STUDIES ON CHITINOLYTIC BACTERIA IN INTESTINAL TRACT OF MARINE FISHES AND SHRIMPS

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INTRODUCTION

Microorganisms excrete a wide variety of Chitinolytic enzymes, which are also found in mammalian systems. commercially they are very important and isolated from various living sources such as fishes, Shrimps, Insects, animals, bacteria and fungi. Chitinase are ubiquitous in nature, being found in eukaryotes, prokaryotes, archaea and viruses (Suzanne et al., 2001). It is believed that stomach chitinase have an indirect digestive function, helping to breakdown the exoskeleton of prey, which allows other digestive enzymes access to soft inner tissue (Fange and Grove, 1979; Lindsay, 1984; Clark Quayle et al., 1998). Chitosan is one of the most abundant biomasses on earth chitooligosaccharides from chitosan polymer have become a remarkable resource for the development of functional foods, artificial skin, medicine and other materials attempted by methods such as the treatment of chitosan polymer by chemicals or enzyme synthetic production of chitooligosaccharide from as the treatment of chitosan polymer.

MATERIALS AND METHODS

Isolation and identification

Fresh marine fish were collected, washed with water, surface sterilized with alcohol, and again washed with sterile saline. The intestine of the fish is taken by dissection process. The intestine is homogenized by using mortar and pestle. The homogenized sample is used for serial dilution technique. The isolates was identified based on cellular morphology, Gram staining, endospore staining and biochemical tests and further it was screened and its phenotypic character was confirmed by molecular methods (16sR RNA)

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Optimization of Chitosanase

Effect of pH on Chitosanase Production

The isolates such as SSPZ11, SSPZ15, and KPP20 were inoculated in chitosan minimal salt broth at different pH ranges from pH 4 to 9 and incubated at 37°C for 48 hours. The chitosanase production was observed in spectrophotometer at OD of 560nm.

Effect of Various Nitrogen Source on Chitosanase Production

The isolates such as SSPZ11, SSPZ15, KPP20 were inoculated in the chitosan minimal salt broth supplemented with various nitrogen source such as tryptone, peptone, yeast extract, beef extract, ammonium chloride, 40% urea inoculated at 37°C for 48 hours. The chitosanase activity was observed in spectrophotometer at OD of 560nm.

Genome Sequencing

The following cultures SSPZ11, SSPZ15, KPP20 were subjected for genome sequencing and the sequencing was done in PACE Microbial technology, Puducherry.

RESULTS AND DISCUSSION

Morphological and Physiological Characteristics

Minimal Salt Agar Medium

The chitinase producing bacterial strains were isolated from fish sample and different fish using minimal salt agar medium observed for clear zone.

Table no 1 Chitinolytic Bacteria Isolated from Various Fishes & Shrimps

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<th>S.No</th>
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Optimization of Chitosanase Enzyme at Various pH

The isolates such as SSPZ11, SSPZ15, KPP20 were inoculated in chitosan minimal salt broth at different pH 4 to 9 and incubated at 37°C for 48 hours. The chitosanase production was observed in spectrophotometer at OD 560nm.

Table 3 Effect of Various pH on Chitosanase Production By Isolate SSPZ11

<table>
<thead>
<tr>
<th>S.NO</th>
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Graph 1 Effect of various pH On Chitosanase Production By Isolate SSPZ11

Table 4 Effect of Various pH on Chitosanase Production By Isolate SSPZ15

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Table 2 Morphological & Biochemical Characteristic Results of Various Isolates

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26278 | Page
Effect of Various pH on Chitosanase Production By Isolate SSPZ15

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Effect of Various pH on Chitosanase Production by Isolate SSPZ15

Optimization of Various Nitrogen Source on Chitosanase Production

The isolates such as SSPZ11, SSPZ15, KPP20 were inoculated in the chitosan minimal salt broth supplemented with various carbon source such as ammonium chloride, peptone, Tryptone, 40% urea, beef extract, yeast extract and incubated and incubated at 37°C for 48 hours. The chitosanase activity was observed in spectrophotometer at OD of 560nm.

Effect of Various Nitrogen Source on Chitosanase Production by Isolate SSPZ15

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<tr>
<td>6</td>
<td>Yeast extract</td>
<td>0.8840</td>
</tr>
</tbody>
</table>

Effect of Various Nitrogen Source on Chitosanase Production by Isolate SSPZ15

Table 6 Effect of Various Nitrogen Source on Chitosanase Production by Isolate SSPZ15

<table>
<thead>
<tr>
<th>S.No</th>
<th>Chitosan Minimal Salt Broth At Various pH</th>
<th>OD AT 560nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ammonium chloride</td>
<td>1.3087</td>
</tr>
<tr>
<td>2</td>
<td>Peptone</td>
<td>2.6804</td>
</tr>
<tr>
<td>3</td>
<td>Tryptone</td>
<td>3.0000</td>
</tr>
<tr>
<td>4</td>
<td>40% urea</td>
<td>2.9456</td>
</tr>
<tr>
<td>5</td>
<td>Beef extract</td>
<td>3.0000</td>
</tr>
<tr>
<td>6</td>
<td>Yeast extract</td>
<td>3.0000</td>
</tr>
</tbody>
</table>

Effect of Various Nitrogen Source on Chitosanase Production by Isolate SSPZ15

Table 7 Effect of Various Nitrogen Source on Chitosanase Production by Isolate SSPZ15

<table>
<thead>
<tr>
<th>S.No</th>
<th>Chitosan Minimal Salt Broth At Different Nitrogen Source</th>
<th>OD AT 560nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ammonium chloride</td>
<td>0.8026</td>
</tr>
<tr>
<td>2</td>
<td>Peptone</td>
<td>3.0000</td>
</tr>
<tr>
<td>3</td>
<td>Tryptone</td>
<td>3.0000</td>
</tr>
<tr>
<td>4</td>
<td>40% urea</td>
<td>2.5873</td>
</tr>
<tr>
<td>5</td>
<td>Beef extract</td>
<td>3.0000</td>
</tr>
<tr>
<td>6</td>
<td>Yeast extract</td>
<td>3.0000</td>
</tr>
</tbody>
</table>
**Chitosan Minimal Salt Agar**

Plate 1 Shows the chitinase producing bacteria forming clear zone

**Hicrome Bacillus Agar**

Plate 2 Shows the growth of different *Bacillus* species forming colourful colonies

**Cetrimide Agar Base**

Plate 3 Shows the Pseudomonas species green colour formation in Cetrimide agar

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**Enzyme Immobilization**

Plate 4 Shows the chitinase immobilized beads

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**Results of Genome sequencing**

The following cultures SSPZ11, SSPZ15, KPP20 were subjected for genome sequencing and the results were identified by molecular method such as, SSPZ11- *Pseudomonas* sp, SSPZ15- *Exiguobacterium* sp, KPP20- *Pseudomonas* sp.

**Sequencing**

The purified PCR products of approximately 1,400bp were sequenced by using the primers (785 F 5’ GGA TTA GAT ACC CTG GTA 3’ and 907 R 5’ CCG TCA ATT CCT TTR AGT TT 3’). Sequencing were performed by using Big Dye terminator cycle sequencing kit (Applied BioSystems, USA). Sequencing product were resolved on an Applied BioSystems were model 3730XL automated DNA sequencing system (Applied BioSystems, USA).

**Phylogenetic Tree Construction**

The culture sequence obtained were subjected to BLAST analysis, the phylogenetically similar type strains sequences and other phylogenic related sequence were selected from the Gen Bank and they were subjected to multiple sequence alignment and then align sequences were trimmed to similar length in nucleotides and were subjected to phylogenetic tree (neighbour joining) construction using MEGA 6. In the tree the number at the nodes indicate levels of the bootstrap support [high bootstrap values (close to 100%) meaning uniform support] based on a neighbour-joining analysis of 1,000 re-sampled data sets. The bootstrap values below 50% were not indicated. Bar 0.005 substitutions per site.
The aim of my present study was Isolation and Characterization of chitosanase producing bacteria from gut of fish and shrimp. For this study 5 fishes and shrimp were collected from the Cuddalore and Pazhaiyar fish market. From these studies it is very clear different bacterial sp is predominant in the gut of fish and intestine. Today most of the country relies on microbial enzymes for commercial exploitation chitosanases have diverse role in day life, for example chitosanases are employed in various industries like beverages, health, medicine. These isolates can be used for further studies and the gene responsible for chitosanase production can be identified, cloned and expressed to get increased production of chitosanase.

**DISCUSSION**

The following cultures SSPZ11, SSPZ15, KPP20 were subjected for genome sequencing and the results were identified by molecular method such as, SSPZ11-Pseudomonas sp, SSPZ15-Exiguobacterium sp, KPP20-Pseudomonas sp.

**The present study determines** Isolation and characterization of chitosanase producing microorganism from various homogenized fish gut samples. 34 bacterial species were isolated and screened. Three bacterial species (SSPZ11, SSPZ15 and KPP20) showed higher production of chitosanases, the optimization of chitosanases at various pH, nitrogen source, was standard. To confirm the bacterial species it was further indentified by genome sequencing methods.

**Significant and impact of the study**

The present study determines Isolation and characterization of chitosanase producing microorganism from various homogenized fish gut samples. 34 bacterial species were isolated and screened. Three bacterial species (SSPZ11, SSPZ15 and KPP20) showed higher production of chitosanases, the optimization of chitosanases at various pH, nitrogen source, was standard. To confirm the bacterial species it was further indentified by genome sequencing methods.

**Chitosan** has a number of commercial and possible biomedical uses. It can be used in agriculture as a seed treatment and biopesticide, helping plants to fight off fungal infections. In winemaking it can be used as a fining agent, also helping to prevent spoilage. In industry, it can be used in a self-healing polyurethane paint coating. In medicine, it may be useful in bandages to reduce bleeding and as an antibacterial agent; it can also be used to help deliver drugs through the skin.

It is very clear different bacterial sp is predominant in the gut of fish and intestine. Today most of the country relies on microbial enzymes for commercial exploitation chitosanases have diverse role in day life, for example chitosanases are employed in various industries like beverages, health, medicine. These isolates can be used for further studies and the gene responsible for chitosanase production can be identified, cloned and expressed to get increased production of chitosanase.
References


24. Yeon jin ghoi, Eun Jung Kim, Zhe Piao, Young Chul Yun and Yong Chul Shin. Purification and characterization of chitosanase from *Bacillus* sp. Strain KCTC-0377BP and its application for the production of Chitosan Oligosaccharides. *Journal of applied and environmental microbiology* are provided here courtesy of American society for microbiology (ASM).