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Enzyme production by a fungoid marine protist, *Thraustochytrium striatum*

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Abstract

Thraustochytrium striatum is a fungoid marine protist and was shown to be a promising enzyme producer for potential industrial applications. This research aimed at studying extracellular enzymes secreted by *T. striatum* under different conditions with specific objectives to qualitatively identify enzymes, quantify the cell growth and enzyme production, correlate enzyme production with extracellular polymeric substances (EPS), and examine the induction of enzyme by polysaccharide substrates. The qualitative analysis showed that *T. striatum* can produce at least seven extracellular enzymes including lipase and six polysaccharases (i.e., amylase, CMCase, xylanase, chitinase, pectinase, and κ-carrageenase). The carbon and nitrogen concentrations and salinity significantly affected the kinetics of enzyme production. *T. striatum* produced decent amount of polysaccharases at all conditions, but negligible lipase. Amylase was the predominant enzyme and reached the highest activity of 750 U/L with glucose = 30 g/L, nitrogen source = 6 g/L and salinity = 100% sea water. Enzymes appeared to correlate with the production and monosaccharide composition of EPS. Enzyme-specific polysaccharide substrates including starch, CMC, xylan, κ-carrageenan, pectin, and chitin did not induce the production of corresponding enzymes by *T. striatum* while carbon starvation condition resulted in comparable enzyme activities, which indicated that enzymes from *T. striatum* were constitutive.

Keywords: Catabolite repression; Extracellular enzyme; Extracellular polymeric substances; Marine protist; Substrate induction; *Thraustochytrium striatum*

Introduction

Thraustochytrids are osmo-heterotrophic unicellular marine protists and have been found in various habitats, including plant detritus (e.g., mangrove and brown algae), fecal pellets of zooplankton, rocky shores, coral reefs, salt marshes, sandy sediment, coastal waters, and deep sea (Raghukumar and Raghukumar, 1999; Raghukumar et al. 1994, 1995). They have recently received extensive studies as a promising source of lipids, especially docosahexaenoic (DHA) and eicosapentaenoic (EPA) acids due to their superior growth rate and oil yield/content

Abbreviations: ASW, artificial sea water; CMC, carboxymethyl cellulose; DCM, dry cell mass; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; EPS, extracellular polymeric substances; DDI, deionized distilled water; MWCO, molecular weight cutoff; NAG-N, acetyl-D-glucosamine; PGA, polygalacturonic acid; pNPB, p-nitrophenyl butyrate; STM, standard medium; YEP, yeast extract and peptone.

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(Fan et al. 2000). In addition, thraustochytrids have been recognized to play an important role in the degradation and mineralization of highly refractory organic matter in marine ecosystems because of their ability to decompose plant detritus by means of extracellular hydrolytic enzymes, such as amylase, cellulase, lipase, protease, pectinase, chitinase, κ-carrageenase, and xylanase (Bongiorni et al. 2005; Nagano et al. 2011; Taoka et al. 2009). Therefore, an emerging interest in thraustochytrids is their diverse extracellular enzymes. Compared to traditional chemical catalysts, enzyme-catalyzed reactions can occur under much milder conditions, e.g., lower operating temperature, neutral pH condition, lower energy cost, lower environmental impact, and/or less impurity in the products. Currently, the majority of the industrial enzymes are from bacteria and fungi, but research is lacking on the marine protist enzymes toward their production and applications. The enzymes from thraustochytrides may possess uniqueness and potential for industrial applications, including detergent, fuel, food, animal feed, beverage, textile, paper, chemical, cosmetics, and pharmaceuticals. Therefore, it is interesting and important to examine enzymes from thraustochytrids from the physiological and industrial viewpoints.

Bongiorni et al. (2005) studied eleven thraustochytrid strains and their enzymes and found that different strains displayed different spectra and intensity of enzymatic activities. All investigated strains exhibited capability of degrading a large variety of substrates via a wide spectrum of enzymes including lipase, esterase, arylamidase, alkaline phosphatase, acid phosphatase, and protease. However, only a few of the strains generated carbohydrate degradation enzymes. In the investigation of enzymes produced by six strains of three thraustochytrid genera, *Thraustochytrium*, *Schizochytrium* and *Aurantiochytrium*, Taoka et al. (2009) detected five to eight kinds of extracellular enzymes depending on the species, but no cellulase from any of the strains. All the strains formed protease, lipase, urease, phosphatase, and α-glucosidase, however, only genus *Thraustochytrium* secreted amylase. These researchers also found that chitinase was detected only in *T. striatum*, and enzymes of genus *Aurantiochytrium* did not contain gelatinase activity. More recently, Nagano et al. (2011) observed carboxymethyl cellulase (CMCase) produced by fourteen out of the nineteen strains of eight thraustochytrid genera examined except for genus *Aurantiochytrium*. Kanchana et al. (2011) attempted to optimize the production of alkaline lipase from two strains of *Thraustochytrium* and indicated that lipase was substrate-inducible enzyme because the presence of glucose in the medium had strong inhibition on enzyme production, while olive oil enhanced lipase production significantly. Other conditions, such as time, temperature, pH, salinity, and concentration of nitrogen source also had significantly effects on lipase production. The optimum conditions [i.e., T = 30 °C, pH = 6.0, t = 168 h, and salinity = 3.4% (w/v)] resulted in a three-fold increase of lipase production compared to the standard conditions [T = 4 °C, pH = 4.0, t = 24 h, and salinity = 1% (w/v)] (Kanchana et al. 2011). Devasia and Muraleedharan (2012) demonstrated that twelve isolates of *Thraustochytrium* can produce a variety of polysaccharide-degrading enzymes such as amylase, xylanase, cellulase, pectinase, and agarase. The production of such enzymes was observed in the presence of different carbon sources, which reflected that the enzyme production by these marine protist isolates appeared to be constitutive (Devasia and Muraleedharan, 2012).

Although thraustochytrid protists exhibited potentials to be promising enzyme producers, very limited research has been done to comprehensively study enzyme production by thraustochytrids compared to bacteria and fungi. Based on previous research, the variety and activity of thraustochytrid enzymes appear to vary significantly among different genus and species. Most studies on the extracellular enzymes of thraustochytrids were focused on qualitative identification. The kinetics of enzyme production from thraustochytrids is also lacking, and the effects of operation conditions and carbon/nitrogen concentration on the enzyme production were understudied. The polysaccharide substrate-induced production of specific enzymes received little research. In addition, thraustochytrid protists were found to produce a large amount of extracellular polymeric substances (EPS) which could play an important role of energy/carbon sinks to be degraded by extracellular enzymes to support cell growth and also provide extracellular matrix where enzymes interact with substrates (Jain et al. 2005; Lee et al. 2014; Xiao and Zheng, 2016). A thraustochytrid strain of interest in the present study is *T. striatum* ATCC 24473 that was often found to thrive on dead autochthonous, allochthonous and plant material such as macroalgae and submerged mangrove leaves, which suggests that *T. striatum* ATCC 24473 plays an important role as saprobes by virtue of extracellular enzymes to degrade detritus (Raghukumar, 2002). It was reported to be a superior enzyme producer which produced the most kinds of enzymes, such as polysaccharases, lipase, proteases, urease, and others (Taoka et al. 2009). Such a wide spectrum of enzymes suggests that *T. striatum* be able to utilize various carbon (e.g., lignocellulosic biomass) and nitrogen sources to produce enzymes of broad applications, e.g., biofuels and bioproducts. However, *T. striatum* did not attain much research attention on its enzyme production.

T. striatum could be a new potential enzyme producer for the production of hydrolytic enzymes (e.g., high salinity tolerance enzymes) for lignocellulosic biomass degradation. Thus, we were interested in studying enzymes produced by *T. striatum*. The objectives of this study are to: (1) qualitatively identify seven different extracellular enzymes including lipase and six polysaccharases (i.e., amylase, CMCase, xylanase, chitinase, pectinase, and κ-carrageenase); (2) study the kinetics of all seven enzymes under different concentrations of glucose, nitrogen and salinity; (3) attempt to correlate enzyme production with EPS and their monosaccharide components; and (4) examine temporal enzyme production induced by polysaccharide substrates, including starch, car-