroscopy (BRUKER RFS 27: FT-Raman Spectrometer, SAIF, IIT Madras, India).

RESULTS AND DISCUSSION

Chitinases are considered useful in the management of chitinous wastes of sea food manufacturing industries (Nawani and Kapadnis 2003). In this study, each 1000 g of the chitinous wastes from crab and shrimp that were processed for the production of GlcNAc yielded varying amounts of chitin and chitosan. Crab wastes yielded a maximum of 150 g of chitin while shrimp wastes produced 140 g and their further processing yielded 130 and 100 g chitosan, respectively. The morphological and physiological characteristics of the soil *Streptomyces* sp. SJKP9 used in the study are given in **Table 1**. Similarly, **Fig. 1A** and **1B** show the scanning electron microscopic (SEM) images of the mycelial mass and curled spore morphology respectively.

The time course of chitinase production by *Streptomyces* sp. SJKP9 is presented in **Fig. 2**. Chitinase activity increased gradually from day 1 to day 6 and maximum enzyme production was observed on day 7. There was a decrease in enzyme activity after day 8. Similar observations were made by Young and Bell (1985) and Neugebour *et al.* (1991) during production of chitinase from *S. marcescens* and *S. lividans*, respectively. The growth of *Streptomyces* sp. SJKP9 was initially slow but became exponential after 84 h. Chitinase production increased in an exponential phase and further accelerated during the stationary phase, similar to the work of Mane and Deshmukh (2009) in *Streptomyces canus*, *Streptomyces pseudogriseolus* and *Micromonospora brevicatiana*.

The chitin produced from the two sources of chitin wastes of the study were tested for GlcNAc production by an enzymatic method. 20 g of chitin each from crab and shrimp produced 12 and 9.6 g of GlcNAc, respectively by the action of 4.4 ml of crude chitinase enzyme produced by

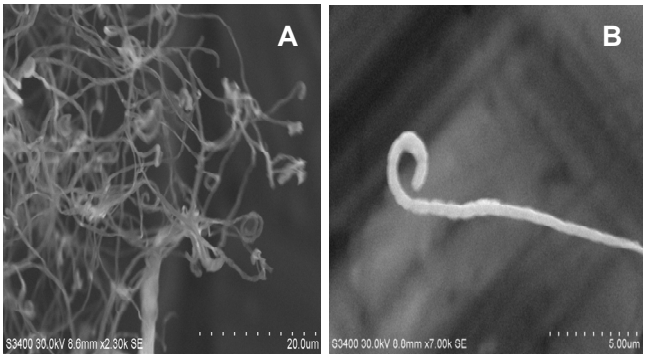

Fig. 1 Scanning electron microscopic (SEM) images of *Streptomyces* sp. SJKP9 (**A**) mycelium and (**B**) sporophore.

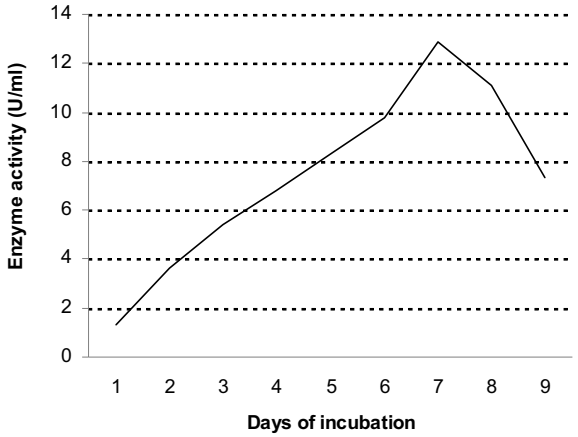

Fig. 2 Chitinase production of *Streptomyces* SJKP9.

Streptomyces sp. SJKP9. GlcNAc is a component of proteoglycan and is a material of focus for the treatment of osteoarthritis (Aiba 2005).

In order to investigate the functional groups of the extracted GlcNAc from crab which produced greater amounts of GlcNAc, the FTIR spectrum was measured in KBr pellets (**Fig. 3**). There was a strong adsorption band at 3459 cm^{-1} and the one at 1420 cm^{-1} seems to be GlcNAc. The band at 3326 cm^{-1} is for the NH stretch and primary and secondary amines and amides, proving that the compound is GlcNAc. This was analogous to the investigations of Brugenerotto *et al.* (2001).

ACKNOWLEDGEMENTS

The authors are thankful to RSIC, IIT-M for the FTIR analysis and CAS in Botany, University of Madras for SEM analysis. We also acknowledge the Management, D.G. Vaishnav College, (Chennai, India) for their support. Finally, the authors thank Dr. Jaime A. Teixeira da Silva for extensive improvements to language, grammar and structure.

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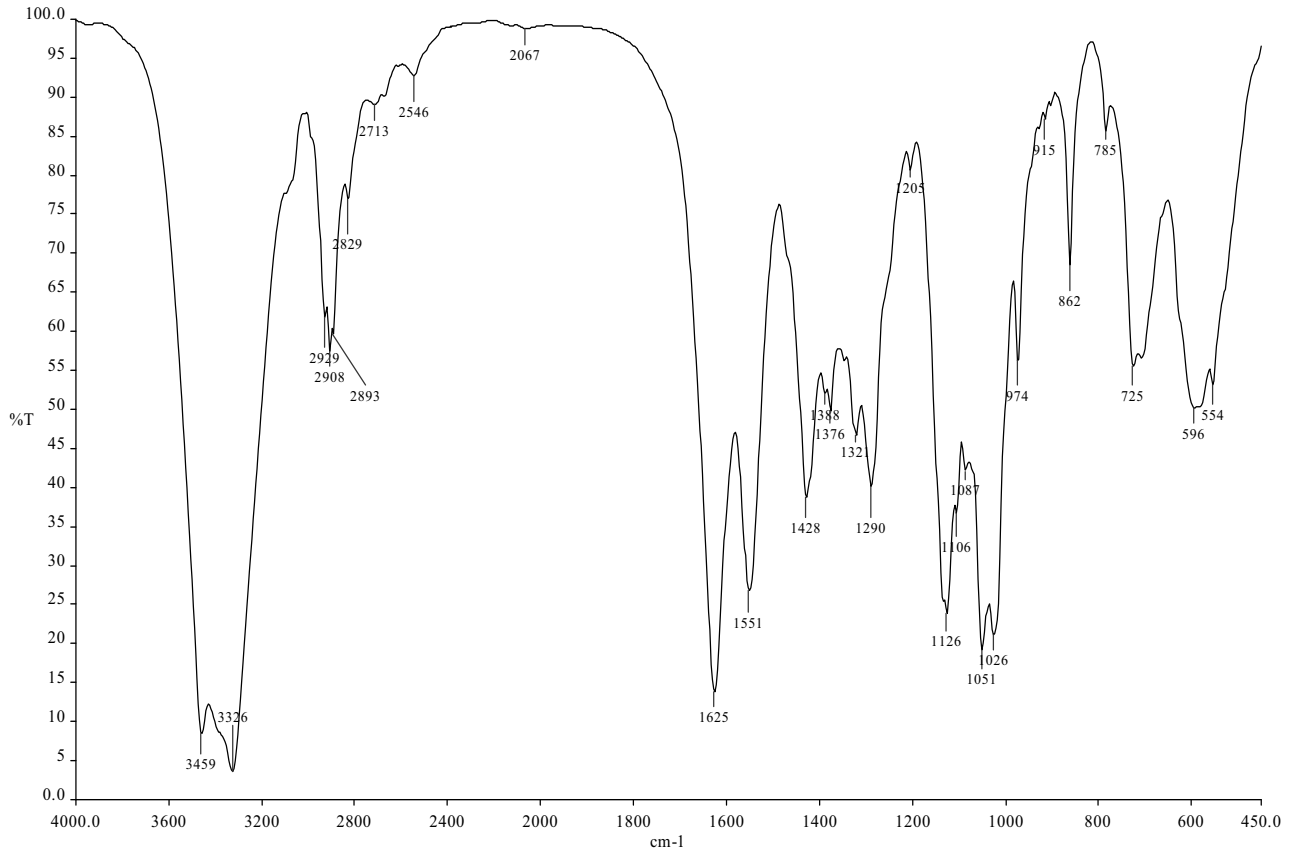

Fig. 3 FTIR analysis of N-acetylglucosamine.

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