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Optimization of Physical Parameters for Chitinase Production from *Serratia marcescens*

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ABSTRACT

Microorganisms have been a source of many industrial enzymes. They have been the target for enzymes for many decades. Many of the biochemical pathways of microorganisms have been extensively studied and evaluated by using large scale screening procedures. In the present study, screening was done to isolate probable candidates that produce extracellular chitinase. After screening, *Serratia marcesens* was identified based on morphological and biochemical assays. The optimum conditions for chitinase production were analyzed using a spectrophotometer. The optimum chitinase production was observed at the following conditions: temperature-37°C, pH-8.0, rpm-250, incubation period-24 hr and colloidal chitin concentration-1%. The chitinase activity was analyzed by plate assay method.

Keywords: chitinase, *Serratia marcesens,* optimum conditions, enzyme production

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INTRODUCTION

Chitin was discovered in 1811 by Henri Braconnot, a French professor of natural history [1] and he had named it as fungine. In 1823, Odier found the same material in insects and plants and named it chitine [2]. It is a polysaccharide that is composed of β-(1,4)-N-acetyl-D-glucosamine units and is a constituent of shells of crustaceans, insect exoskeleton and fungal cell walls. The deacetylated form of chitin is called as chitosan which is formed when chitin is heated with a chemical solution. Chitosan has some advantages over chitin because it is more water-soluble [3].

Chitinases are enzymes that catalyze the hydrolysis of β-1,4-*N*-acetylglucosamine linkages present in chitin. They have widespread application in pharmaceutical, food and cosmetic industries. It is difficult to obtain pure chitin, through conventional techniques. *E.coli* cells have been engineered to overcome this problem (B). The plant chitinases inhibit the growth of fungal pathogens [4]. The insect chitinases play a major roles in ecdysis, and also has a defensive role against their own parasites [4,5]. Chitinases in crustaceans are induced before molting process in the integument [5]. Chitinases are present in many microorganisms [3,5].

Chitinases have been isolated from *Streptomyces sp* [6,7,8]. *Alcaligenes xylosoxydans, Serratia marcescens* [9,10], *Lactobacillus plantarum*, *Penicillium chrysogenum*, *Trichoderma harzianum* and *Paenibacillus* [11]. *Serratia marcescens* is a gram negative, rod shaped bacterium belonging to the family Enterobacteriaceae. It is involved in causing various nosocomial infections [12]. It is commonly found in the respiratory and urinary tracts of hospitalized adults and in the gastrointestinal system of children. In the present study the optimum conditions necessary for the production of chitinase form *Serratia marcescens* was investigated using various parameters.

MATERIALS AND METHODS

Serratia marcescens was isolated from the soil sample that was collected from the vicinity of Tambaram Fish Market, Chennai, Tamil Nadu, India. It was identified using gram staining, microscopic and biochemical analysis [13].

Media optimization

Media optimization was done to standardize the conditions favouring the bacterial growth and to get high yield of the enzyme chitinase for the strain isolated and screened on chitin agar plate for maximum chitinolytic activity. It was done by changing one independent variable while fixing all the others at a constant level. The experiment was carried out by maintaining the culture in mineral salt solution (NaCl -5 g, $CaCl_2$-0.2 g, $MgSO_4$-0.2 g, K_2HPO_4-1.25 g, distilled water-1 L) and the optimum conditions were analyzed by varying the parameters like carbon source (colloidal chitin), pH, temperature, rpm and incubation time. Temperature was maintained at 37°C, pH of 7.2, rpm zero (static condition), chitin

concentration of 1% and incubation time of 24 hr as non varying parameters in all those different sets of conditions where variation of another parameter was desired.

Effect of concentration of colloidal chitin on chitinase production

The carbon source used was colloidal chitin. Colloidal chitin was prepared from purified chitin. Mineral salt solution (10 ml) was taken in five culture flasks and 0.2%, 0.4%, 0.6%, 0.8% and 1.0% of colloidal chitin was added. The pH was maintained at 7.2. All flasks were inoculated with 0.1 ml (1% v/v) of the test culture. Following inoculation the flasks were incubated at 37°C for 24 hr and the chitinase activity was determined by plate assay method.

Effect of pH on chitinase production

Five pre-sterile culture flasks were taken and 10 ml of mineral salt solution supplemented with 1% chitin was added. The pH was adjusted to 5.0, 6.0, 7.0, 8.0, 9.0. All flasks were inoculated with 0.1 ml (1% v/v) of the test culture. Following inoculation the flasks were incubated at 37°C for 24 hr and the chitinase activity was determined by plate assay method.

Effect of temperature on chitinase production

Five pre-sterile culture flasks were taken and 10 ml of mineral salt solution supplemented with 1% chitin was added. All flasks were inoculated with 0.1 ml (1% v/v) of the test culture. Following inoculation the flasks were incubated at different temperatures namely, 25°C, 30°C, 37°C, 45°C and 50°C for 24 hr and the chitinase activity was determined by plate assay method.

Effect of rpm on chitinase production

Mineral salt solution (10 ml) supplemented with 1% chitin was taken in five sterile flasks. All flasks were inoculated with 0.1 ml (1% v/v) of the test culture. Following inoculation the flasks were incubated at 37°C for 24 hr in a shaker by varying the rpm (100, 150, 200, 250 and 300) and the chitinase activity was determined by plate assay method.

Effect of incubation period on chitinase production

Five clean culture flasks were taken and 10 ml of mineral salt solution supplemented with 1% chitin was added. The pH of the media was maintained at 7.2. All flasks were inoculated with 0.1 ml (1% v/v) of the test culture. Following inoculation the flasks were incubated at 37°C for 18 hr, 24 hr, 30 hr, 36 hr and 42 hr and the chitinase activity was determined by plate assay method.

RESULT AND DISCUSSION

Soil sample was collected from a fish market and after culture it was narrowed down to four isolates I-1, I-2, I-3, I-4 based on their ability to produce the zone of chitinolysis [13]. Out of them, isolate I-3 showed maximum zone of chitinolysis followed by I-4, I-1 and I-2. I-3 was thus chosen for further characterization by biochemical tests and optimization studies.

Media optimization

The optimization was carried out by altering the physical parameters like pH, temperature, incubation time, rpm and carbon source of the culture media. The bacterial growth was estimated by finding the OD values.

Colloidal chitin concentration

Colloidal chitin in different concentrations was used to determine the maximum concentration of chitinase activity. When colloidal chitin was used at a concentration of 1% of the salt supplemented medium (broth), the OD obtained was the maximum, thereby indicating the optimum concentration of colloidal chitin for chitinase (**Fig-1**). Colloidal chitin (0.1-2%) was used as the carbon source in most cases for the production of chitinase [7,11,14,15].

Chitinase was also produced by solid state fermentation. The substrates used are rice bran [9], sugarcane bagasse [10] and crude shrimp waste [16]. The best nitrogen sources were found to be malt extract [14], yeast extract [15] and urea [11].

Optimum pH

Sodium phosphate buffer at different pH was used to find the optimum pH for chitinase activity and bacterial growth. The culture showed maximum OD value at pH 8.0 (**Fig-2**). The optimum pH varied from 5 to 7.9 and it greatly depended on the species and media composition [7-10,14-16].

Optimum temperature

Optimum temperature was determined and identified to be 37°C by plotting the graph of OD values vs temperature (**Fig-3**). The optimum temperature in similar work ranged from 28°C to 35°C. However 30°C seemed to be ideal [7-10,14-16].

Optimum rpm

The optimum rpm for the maximum growth and chitinase activity was found to be 250 rpm (**Fig-4**). There weren't enough reports specifying the optimum rpm for chitinase production.

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Incubation Time

The optimum incubation time for chitinase production was found to be 24 hr as shown in **Fig- 5**. There are reports specifying the optimum incubation time to be 72 hr [15].

Much attention has been paid to chitinase research, but its substrate seems to have been neglected or, at least, underestimated. If we are going to use the huge amount of chitin produced annually in nature, chitin research needs an increase in funding to develop equipment to harvest chitin from its diverse sources, improve the purity of obtained chitin, and produce novel materials with new applications from this environmentally friendly biopolymer.

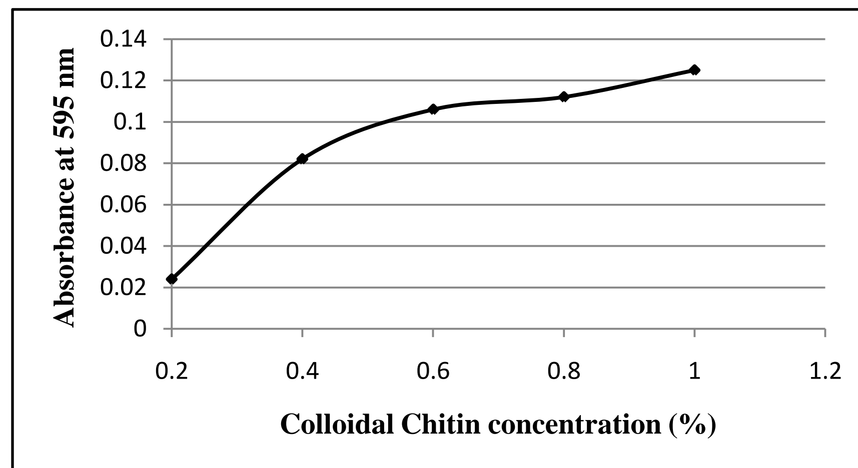

Fig-1 Effect of colloidal chitin concentration on chitinase production

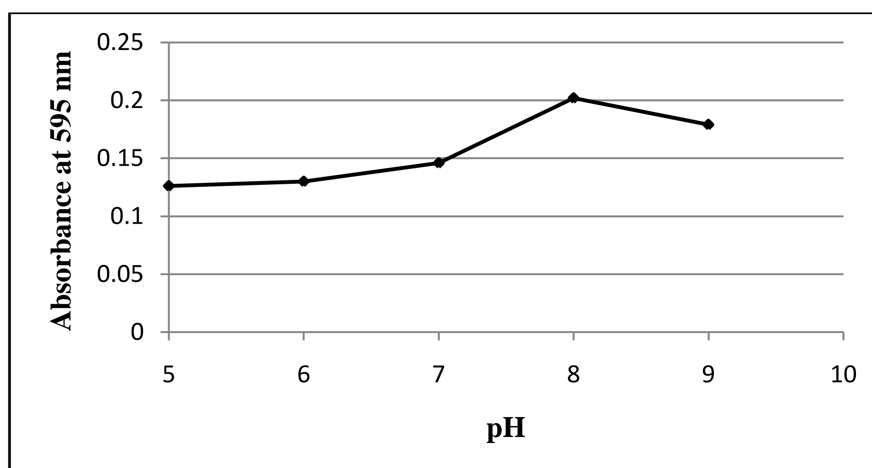

Fig-2 Effect of pH on chitinase production

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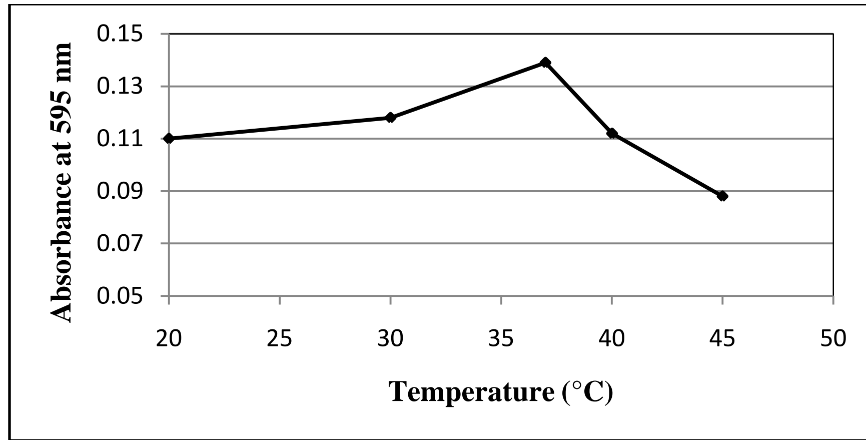

Fig-3 Effect of temperature on chitinase production

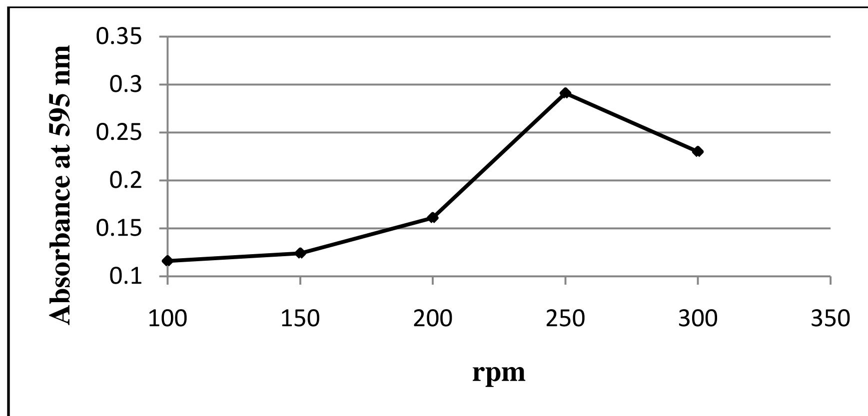

Fig-4 Effect of rpm on chitinase production

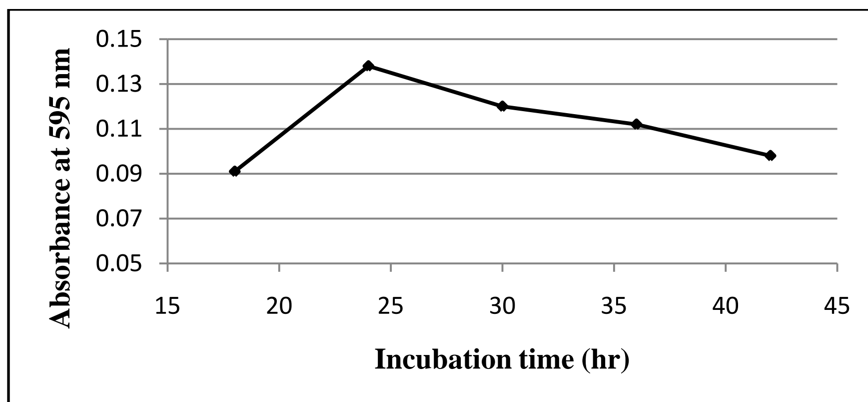

Fig-5 Effect of incubation time on chitinase production

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CONCLUSION

In summary, *Serratia marcescens* is capable of producing enzyme chitinase and degrade chitin at substrate level but still a lot more need to be studied to harness it at commercial scale and utilize it for the mass scale degradation of chitin wastes generated in the sea-food industries. In the current study, the parameters for optimum growth conditions were found out which needs to be studied further to correlate the bacterial growth with chitinase activity. The pilot scale production of chitinase, fermentation methodology to be adopted for maximum production and assay of enzyme kinetics at various steps is still to be studied.

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