Available online at www.sciencedirect.com

ScienceDirect

http://www.elsevier.com/locate/biombioe

Isochrysis sp. IOAC724S, a newly isolated, lipid-enriched, marine microalga for lipid production, and optimized cultivation conditions

Qian Liu, Tong Pang, Ling Li, Jianguo Liu, Wei Lin*

Institute of Oceanology, Chinese Academy of Sciences, 7 Nanhai Road, Qingdao, China

ARTICLE INFO

Article history:
Received 29 December 2011
Received in revised form
5 November 2013
Accepted 8 November 2013
Available online 8 December 2013

Keywords:
Marine microalgae
Isochrysis sp. IOAC724S
High temperature tolerance
Fatty acid
Lipid
Optimization

ABSTRACT

An oleaginous, unicellular, marine microalga termed IOAC724S was isolated from the South China Sea. Morphology and genetic analyses indicated it belongs to the genus *Isochrysis*. Gas chromatography (GC) results showed that more than 10 types of fatty acids existed in *Isochrysis* sp. IOAC724S and that 90% of them were suitable for lipid production. The culture conditions suitable for cell growth were progressively optimized through photosynthetic and respiratory analyses. The optimal culture conditions were: photon flux 200–500 μmol m^{-2} s^{-1}, temperature 35 °C during daytime and 24 °C at night, pH value between 7 and 8, $NaNO_3$ 160 g m^{-3} and $NaH_2PO_4 \cdot 2H_2O$ 80 g m^{-3} for starting culture. When microalgal cultures were exposed to these optimal conditions, the specific growth rate reached to 0.26 d^{-1} on average and 1.0 d^{-1} in MAX. Lipid production was optimized through nutrient starvation processes, including nitrate or phosphate deprivation and simultaneous nitrate and phosphate deprivation. The highest lipid mass fraction of dry cell weight (about 55.6%) was obtained after the stationary phase algal culture was transferred into phosphate-free medium for 3 days. GC data demonstrated that the enhancement of lipid accumulation in algal cells maintained under nutrient starvation came mainly from an increase of C16:0 and C18:1 fatty acids; however, the lipids with a chain length appropriate for fuel use (C14 to C18) were unchanged at 90% mass fraction of the dry cell weight. Based on these good characteristics, *Isochrysis* sp. IOAC724S appeared to be a strong candidate for lipid production.

1. Introduction

Biodiesel has become more attractive recently owing to its non-toxic, biodegradable and renewable characteristics. Thus far, biodiesel production has mainly utilized food crops, such as soybean, rapeseed and sunflower [1]; however the need for arable land, fresh water and food is so great that new bio-resources need to be found as substitutes for the land and crops currently utilized. Microalgal lipids are regarded by many as the most promising feedstock for future, sustainable, biodiesel production because of high algal growth rates and photosynthetic efficiencies compared with conventional terrestrial plants [2]. Microalgae have been claimed to be up to 20 times more productive per unit area than the best oil-seed crop [3]. Oil content of 20–30% can easily be attained in several microalgal species such as *Chlorella* sp. [4,5], *Isochrysis* sp., *Phaeodactylum tricornutum* [6], *Dunaliella* sp. [7,8], *Nannochloris* sp. [9,10] and *Oocystis minuta* [11]. *Botryococcus braunii* was considered to have the highest (86%) hydrocarbon content of

* *Corresponding author*. Tel./fax: +86 532 82898709.
E-mail address: jgliu@qdio.ac.cn (J. Liu).
0961-9534/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biombioe.2013.11.003

dry cell weight (DCW), when it was in its resting state [12]. However, a poor growth rate of just 0.09 d^{-1} hindered industrial development of *B. braunii* for biodiesel production [13]. Among these microalgae, marine species have some advantages such as their use of seawater rather than fresh water and the availability of millions of hectares of non-agricultural saline-alkali soil around the world that could be used for cultivation sites, which these advantages could make the dream of having biodiesel production no longer competing with food crops for arable land come true.

Isochrysis sp. IOAC724S, a newly isolated, oleaginous, marine microalga in our laboratory, exhibited fast growth under a wide range of temperature, pH, nitrogen and phosphate conditions, and was considered a potential bio-resource for commercial production.

2. Materials and methods

2.1. Algal strain

An axenic culture of strain IOAC724S was obtained from the algal collection (IOAC) of the Institute of Oceanology, Chinese Academy of Sciences (IOCAS). The strain IOAC724S was originally isolated from the South China Sea (18°64′N, 110°51′E) by using the following methods. Water samples collected from natural seawater were shipped to IOCAS. The water sample was serially diluted with L_1 seawater medium [14], and then further purified by repeated isolation from plates until a single colony was obtained.

The colony of strain IOAC724S was selected and transferred to L_1 liquid medium for further experimentation. The algal suspension was cultured in 500–5000 mL triangle flasks at 24 ± 0.2 °C in the light incubator. Illumination of 40 μmol photon m^{-2} s^{-1} was provided by white fluorescent lamps on a 12L/12D cycle. Light intensity was measured by a photometer at the surface of the culture. During the culture periods, the algal cultures were manually shaken 2–3 times a day to avoid sticking.

2.2. Scanning electron microscopy observation

Glutaraldehyde-seawater solution was filtered through a 0.45 μm micro-membrane. Prefiltered glutaraldehyde (2.5 mL) was dripped into a continuously shaken flask containing 100 mL of stationary phase algal culture. Then, the mixture was centrifuged at 141 g for 5 min, and the algal residue was kept at 4 °C overnight. The algal samples were dehydrated stepwise in 30%, 50%, 70%, 80%, 90% and 100% ethanol, with a 15 min interval between each step. The dehydrated algal samples were placed in isoamyl acetate for 30 min, critical point dried and finally gold coated using an ion plating machine. The algal cell was then observed by scanning electron microscopy (SEM KYKY-2800B).

2.3. DNA isolation and sequencing

Cultures were harvested by centrifugation. DNA was extracted using a Universal Genomic DNA Extraction Kit Ver.3.0 (TaKaRa, Dalian). The primers used for amplification of the small subunit rRNA coding gene (SSU rDNA) were SSUF (forward): 5′ AACCTGGTTGATCCTGCCAGT 3′, and SSUR (reverse): 5′ TGATCCTTCTGCAGGTTCACCTAC 3′ [15]. The program used for PCR was 94 °C for 5 min; 35 cycles of 94 °C for 40 s, 55 °C for 40 s and 72 °C for 100 s; and a final 10 min elongation at 72 °C. The PCR product was purified and sequenced by Sangon Biotech (Shanghai) Co., Ltd. with an ABI3730xl sequencer. The overlapping fragments were assembled using the Contig Express program in the Vector NTI software package (Version 9).

2.4. Sequence analysis

The SSU rDNA sequence was analyzed using the BLAST algorithm at the National Centre for Biotechnology Information (http://www.ncbi.nlm.nih.gov/blast). Seventeen relevant sequences were obtained from GenBank for phylogenetic analysis. The ClustalW Multiple Alignment program (http://www.ebi.ac.uk/clustalw/) was used to create the multiple sequence alignment. A neighbor-joining (NJ) algorithm-based [16] unrooted phylogenetic tree was constructed using the Kimura 2-parameter model [17] by MEGA 5.05 [18]. The reliability of the branching was tested by bootstrap re-sampling (1000 pseudo-replicates).

2.5. Specific growth rate and biomass measurement

Absorbance at 750 nm wavelength was determined by using a 722s spectrophotometer. The specific growth rate was calculated according to the daily changes of absorbance. Biomass was determined gravimetrically according to Liu et al. [19]. One liter of algal suspension in stationary phase was centrifuged at 250 g for 5 min. The algal pellet was then vacuum freeze-dried at −50 °C until reaching a constant weight. The algal powder was stored at −20 °C for total lipid and fatty acid analysis.

2.6. Total lipid extraction and fatty acid analysis

Total lipid was extracted according to the modified Bligh and Dyer's method [20]. About 25 mg algal powder, 2 mL chloroform and 1 mL methanol were added to a plastic centrifuge tube, and mixed well by vortex for 2 min and then kept at 25 °C for 24 h. The mixture was centrifuged at 134 g for 10 min. The supernatant was transferred into a pre-weighed vial (W_1). Another 1 mL of chloroform-methanol (2:1) was added to the plastic centrifuge tube. The algal residue was fully suspended by vortex and centrifuged again as mentioned above. After that, the supernatants were combined and evaporated in an oven at 70 °C until reaching a constant weight (W_2). The total lipid content was calculated according the following equation: total lipid content (% of dry cell weight) = $(W_2 - W_1) \times 100 \div$ the weight of algal powder.

Fatty acids in dry algal powder were extracted and transesterificated by a 2% H_2SO_4-methanol solution according to Liu [19]. Computer-controlled gas chromatographic (GC 112A, SPSIC) analysis was performed on a capillary column (30 m × 0.32 mm × 0.25 μm, Omegawax™320). The column temperature increased from 60 °C to 150 °C at a rate of 20 °C min^{-1}, and then was kept at 150 °C for 2 min. After that, the temperature was gradually raised to 265 °C at a rate of