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Optimization of culture conditions for growth of the *Aurantiochytrium* sp. shy, isolated from the Persian Gulf

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Abstract

In this study, a native microalgae strain with a high capability of DHA production has been isolated and purified from mangrove shores in the Persian Gulf. Morphological and molecular identification based on 18S rRNA gene showed that the isolated strain belongs to *Aurantiochytrium* genus. In order to optimize the growth of this strain, various environmental factors were examined, including temperature (15, 20, 25, 30 and 35°C), salinity (10, 30 and 50 g L^{-1}) and different concentrations of nitrogen sources of yeast extract, peptone, meat extract and monosodium glutamate, each of which was tested at the concentration of 10 g L^{-1}. The findings show that the optimal growth conditions occur at temperatures of 25-30 °C, pH equal to 6 and in the presence of meat extract as the nitrogen source. In these conditions, the cellular biomass weight, fatty acids and DHA amounts obtained were 7.34, 2.25 and 0.4 g L^{-1}, respectively. These results imply that the new isolated strain of *Aurantiochytrium* sp. shy it can be suitable for lipid production.

Keywords: Isolation, *Aurantiochytrium* sp., Optimization, Biomass, Microalgae, Heterotrophic.

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Introduction

The Docosahexaenoic Acid (DHA) is an essential fatty acid for the human body, especially for the brain and eyes, in that their role in the evolution of the nervous system and vision during prenatal, infancy and even adulthood periods is approved. These fatty acids also play an effective role in preventing cardiovascular diseases (Yang *et al*., 2010). Several researches proved that the daily use of DHA prevents diseases such as asthma, increases blood pressure, heart attacks and some cancers (Jakobsen *et al*., 2008). So far, the main commercial resource of DHA has been fish oil, however, pollution of water resources and marine ecosystems, the presence of heavy metals and the high costs of purification operations, as well as the bad taste it has for some consumers have decreased its intake (Li *et al*., 2008). Accordingly, researchers seek alternate resources for Omega 3 fatty acids (DHA). Oil producer microorganisms like microalgae, fungi, yeast and bacteria are some of these resources. These microorganisms produce biocompounds with various applications, including biofuel, proteins, enzymes, pigments and exopolysaccharides, besides fatty acids under appropriate environmental conditions. Since the lifecycle of these microorganisms is short, we can easily increase production by controlling the optimal environmental conditions in a short period of time (Li *et al*., 2008). The oil produced by unicellular beings Single Cell Oil (SCO) does not have the issues of fish oil and is produced in controlled environments (Qu *et al*., 2011). Marine microalgae such as *Aurantiochytrium* (Yokoyama and Honda, 2007) and *Crypthecodinium cohnii* are good producers of human grade DHA, due to the lack of toxins such as domoic acid and prymnesin (Ratledge, 2003). These two strains are capable of storing 50-80% of fat on dry cellular weight basis, which a major part of it being DHA (Jakobsen *et al*., 2008).

Oil analysis of this component shows that it can produce 178.32 mg L^{-1} of saturated fatty acid and 277.48 mg of unsaturated fatty acid with several double bonds of DHA, Eicosapentaenoic Acid (EPA) and Docosapentaenoic Acid (DPA) types for each gram of produced oil (Fekrat and Shakeri, 2015). The *Aurantiochytrium* microalgae oil contains 46% Palmitic acid fatty acids, which is suitable for biofuel production (Shahryar, 2015) as it has a high cetane number, high stability and low iodine content. Several researches have been conducted aiming to increase the fat content and biomass, one of them being the culture with fed batch method, with two carbohydrate sources of glucose and acetic acid, for two strains of *Aurantiochytrium* sp. and *C. cohnii* (De Swaaf *et al*., 2003; Nagano *et al*., 2009). Researchers have focused on the *Aurantiochytrium* sp. during recent years, and since an optimal culture medium for this species has not been approved, there is a need for further studies in order to achieve the highest amount of biomass and DHA production (Hong *et al*., 2011b). As the new isolated strain from the Persian

Gulf is native, we have started a survey to understand their physiology and growth conditions. This study aimed to identify, purify and investigate the effects of culture medium conditions (pH, salinity, temperature and nitrogen source type) on the amount of the produced biomass, and then measure the cellular bulk and amount of fat and fatty acids in optimal conditions.

Materials and methods

Chemicals

The chemicals, including meat extract, monosodium glutamate, yeast extract, peptone, trypton, $MgSO_4.7H_2O$, $KHPO_4$, $NaHCO_3$, $MnCl_2.4H_2O$, $FeCl_3.6H_2O$, $ZnSO_4.7H_2O$, $CoSO_4.5H_2O$, $CuSO_4.6H_2O$ and agar were purchased from the Merck Company, thiamin hydrochloride, biotin and cyanocobalamin (B12) vitamins from Applichem, sea salt from U.S Kent Co, and glucose from Caspian Co. of Iran.

Preparation, purification and characterization of the strain

Plant leaves of *Avicenia marina* (Forssk) Vierh gathered from the Persian Gulf mangrove shores were transferred to Algae Laboratory, Iranian Biological Resources Center, in sterile plastic containers. The leaves were washed 4-5 times in mangrove sterile water with Penicillin G and streptomycin sulfate at the concentration of 0.25-0.5 mg L^{-1} to suppress bacterial growth (Kamlangdee and Fan, 2003).The washed leaves were cut in 1-1.5 cm pieces and placed in YEP standard solid medium containing yeast extract 1 g L^{-1}, peptone 1 g L^{-1} and agar 10 g L^{-1} at pH equal to 6. The plates were incubated for 24-72h at 25 °C in an incubator. In microscopic observations, *Thraustochytrid* moving cells were visible. Afterwards, the leaves were removed from the plate and the colonies were transferred to a new sterile YEP medium. This was repeated several times to complete the purification process. Upon completion of the purification process, the stock was maintained on YEP agar slants and sub-cultured every 15 days (Fan et al., 2002). The newly isolated strain *Aurantiochytrium* was preserved in 30% (v v^{-1}) glycerol at -70°C for long term preservation (Ranasinghe, 2013).

Culture medium

Two culture media were used as the pre-culture and the main culture media. The pre-culture medium contained sea salt 28 (ppt), yeast extract 1 g L^{-1}, tryptone 1 g L^{-1} and glucose 20 g L^{-1} (Kamlangdee and Fan, 2003; Manikan *et al*., 2015). The culture were incubated at 28 °C at 150 rpm for 24-48 hours and then inoculated to the main culture medium with a volume ratio of 10%. In the main medium, treatments (excluding testing the effects of salt) of 50:50 mangrove water and distilled water were used.

Morphological and molecular identification of isolated

Morphological identification of the *Aurantiochytrium* sp. was performed with Nikon 80i microscope (Japan) and morphological characters were described with valid keys (Ranasinghe,

2013). Primary identification comprised microscopically features, form and color of the colony. The cells were observed for 48 h after inoculation and different cell morphologies were studied in the life cycle of *Aurantiochytrium*.

DNA extraction, PCR and cloning of 18SrRNA

In order to determine the taxonomy of the isolate with molecular tools by using preserved genomic sequence analysis 18S rRNA, 3 steps, including genomic extraction, genomic region amplification and sequence analysis were performed.

Biomass was obtained from fresh culture by centrifugation at 5000g in 5 min. DNA extraction was carried out with modifications based on SDS shock and freeze-thaw method. Molecular identification of this strain was carried out based on 18S rRNA gene. Universal primers for 18S rRNA gene were used; SSU1-478 (5'-GGGACAGTTGGGGGTATTCGTA-3') (Forward) and ITS1DR (5'-CCTTGTTACGACTTGACCTTCC-3') (Reverse). PCR reaction was initiated in the primary denaturation step at 94 °C for 90 seconds and continued for 35 cycles: denaturation step: 94°C for 50 seconds, annealing step at 52°C for 50 seconds, and extension step at 72°C for 50 seconds and a terminal extension was in 72°C for 7 minutes. Quality of products obtained by polymerase chain reaction was investigated on 1% gel Agarose. After completing the reaction, the vials were kept at -20 °C. PCR product ligated to PTZ57R/T vector and plasmid were extracted from transformed colonies with GeneJET Plasmid Miniprep Kit. The sequencing was carried out by Bioneer Company (Korea).

The sequence was first edited with Chomas Pro 1.7.4 and then analyzed with BLASTN in NCBI. Similar sequences (from NCBI/nucleotide) (table1) were aligned and edited with a muscle algorithm (Aliview1.17.1), and a phylogenetic tree was constructed with Mega 7 using the neighbor joining method and 1000 replicates.

Table 1: Strains used in the molecular phylogenetic analyses with sequence accession numbers.

Taxon	Strain	Accession number	References
Aurantiochytrium limacinum	NIBH SR21	AB022107	(Honda *et al*., 1999)
Schizochytrium minutum	KMPB N-BAp-77	AB022108	(Honda *et al*., 1999)
Schizochytrium aggregatum	ATCC 28209	AB022106	(Honda *et al*., 1999)
Thraustochytrium aureum	ATCC 34304	AB022110	(Honda *et al*., 1999)
Thraustochytrium striatum	ATCC 24473	AB022112	(Honda *et al*., 1999)
Aurantiochytrium mangrovei		DQ367049	–
Aurantiochytrium sp.	SEK 217	AB290572	–
Aurantiochytrium sp.	SEK 218	AB290573	(Yokoyama and Honda, 2007)
Aurantiochytrium sp.	SEK 209	AB290574	(Yokoyama and Honda, 2007)
Crypthecodinium cohnii	ATCC 30336	FJ821501	(Yang *et al*., 2010)
Thraustochytrium sp.	ATCC 26185	FJ821482	(Yang *et al*., 2010)
Thraustochytrium multirudimentale	KMPB N-BA-113	AB022111	(Honda *et al*., 1999)
Oblongichytrium sp.	SEK 347	AB290575	–
Japonochytrium sp.	ATCC 28207	AB022104	(Honda *et al*., 1999)
Thraustochytrium aggregatum	KMPB N-BA-110	AB022109	(Honda *et al*., 1999)
Aurantiochytrium sp.	15A-8a	AB810998	
Aurantiochytrium sp.	SK4	FJ560900	-
Aurantiochytrium sp.	18W-5a	AB811024	(Nakazawa *et al*., 2014)

Table 1 continued:

Aurantiochytrium sp.	15A-11a	AB811006	(Nakazawa *et al*., 2014)
Aurantiochytrium sp.	15A-8a	AB811004	(Nakazawa *et al*., 2014)
Aurantiochytrium sp.	TF49	KM023693	–
Aurantiochytrium sp.	TF94	KM023705	–
Aurantiochytrium sp.	TF59	KM023711	–
Aurantiochytrium sp.	15A-10a	AB811005	(Nakazawa *et al*., 2014)
Aurantiochytrium sp.	TA4	KJ938302	(Shahryar, 2015)
Aurantiochytrium sp.	SHY	KY677759	This study
Thraustochytrium sp.	DBTIOC-14	KF668629	–
Thraustochytrium kinneii	NIOVT12-DPSgF10	KF460465	–
Aurantiochytrium limacinum.		DQ023611	–

Determination of dry cell weight (DCW)

To determine the dry weight of biomass, 10 ml culture medium was centrifuged (SIGMA UK-16) at 7000 rpm for 5 minutes and wet biomass was dried in the oven at 60 °C for 12 hours to reach a constant weight. The dry cell weight is expressed as triad mean per liter of culture medium. Some part of the wet biomass was freeze dried at -55°C (Operon apparatus- South Korea machine) for long-term conservation, prevention of pollution, enzymatic and microbial decay and GC analysis.

Lipid staining

0.3 g Sudan Black (solution with 70% ethanol) was added to the biomass for 15 minutes and then washed. Afterward safranin was added for 30 seconds. Slides were assessed with an optical microscope with a magnification of 100 and imaging was performed (Manikan *et al*., 2015).

Lipid extraction and analysis of fatty acids

Lipid extraction was performed according to the Bligh & Dyer method using the solvent chloroform-methanol. Extracted material in the first evaporation was placed in the oven at 105°C for an hour to reach a constant weight. To determine the composition of fatty acids in the obtained lipid with gas chromatography, it is necessary to convert fatty acids to methyl esters of fatty acids (FAMEs). In this study, the quick trans-methylation method in alkaline conditions was used for conversion. So, 0.1 g microalgae oil with 2 mL isooctane and 0.1 mL methanol-potassium hydroxide 2^{M} solution were added to a test tube and stirred for one minute. After precipitating glycerol and becoming a transparent mixture, 2 mL sodium chloride solution 28wt% was added to the remaining mixture. Isooctane was separated and 1g sodium hydrogen sulfate was added and stirred. This mixture was injected into the GC (Agilent 6890- USA) in order to analyze the profile of fatty acid. The device was equipped with a Capillary Column 100 m, 0.25 μm, 0.2 μm. Column temperature increases from 110 °C to 230 °C at a rate of 4 °C min^{-1} and is maintained constant at 230 °C for 10 minutes. Injector and detector temperatures were set to 280 °C and 320 °C, respectively. Hydrogen was used as a carrier gas (Bligh and Dyer, 1959).

Culture medium optimization

The microalgae *Aurantiochytrium* sp. shy was tested at different temperatures (15, 20, 25, 30 and 35°C), pH (6, 7, 8), salinity (10, 30 and 50 ppt) using different levels of nitrogen source including yeast extract, peptone, meat extract and monosodium glutamate, each at a concentration of 10 g L^{-1} in order to study the effects of various factors on biomass content. All experiments were performed in a Wised shaker incubator (South Korea) and shakers in all the experiments were set at 170 rpm.

Results

Isolation and purification

24 hours after culturing mangrove leaves in the YEP medium, clearly a discoloration was observed in the marginal parts of the leaves. In the microscopic investigation of the marginal parts of the leaves, abundant moving cells (zoospore) aggregated and formed colonies and many branched fungi were observed. Using two antibiotics, penicillin G and streptomycin sulfate, almost inhibited the bacterial activities. Considering the rapid growth of fungi, isolating process of *Aurantiochytrium* colony was faster, therefore by accelerating the process of isolation and repeated relocation of *Aurantiochytrium* sp. shy colony to new YEP plates, pure and fresh cultures were obtained eventually. In order to accurately assess the cells, they were cultured in the solid medium and in liquid medium.

Morphological description

The spherical vegetative cells of *Auranthiochytrium* strain shy with two flagellates and 3 to 7µm diameter were observed under a microscope. The triad and tetrad forms of cells were seen due to binary division.

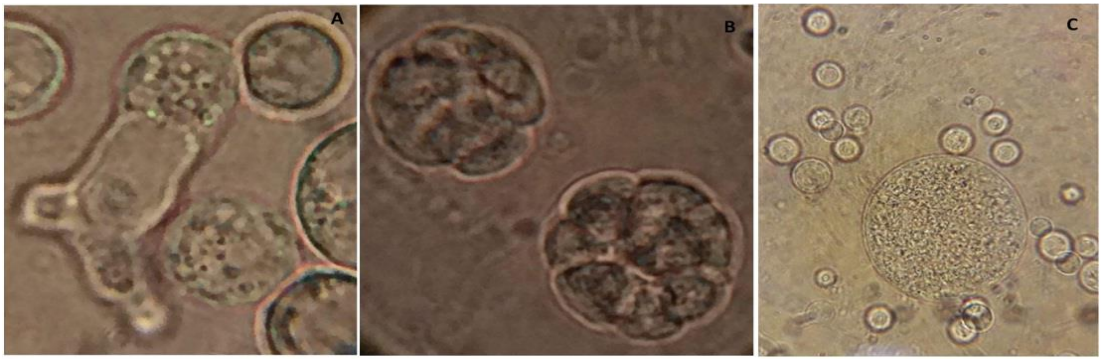

Figure 1: The morphological picture of *Auranthiochytrium* strain shy: a, The amoeboid cells, b, The cell cluster, c, The zoospore.

The globose or ovoid zoospores lost their flagellum and developed into vegetative cells. The vegetative cells shifted to cell clusters or zoosporangium. The cell clusters changed into amoeboid cells and the zoosporangium released zoospore and the cycle repeated. In contrast to *Thraustochytrids*, no ectoplastic nets were seen. The size and shape of the zoosporangium is an important feature

in the morphological identification of the genus (Fig.1).

Fresh and pure colonies were shiny and orange in color. In the broth of YEP, these colonies were observed in a clump, mass and white state, but in the YEP solid medium they were transparent, however, in solid medium containing (50% to 50% distilled water and mangroves water, 20 g L^{-1} glucose, 5 g L^{-1} yeast extract, 10 g L^{-1} tryptone and 13 g L^{-1} agar they were in orange color with a transparent glaze.

Molecular identification of Aurantiochytrium sp.

Morphological and ecological characters showed that strain SHY is near to photosynthetic fungi-like microorganisms, with similar traits to *Schizochytrium* genus. This genus was separated from *Schizochytrium* in 2007 (Yokoyama and Honda, 2007). BLAST analysis showed this strain has high similarity (97 bootstrap) to *Aurantiochytrium* sp. TA4. The *Aurantiochytrium* has differentiated in the cladogram and separated from other genera. The *Aurantiochytrium* isolated was inserted to this big clad beside the *Aurantiochytrium* sp. TA4. As depicted in the phylogenetic tree (Fig. 2) the strains belonged to the *Aurantiochytrium* clade with high bootstrap value (100), and with 91% value near to *Schizochytrium aggregatum*.

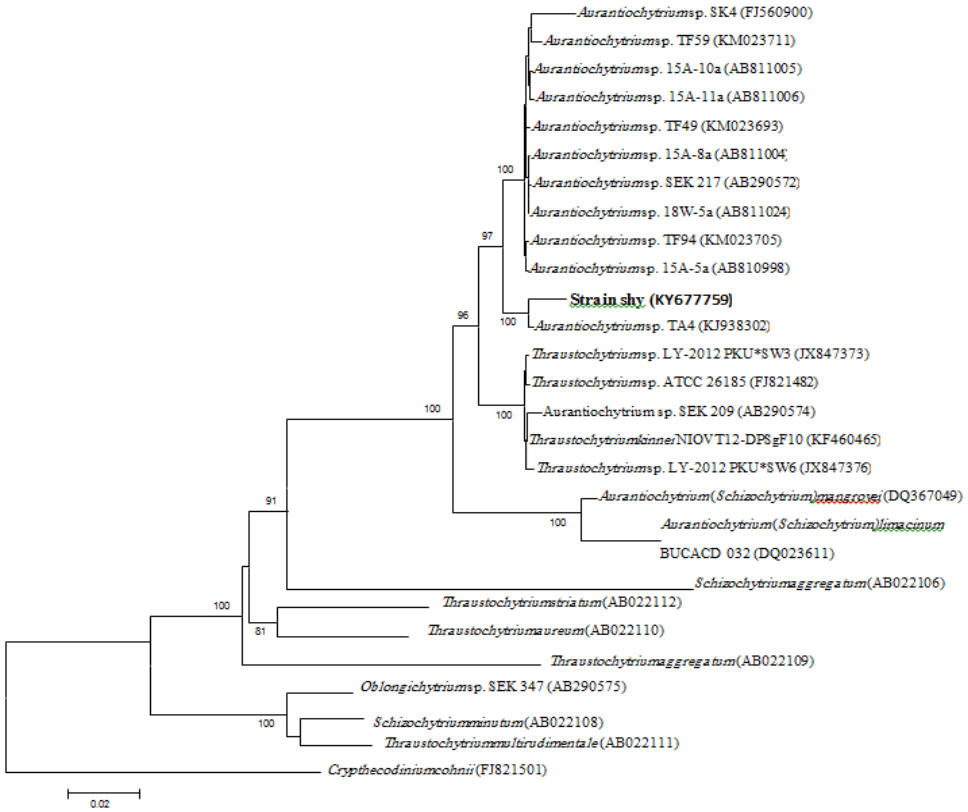

Figure 2: Molecular identification and phylogenetic tree.

pH optimization

The effect of different pH on the produced biomass is shown in Fig. 3. The highest amount of biomass equal to 6.77 g L^{-1} was observed at pH equal to 6. By increasing the pH, the amount of biomass production decreases.

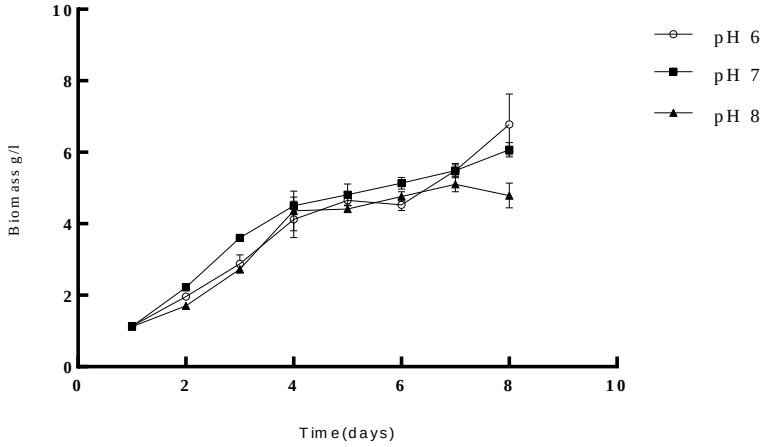

Figure 3: The effect of different pH on the biomass formation of *Aurantiochytrium* sp. Shy during heterotrophy cultivation.

Optimization of nitrogen sources

The effect of nitrogen source on biomass production is shown in Fig.4. The greatest amount of biomass produced in the presence of meat extract as a nitrogen source was 7 g L^{-1}. No significant difference between the results of pepton and yeast extract was observed. MSG had minimum biomass in comparison with pepton, meat extract and yeast extract and growth was poor.

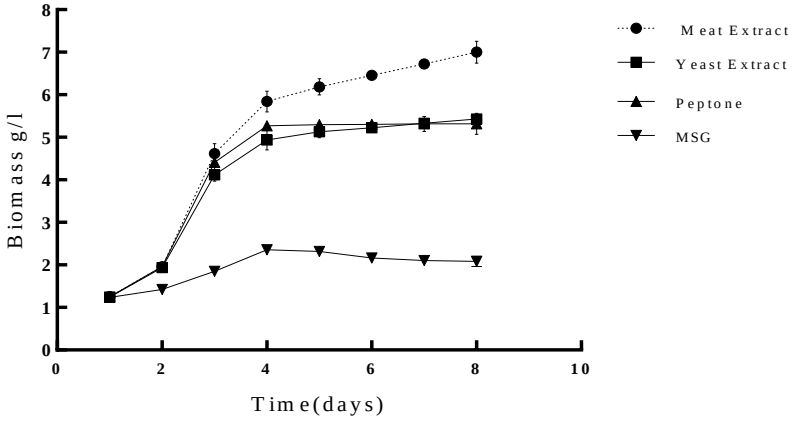

Figure 4: Effect of different nitrogen sources on the biomass formation of *Aurantiochytrium* sp. shy during heterotrophy cultivation.

Temperature optimization

To evaluate the effect of temperature on the amount of biomass produced by microalgae *Aurantiochytrium* sp. shy, tests were carried out at different temperatures of 10, 15, 20, 25, 30 and 35 °C. The results are shown in Fig. 5. The highest amount of dry biomass (DCW) was observed at 25°C at 8.01 g L^{-1}. After 5 days at the temperature of

10°C, no cell growth was observed (data not shown). At 15°C cell growth was weak and its lag phase was long. Increasing the temperature from 15 °C to 25 °C led to a 1.38 times increase in the amount of biomass and a change in temperature of 25°C to 35°C led to a 1.59 times decrease in the amount of biomass (Fig. 5).

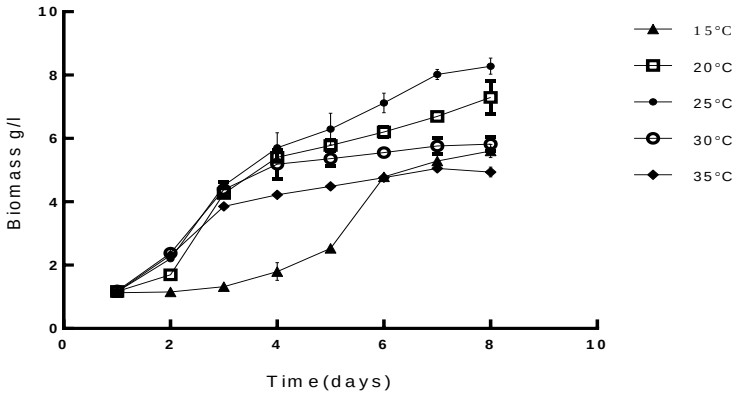

Figure 5: Effect of different temperatures on biomass formation of *Aurantiochytrium* sp.shy during heterotrophy cultivation.

Salinity optimization

To evaluate the effect of salinity on the biomass production, experiments were carried out in three different concentrations of 10, 30 and 50 ppt. The results are shown in Fig. 6. The highest biomass of 6.61 g L^{-1} was obtained in the salinity of 50 ppt. There is no significant difference between the results in the range of 10 to 50 ppt.

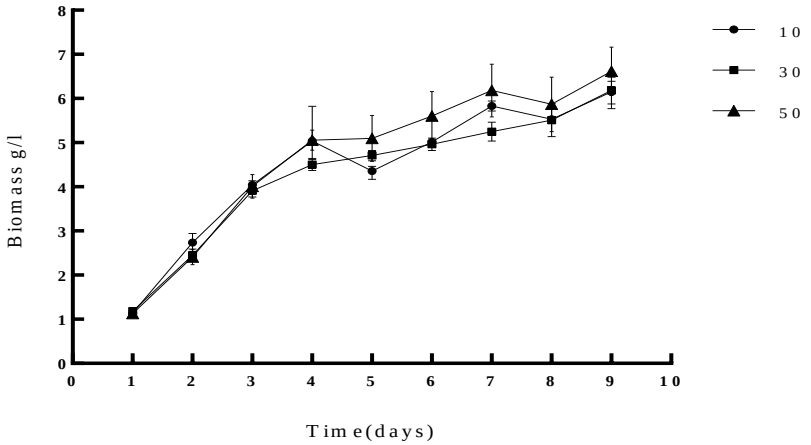

Figure 6: Effect of salinity on the amount on biomass formation of *Aurantiochytrium* sp. shy during heterotrophy cultivation.

Cultivation of the new strain Aurantiochytrium sp. shy *in optimal condition*

After optimization of environmental parameters the new strain *Aurantiochytrium* sp. shy was cultivated in optimal conditions

(temperature 25-30°C, pH equal to 6, meat extract and sea salt 50 ppt).

The biomass, total lipid and DHA concentration were assessed. In this case, the highest biomass and lipids obtained were 7.34 and 2.25 g L^{-1} respectively. In this condition, the amount of DHA was 400 mg L^{-1}. Fig. 7 shows the microalgae growth curve in the optimal conditions of the culture medium.

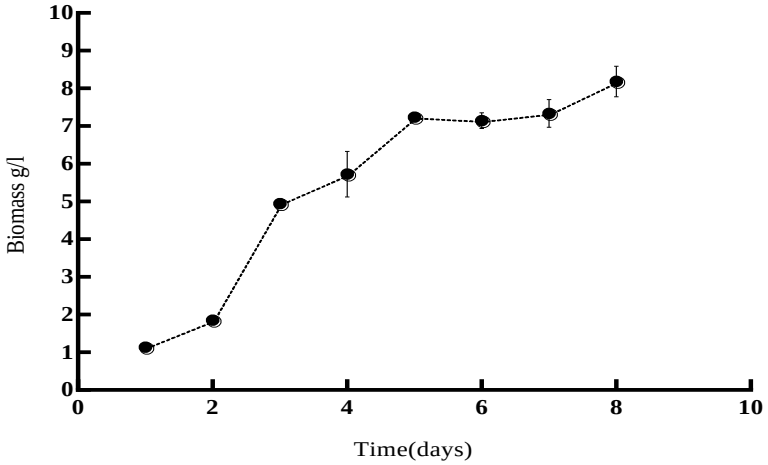

Figure 7: *Aurantiochytrium* sp.shy microalgae growth curve in optimal heterotrophy cultivation.

Discussion

Auranthiochytrium sp. belongs to the *Thraustochytrids* or *Thraustochytriacea* family from Chromista kingdom with traits similar to microalgae and fungi (Burja *et al*., 2006). The *Schizochytrium* genus with high DHA potential production is situated in this family beside *Auranthiochytrium*. Kamlangdee and Fan (2003) reported the amount of biomass of five-strains 1N-, 2N-, 5N-, 6N- and 9N- of *Schizochytrium* was the best at pH 6 (Kamlangdee and Fan, 2003). Mang Gao (2013) investigated the effect of pH on the production of biomass from microalgae *Aurantiochytrium* sp. SD116 and reported the highest amount of biomass at pH 7 (Gao *et al*., 2013). The initial pH value of the culture medium is effective on the metabolism and function of cell membranes such as the absorption of nutrients and bio-synthesis. Experiments show that most strains of microalgae *Aurantiochytrium* have the best performance in slightly acidic to neutral pH for biomass production (Wu *et al*., 2005; Yang *et al*., 2010; Hong *et al*., 2011a; Hong *et al*., 2011b). In the strain *Aurantiochytrium* SD116, research reports revealed that part of the carbon sources at pH less than optimum are used in maintaining the stability of physiological conditions rather than growth and lipid production (Gao *et al*., 2013) and also the amount of glucose consumption decreases in alkaline conditions (Wu *et al*., 2005).

Aurantiochytrium strains commonly contain 14-20% of nitrogen in their

molecular structures, which are mainly in the form of proteins and nucleic acids, therefore the presence of nitrogen sources is essential for the proliferation of these strains (Wen and Chen, 2001).

The presence of nitrogen, causes rapid growth of microorganisms in the early stages (Yokochi *et al*., 1998). Nitrogen is necessary for the synthesis of amino acids. Nitrogen sources are divided into two categories: simple and complex; in the first case, the source of nitrogen is in the form of specific molecules such as ammonia and nitrate (inorganic), urea and monosodium glutamate (organic); but in the second case, in addition to the nitrogen, it contains other materials including yeast extract, meat extract, peptone, tryptone and corn steep liquor (Chen *et al*., 2010).

Simple nitrogen sources are easily used in the early stages of logarithmic growth *Aurantiochytrium* strain (Marchan *et al*., 2017). Nitrogen ions in nitrite salts, before any metabolism within the cell are converted to ammonia. The intracellular ammonia leads to produce monosodium glutamate and after reaction with it, glutamine is produced (Magasanik and Kaiser, 2002). Glutamate and glutamine play a major role in the metabolism of nitrogen and convert to other nitrogen-containing materials. In a test, simple nitrogen sources were used for the culture and growth of *Aurantiochytrium* and the highest biomass content obtained was equal to 9.82 g L^{-1} using the nitrogen source monosodium glutamate (Jakobsen *et al*., 2008; Chen *et al*., 2010). Increasing the concentration of peptone leads to increase the DCW and its maximum value observed in the concentration of 6 g L^{-1} of peptone was equal to of 8.81 g L^{-1} (Chen *et al*., 2010). The complex nitrogen sources in addition to nitrogen, contains a lot of protein, peptide and free amino acids, a small amount of sugar, fat, vitamins and growth factors, therefore, the production of biomass and storage of fat in this type of biomass is more than other ones (Chen *et al*., 2010). After decreasing the concentration of nitrogen sources in the growth medium and increasing C/N aspect, the synthesis of proteins and nucleic acids became limited and carbon sources provided required energy for maintenance and converted into lipids while metabolized (Bellou *et al*., 2016).

Yeast extract has the highest production of biomass when different nitrogen sources are used in *Aurantiochytrium* sp. SD116 culture (Gao *et al*., 2013). Gaining the yeast extract concentration from 0.5 to 3 g L^{-1} leads to increase in the DCW, but when it is raised more, the DCW increases slightly. Since yeast extract is a rich source of trace elements such as adenine and pantothenate and these are cofactors of ATP and CoA which have a good influence on the growth of microalgae *Aurantiochytrium*(Chen *et al*., 2010).

In the *S. Limacinum*, when the temperature was raised from 30 °C to 37 °C, the biomass declined 2.6 times (Zhu *et al*., 2007). *Aurantiochytrium* strains are always inhabited in brackish mangrove areas. In these areas, the daily and seasonal changes in temperature is very high. So *Aurantiochytrium* strains can be adaptable to temperature changes. Some strains such as investigated strains

in this study *Aurantiochytrium* sp.shy and *Aurantiochytrium* sp. SK02 in a temperature range of 15 to 35°C and some others like *Aurantiochytrium* 4w-1bin the range of 10 to 35°C have growth capability and this has been approved by most researchers (Zhu *et al*., 2007). At temperatures above 35 °C not only because of heat stress, but also changes in lipids -which enables microorganisms to live in environmental deficiency- have a negative effect on the metabolites formation (Zhu *et al*., 2007). Temperature is one of the most important factors affecting the culture medium and has a significant impact on the growth and fatty acid compositions in various microorganisms. Taoka *et al*. (2009) examined the effect of temperature on the amount of biomass of microalgae *Aurantiochytrium* sp. mh0186 in the range of 5 to 40°C. The results showed that, in 5 and 40 °C as expected, there was no growth and in the temperature of 10°C growth was weak and in the range of 15 to 30 °C growth was very favorable. The growth in 35 °C was 70% of the average growth in the temperature range of 15 to 30°C (Taoka *et al*., 2009). Gao *et al*. (2013) reported the effect of environmental temperature of the culture of microalgae biomass production *Aurantiochytrium* sp. SD0116 in the temperature range of 20 to 37°C. By varying the temperature from 20 to 28°C, small changes in the DCW were observed and maximum value reported was equal to 29.56 g L^{-1} at the temperature of 28°C, but by changing the temperature to 37 °C, the amount of biomass declined and reached 9.24 g L^{-1} (Gao *et al*., 2013). Also Nakazawa *et al*. (2012) investigated the influence of temperature on the production of microalgae biomass *Aurantiochytrium* sp. 4W-1b in the temperature range of 10 to 35°C. In this temperature range, the strain had sufficient biomass production, and the highest amount observed in the range of 15 to 25 °C was equal to 13 g L^{-1} and the doubling time was 2.1 hours (Nakazawa *et al*., 2012). Also, Chodchoey *et al.* (2012) studied the amount of DCW produced by microalgae *Aurantiochytrium* SK02 within the temperature range of 12 to 35°C. Based on their findings, the amount of DCW in the range of 15 to 30°C were almost similar and the maximum value was observed at 30°C (Chodchoey and Verduyn, 2012). Generally, the microorganism exothermic growth is affected by environmental temperature and appropriate temperature for growth depends on membrane fluidity (Wada *et al*., 1987). The results show that *Aurantiochytriumsp.*shy grows in a wide range of temperatures (15-35 °C) well.

In a study on the effectiveness of salinity on *S. Limacinum* strain, Zhu *et al*. (2007) found that a salinity below 9 g L^{-1} has created a state of stress which leads to a cessation in the metabolites and growth of microorganisms (Zhu *et al*., 2007).

Gao *et al*. (2013) investigated the effect of salinity on the amount of biomass produced by microalgae *Aurantiochytrium* sp. SD116 in the range of 0-60 ppt salinity. They reported a value of 15 ppt as the optimized amount for the production of biomass, while the biomass production was not

different in the range of 15-60 ppt salt concentration (Gao *et al*., 2013). *Aurantiochytrium* sp.SD116 and *A. limacinum* SR21 strains have capable of growth in fresh water (Yokochi *et al*., 1998; Hong *et al*., 2011b). Kyochan Kim *et al*. (2013) studied the impact of sea salt concentrations on the production of biomass from microalgae *Aurantiochytrium* sp. KRS101 in the range of 2-20 ppt and reported the maximum amount of biomass was observed in the concentration of 20 ppt (Kim *et al*., 2013). Nagano *et al*. (2009) assessed the growth of *A. limacinum* mh0186 microalgae in the range of 0-70 ppt and reported the amount 17.5 as an optimal value. This strain has no growth in fresh water. They eliminate magnesium sulfate from the sea water composition and observed that the growth is limited, so they figured out the sulfate ion was not suitable for strain growth (Nagano *et al*., 2009). Hong *et al*. (2011 b) studied the effect of sea salt concentration on the amount of DCW in microalgae *Aurantiochytrium* sp.KRS101 in the range of 2-50 ppt and observed that in this range of concentration, the DCW remain rather constant (Hong *et al*., 2011b).

Decreasing or lack of growth in an environment with low salinity, usually is because of two reasons; lack of ions and an increase in osmotic pressure (Kim *et al*., 2013). Sea water contains large amounts of sodium ions, potassium, calcium and magnesium, which are necessary for the growth of marine organisms (Bahnweg, 1979). Phosphate is a necessary compound for rapid growth of thraustochytrids and high amount of sodium ions lead to phosphate absorption by the marine organism. (Garrill *et al*., 1992). By increasing the concentration of sea salt, extracellular osmotic pressure reduces and rupture of the cell membrane decreases, and as a result the biomass production increases (Hong *et al*., 2011b). It should be mentioned that strains of marine mangroves areas have the ability to grow in environments with different salinities. Most *Aurantiochytrium* strains show the highest biomass in the range of 10-20 ppt salinity (Kim *et al*., 2013). The results show that there are no significant changes in biomass production in this strain with the mention concentration of sea salt.

The results showed that *Aurantiochytrium* sp. shy strain is appropriate for commercial production of biomass and related products. The results also illustrated that the production of the biomass and lipids can be increased by creating an appropriate culture conditions.

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References

Bahnweg, G., 1979. Studies on the physiology of Thraustochytriales. I. Growth requirements and nitrogen nutrition of *Thraustochytrium* spp., *Schizochytrium* sp., *Japonochytrium* sp., *Ulkenia* spp., and *Labyrinthuloides* spp. *Veroff Inst*

Meeresforsch Bremerhaven, 17**,** 245-268.

Bellou, S., Triantaphyllidou, I.E., Aggeli, D., Elazzazy, A.M., Baeshen, M.N. and Aggelis, G., 2016. Microbial oils as food additives: recent approaches for improving microbial oil production and its polyunsaturated fatty acid content. *Current Opinion in Biotechnology,* 37**,** 24-35.

Bligh, E.G. and Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology,* 37**,** 911-917.

Chen, G., Fan, K.W., Lu, F.P., Li, Q., Aki, T., Chen, F. and Jiang, Y., 2010. Optimization of nitrogen source for enhanced production of squalene from thraustochytrid *Aurantiochytrium* sp. *New Biotechnology,* 27**,** 382-389.

Chodchoey, K. and Verduyn, C., 2012. Growth, fatty acid profile in major lipid classes and lipid fluidity of *Aurantiochytrium mangrovei* Sk-02 as a function of growth temperature. *Brazilian Journal of Microbiology,* 43**,** 187-200.

De Swaaf, M.E., Sijtsma, L. and Pronk, J.T., 2003. High-cell-density fed-batch cultivation of the docosahexaenoic acid producing marine alga *Crypthecodinium cohnii*. *Biotechnology and Bioengineering,* 81**,** 666-672.

Fan, K., Vrijmoed, L. and Jones, E., 2002. Physiological studies of subtropical mangrove thraustochytrids. *Botanica Marina,* 45**,** 50-57.

Gao, M., Song, X., Feng, Y., Li, W. and Cui, Q., 2013. Isolation and characterization of Aurantiochytrium species: high docosahexaenoic acid (DHA) production by the newly isolated microalga, Aurantiochytrium sp. SD116. *Journal of Oleo Science,* 62**,** 143-151.

Garrili, A., Clipson, N. and Jennings, D., 1992. Preliminary observations on the monovalent cation relations of Thraustochytrium aureum, a fungus requiring sodium for growth. *Mycological Research,* 96**,** 295-304.

Honda, D., Yokochi, T., Nakahara, T., Raghukumar, S., Nakagiri, A., Schaumann, K. and Higashihara, T. 1999. Molecular phylogeny of Labyrinthulids and Thraustochytrids based on the sequencing of 18s ribosomal RNA gene. *Journal of Eukaryotic Microbiology,* 46**,** 637-647.

Hong, D.D., Anh, H.T.L. and Thu, N.T.H., 2011a. Study on biological characteristics of heterotrophic marine microalga—schizochytrium mangrovei PQ6 isolated from Phu Quoc Island, Kien Giang Province, Vietnam. *Journal of Phycology,* 47**,** 944-954.

Hong, W.K., Rairakhwada, D., Seo, P.S., Park, S.Y., Hur, B.K., Kim, C.H. and Seo, J.W., 2011b. Production of lipids containing high levels of docosahexaenoic acid by a newly isolated microalga, *Aurantiochytrium* sp. KRS101. *Applied Biochemistry and Biotechnology,* 164**,** 1468-1480.

Jakobsen, A.N., Aasen, I.M., Josefsen, K.D. and Strom, A.R., 2008. Accumulation of docosahexaenoic acid-rich lipid in thraustochytrid Aurantiochytrium sp. strain T66: effects of N and P starvation and O2 limitation. *Applied Microbiology and Biotechnology,* 80**,** 297.

Kamlangdee, N. and Fan, K., 2003. Polyunsaturated fatty acids production by Schizochytrium sp. isolated from mangrove. *Songklanakarin Journal of Science and Technology,* 25**,** 643-650.

Kim, K., Kim, E.J., Ryu, B.G., Park, S., Choi, Y.E. and Yang, J.W., 2013. A novel fed-batch process based on the biology of *Aurantiochytrium* sp. KRS101 for the production of biodiesel and docosahexaenoic acid. *Bioresource technology,* 135**,** 269-274.

Li, Q., Du, W. and LIU, D., 2008. Perspectives of microbial oils for biodiesel production. *Applied Microbiology and Biotechnology,* 80, 749-756.

Magasanik, B. and Kaiser, C.A. ,2002. Nitrogen regulation in Saccharomyces cerevisiae. *Gene,* 290, 1-18.

Manikan, V., Nazir, M.Y.M., Kalil, M.S., Isa, M.H.M., Kader, A.J.A., Yusoff, W.M.W. and Hamid, A.A., 2015. A new strain of docosahexaenoic acid producing microalga from Malaysian coastal waters. *Algal Research,* 9**,** 40-47.

Nagano, N., Taoka, Y., Honda, D. and Hayashi, M., 2009. Optimization of culture conditions for growth and docosahexaenoic acid production by a marine thraustochytrid, <I>Aurantiochytrium limacinum</I> mh0186. *Journal of Oleo Science,* 58**,** 623-628.

Nakazawa, A., Kokubun, Y., Matsuura, H., Yonezawa, N., Kose, R., Yoshida, M., Tanabe, Y., Kusuda, E., Van Thang, D., Ueda, M., Honda, D., Mahakhant, A., Kaya, K. and Watanabe, M.M., 2014. TLC screening of thraustochytrid strains for squalene production. *Journal of Applied Phycology,* 26**,** 29-41.

Nakazawa, A., Matsuura, H., Kose, R., Ito, K., Ueda, M., Honda, D., Inouye, I., Kaya, K. and Watanabe, M.M., 2012. Optimization of biomass and fatty acid production by *Aurantiochytrium* sp. strain 4W-1b. *Procedia Environmental Sciences,* 15**,** 27-33.

Qu, L., Ji, X.J., Ren, L.J., Nie, Z.K., Feng, Y., Wu, W.J., Ouyang, P.K. and Huang, H., 2011. Enhancement of docosahexaenoic acid production by Schizochytrium sp. using a two-stage oxygen supply control strategy based on oxygen transfer coefficient. *Letters in Applied Microbiology,* 52**,** 22-27.

Ranasinghe, C.P., 2013. Straminipilan protists: a study of their taxonomic position, morphology and abiotic stress-mediated gene expression. Queensland University of Technology. pp. 44-45.

Ratledge, C., 2003. An opinion on the safety data for the DHA-rich oil from Schizochytrium sp., also known as DHASCO-S. GRAS Notification for

DHA Algal Oil Derived from *Schizochytrium* sp, 5 P.

Shahryar, F.F.A.S., 2015. Investigation and Production of omega3 rich in docosahexaenoic acid by native strain of Aurantiochytrium TA4. *Biological Journal of Microorganism* 4**,** 9-24.

Taoka, Y., Nagano, N., Okita, Y., Izumida, H., Sugimoto, S. and Hayashi, M., 2009. Influences of culture temperature on the growth, lipid content and fatty acid composition of *Aurantiochytrium* sp. strain mh0186. *Marine Biotechnology*, 11**,** 368-374.

Wada, M., Fukunaga, N. and Sasaki, S., 1987. Effect of growth temperature on phospholipid and fatty acid compositions in a psychrotrophic bacterium, *Pseudomonas* sp. strain E-3. *Plant and Cell Physiology*, 28**,** 1209-1217.

Wen, Z.Y. and Chen, F., 2001. Optimization of nitrogen sources for heterotrophic production of eicosapentaenoic acid by the diatom Nitzschia laevis. *Enzyme and microbial technology*, 29**,** 341-347.

Wu, S.T., Yu, S.T. and Lin, L.P., 2005. Effect of culture conditions on docosahexaenoic acid production by *Schizochytrium* sp. S31. *Process Biochemistry*, 40**,** 3103-3108.

Yang, H.L., Lu, C.K., Chen, S.F., Chen, Y.M. and Chen, Y.M., 2010. Isolation and characterization of Taiwanese heterotrophic microalgae: screening of strains for docosahexaenoic acid (DHA) production. *Marine Biotechnology*, 12**,** 173-185.

Yokochi, T., Honda, D., Higashihara, T. and Nakahara, T., 1998. Optimization of docosahexaenoic acid production by *Schizochytrium limacinum* SR21. *Applied Microbiology and Biotechnology*, 49**,** 72-76.

Yokoyama, R. and Honda, D., 2007. Taxonomic rearrangement of the genus Schizochytrium sensu lato based on morphology, chemotaxonomic characteristics, and 18S rRNA gene phylogeny (Thraustochytriaceae, Labyrinthulomycetes): emendation for Schizochytrium and erection of Aurantiochytrium and Oblongichytrium gen. nov. *Mycoscience*, 48**,** 199.

Zhu, L., Zhang, X., Ji, L., Song, X. and Kuang, C., 2007. Changes of lipid content and fatty acid composition of Schizochytrium limacinum in response to different temperatures and salinities. *Process Biochemistry*, 42**,** 210-214.