CHITINASE PRODUCING POTENTIAL OF ACTINOMYCETES FROM THE EXTREME SALINE-ALKALINE ENVIRONMENT OF LONAR LAKE.

Prasad P. Loni* and Shyam S. Bajekal
Department of Microbiology, Yashwantrao Chavan College of Science, Karad<br>Vidyagan, Karad – 415 124 Maharashtra, (INDIA)<br>

Received April 24, 2009 Accepted July 13, 2009

ABSTRACT
Chitinases are a group of enzymes that act on chitin, the second most abundant natural polymer after cellulose. Like many other enzymes, chitinases are also today finding an increasing array of applications from their use as biocontrol agents against phytopathogenic fungi, for the generation of fungal and yeast protoplasts and for the treatment of seafood waste. Among enzymes, those from extremophilic organisms are also finding increasing use today and chitinases are no exception.

Actinomycetes are a very sturdy group of microorganisms found in a myriad of environments including some extreme ones and are known to be major producers of chitinases. The Lonar Lake environment is known to be one such extreme environment with its highly alkaline pH around 9.5-10.0 and high salinity. It is the third largest natural salt water lake in the world and is formed in a meteorite impact crater, the only one formed in basaltic rock.

The littoral soil of this lake was screened for Actinomycetes producing chitinase. Among the several isolates found to be potent chitinase producers, productivity studies were carried out on six of these isolates with a view to explore their commercial potential as also their prospective applications in various fields such as biocontrol, etc.

Key Words: Chitin, Chitinase, Actinomycetes,

INTRODUCTION
Chitinases are the group of hydrolytic enzymes that catalyse depolymerisation of chitin, the second most abundant natural polymer. Chitinases have many industrial and agricultural applications. This property of chitinase to degrade chitin makes them valuable in the field of pest control and pollution abatement. More specifically they can be applied as biocontrol agents against phytopathogenic fungi and treatment of seafood waste management.

Chitinase carry out breakdown of chitin which is a linear β-1,4 homopolymer of N-acetylglucosamine found naturally in crustaceans such as crab, shrimps, lobsters also in insect exoskeleton, fungal cell walls etc. Chitinase play an important role in the carbon cycle of biosphere to maintain the ecosystem. In India 60000 to 80000 tonnes of chitinous wastes are produced annually. Therefore to maintain the environmental balance of the C:N ratio this high amount of chitin should be degraded hence maintaining the carbon nitrogen ratio evenly. There are

* Author for correspondence
lots of conventional and traditional methods to degrade the chitinous waste but the biological i.e., the enzymatic method for degrading chitinous waste is more ecofriendly and comparatively economic.

The chitinase enzyme is produced by microorganisms including bacteria, fungi, actinomycetes and diverse range of life forms such as snails, insects, plants etc. Among the microorganisms more than 90 percent of chitinolytic populations are actinomycetes. Actinomycetes are very sturdy group of microorganisms found in myriad of environments including the extreme environments. The chitinases produced from such actinomycetes are reported to have high applicability in their uses. Thus the study is focused on the chitinase produced by the actinomycetes isolated from the extreme saline alkaline environment of Lonar Lake.

Lonar lake is situated in Buldhana district of Maharashtra. It is the only lake in the world formed by meteorite impact in basalt rock. The environment of the Lake i.e. the water and littoral soil is extreme with respect to highly alkaline pH (around 9.5 to 10.0) and high salinity. The actinomycetes in this environment and their enzymes too are unique as adapted to the alkaline pH.

Amongst the several screened actinomycetes from the littoral soils of the lake, six of the potent chitinase producing actinomycetes were studied in detail. The study was explored for enzyme production and their application in biocontrol as well.

**MATERIAL AND METHODS**

**Soil sample collection**

Soil samples were collected from the selected sites from the Littoral zone of the Lake. All the samples were collected in sterile polythene bags and maintained properly until use.

**Isolation of Chitin degrading actinomycetes**

Isolation of chitinolytic Actinomycetes was done on Chitin agar with pH-10 by streak plate technique. The plates were incubated at 37°C for 5 days. After the incubation, the colonies showing large zone of clearance (CZ) around them indicative of chitinolysis. They were purified by repeated streaking on the same medium two times and then preserved on slants of the same medium at 4°C. These selected colonies were confirmed by colony morphology for actinomycetes and used for further study.

**Chitinase production**

Production of chitinase was done in aliquots of colloidal Chitin broth with pH-10. One hundred milliliter of broth in Erlenmeyer flasks of 250 ml capacity were inoculated with 1ml spore suspension (adjusted to 1.0 OD 600) of the isolates. Flasks were placed in rotary incubator at 150 rpm and 40°C and analyzed for chitinase production at 24hr intervals. The cultures broth was then centrifuged at 5000 rpm for 10 min to remove cell mass. The cell free supernatant was used for enzyme extraction by 40% to 60% of ammonium sulphate and kept at 4°C overnight. The precipitate was separated by centrifugation at 1000 rpm for 10 min and dissolved in phosphate buffer (pH 9.0) followed by dialyses against distilled water. This crude enzyme was used and preserved in plastic bottles at 0°C.

**Chitinase assay**

The chitinase assay system of Monreal and Reese estimating reducing sugars released by enzyme action was adapted for study. In 2.5 ml buffer 2.5 ml substrate (swollen chitin) was added followed by 0.5 ml of crude enzyme. The tube was incubated at 37°C in water bath for 1 hr. The reaction was arrested with 3.0 ml of
10% dinitro salicylic acid (DNSA). And then heated in boiling water bath for 10min. The coloured solution was centrifuged at 5000 rpm for 5min and absorbance of the supernatant was measured at 540 nm wavelength in a systronics spectrophotometer. The reducing sugar was estimated from the glucose curve. One unit of enzyme is defined as the amount of enzyme which catalyses the release of 1μM of reducing sugar per minute under assay conditions.

RESULTS AND DISCUSSION

Isolation of chitinolytic actinomycetes

In all, obtained actinomycetes strains from the littoral soils samples of Lonar lake, six of them (S-1-2, S -1-3, St-I-1, St-I-4, SER-3 and SER-4) potent chitinase producing actinomycetes were selected for further enzyme production. Studies on the basis of the zone of clearance around their colonies and the CZ/CS ratio was also done. (Table 1)

Table 1 : Chitinase activity of isolates on solid chitin agar

<table>
<thead>
<tr>
<th>Code Name</th>
<th>Colony size (CS) in mm</th>
<th>Zone of clearance (CZ) in mm</th>
<th>CZ/CS Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-1-2</td>
<td>3</td>
<td>6</td>
<td>2.0</td>
</tr>
<tr>
<td>S-1-3</td>
<td>3</td>
<td>7</td>
<td>2.33</td>
</tr>
<tr>
<td>St-I-1</td>
<td>2</td>
<td>6</td>
<td>3.0</td>
</tr>
<tr>
<td>St-I-4</td>
<td>4</td>
<td>5</td>
<td>1.25</td>
</tr>
<tr>
<td>SER-3</td>
<td>5</td>
<td>8</td>
<td>1.66</td>
</tr>
<tr>
<td>SER-4</td>
<td>2</td>
<td>5</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Among these six isolates again, the most potent is found to be St-I-1 which has maximum CZ/CS ratio followed by other isolates.

Chitinase Productivity

The chitinase production on rotary shaker was done for a period of 10 days with monitoring of enzyme production at 24hr intervals. It was found that enzyme production for all the isolates was maximum on the 8 day of incubation after which it decreased drastically. (Readings after 48 hrs are presented in Fig.1

The most potent chitinase producer is seen to be the isolate St-I-1 that produces 1.2 U/ml, followed by S-1-3 and SER-4(0.44 U/ml), S-1-2(0.40 U/ml),SER-3 (0.38 U/ml) and St-I-4 producing the least enzyme at 0.30 U/ml.

CONCLUSION

From this study we propose that the actinomycetes from the extreme environment of Lonar lake have high potential of producing chitinase enzyme. The production of enzyme of all the six isolates was maximum at pH 10 and on 8th day of fermentation. Thus such high amount of alkaline chitinase produced by actinomycetes could have application in seafood waste composting and Biocontrol.
ACKNOWLEDGEMENT

The authors are thankful to the Head of the Department, Management and Administration of Yashwantrao Chavan College of Science, Karad for providing the necessary facilities in the Microbiology Department for this work.

REFERENCES


