Extracellular Enzymes Produced by Marine Eukaryotes, Thraustochytrids

Yousuke Taoka,1 Naoki Nagano,1 Yuji Okita,2 Hitoshi Izumida,2 Shinichi Sugimoto,2 and Masahiro Hayashi1,1

1Laboratory of Marine Bioscience, Department of Biological Production and Environmental Science, Faculty of Agriculture, University of Miyazaki, I-1 Gakuenkibanadai-nishi, Miyazaki 889-2192, Japan
2Nippon Suisan Kaisha, Ltd., 2-6-2 Otemachi, Chiyoda-ku, Tokyo 100-8686, Japan

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Extracellular enzymes produced by six strains of thraustochytrids, Thraustochytrium, Schizochytrium, and Aurantiochytrium, were investigated. These strains produced 5 to 8 kinds of the extracellular enzymes, depending on the species. Only the genus Thraustochytrium produced amylase. When insoluble cellulose was used as substrate, cellulase was not detected in the six strains of thraustochytrids. This study indicates that marine eukaryotes, thraustochytrids, produced a wide variety of extracellular enzymes.

Key words: extracellular enzymes; thraustochytrids; Thraustochytrium; Schizochytrium; Aurantiochytrium

Thraustochytrids are marine eukaryotes. Studies of thraustochytrids have focused on their high accumulation of fatty acids, especially docosahexaenoic acid (DHA), in the cells, their niche in the marine ecosystem, their pathogenicity against seaweed and seagrass, and their phylogenetic classification.1,4) But studies of extracellular enzymes from thraustochytrids are scarce. Some polysaccharide hydrolyzing enzymes, such as cellulase and glucanase produced by thraustochytrids have been reported.5,6) Recently, Damera and Raghukumar7) investigated the extracellular enzymes produced by aplanochytrids, but varieties and characteristics of these enzymes are not well known. Perhaps unique and potential enzymes for industrial application are present in these enzymes. Furthermore, information on kinds of enzymes produced by each strain of thraustochytrids is lacking. Therefore, from the physiological and industrial viewpoints, it is very interesting and important to conduct research on enzymes from thraustochytrids. In this study, extracellular enzymes produced by various strains of thraustochytrids were investigated.

Aurantiochytrium limacinum ATCC MYA-1381, Schizochytrium aggregatum ATCC 28209, Thraustochytrium striatum ATCC 24473, T. roseum ATCC 28210, T. aureum ATCC 34304, and Aurantiochytrium sp. strain mh0186 identified based on the sequence information of the 18S rRNA gene (DDBJ, accession no. AB362211), were used in this study. Strains were maintained on B1 agar medium consisting of 2 g of polypeptone, 2 g of yeast extract, 5 g of glucose, and 1,000 ml of 1/2 artificial seawater (ASW) (pH 7.0).

The production of nine extracellular enzymes was investigated in all test strains of the thraustochytrids. The activity of each enzyme, protease, amylase, lipase, gelatinase, urease, phosphatase, cellulase, chitinase, and α-glucosidase was measured according to methods described in these previous reports.

The results of the enzyme assays are shown in Table 1 and Fig. 1. Protease activity, lipase activity, phosphatase activity, urease activity, and α-glucosidase activity were detected in all the strains. Amylase activity was detected only in the genus Thraustochytrium. Gelatinase activity was detected in all strains, except for A. limacinum and Aurantiochytrium sp. Chitinase was detected only in T. striatum (radius of clear zone, 6.2 mm). Cellulase was not detected in any of the strains. In a comparison of clear zones showing enzyme activity on agar plates, T. roseum showed the strongest activity in the case of protease, while those of A. limacinum and Aurantiochytrium sp. were very weak (Fig. 2a). In the case of amylase, T. roseum showed the strongest activity (Fig. 2b).

It has been reported that thraustochytrids produce degradative enzymes,5,6,15) Damare and Raghukumar7) have investigated four extracellular enzymes, protease, amylase, lipase, and chitinase, produced by 14 strains of aplanochytrids, and found that the aplanochytrids used in their study produced only protease. Sharma et al.16) have reported that two thraustochytrids, Labyrinthulaoides minuta and Ulkenia visurgensis, produced protease and utilize casein and gelatin as carbon sources. In the present study, gelatinase was also detected in these four strains. Sharma et al.16) investigated six enzymes, cellulase, xylanase, laminarinase, polygalacturonase, amylase, and alginate hydrolase, in culture filtrates produced from thraustochytrids, and found that thraustochytrids used in their study produced only protease. In this study, three strains of the genus Thraustochytrium (T. aureum, T. roseum, and T. striatum) produced amylase, but three strains formerly classified in the genus Schizochytrium (S. aggregatum, A. limacinum, Aurantiochytrium sp.) did not produce it. Amylase appeared to be produced by the genus Thraustochytrium, although Raghukumar et al.15) confirmed amylase production from S. mangrovei. Further study is required to clarify this point.

Cellulase was not detected in any of the six strains of thraustochytrids tested. As positive control, a cellulase-producing bacterium isolated from Kagoshima Bay, Japan, was used. Thraustochytrids were thought to be incapable of utilizing cellulose as a carbon source.17) Cellulase production by S. mangrovei and S. aggrega-

1 To whom correspondence should be addressed. Tel./Fax: +81-985-58-7225; E-mail: hayash-m@cc.miyazaki-u.ac.jp
tum has, however, been observed. In a study by Bremer and Talbot, carboxymethyl cellulose (CMC) was used as a substrate for cellulase production by S. aggregatum. Our results differ with those of Bremer and Talbot, probably because we used an insoluble substrate, cellulose powder, and they used a soluble substrate, CMC.

Chitin is an abundant structural polysaccharide that is a constituent of the exoskeleton of zooplankton and invertebrate larvae. Chitinase was detected in small amounts only in T. striatum. There has been no study of chitinase production by thraustochytrids. In the study by Damare and Raghumar, no production of chitinase in 14 strains of aplanochytrids was observed. A small fraction of thraustochytrids might be responsible for chitin degradation in the marine environment.

Phosphorus is nutritionally very important to all organisms. Bongiorni and Dini confirmed a significant relationship between total phosphorus and thraustochytrid densities on the Mediterranean coast. In the marine environment, thraustochytrids might utilize phosphorus released from dissolved organic phosphorus in seawater by phosphatase, and might play a role in the phosphorus cycle.

### Table 1. Detection of Extracellular Enzymes Produced by Six Strains of Thraustochytrids

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Genus Thraustochytrium</th>
<th>Genus Schizochytrium</th>
<th>Genus Aurantiochytrium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ATCC 34304</td>
<td>ATCC 28210</td>
<td>ATCC 24473</td>
</tr>
<tr>
<td>Protease (casein hydrolysis)</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Amylase (starch hydrolysis)</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lipase (Tweed 80 hydrolysis)</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Gelatinase (gelatin liquefaction)</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Urease</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phosphatase</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cellulase</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Chitinase</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>α-Glucosidase</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

(+ ) positive and (−) negative results of enzyme assay were decided as follows: positive results mean protease, zone of clearance around colonies upon the addition of 10% trichloroacetic acid; amylase, zone of clearance around colonies upon the addition of Lugol’s iodine solution; lipase, opaque zone around colonies; gelatinase, liquefaction of gelatin under cool temperatures; urease, medium to red color change; phosphatase, medium to pink color change around the colonies upon the addition of ammonia vapor; cellulase, zone of clearance around colonies; α-glucosidase, color change of medium.

All enzyme detection was done in duplicate (n = 2).
α-Glucosidases are enzymes that hydrolyze α-glucosidic binding from the non-reducing end of oligosaccharides and polysaccharides, with release of α-glucose.20) All the strains tested in this study produced α-glucosidase.

Urease, an ammonium permerase, catalyzes the hydrolysis of urea to ammonia and carbon dioxide. This enzyme was detected in all the strains tested. If urease is available in the growth environment, thraustochytrids can probably grow on it as a nitrogen source, and thus partly contribute to the nitrogen cycle in the marine ecosystem.

The results of a series of enzyme detection assays indicated that thraustochytrids produce diverse extracellular enzymes. It has been thought that thraustochytrids can play an important role in carbon cycle of the marine ecosystem within the microbial loop.21) Diverse enzymes produced by them probably contribute to the microbial food chain in the marine ecosystem. This study indicates that thraustochytrids have the potential to produce a wide variety of enzymes.

References