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Isolation and molecular characterization of *Thraustochytrium* strain isolated from Antarctic Peninsula and its biotechnological potential in the production of fatty acids

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ABSTRACT

Thraustochytrids are unicellular protists belonging to the Labyrinthulomycetes class, which are characterized by the presence of a high lipid content that could replace conventional fatty acids. They show a wide geographic distribution, however their diversity in the Antarctic Region is rather scarce. The analysis based on the complete sequence of 18S rRNA gene showed that strain 34-2 belongs to the species *Thraustochytrium kinnei*, with 99% identity. The total lipid profile shows a wide range of saturated fatty acids with abundance of palmitic acid (16:0), showing a range of 16.1–19.7%. On the other hand, long-chain polyunsaturated fatty acids, mainly docosahexaenoic acid and eicosapentaenoic acid are present in a range of 24–48% and 6.1–9.3%, respectively. All factors analyzed in cells (biomass, carbon consumption and lipid content) changed with variations of culture temperature (10 °C and 25 °C). The growth in glucose at a temperature of 10 °C presented the most favorable conditions to produce omega-3fatty acid. This research provides the identification and characterization of a *Thraustochytrids* strain, with a total lipid content that presents potential applications in the production of nutritional supplements and as well biofuels.

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Introduction

The microorganisms belonging to the Labyrinthulomycetes class and family *Thraustochytriaceae* are unicellular protists which are present in marine ecosystems. They have a key role on the initial stage of the microbial chain food, as organic matter degraders.[1,2] These microorganisms have been studied at the morphological, ecological and biotechnological levels.[3,4] Due to the presence of a high lipid content that could replace conventional sources of fatty acids; the *Thraustochytriaceae* family is of great interest.[5–8] Particularly, the most studied metabolites are docosahexaenoic acid (C22:6, DHA) and eicosapentaenoic acid (C20:5, EPA).[9,10] These essential biomolecules of the omega-3 family are involved in the physiological development of children and adults, cholesterol regulation, prostaglandin, thromboxane and leukotriene biosynthesis. Furthermore, they have a preventive role on different pathologies such as arteriosclerosis, asthma, thrombosis, arthritis and a wide range of tumors.[11–13] Besides, a variety of biomolecules with unknown functions that could have a potential biotechnological application, such as extracellular polysaccharides, carotenoids, squalene, enzymes, osmolytes, unsaturated and saturated fatty acids could be used as biofuel.[14] Taxonomic classification of the *Thraustochytriaceae* family comprises the genera *Aplanochytrium*, *Ulkenia*, *Thraustochytrium*, *Japonochytrium*, *Aurantiochytrium* (also known as *Schizochytrium*), *Botryochytrium*, *Parietichytrium* and *Sicyoidochytrium*.[15,16] Their geographic distribution includes the North Sea, India, Indonesia, Japan, Australia, South America and the Antarctic Continent.[17,18] The latter place presents poor studies of the marine microbial diversity and the number of species varies widely between taxons.[19–21] Currently, in the diversity studies of the *Thraustochytrids* from Antarctic waters two species have been described: *Thraustochytrium antarticum* (Southeastern Indian Ocean) and *Thraustochytrium rossii* (Southwestern Pacific Ocean).[23] More recently, only one microorganism has been characterized by molecular phylogeny; *Aplanochytrium stocchinoi* (Terra Nova Bay).[23]

Microorganisms have been used as alternative fatty acids source in bacteria, fungi, yeasts and microalgae.[24–26] Protist species such as *Crythecodiniumcohnii*, *Schizochytrium* sp. and *Ulkenia* sp. have been characterized by their rapid growth, high photosynthetic activity and high production of biomass.[27–29] In *vitro* culture of these microorganisms with high carbon/nitrogen ratio favors lipids accumulation and a decrease in cell development.[29] However, this is not clearly established, since in *Schizochytrium* strains, the fatty acids increase is associated with biomass growth.[30] Besides, *in vitro* studies with carbon and nitrogen sources could favor the biomass production as lipid contents, because these molecules are linked to the development of a biological model successful on fatty acids production.[18,29]

This study provides a biological and biotecnological focus, using *in vitro* studies of a strain belonging to the *Thraustochytriaceae* family isolated from the Antarctic coast of King George Island. The objective of this research is based on the morphology and taxonomic classification (18S rRNA gene) studies of the culture parameters such as the carbon source and the temperature on biomass production with high content of essential fatty acids. This research offers new information about the biodiversity of Labyrinthulomycetes class in the Antarctic continent and their potential application as a biotechnological tool on LC-PUFAs or biofuels production.

Materials and methods

Isolation and culture conditions

Two samples were collected on the coast of King George Island, specifically on coordinates S 62° 12′ 34.8″ W 58° 55′ 34.3″ and the microorganisms were isolated from the water column (2.4 °C and pH 8.1). Thraustochytrids were obtained using the pine polen method.[31] Then, they were incubated at different temperatures (10 °C and 25 °C) and observed using an optic microscope during 10 days to improve microorganism fixation. Inocules were cultivated in liquid medium using Honda et al. (1998) modified protocol (0.2% yeast extract and 0.2% sodium glutamate) in artificial seawater with 5% glucose or 5% starch, at the same temperature conditions during 8 days.[32] Finally, the samples were lyophilized, centrifuged and stored at −20 °C for posterior analysis. Morphological analysis was performed with an Olympus CX21 light microscope (Tokyo, Japan).

DNA extraction and amplification of the 18S rRNA gene

DNA extraction was performed using Mo and Rinkevich[33] modified protocol. Cultures were centrifuged at 14,000 rpm for 3 min, supernatant was discarded, and buffer extraction was added on pellet (0.2 M Tris–HCl pH 8.0, 1.4 M NaCl, 0.1 M EDTA and 1.5% SDS) and sonicated for 30 s. Then, the solution was centrifuged at 12,000 rpm and the supernatant was transferred to a new tube to obtain the DNA using the phenol:chloroform solution (5:1, pH 4.8). The mixture was centrifuged at 13,000 rpm for 5 min. The aquose phase was precipitated with ethanol at 4 °C overnight. Finally, the resulting pellet was suspended on nuclease free water and quantified in Infinity200 Pro NanoQuant (TECAN) and stored at −20 °C.

PCR amplification of 18S gene was carried out using comercial kit GoTaq® Green Master Mix (Reaction Buffer pH 8.5, 400 μM of dNTPs Promega and 3 mM $MgCl_2$), and Genomic DNA (with an average ratio 260/280 of 1.6) and 1 mM of specific primers (FA1 5′-AAAGATTAAGCCATGCATGT-3′, RA1 5′-AGCTTTTTAACTGCAACAAC-3′; FA2 5′-GTCTGGTGCCAGCAGCCGCG-3′, RA2 5′-CCCGTGTTGAGTCAAATTAAG-3′; FA3 5′-CTTAAAGGAATTGACGGAAG-3′ and RA3 5′-CAATCGGTAGGTGCGACGGGCGG-3′).[34] Thermocycling profile was performed with an initial denaturation step of 3 min a 95 °C, 35 cycles of amplification (1 min 94 °C, 1 min 53 °C and 1 min 72 °C), and final elongation at 72 °C of 10 min. The PCR fragments were visualized on 1.2% agarose gel using 10 μg/mL of ethidium bromide.

Sequencing and molecular phylogenetic analysis

The 18S rRNA fragments were amplified and then sequenced using automated DNA sequencer ABI-Prism (Pontifical Catholic University of Chile, Chile). For identification, a search

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