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Isolation and screening of chitinase producing *Serratia marcescens* from soil

L Jeyanthi Rebecca*, G Susithra, S Sharmila and Merina Paul Das

Dept. of Industrial Biotechnology, Bharath University, Selaiyur, Chennai

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ABSTRACT

In the present study an attempt was made to isolate organisms that would synthesize chitinase which can be used for the effective biodegradation of a vast majority of crustacean waste. Out of the four isolates screened, the isolate I-3 showed maximum chitinase activity and was selected for further research. The isolate I-3 was identified as Serratia marcescens using the standard identification parameters like gram staining followed by the biochemical assays. Further characterization and purification of chitinase needs to be carried out.

Keywords: chitinase, chitin, *Serratia marcescens*, isolation, screening

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INTRODUCTION

Chitin is one of the most abundant renewable biopolymer on earth that can be obtained as a cheap renewable biopolymer from marine sources [1]. It is biocompatible, biodegradable and bio-absorbable, with antibacterial and wound-healing abilities and low immunogenicity; therefore there have been many reports on its biomedical applications [1, 2]. Chitosan, a derivative of chitin, was produced in 1859, and since then, research has been conducted to learn about the properties of chitin and chitosan and develop commercial application for their large scale uses. Chitosan has some advantages over chitin because it is more water-soluble [3, 4].

Chitin is a polysaccharide composed of β-(1,4)-N-acetyl-D-glucosamine units. Chitinases belong to glycosyl hydrolase families 18 and 19 according to the classification made by Henrissat and Bairoch. *E. coli* has been engineered to produce chitobiose [5]. Chitinases are enzymes that catalyse the hydrolysis of β-1,4-*N*-acetylglucosamine linkages present in chitin. As chitin is a major component of fungal cell walls, and is absent in plants, chitinases play a role in plant defence against pathogens. Supportive evidence for the defensive role of chitinases includes chitinase inhibition of fungal growth *in vitro* [4] enhanced resistence to pathogens in plants that constitutively express high levels of chitinase [6], and visualization of *in vitro* chitin breakdown [7].

Serratia marcescens is a gram-negative, rod-shaped, motile bacterium belonging to the family Enterobacteriaceae. A human pathogen, *S. marcescens* is involved in nosocomial infections, particularly catheter-associated bacteremia, urinary tract infections and wound infections, [4, 6, 8] and is responsible for 1.4% of bacteremia cases in the United States [9, 10]. It is commonly found in the respiratory and urinary tracts of hospitalized adults and in the gastrointestinal system of children. It can grow in temperatures ranging from 5–40°C and in pH levels ranging from 5 to 9. It is differentiated from other Gram-negative bacteria by its ability to perform casein hydrolysis, which allows it to produce extracellular metalloproteinases which are believed to function in cell-to-extracellular matrix interactions [11]. The present study was carried out to isolate and screen for the presence of chitinolytic organisms.

EXPERIMENTAL SECTION

Almost all industrial microbiology processes require the initial isolation of cultures from nature by a suitable method, followed by small-scale cultivation and optimizations before any large scale production can become a reality. This project report gives the detailed study of the sequential steps involved in isolation and screening of chitinase producing microorganism present in soil, followed by its optimization for maximum chitinase production.

Isolation of Chitinase Producers from the Environment:
The first stage in the screening of microorganisms for potential industrial application is their isolation. Soil sample was collected from the ground near Tambaram Fish Market. The debris from the top of the soil was swept off and the soil was collected from a depth of two inches using a spatula and stored in a ziplock bag. An aliquot of 0.1ml of the serially diluted sample (from 10^{-6} and 10^{-7} dilutions) was transferred to chitin agar plates using spread plate method. The plates were maintained at a temperature of 37ºC for 24 hr. The colonies obtained through spread plate method were sub-cultured in nutrient agar slants, in duplicates, incubated at 37°C for 24 hrs and then stored at 4^{o}C.

Screening:
The pure colonies maintained on nutrient agar slants were streaked on colloidal chitin agar medium plates (1%) and then selected based on their ability to hydrolyze and grow on chitin plates due to secretion of chitinase. The plates were then kept for incubation at 37°C for 24 hrs. The zone of clearance was measured and the cultures giving significant results were selected for further analysis.

Identification of the organism:
The identification of the organisms was done primarily by studying the colony morphology followed by gram staining. After screening and observing the colony morphology, the culture giving the maximum zone of clearance was subjected to various biochemical tests like indole test, methyl red test, Voges-proskauer test, citrate utilization test, catalase or hydrogen peroxide test, Starch hydrolysis test, Gelatin test and motility test to confirm the characteristic features of the microorganism producing chitinase.

RESULTS AND DISCUSSION

Upon performance of serial dilution and spread plate method, different bacterial colonies were observed on the nutrient agar plates. Subculture of these colonies from pure cultures on 1% chitin agar plate, showed four colonies with promising growth. These strains were selected for further studies and their colony morphology was observed. When subjected to gram staining, all the colonies were found to be of gram negative rods with different arrangement of colony (Table-1).

Isolation and Screening of Chitinase Producers
The four isolates numbered as I-1, I-2, I-3, I-4 and were subcultured in nutrient agar slants in duplicates and stored at 4°C (Table-1). The colour of the colonies ranged from white to red. Each colony had a distinct shape. The colony morphology study was followed by the chitin hydrolyzing test to confirm the production of chitinase enzyme. All the four isolates subjected to screening by streaking on chitin agar medium when allowed to grow on 1% colloidal chitin demonstrated the zone of chitinolysis following 24 hrs of incubation at 37^{o}C.

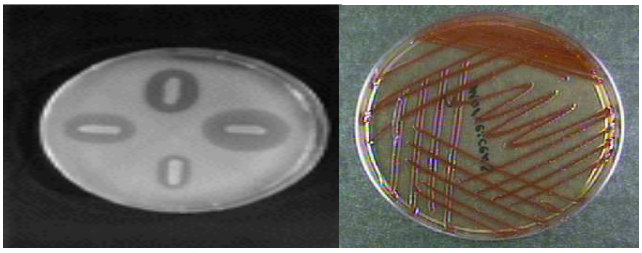
Table-1: Colony morphology of the four isolates from soil

Isolate No	Size	Shape	Margin	Texture	Colour	Gram's reaction
I-1	Large	Circular	Smooth	Mucoid	White	Gram negative rods
I-2	Medium	Powdery	Smooth	Non mucoid	Grey White	Gram negative rods in chains with endospores
I-3	Small	Concave	Smooth	Mucoid	Orange to Red	Gram negative small rods
I-4	Medium	Circular	Smooth	Non mucoid	White	Gram negative slender long rods

Out of the four isolates, isolate I-3 showed maximum zone of chitinolysis (1.8 cm) followed by I-4 (1.2 cm) and I-1 (1.0 cm) (Table-2; Fig-1A). The isolate I-2 showed the least activity. Hence I-3 was chosen for further characterization by biochemical tests.

Table 2 : Chitin Hydrolysis Test

Culture No	Zone Of Chitinolysis (in cms)
1	1.0
2	0.7
3	1.8
4	1.2



A **B**

Figure 1: A. zone of chitinolysis; B. pure culture of the isolate I-3

Biochemical tests:

The results of the biochemical tests for the isolate I-3 are summarized in Table-3. The isolate I-3 showed positive result for the indole test, citrate utilization test and for the hydrolysis of chitin, starch and gelatin. It showed negative results for all the other tests. The results of the biochemical tests confirmed that the isolate I-3 was that of *Serratia marcescens*. The pure culture of the isolate I-3 is shown in Fig-1B. '

Table-3: Biochemical tests for the selected isolate I-3

S.No	Biochemical Tests	Isolate I-3
1	Chitin hydrolysis	+
2	Starch hydrolysis	+
3	Gelatin hydrolysis	+
4	Indole Test	+
5	Methyl Red Test	-
6	Voges – Proskauer Test	-
7	Citrate Utilisation Test	+
8	Catalase Test	-
9	Motility test	Motile

Serratia marcescens and different species of *Bacillus* has been reported to produce chitinase [12, 13, 14] and the result of the present study also confirms the same.

CONCLUSION

In summary, *Serratia marcescens* is capable of producing enzyme chitinase and degrade chitin at substrate level but still a lot more is needed 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. 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. Despite the multiple potential applications of chitin, we believe that the most promising future applications are in the field of nanobiotechnology, which involves drug, gene delivery and scaffold for tissue engineering, and as chito-oligosaccharides in medicine and agriculture.

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