Protist, Vol. 169, 451–465, xx 2018
http://www.elsevier.de/protis
Published online date 4 June 2018

Protist

ORIGINAL PAPER

Comprehensive Study of Cultivation Conditions and Methods on Lipid Accumulation of a Marine Protist, *Thraustochytrium striatum*

Rui Xiao, Xiang Li, and Yi Zheng[1]

Department of Environmental Engineering and Earth Sciences, Clemson University, 342 Computer Court, Anderson, SC 29625, USA

Submitted March 8, 2018; Accepted May 24, 2018
Monitoring Editor: Saul Purton

This research studied the influences of cultivation conditions (carbon/nitrogen source concentration, initial pH, salinity, and rotation speed) on cell growth and fatty acid (FA) production/composition of a marine protist, *Thraustochytrium striatum*. Fed-batch was also studied to improve cell growth and FA production. The optimum cell growth (∼5 g/L dry cell mass, DCM) occurred under the cultivation conditions of T = 25 °C, glucose = 30 g/L, yeast extract/peptone (YEP) = 4 g/L, salinity = 100% of seawater, pH = 6-7, and rotation speed = 120 rpm. Starch/glycerol and yeast extract were the best carbon and nitrogen sources, respectively for achieving the maximum cell growth. Low carbon/nitrogen (C/N) ratio benefited cell growth while high C/N ratio was conducive to FA accumulation. The maximum lipid content of 25% (g/g DCM) was obtained at glucose/YEP of 30/1 (w/w). Starch and ammonia chloride were suggested to be used as carbon and nitrogen sources. Compared to batch, fed-batch increased FA content significantly from 27 to 38%, primarily including 35% of C16:0, 42% of C18:1, 9% of C18:2 and 5% of EPA/DHA. The major FAs of *T. striatum* were palmitic, stearic, oleic, and linoleic acids along with a small amount of docosahexaenoic and eicosapentaenoic acids, which suggests lipid from *T. striatum* be suitable for biodiesel production.

Key words: Lipid; polyunsaturated fatty acid; extracellular enzyme; marine protist; fed-batch; *Thraustochytrium striatum*.

[1]Corresponding author; fax +1 864 656 1041
e-mail zheng9@clemson.edu (Y. Zheng).

Abbreviations: ASW, artificial sea water; DCM, dry cell mass; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; ARA, arachidonic acid; FAME, fatty acid methyl ester; LC-PUFA, long chain polyunsaturated fatty acid; DDI, deionized distilled water; MSG, monosodium glutamate; TFA, total fatty acid; YE, yeast extract; YEP, yeast extract and peptone.

https://doi.org/10.1016/j.protis.2018.05.005

Introduction

Thraustochytrids, marine heterotrophic protists, have recently received a great deal of research as oleaginous microorganisms for lipid production especially for long chain polyunsaturated fatty acids (LC-PUFAs) such as docosahexaenoic acid (DHA, C22:6n-3) and eicosapentaenoic acid (C20:5n-3, EPA). DHA and EPA can be used as pharmaceuticals and nutraceuticals as well as additives for aquaculture (Spolaore et al. 2006). In addition to LC-PUFAs, thraustochytrid lipid also contains a large quantity of monounsaturated (e.g., palmitoleic and oleic acids) and saturated fatty acids (e.g., palmitic acid), which make thraustochytrids exceptionally promising lipid producers for biofuel production (Gouveia and Oliveira 2009).

The most studied lipid-producing thraustochytrid species belong to the genera *Schizochytrium* (known as *Aurantiochytrium* after 2007), *Ulkenia* and *Thraustochytrium* (Raghukumar 2002). Of these protists, *Schizochytrium* and *Ulkenia* have been developed as alternative commercial sources of DHA for infant formula, food and/or feed (Gunstone 2006). Depending on the species and strain, *Schizochytrium* cell mass concentration can be very high, reaching up to 140 g/L (Chi et al. 2009). Major fatty acids of *Schizochytrium* included palmitic acid and DHA (~50% of total fatty acids, TFAs) (Chi et al. 2007; Ganuza and Izquierdo 2007; Liang et al. 2010; Ryu et al. 2013). The highest reported DHA concentration was produced by *Aurantiochytrium* sp. SD116, reaching 17.4 g/L (35% of TFAs) which was accompanied by palmitic acid (26% of TFAs) (Gao et al. 2013). Compared to *Schizochytrium*, lipids from genus *Ulkenia* can also contain up to 50% DHA (FDA 2010) with similar fatty acid composition (mainly in palmitic acid and DHA), but their cell mass concentration is lower in general. For instance, a native thraustochytrid strain with high similarity to *Ulkenia* sp. showed DHA and palmitic acid levels of 46% and 30% in TFAs, similar to the ones reported for *Ulkenia* sp. at industrial scale (Kiy et al. 2005; Quilodrán et al. 2010). *Ulkenia* sp. and *Schizochytrium* sp. have been found to grow much faster and achieve higher cell mass than *Thraustochytrium* sp. (Fan and Chen 2007), which may be the reason underlying the apparent preferential utilization of the former two in industrial LC-PUFA production (Martins et al. 2013). The previously studied *Thraustochytrium* strains mainly include *T. arueum*, *T. roseum*, *T. striatum*, and *Thraustochytrium* sp. ONC-T18 (Shene et al. 2013). Typically, the cell mass concentration of *Thraustochytrium* strains is less than 10 g/L (Anbu et al. 2007; Bajpai et al. 1991; Iida et al. 1996; Jeh et al. 2008; Li and Ward 1994; Taoka et al. 2011) except for *Thraustochytrium* sp. ONC-T18 that showed the best performance with superior cell mass up to 28 g/L and DHA content of 31.4% of TFA (i.e., 4.6 g/L) (Burja et al. 2006, 2007) The common fatty acids produced by these strains include palmitic acid, stearic acid, oleic acid, EPA, docosapentaenoic acid (DPA), and DHA (Anbu et al. 2007; Bajpai et al. 1991; Burja et al. 2006, 2007; Iida et al. 1996; Jeh et al. 2008; Li and Ward 1994; Shene et al. 2013; Taoka et al. 2011).

Of *Thraustochytrium* strains, *T. striatum* gained much less research attention than other strains due to their lower yield of DHA and EPA. Li and Ward (1994) reported that *T. striatum* ATCC 24473 was able to produce 34% (of TFAs) of palmitic acid and 48% of oleic acid but only 0.75% of DHA (22:6). This high yield of palmitic and oleic acids indicates the potential of *T. striatum* for use in biodiesel production. Our recent research on *T. striatum* ATCC 24473 found this strain is highly versatile that it can use a broad range of carbon sources including monomeric 5C/6C sugars, glycerol, organic acids, and various polysaccharides under heterotrophic condition, which could be ascribed to its capability of producing diverse extracellular enzymes. The most attractive fact is that this strain can metabolize aromatic compounds as sole carbon and energy sources to accumulate lipids and pigments under certain stress conditions, which implies the potential of this strain for lignocellulosic biomass degradation for biorefinery applications and bioremediation for pollution control. *T. striatum* can also use a variety of nitrogen sources alternative to yeast extract and peptone, including urea, ammonia chloride, nitrate, etc. with ammonia chloride the best for lipid accumulation. From the product point of view, this strain can produce not only lipid, but also protein, carbohydrate and carotenoids as well as bioactive extracellular cellular compounds (Xiao et al. 2018).

Therefore, it is noteworthy to conduct further research on *T. striatum* to explore its potential applications. One of focuses of this research was to examine the influence of critical cultivation conditions on cell growth and lipid production, including the source and concentration of carbon and nitrogen, temperature, salinity, pH, and rotation speed (Shene et al. 2013). Except for cultivation conditions, the cultivation method can also affect microbial cell growth and product yield. For instance, fed-batch operation has been used to enhance cell growth and product yield (Cheirsilp