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Organized by

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Rajarshi Shahu Mahavidyalaya (Autonomous),
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Isolation of Chitinolytic *Pseudomonas*species from the shrimp shell waste

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Abstract
The enzyme chitinase, produced by various bacteria in both terrestrial and marine environments is used for the degradation of chitin which possesses several industrial applications. The biological applications of these enzymes have been exploited in the food and pharmaceutical industries. In the present study, 5 *Pseudomonas* isolates were screened for chitinolytic activity, and on the basis of the chitin hydrolysis zone,*Pseudomonas* isolates were selected for chitinase production on chitinase agar media.
By study obtained the *Pseudomonas RSML-07*produced the chitinase in sub-merged fermentation were 29 EU/ml in the crude extract by using a liquid medium modified with 0.1%glucose (w/v) containing2% colloidal chitin KNO_3-10g, KH_2PO_4-5g, $MgSO_4$-2.5g, $FeCl_3$-0.02 g L^{-1}. Chitinase production started after 24 h of incubation and reached maximum levels during the fourth day of cultivation at 30˚C.
After the production, Chitinase was purified from crude enzyme extract by ammonium sulfate precipitation and dialysis. The enzyme was purified using 70% ammonium sulfate precipitation and dialyzed against 0.02 M Tris buffer of pH 8.8 for overnight at 4˚C.
The present study provides a suitable medium for enhancing chitinase production by *Pseudomonas RSML-07*.
Keywords: Chitinase, *Pseudomonas RSML-07,* Purification.

Introduction

Recently, the advent of biotechnology has been a growing interest in and demand for enzymes with novel properties. Chitin is utilized as a structural component by most species alive today. It occurs in the exoskeleton material of crustaceans such as crabs, lobsters, shrimps, prawns, and crayfish. Chitin holds great economic value due to its versatile biological activities and chemical applications, mainly in medical (A.LSvitil, 1997 &R. Murugan,2004) and pharmaceutical areas(A.V Yadav,2004 &H.Takeuchi,2001). The study of chitinases is important in the application of biological control (Y. Kato,2003) Especially it is a potent antifungal agent through chitin degradation activity (S.N Zhou,1999) Chitinase has found extensive use in the

preparation of protoplasts from fungi, a technique of increasing importance in biotechnology(N. Mathivanan, 1998). Chitinase is widely distributed in bacteria, actinomycetes, and plants(J.Peberdy 1983 , M.A Pisano, 1992) Most abundant of chitinase producing bacteria such as *Aeromonas sps, Clostridium sps, Vibrio sps, Streptomyces sps, Beneckeasps, Achromobactersps, Alginomonassps, Pseudomonas sps, Clostridium sps*etc(T. Watanable,1990). Hence an attempt has been made to isolate and characterize the chitinase-producing bacteria from the sediment of shrimp pond.

The presence of chitinase was first described in 1911 by Bernard who found a thermos-sensitive and diffusible antifungal factor in orchid bulbs and in 1929 by Karrer and Hofmann who discovered chitinase in a snail (Flach et al., 1992).

Chitinolytic enzymes have also been applied in human therapeutic studies with chitin residues such as pentacetylchitopentaose (CHP) as well as hexaacetylchitohexaose showing antitumor activity (Patil *et al.* 2000). The dimeric substrate, diacetylchitobioside (CHB) has been widely used as a starting material for the synthesis of biologically active compounds (Terayama*et al.* 1993). Kobayashi *et al*. (1996) employed a *Bacillus* chitinase in the generation of both regio and stereoselective glycosidic bonds between chitobioside units. The artificial chitin produced in this manner has found application in numerous scientific studies and in the production of multifunctional substances (Kobayashi *et al.* 1996)

Materials and Methods

Sample collection:

The shrimp shell was collected from the *white leg shrimp* Marine Shrimp Shell Wastes *which is collected* from the fish market, Latur, Maharashtra.

Isolation of *Pseudomonas* strains:shrimp shell was diluted in sterile saline waterand spread on sterile pseudomonas isolation agar (Hi-Media Ltd, Mumbai, India).

The plates were incubated atroom temperature for 24 to 48 h. After incubation, pigmented colonies were pointed out, selected, streak inoculated on nutrient agar slants and incubated at 28^0C. After growth, the slants were then preserved in the refrigerator for further studies.

Screening and Identification of the bacteria

The*Pseudomonas* strains were isolated and identified through its morphological and biochemical properties according to Bergey's manual of systematic Bacteriology.

Chitinase Production:

For the production of chitinase, strain was grown in 100 ml of fresh medium (2% w/v chitin; KNO_3-10g, KH_2PO_4-5g, $MgSO_4$-2.5g, $FeCl_3$-0.02 g L^{-1}pH 6.0) in a 250 ml Erlenmeyer flask at 28°C. For reflecting the growth of the culture in this medium OD at 660 nm was taken using the blank as a medium in which no inoculum was added. The supernatant (enzyme) was collected from 3-day-old cultures by centrifuging the mixture at 12,000 g for 15 minutes.

Measurement of enzyme activity

Chitinase activity was measured with colloidal chitin, as the substrate. Enzyme solution (0.5 ml) was added to 1.0 ml of the substrate solution, which contained a 1.5 % suspension of each of the colloidal chitin prepared in a phosphate buffer (50 mM, pH 6.0) separately and the mixture was incubated at 37°C for 15 minutes. After centrifugation, the amount of reducing sugars produced in the supernatant was determined by dinitrosalicylic acid (DNSA) method. (Hiroshi Tsujibo,1991)using N-acetyl glucosamine as a reference compound (G.L. Miller,1972). One unit of chitinase activity was defined as the amount of the enzyme that produced 1 µ mol of reducing sugar per minute.

Purification of chitinase

After the production, Chitinase was purified from crude enzyme extract by ammonium sulfate precipitation and dialysis. The enzyme was purified using 70% ammonium sulfate precipitation and dialyzed against 0.02 M Tris buffer of pH 8.8 for overnight at 4˚C by the method of Imoto and Yagishita (T. Imoto, K. Yagishita.1971).

Results and Discussion

In the present study, 5 *Pseudomonas* isolates were screened for chitinolytic activity, and on the basis of the chitin hydrolysis zone (Fig:3), *Pseudomonas* isolates were selected for chitinase production on chitinase agar media. On the chitin agar clear zone producing *Pseudomonas* RSML07 (**Figure 3**) was selected for further chitinase study and the organism was identified as *Pseudomonas* RSML07 through the biochemical test (**Table 1**)

By study obtained the *Pseudomonas* RSML-07produced the chitinase in sub-merged fermentation were 29 EU/ml in the crude extract by using a liquid medium modified.

After the production, Chitinase was purified from crude enzyme extract by ammonium sulfate precipitation and dialysis. The enzyme was purified using 70% ammonium sulfate precipitation and dialyzed against 0.02 M Tris buffer of pH 8.8 for overnight at 4˚C.

Almost all of the reported chitinase-producing strains could use chitin or colloidal chitin as a carbon source (Wang et al., 1995). *Pseudomonas* RSML07 expresses maximum chitinase around the six days of fermentation in most cases and a chitin concentration of 2% was found to be the most suitable concentration for chitinase production (Thiagarajan et al., 2011).

Table 01-: Biochemical characteristics of *Pseudomonas*isolates.

Sr.No.	Isolate code	Biochemical test			
		Indole	Citrate	Catalase	Oxidase
1	RSML01	-	+	+	+
2	RSML02	-	+	+	+
3	RSML07	-	+	-	+
4	RSML14	-	+	+	+
5	RSML17	-	+	+	+

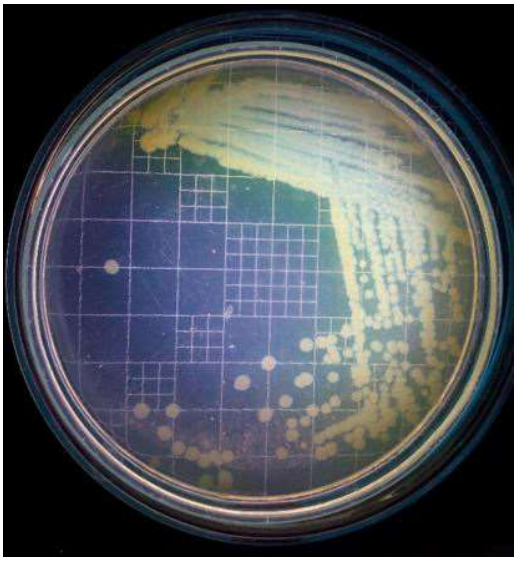

Fig.1: Pigmented growth of *Pseudomonas* on agar medium

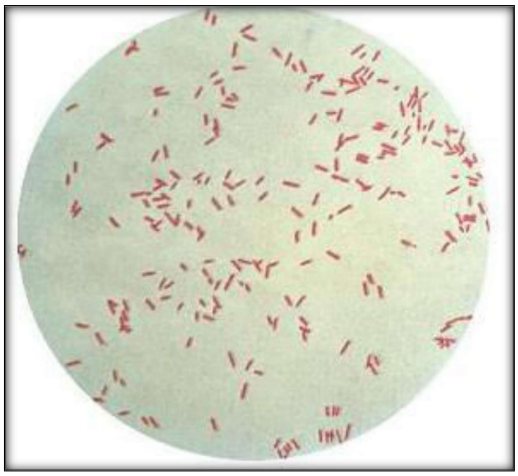

Fig.2: Morphological features of *Pseudomonas* under a light microscope (100 X).

Figure 3: Chitinase Agar showing halo zone by *Pseudomonas* RSML07

Conclusion:

The present study, confirmed that the strain *Pseudomonas* RSML07 isolated from the Marine Shrimp Shell Wastes has chitinase activity. By study obtained the *Pseudomonas* RSML-07produced the chitinase in sub-merged fermentation were 29 EU/ml in the crude extract by using a liquid medium.

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