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Isolation and characterization of chitinase from bacteria of Shrimp pond

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ABSTRACT

The enzyme chitinase, produced by various bacteria in both terrestrial and marine environment is used for the degradation of chitin which possesses several industrial applications. The biological applications of these enzymes have been exploited in food and pharmaceutical industries. Chitin degrading bacteria which produced an extracellular chitinase was isolated from the shrimp pond sediment and identified as Vibrio alginolyticus. Chitinase activity for this strain was 0.08µg/ml/minute. The optimum pH and temperature for chitinase activity was 8 and 45ºC respectively. The molecular mass of the chitinase identified was 62KDa.

Key words: Chitinase, Purification, SDS-PAGE, *Vibrio* species

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INTRODUCTION

Recently, 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 hold great economic value due to their versatile biological activities and chemical applications, mainly in medical [1,2] and pharmaceutical areas [3,4]. Study of chitinases is important in the application to biological control [5]. Especially it is a potential antifungal agent through chitin degradation activity [6]. Chitinase have found extensive use in preparation of protoplasts from fungi, a technique of increasing importance in biotechnology [7]. The Chitinase is widely distributed in bacteria, actinomycetes and plant [8,9]. Most abundant of chitinase producing bacteria such as *Aeromonas sps, Clostridium sps, Vibrio sps, Streptomyces sps, Beneckea sps, Achromobacter sps, Alginomonas sps, Pseudomonas sps, Clostridium sps* etc.[10]. Hence an attempt has been made to isolate and characterize the chitinase producing bacteria from the sediment of shrimp pond.

MATERIALS AND METHODS

Sample collection

The sediment was collected from the colachel shrimp pond in Kanyakumari district, Tamil Nadu.

Screening and Identification of the bacteria

The bacteria were isolated and chitinase producing bacteria was 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 (3% w/v chitin; 0.1% KH_2PO_4; 0.05% $MgSO_4.7H_2O$; 50mM Sodium Phosphate buffer, pH 6.0) in a 250 ml Erlenmeyer flask at 30°C. For reflecting the growth of the culture in this medium OD at 660 nm was taken using blank as 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 20 minutes. The enzyme was concentrated by condensing the solution in order to reduce its volume.

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 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. [11] using N-acetyl glucosamine as a reference compound [12]. One unit of chitinase activity was defined as the amount of the enzyme that produced 1 µ mol of reducing sugar per minute.

Effect of pH and temperature on the chitinase activity
The effect of pH on the activity of chitinase was determined at different pH (5 to 9) by using standard buffer solutions. The optimal temperature for the chitinase activity was determined in the range of 20-50^{0}C under standard assay conditions.

Purification of chitinase
The chitinase was purified by the method of Imoto and Yagishita [13].

SDS- PAGE
SDS- PAGE was carried out by the modified method of Ohtakara [14].

RESULTS AND DISCUSSION

Chitinase producing bacteria was isolated from the shrimp pond sediment and identified. The effect of pH and temperature on chitinase activity was determined. The purified chitinase was characterized by SDS PAGE.

Identification of bacteria
On the chitin agar clear halo zone producing organism (Fig.1) was selected for further chitinase study and the organism was identified as *Vibrio alginolyticus* through the biochemical test (Table1).

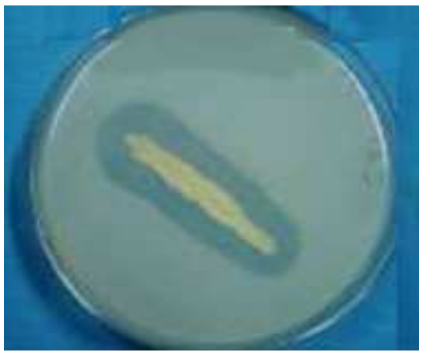


Fig.1: TCBS agar showing halo zone by *Vibrio alginolyticus*

The chitinase activity of the isolate *V.alginolyticus* was 0.08 µg/ml/ minute. Almost all *Vibrio* species showed chitinase producing activity specifically *V.harveyi* , *V.furnisii* [15] and *V.parahaemolyticus* [16] were showed more chitinase producing activity.

The effect of pH on the chitinolytic activity was studied with varying buffer (pH 5-9). The maximum activity of chitinase was observed at pH 8.0 (Fig.2). The marine bacterial chitinase showed broader pH optima or more active in neutral or slightly alkaline conditions [17,18].

Table 1: Characteristics of *V. alginolyticus*

Test	Reaction
Fermentation/Oxidation	
Glucose	+
Mannitol	+
Sucrose	+
Amygdalin	+
Inositol	-
Sorbitol	-
Rhammose	-
Mclibiose	-
Arabinose	-
Voges-Proskauer	-
Lysine decarboxylase	+
Ornithine decarboxylase	+
Citrate utilization	+
Tryptophan deaminase	+
Indole production	+
Gelatinase	+
Oxidase	+
B-galactosidase	-
H_2S production	-
Urease	-
Nitrate/ Nitrite reduction	-

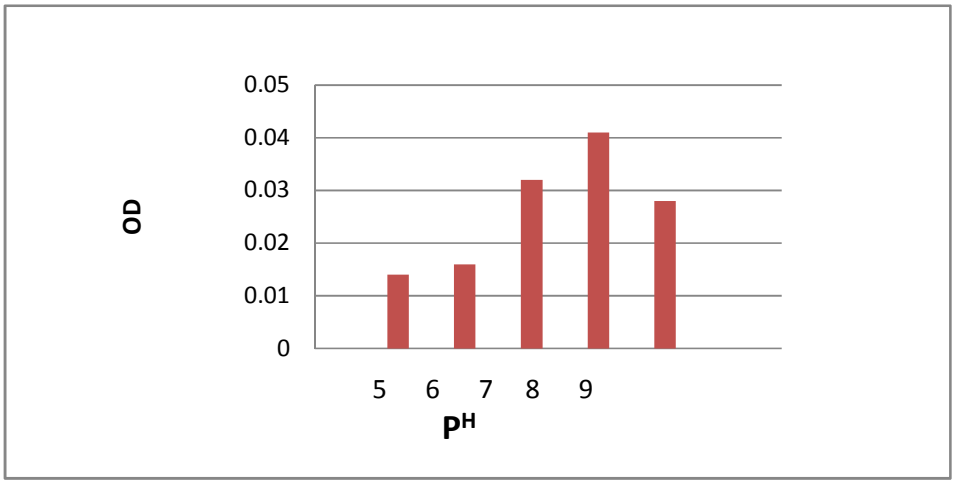


Fig.2: Effect of P^H on chitinase activity

The optimum temperature for chitinase was 45^0C under the standard assay conditions. (Fig.3). The similar temperature optimum was observed in marine bacteria, *Aeromonas hydrophila* [19], *Alteromonas* sp. [18] and *Pseudomonas aereginos* [20].

Determination of molecular weight

The purification of extracellular chitinase from *Vibrio alginolyticus* was effectively concentrated by salting-out method with ammonium sulphate and the eluted product from the column was lyophilized. The molecular weight of lyophilized chitinase was estimated by SDS-PAGE. Through gel documentation the molecular weight of chitinase was identified as 62KDa (Fig.4). The molecular weights of the chitinase from marine bacteria are mostly around 60KDa [17,19,20].

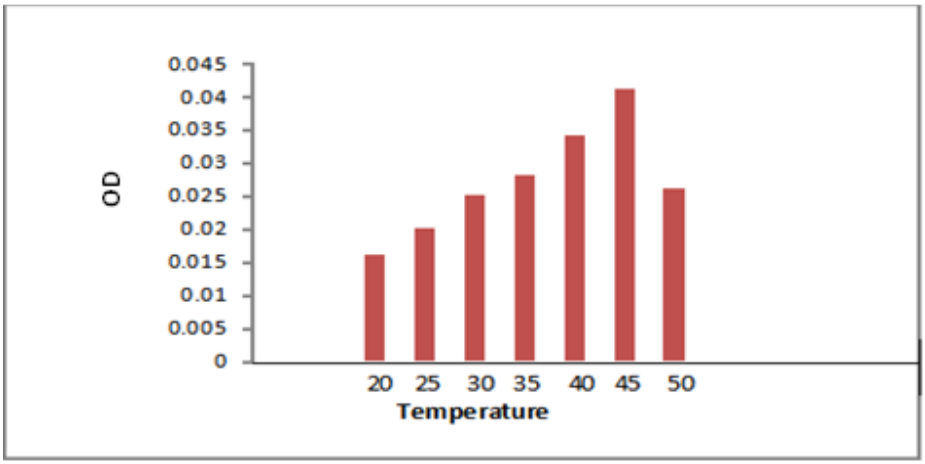
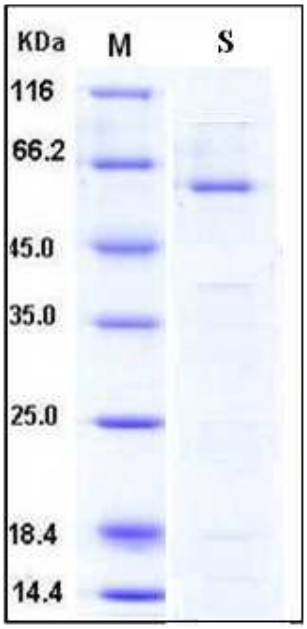


Fig. 3: Effect of temperature on chitinase activity



M-Marker; S-Sample

Fig. 4: SDS PAGE of purified chitinase

CONCLUSION

From the present study it confirmed that the strain *Vibrio alginolyticus* isolated from the shrimp pond sediment have chitinase activity. The enzyme chitinase can be immobilized to retain its activity for prolonged period and can be used in processing chitinase material and producing defined oligosaccharides.

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