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Production Of Chitinase Enzyme From Fish Waste

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ABSTRACT

Fishery processing industries generate large amounts of products. The disposal of these waste represents an increasing environmental and health problem. To avoid wasting of these by products, various disposal methods have been applied including insulation, fermentation hydrolysate and fish oil production. Fish by products provide an excellent nutrient source for microbial growth useful in enzyme production process, which is largely governed by the cost related to growth media. Recently environmental regulations are becoming stricter, requiring new disposal methods based on fact that fish waste may considered as an important of protein, lipids and material with high biological value. In this current study, fish waste was prepared and tested as growth substrate for microbial enzymes production. Three isolates were isolated from soil which produced chitinase enzyme. Chitinase enzyme was purified and activity which was confirmed by standard enzyme assays and thin layer chromatography.

Keywords: chitin, enzyme assay, thin layer chromatography

Introduction: [1] [5][[8]9] [14] [15][16]

India is a major producer of fish. India holds second ranks in the world after China; contributing to 5.68% of global fish production. The country has a long coastline of 8118 km and inland fishery resource include 1.96 lakh km stretch of rivers and canals, 29.07 lakh hector reservoirs 24.40 lakh hector ponds and tanks . In recent years, total fish production is 9.58 million metric tons with a contribution of 6.14 million metric tons from inland sector and 3.44 million metric tons from marine sector respectively .

Approximately 131 (85%) million tonnes of fish were directly utilized as food and the rest (15%) was underutilized as live bait for fishing, ornamental products (pearls and shells), feed for carnivorous farmed species and marine worm. The production of fish in China Indonesia, India and Russia has increased while fish production decreased in other countries over the ten year period. About 75% fish resource was used for human

consumption and remaining 25% is consider as waste. According to the Food and Agricultural Organization of the United Nations, in appropriate terms over 100 million metric tons of fish waste or discard in water body is generated worldwide annually, with only a small portion used in the production of fishmeal and fish oil.

In India, it was estimates that 3, 02,750 tonns of (both processing and pre-processing taken together) waste was generated. In the context of environmental pollution waste generated in fish processing industry is a matter of great concern. Among the maritime state the largest waste generation was observed from Gujarat (30.51%) followed by Maharashtra (23%) and Kerala (17.5%).

Chitin is the insoluble linear β 1, 4 linked polymer of N-acetylglucosamine. It is an important constituent of outer layer of many organisms, exoskeleton elements of some animals including fish, worms, arthropods, such as crustaceous, crabs, lobsters, shrims, insects and octopus. [17][18]

Chitinase enzymes are chitinolytic enzyme it degrade chitin and they are produced by various organism such as viruses, bacteria, insects, higher plants and animals. [17][18]

Chitinase are types of enzymes that hydrolyes chitin by cleaving its β-1,4 N glycosidic bond generating soluble chitooligosaccharides of low mass multimer Chitinases have several field applications. Chitinases are attaining prominence in the field of biotechnology applied in waste management, pest control in agriculture, and human health care. [17][18]

In this study, the three selected isolates isolated from soil were grown in 100ml of sterile basal medium with fish powder , and the flasks kept on orbital shaker and incubate it for three to four days at 37°C at pH 7. After incubation, culture broth is centrifuged at 1200 rpm for 20 minutes. Supernatant used for further purification of chitin and enzyme assay and TLC of chitin was done for confirmation of enzyme activity.

MATERIALS AND METHODS

1.Collection of sample:

Mackerel (*Rastrelliger kanagurta*) were used in these experiments because nutritional value of Mackerel fish is more than other fishes. Fish waste which consists of head, bones, fins and viscera were collected from Hadapsar, (Pune) fish market. These fish waste was washed thoroughly and blended. Then stored at 0^0C until use.

2: Production of Chitinase enzyme:

2.1: Collection of sample:-

R.kangurta fish waste was washed thoroughly with tap water and sun dried. Then this dried material was milled to powder.

2.2: Isolation and screening of Chitinase producing strains: [9]

3. Preparation of colloidal chitin: -[1]

A simplified and efficient method of preparing colloidal chitin from crude chitin. It modified some step for existing techniques to provide significant saving in efforts and materials for colloidal chitin preparation. 20 gm crude chitin treated with 150ml of (12M) concentrated HCl was added slowly with continuous stirring then it was treated with 2lit. ice cold distilled water. Change the water after every two hours of intervals until the pH become 7. Obtained colloidal chitin was pressed between filter paper to remove excess moisture and autoclave and stored at 4° C.

4. Enrichment:

Soil was collected from three different areas. 0.1 gm of soil was mixed with 9ml of water and then it was spread on the sterile basal medium with colloidal chitin agar plates and the plates were incubated at 37°C for 24 to 72 hours. For isolation of microorganisms we used basal medium with colloidal chitin agar.

5: Production and extraction of Chitinase enzyme:-

Assay for Chitinase enzyme:

The three selected isolates were grown in 100ml of sterile basal medium with colloidal chitin broth and sterile basal medium with fish powder broth simultaneously, and the flasks kept on orbital shaker and incubate it for three to four days at 37°C at pH 7. After incubation, culture broth is centrifuged at 1200 rpm for 20 minutes. Supernatant used for further purification.

6: Purification of Chitinase:- [9]

7: Thin layer chromatography of Chitinase:- [9]

0.1ml enzyme was incubated with 0.1ml of substrate solution (0.1gm colloidal chitin in 10ml of 50mM phosphate buffer) at RT for 1 hrs. The reaction mixture was loaded on silica gel sheet. Silica gel is kept in solvent (up to 2/3rd solvent is run). Spraying reagent was sprayed on silica gel sheet. Heating it in an oven at 110°C for 15-20 minutes.

RESULT AN DISCUSSION

Isolation and screening of chitinase producing strain

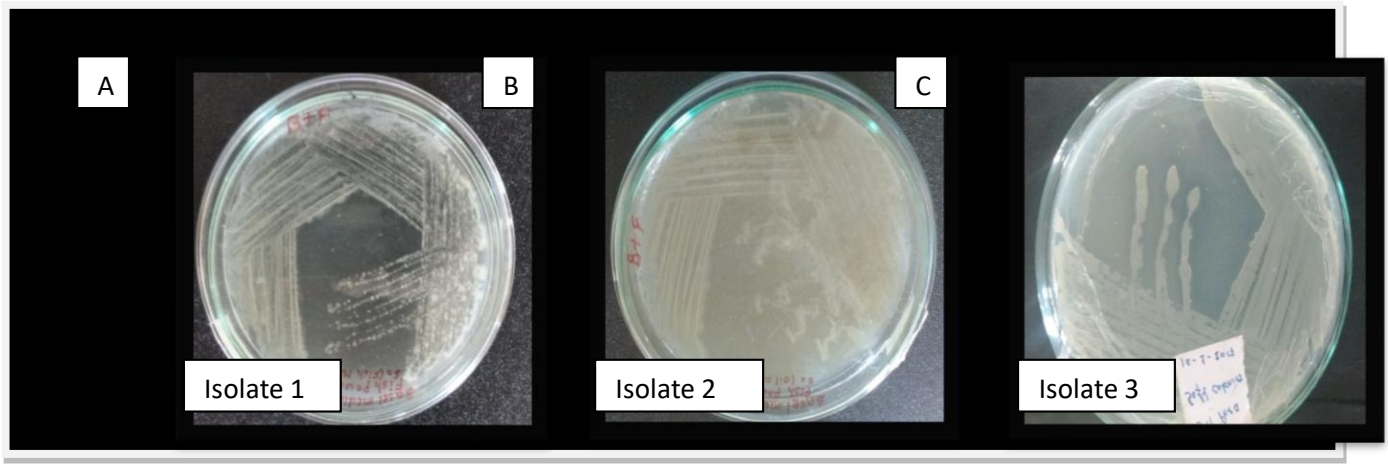

Fig.1: Isolation of chitinase producing strain. A] Isolate 1, B] Isolate 2, C] Isolate 3

From soil sample chitinase producing bacteria were isolated on basal medium with colloidal chitin and isolates from colloidal chitin plate were grown on basal medium with fish waste powder.

Chitinase producing strain isolated from basal medium with colloidal chitin was transferred on basal medium with fish waste powder and observed for production of enzyme by Lugol's Iodine solution. Zone of clearance (Fig 2) was observed around (A)Isolate 1,(B) Isolate 2, (C) Isolate 3.

Detection of chitinase producing strain on basal medium with fish waste:

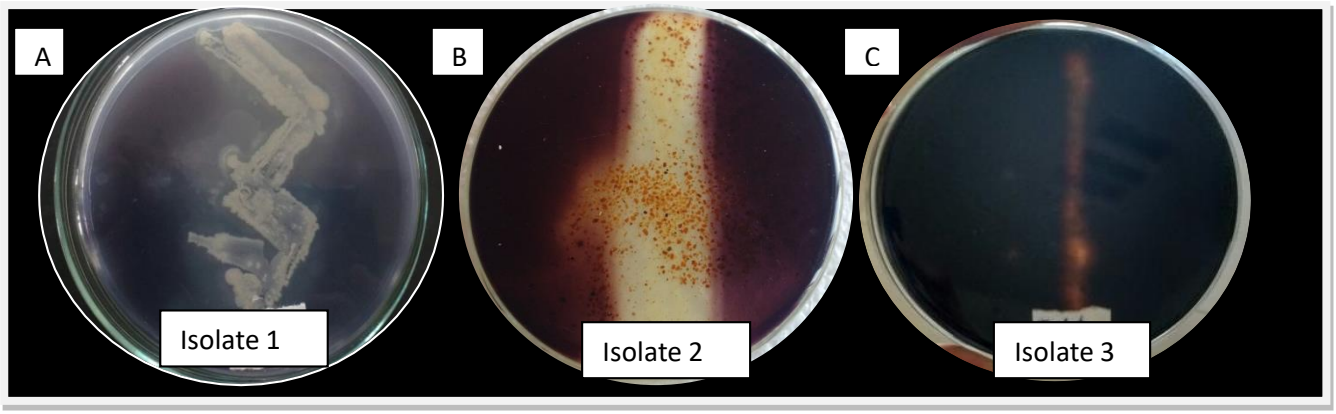

Fig.2. Detection of chitinase activity on Basal medium with fish waste

A] Isolate 1 B] Isolate B C] Isolate 3

Purification chart of chitinase enzyme extracted from isolate 1 shows maximum specific activity 43.6 and yield 15.6% , isolate 2 shows maximum specific activity 2.96 and yield 75.4 % .and isolate 3 shows maximum specific activity 118.5 and yield 31.9 % .

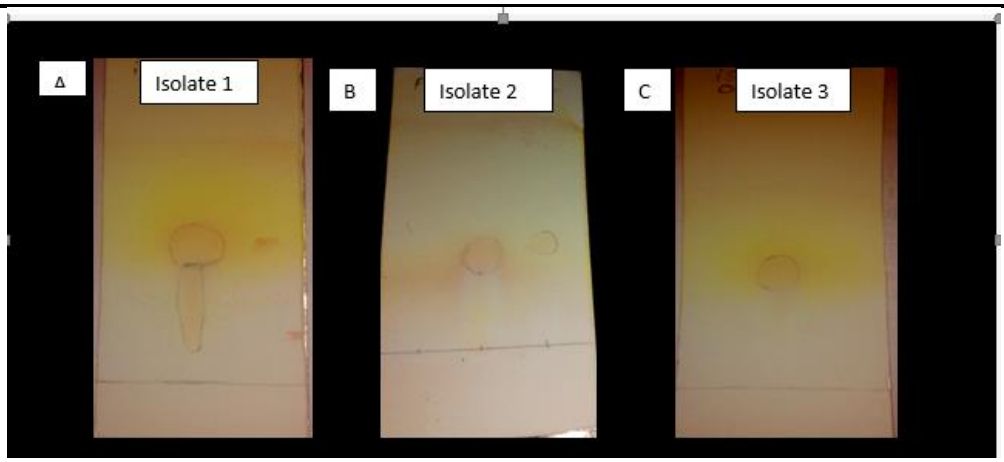

Fig 3: Thin layer chromatography of a. Isolate 1 purified enzyme b. Isolate 2 purified enzyme c. Isolate 3 purified enzyme. Standard sugars were also placed as control.

According to standard Rf value all isolates may contain sugar D-Ribose , D-Arabinose and D-Glucose. These sugars are the part of chitooligosaccharides present in enzyme chitinase. Hence presence of theses sugars confirms the production of chitinase enzyme.

In this study, fish waste was the best substrate supplement under solid-state fermentation. Our results agree with others. e.g., San-Lang Wang. Tao- Jen Chang et . al.reported that fish residue was the best substrate supplement for chitinase production by *Serratia sp.* TKU016. Fish waste is inexpensive, abundant, and easily available, and supplies the microorganism with better nutrition. Moreover, the use of purified chitin enhances the cost of enzyme production which is a major limitation to the economic feasibility of the bioconversion and utilization of lignocellulosic materials.

All three isolates isolated from soil has the capability for the production of chitinase on solid cultivation using fish waste as substrate. These bacteria should be further identified and can be useful for treatment of chitinolytic waste and could be an ideal candidate for biological control of various crop pathogens. Further research should concentrate on the purification and characterization of chitinolytic enzymes in order to study their role as a biocontrol agent.

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