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Optimization of biomass and fatty acid production by *Aurantiochytrium* sp. strain 4W-1b

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Abstract

The biomass and lipid productions by a newly isolated *Aurantiochytrium* strain, 4W-1b, were investigated at different temperatures and glucose concentrations. The maximum biomass was produced at 15–25°C. The biomass, lipid, and fatty acid productions were the maximum in a 6% glucose medium. The lipid and fatty acid productions were estimated to be approximately 11 g/L and 9 g/L, respectively, with the highest yields of docosahexaenoic acid (22:6; 1.5 g/L) and palmitic acid (16:0; 4.8 g/L). The 4W-1b strain is considered to have a high potential for uses in various industrial sectors, including fuel, health supplements, soap, and food oil companies.

Keywords: Aurantiochytrium; biomass; fatty acids; glucose; Labyrinthulomycetes; thraustochytrid

1. Introduction

Fatty acids, especially polyunsaturated fatty acids (PUFAs), such as docosahexaenoic acid (DHA), are in great demand by industry because of their beneficial effects on human health. Fish oil is the conventional source of commercially available PUFAs; however, its low content makes it difficult to obtain PUFAs in purified form. Some thraustochytrid species (Labyrinthulomycetes [3]) have high fatty

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acid content and are expected to be useful sources for microbial fatty acid production [5, 6, 8, 9]. The effect of culture conditions on lipid and DHA productions by the *Aurantiochytrium* NIBH SR21 strain has already been reported [11], and the SR21 strain has been suggested to be the most promising resource for microbial DHA production.

We isolated a new *Aurantiochytrium* strain, 4W-1b, which contains a similar or higher level of biomass and fatty acids than the SR21 strain. In this study, we compared the growth and lipid production ability of this strain with those of the SR21 strain. We also determined the culture conditions suitable for fatty acid production by the 4W-1b strain.

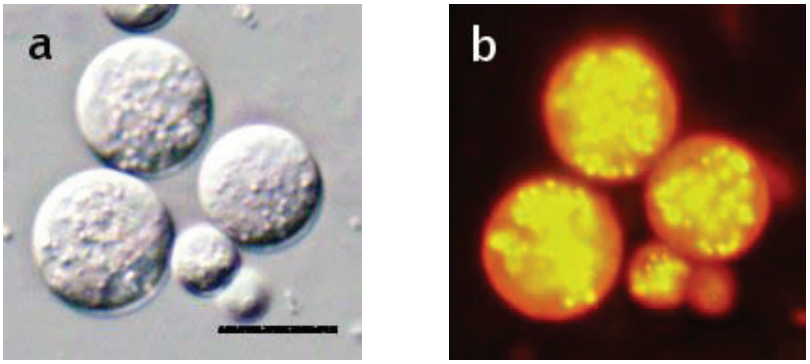

Fig. 1. Vegetative cells of *Aurantiochytrium* strain 4W-1b. (a) light micrograph; (b) fluorescent micrograph stained with Nile Red (Yellow fluorescence: neutral lipids. Red fluorescence: polar lipids). Scale bar, 20 μm.

2. Materials and Methods

2.1 Microorganisms

Aurantiochytrium sp. strain 4W-1b (Figs. 1a, 1b) was isolated from a mangrove coastal area in Okinawa Prefecture, Japan. *A. limacinum* strain NIBH SR21 ("SR21", IFO 32693 [9, 12]) was used as a control in the experiments. The organisms were maintained on agar plates containing modified GPY medium, with 2% glucose (Wako Pure Chemicals, Ltd., Japan), 1% tryptone (Difco, USA), 0.5% Bacto™ Yeast Extract (Becton, Dickinson and Co., USA), and 1.5% agar (Wako Pure Chemicals, Ltd.), at half the concentration of natural seawater.

2.2 Culture conditions

Aurantiochytrium sp. strain 4W-1b and *A. limacinum* strain SR21 were precultured in 500 mL conical flasks containing 200 mL of GPY liquid medium [10] with rotational shaking (130 rpm) at 25°C for three days. Parts of each preculture were transferred into 500 mL conical flasks containing GPY liquid medium with the same amount and component as preculture or 200 ml of AF-6 liquid medium [1] with 2% glucose, followed by culture with reciprocal or rotational shaking (100 strokes/min or 130 rpm) to examine growth and oil production. To test the effect of temperature, 400 μL of the GPY precultures was transferred into 200 ml of fresh GPY liquid medium and incubated at 10°C, 15°C, 20°C, 25°C, 30°C, and 35°C until the growth reached the early stationary phase. Growth was measured by optical density (OD_{660}) using a UV spectrophotometer (UV-1800, Shimadzu, Japan). To investigate the effect of glucose concentrations, 400 μL of the GPY precultures was transferred into 200 ml of flesh GPY liquid media

Table 1. Dry cell weight (DCW) and total lipid content (TL) of *Aurantiochytrium* strains under different culture conditions*.

Strains	AF-6 medium with glucose		GPY medium			
	100 strokes/min (14 days at 25°C)		100 strokes/min (12 days at 25°C)		130 rpm (172 hrs at 25°C)	
	DCW (g/L)	TL (%)	DCW (g/L)	TL (%)	DCW (g/L)	TL (%)
SR21	1.60	5.0	4.60	31.1	5.47	16.9
4W-1b	0.95	20.1	7.20	38.8	9.81	38.2

*Values are expressed as mean of two or triplicates.

containing 3%, 6%, 9%, and 12% glucose and then cultured with rotational shaking (200 rpm) at 25°C for four days. During culture, TAL-S12 (Thomas Kagaku, Japan) or BR-40LF (only for the temperature test; TAITEC, Japan) was used for reciprocal shaking, whereas an orbital shaker, MIR-S100 (SANYO, Japan), was used for rotational shaking.

2.3 Lipid analysis

Cell growth was determined by dry cell weight (DCW). The cells from the 200 mL culture broth were harvested by centrifugation, rinsed twice with water, and then lyophilized and weighed. The dried alga was immersed in chloroform: methanol (2:1; v/v). The fatty acids were directly transmethylated from the extract using 14% BF_3. The esterified fatty acids were extracted with *n*-hexane, and the resultant extracts were applied to a GC-FID (Shimadzu GC-2010) equipped with a DB-5MS capillary column (30 m × 0.25 mm inner diameter; 0.25 mm film thickness; J & W Scientific, Agilent Technologies Japan, LtD). The temperature program rose from 130°C to 270°C with increments of 20°C/min. Peaks were identified using authentic standards of fatty acid methyl esters.

3. Results

3.1 Cell growth and oil production by Aurantiochytrium strains

The biomass and oil content of the 4W-1b and SR21 strains were measured using two different culture media, namely 200 ml of GPY liquid medium containing half-strength of natural seawater, and 200 ml of modified AF-6 liquid medium with 2% glucose. In GPY medium, the growth of the 4W-1b strain was 7.2 g DCW/L at 25°C and the accumulated lipids comprised 38.8% of the DCW. However, its biomass and lipid content decreased to 1/8 and 1/2, respectively, when cultured in modified AF-6 liquid medium (Table 1). The growth and lipid content of the SR21 strain were 4.6 g DCW/L and 31.1% of the DCW, respectively, in GPY medium; however, these decreased to 1/3 and 1/6, respectively in modified AF-6 liquid medium (Table 1). It was noteworthy that the 4W-1b strain decreased its biomass more than its lipid content, whereas the SR21 strain decreased its lipid content more than its biomass. The biomass and lipid content of the 4W-1b strain were higher than those of the SR21 strain in GPY medium. Thus, the lipid productivity of the 4W-1b strain was estimated to be 2.8 g/L, which was much higher than that of the SR21 strain (1.4 g/L). Additional experiments with rotational shaking (130 rpm) for 172 h at 25°C suggested further superiority of the 4W-1b strain, with 1.79 times higher DCW and 4.05 times higher lipid content than the SR21 strain (Table 1). Thus, the 4W-1b strain had 7.0 times higher lipid productivity compared with the SR21 strain.

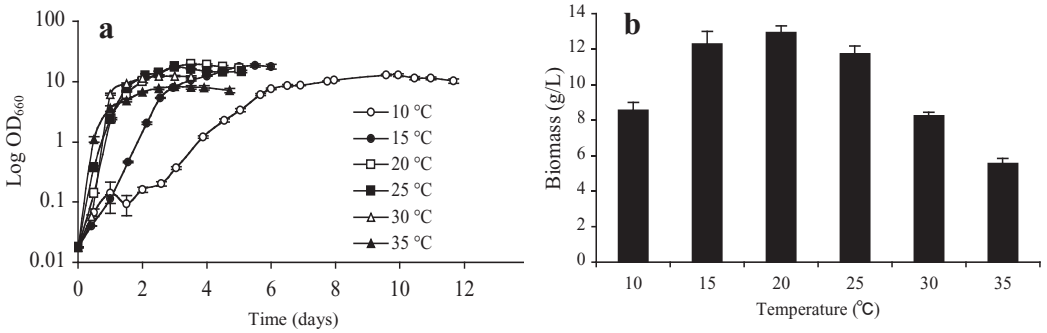

Fig. 2. Cell growth of the 4W-1b strain in basal GPY liquid medium (2% glucose) with 100 strokes/min. (a) Effect of culture temperatures on cell growth of the 4W-1b strain measured by OD_{660}. (b) Biomasses at the maximum cell growth (early stationary phase) at each temperature. Values are represented as mean ± standard deviation of triplicates.

Table 2. Effects of glucose concentration on biomass and lipids of *Aurantiochytrium* sp. strain 4W-1b*.

Glucose concentrations	3%	6%	9%	12%
Biomass (g/L)	9.01 ± 0.62	20.10 ± 5.68	15.06 ± 1.21	15.86 ± 1.41
Lipid productivity (g/L)	4.16 ± 0.48	10.93 ± 2.65	10.98 ± 1.15	8.76 ± 1.79
Total lipid content in dry cells (%)	46.05 ± 2.73	55.42 ± 9.92	73.14 ± 7.59	54.70 ± 6.98
Total fatty acid composition**(%)	60.53 ± 2.43	83.73 ± 3.24	80.83 ± 14.26	72.97 ± 6.69

*Values are expressed as mean ± standarf deviation of triplicates.

**Data as methylesterified samples.

3.2 Effects of culture temperature on cell growth

The effect of temperature on cell growth was tested in the range of 10–35°C. The 4W-1b strain grew well at any of these temperatures. The highest growth rate and growth yield were obtained at 30°C with a doubling time of 2.1 h (Fig. 2a) and at 15–25°C with 12–13 g DCW/L (Fig. 2b), respectively.

3.3 Cell growth and fatty acid production at different glucose concentrations

The biomass of the 4W-1b strain reached the maximum value of 20 g/L at 6% glucose (Table 2). However, the biomass decreased to 15 g/L at 9%–12% glucose. Thus, the total lipid production per unit culture volume was the maximum (approximately 11 g/L) at 6%–9% glucose, although the total lipid content (TL) was the maximum at 9% glucose (73.14% of DCW). The total fatty acid (TFA) content maximum was at 6% and 9% glucose. Consequently, the maximum fatty acid yield was estimated to be 9.15 g/L at 6% glucose because of the highest growth obtained at this concentration (Table 2).

Table 3. Fatty acid composition of *Aurantiochytrium* sp. strain 4W-1b grown on various concentration of glucose*

Glucose concentrations	Fatty acid contents (%)							
	14:0	15:0	16:0	17:0	18:0	22:5n-3	22:6n-3	Others
3%	1.4 ± 0.1	6.6 ± 0.2	46.9 ± 1.6	6.0 ± 0.2	4.1 ± 0.1	6.4 ± 0.3	27.9 ± 1.1	0.7 ± 0.1
6%	4.0 ± 0.2	13.1 ± 1.0	52.6 ± 0.4	4.7 ± 0.2	2.4 ± 0.1	3.9 ± 0.1	17.2 ± 0.6	2.1 ± 1.8
9%	4.7 ± 0.3	14.9 ± 0.4	50.7 ± 0.4	5.0 ± 0.3	2.4 ± 0.6	3.6 ± 0.1	17.5 ± 2.1	1.2 ± 0.8
12%	2.0 ± 0.4	12.2 ± 0.5	47.6 ± 1.5	5.9 ± 0.4	2.8 ± 0.8	4.3 ± 0.4	21.2 ± 1.5	4.0 ± 3.4

*Values are expressed as mean ± standard deviation of triplicates.

The fatty acid composition of the 4W-1b strain was investigated using different glucose concentrations (Table 3). The seven main fatty acids were detected at each glucose concentration and the major fatty acid was palmitic acid (16:0), the content of which did not change significantly at different glucose concentrations, ranging from 46.9% to 52.6%. Polyunsaturated fatty acids (PUFAs) mainly comprised DHA (22:6 n-3) and docosapentaenoic acid (DPA; 22:5 n-3), with the maximum production being 27.9% and 6.4%, respectively, at 3% glucose. The fatty acids of 14:0, 15:0, and 16:0 increased more than the other fatty acids, and the total fatty acid content was higher at 6% and 9% glucose (Table 4).

4. Discussion

The 4W-1b strain showed a wide temperature tolerance, increasing in the range of 10–35°C (Fig. 2). The temperature range for the growth of the 4W-1b strain was slightly wider than that for the SR21 strain [11], in which growth was greatly inhibited at 10°C and 35°C. *Aurantiochytrium* sp. usually inhabits brackish mangrove areas where the temperature fluctuates greatly on a seasonal and daily basis. Thus, the 4W-1b strain may have the potential to be more adaptable to daily and seasonal temperature fluctuations than the SR21 strain, which means it may be more applicable for commercial mass production because of lower energy cost requirements for controlling the temperature.

The highest lipid and fatty acid production, i.e., approximately 11.0 g/L and 9g/L, respectively, was obtained at 6% glucose (Tables 2, 4). However, 12% glucose resulted in a markedly lower lipid and fatty acid productivity (Tables 2, 4), thereby indicating that increasing the carbon concentration was only partially effective in increasing lipid and fatty acid production. Growth of *Thraustochytrium aureum* was also inhibited at a glucose concentration greater than 10 g/L [4] and a similar ineffectiveness at higher glucose concentration, irrespective of the level of inhibition, has been widely reported [11]. In this study, the TL per dry cell weight with 12% glucose was almost the same as that with 6% glucose. Therefore, it was clear that the decreased fatty acid production was mainly because of the decreased biomass, followed by a slight decrease in the fatty acid content (Table 2).

The lipids produced by the 4W-1b strain had a simple fatty acid profile with major constituents of palmitic acid (16:0) as a saturated fatty acid and DHA and DPA as PUFAs (Table 3). The profile is basically similar to that of the SR21strain; however, additional unsaturated fatty acids, such as icosapentaenoic and arachidonic acids, which are produced by many other thraustochytrid strains [2], were not significantly detected. The maximum DHA yield was greater than 1.5 g/L at 6% and 9% glucose (Table 4). DHA yields of the 4W-1b strain were double than those of *T. aureum* [2] and *T. roseum* [7]. In

Table 4. Effect of glucose concentration on the production of TFA and its three major constituents by *Aurantiochytrium* 4W-1b*

Glucose concentrations	3%	6%	9%	12%
Total fatty acid (g/L)	2.52	9.15	8.88	6.39
DHA (22:6 n-3) (g/L)	0.7	1.57	1.55	1.36
DPA (22:5 n-3) (g/L)	0.16	0.36	0.32	0.27
Palmitic acid (16:0) (g/L)	1.18	4.81	4.5	3.04

*Values are expressed as mean of triplicates.

addition, high amounts of palmitic acid (16:0; 4.8 g/L) were obtained at 6% glucose (Table 4), and this compound has potential applications for products such as soap or salad oil.

When cultured with 2% glucose, the 4W-1b strain showed double the lipid productivity of the SR21 strain. A further experiment with rotational shaking (130 rpm) for 172 h at 25°C showed that it produced more than 7.0 times the lipid produced by the SR21 strain (Table 1). The biomass and fatty acid productions of the SR21 strain were approximately 36 g/L and 14 g/L at 9% glucose and sufficient nitrogen (corn steep liquor) and phosphorus (KH_2PO_4) sources [11]. In this study, we used tryptone as a nitrogen source, which was not efficiently utilized by the SR21 strain [11], and we added no inorganic phosphorus source. Thus, the nitrogen and phosphorus sources must have limited the biomass, lipid, and fatty acid productions by the SR21 strain and probably the 4W-1b strain. Given the superior biomass, lipid, and fatty acid productions by the 4W-1b strain than the SR21 strain in this study, the 4W-1b strain would probably have much higher biomass, lipid, and fatty acid productivity, including palmitic acid and DHA, than the SR21 strain even when cultured with sufficient nitrogen and phosphorus sources. Further study is needed to demonstrate the high applicability of the 4W-1b strain for uses in various industrial sectors, including fuel, health supplements, soap, and food oil companies.

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