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Isolation and Identification of *Serratia marcescens* NASC 1 and Optimization of its Chitinase Production

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Abstract

Chitinolytic bacteria were isolated from the soil samples collected in agricultural fields. Among 20 bacterial strains isolated, a potent bacterial isolates, that showed maximum zone of clearance in chitin agar plate was identified as *S. marcescens* by studying cultural, morphological and biochemical characteristics. An optimization of cultural conditions was carried out with different parameters such as different concentration of substrate, various pH and different incubation temperature. The enzyme showed maximum activity at 0.5% chitin concentration, pH 9 and temperature of 37°C. The enzyme could be useful for treatment of chitinous waste and for the production of different products of hydrolysed chitin in various applications.

Keywords: Chitinase, Chitin, *Serratia marcescens*, Chitinolytic activity

INTRODUCTION

Serratia marcescens is one of the most effective Gram-negative bacteria for degradation of chitin. It secretes a variety of extracellular enzymes including chitinase [1]. It is a saprophyte found in water, soil and food. Nosocomial infections due to *serratiamarcescens* are being reported with meningitis, endocarditis, septicaemia, peritonitis respiratory infection and many other conditions. Soil bacteria such as aerobic spore forming bacilli and those found on decaying vegetation such as the *Enterobacter* sp., may also be washed into natural water during the rains. It also named the bacterium *Serratia marcescens*. Gentamycin is used to treat *Serratia* infection. Screening may be one of the most efficient and successful ways of searching for new or suitable microbial enzymes [2].

Chitin is the most plentiful source of a natural organic compound after cellulose [3]. It is a long chain biopolymer containing N-acetyl-D-glucosamine monomers form covalent β-1,4linkages [4]. Chitin in soil can be degraded by a wide variety of microorganism including fungal and bacterial species. Chitinolysis, namely hydrolysis of the glycosidic bonds of chitin by chitinase, is probably the most important pathway of degradation of chitin in soil. Chitin is widely dispersed in the structural components of many organisms that include crustacean and mullosk shells, arthropod exoskeletons and fungal cell walls [5,6]. In chitin containing organisms, chitinase play an important role in normal life cycle functions such as morphogenesis and cell division, whereas plants produce *Chitinase* as part of their defence against fungal pathogens. Many bacteria and fungi contain chitinolytic enzymes to convert chitin into compounds that can serve as energy source.

Chitinase belong to families 18 and 19 of glycosyl hydrolases on the base their amino acid [7]. Chitinase 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 microorganisms such as bacteria, fungi, actionomycetes, yeasts, plants, protozoans, coelenterates, nematodes, arthropods and humans [8- 10]. Chitinase have received increasing attention because of their broad application in the fields of medicine, agricultural, biotechnology, waste management and industrial application, which include antifungal, hypocholestrolemic, anti-hypertensive activities and food quality enhancers [11-14].

This study describes the screening program for isolation of microorganism producing high levels of *Chitinase* from environment sources and the most potent isolate was identified as *Serratia marcescens* NASC 1 and physiochemical parameters for the enhanced production of chitinase are optimized.

MATERIALS AND METHODS

Soil samples

Soil sample (4-5g) was collected from agricultural fields in and around Erode, Tamil Nadu at a depth of 2-5 cm and transferred to a clean polyethylene bags. The samples were further air dried at room temperature and taken for bacterial isolation.

Preparation of colloidal chitin

Colloidal chitin is commonly used as a water insoluble substrate for the study of chitinase. It was prepared from purified chitin according to the method of Roberts and Selitrennikoff. 0.5g of chitin powder was added slowly into 90 ml concentrated HCl and left at 4°C overnight with vigorous stirring. The mixture was added to 500 ml of ice-cold 95% ethanol under vigorous stirring for 30 min and kept overnight at 25°C and then stored at -20°C. The precipitate was collected by centrifugation and washed with sterile distilled water until the colloidal chitin became neutral (pH 7).

Isolation of chitin degrading bacteria

1g of collected soil was suspended in 100 ml of distilled water. Then incubated in an orbital shaker incubator at 28°C with shaking at 200 rpm for 30 min. Serial dilution was made up to 10^{-5} using sterile distilled water and agitated with the vortex at maximum speed. An aliquot of 0.1 ml of each dilution from 10^{-2} to 10^{-5} was taken and spread evenly over the surface of Nutrient agar medium supplemented with 2% colloidal chitin. The inoculated plates were incubated at room temperature for 48 h. Chitin degrading bacteria were identified with distinct zone of clearance around the colony and sub cultured in Nutrient agar slants for further use.

Characterization and identification of strain

Chitinolytic bacteria identification was performed by studying cultural, morphological and biochemical characteristics including Gram staining, motility test, catalase test, oxidase test, indole test, methyl red, Voges proskauer test and citrate test.

Optimization of culture conditions

The optimum cultural condition for the production of chitinase was determined. A loopful of culture was taken and inoculated into 50 ml of broth media with optimized growth conditions such as 0.3% of colloidal chitin, pH 8.0 and temperature 35°C. The medium was incubated for 72 h in a shaking condition.

Extraction of crude enzyme

The broth medium was extracted by muslin cloth and then cell free supernatant was collected by centrifugation at 10,000 rpm for 10 min.

Enzyme assay and protein concentration

The supernatant collected was assayed for chitinase activity by determining the releasing of reducing sugar by DNSA method [15]. Protein concentration of the enzyme was estimated by the

method of Lowery *et al.* [16] and absorbance determined at 660 nm by using bovine serium albumin (BSA) as standard.

Purification by chromotography method

The bacterial cell free fermented media was collected and subjected to different steps of purification. It was saturated to 75% with 2-propanol at a flow rate of 3 ml/min (kept at 0 to -4°C) under continuous stirring. The mixture was allowed to stand at –20°C for 2 h and centrifuged for 15 min at 13 000 rpm and 0°C. The precipitate was then dissolved in 03 M Tris-HCl buffer and dialysed twice against same buffer. The crude enzyme solutions obtained after dialysis were loaded on Sephadex G-200 columns for further partial purification. A fraction of 6 ml was collected throughout with a flow rate of 15 ml/h and then assayed for enzyme activity and protein concentration.

RESULTS

Isolation of chitin degrading bacteria

In the present study, a total of 20 bacterial colonies were isolated from soil samples collected from agricultural field. Among these, one potent bacterial isolate, which can produce maximum zone of clearance on the Nutrient agar plate supplemented colloidal chitin as a sole carbon source (Fig.1).

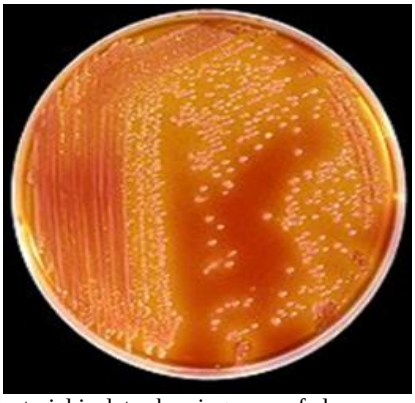

Figure 1: Bacterial isolate showing zone of clearance on colloidal chitin agar plate

Characterization and identification of strain

The potential strain was identified by studying physiological, staining and standard biochemical tests. After the all tests were done, the strain was rather identified using the Bergey's Manual of Determinative Bacteriology. Based on the above results (Table 1), the isolated strain is probably identified as ***Serratia*** sp.

Table 1: Characterization of potential strain

Characterization	Results
Gram reaction	-ve
Cell morphology	Rods
Arrangements	Single/paired
Motility	+
Catalase	-
Oxidase	+
Indole	+
Methyl red	+
Voges proscauer	+
Citrate test	+

'+' Positive; '-'Negative

OPTIMIZATION OF CULTURAL CONDITION

Effect of supplement with chitin

Growth was carried out in a synthetic medium and gradually enriched various ingredients of chitin were seen in 0.5 to 0.2%. It was found that the best activity of chitin 0.57 mg/ml in 0.5 % of chitin supplement. The protein concentration was increased in 0.5% of chitin (Fig. 2).

Effect of pH

The optimum pH to produce chitin was determined by varying the pH of the medium from pH 6 to 9. The result indicated that the chitin was active at pH 6 and achieved its maximum activity at pH 9 (Fig. 3)

Effect of temperature

The optimum temperature to produce chitin was determined by incubating the medium in different temperatures like 21, 27, 30 and 37°C. The results indicated that the maximum activity of enzyme was achieved at 37°C (Fig. 4)

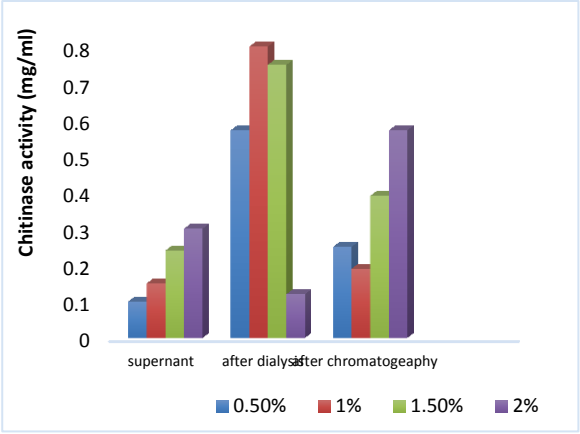

Figure 2: Effect of different substrate concentration chitinase production

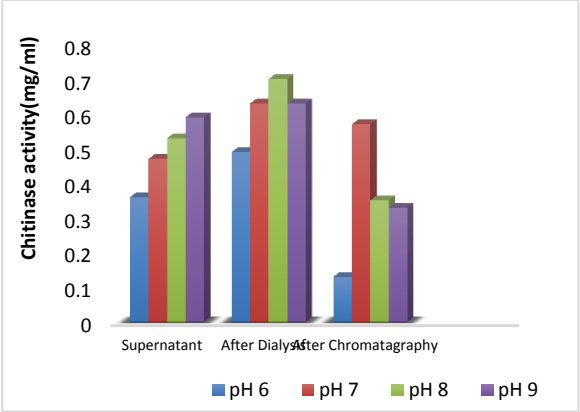

Figure 3: Effect of different pH on chitinase production

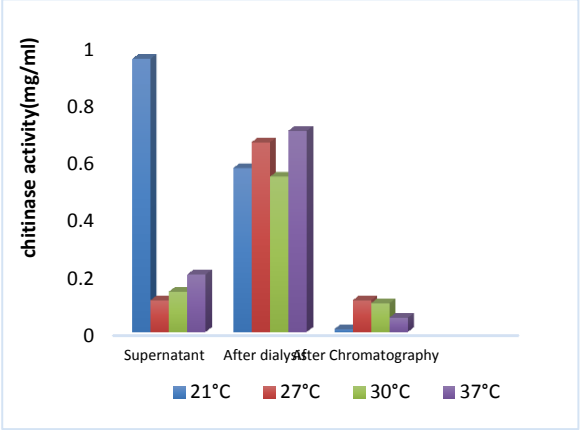

Figure 4: Effect of different temperature on chitinase production

DISCUSSION

Enzymes are extensively used in modern biotechnology, with new application of enzyme being discovered continuously. The development of enzyme products often relies on screening many organisms for an enzyme activity [17]. Almost all the reported chitinase or colloidal chitin as a carbon source [18]. Maximum enzyme production of chitinase was observed from 30 to 40°C [19-21]. Microbial production of chitin has attention in the world wide because of its wide spectrum of application but also for the lacuna of an effective production method. In this study 20 colonies were isolated from different soil sample, they are using colloidal chitin as a sole carbon source and forming halos on chitin containing agar medium. Strains from which the colonies formed large and clear zone was observed. Isolated strain was subjected to taxonomic analysis based on Bergey's manual of systematic bacteriology and identified as *Serratia* sp. Growth was carried out in a synthetic medium and with a various ingredient of chitin (0.5-2.1%) and it was found that the best activity was observed at 0.5%. Also, increased level of protein concentration was observed in 0.5% of chitin. The optimum pH to produce chitin was determined by varying the pH 6-9. The results indicated that chitin is active at pH 6 and achieved its maximum level at pH 9.The production temperature to produce chitin were determined by using different temperature from 21- 37°C. The results indicated that the maximum activity of enzyme was found at 37°C. In purification, crude samples were treated with ammonium per sulphate. The dialyzed samples were eluted in crude in column. The eluted samples were used for enzyme assay protein concentration.

CONCLUSION

The chitinolytic bacteria *Serratia* sp. isolated from soil sample was useful for treatment of chitinous waste and for production of different products of hydrolysed chitin in various applications.

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