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Isolation and characterization of *Aurantiochytrium* species: high docosahexaenoic acid (DHA) production by the newly isolated microalga, *Aurantiochytrium* sp. SD116

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Abstract: A heterotrophic microalga, strain SD116, with the ability to produce high concentrations of docosahexaenoic acid (DHA, C22:6n-3) was isolated from Shuidong Bay, Guangdong Province, China. Nucleotide sequence analysis of the 18S rDNA of SD116 showed that the strain has a close phylogenetic relationship to *Aurantiochytrium* species. The highest rates for growth and DHA accumulation for SD116 were obtained in 6.0% glucose, 2.0% yeast extract, and 50% artificial seawater (ASW) at a pH of 7 at 28℃. The maximum total lipid content reached 56.3% of the dry cell weight (DCW), and the maximum DHA content accounted for 50.9% of the total fatty acid (TFA) content. It was further found that urea may be a potential nitrogen source for industrial fermentation because of its cheap price and ability to induce a relatively high biomass and lipid production capacity. Using 5 L fermenters, the DCW, total lipid content, and DHA yield were found to be 70.43 g L^{-1}, 71.09% of the DCW, and 17.42 g L^{-1} (34.79% of the TFA), respectively. The results show that *Aurantiochytrium* sp. SD116 is a promising candidate for commercial DHA production and could be useful for the synthesis of biomass-related products.

Key words: *Aurantiochytrium* sp., biomass, docosahexaenoic acid, heterotrophic marine algae, lipid

Comprehensive list of abbreviations

ARA: arachidonic Acid
ASW: artificial seawater
DCW: dry cell weight
DHA: docosahexaenoic acid
DO: dissolved oxygen concentration
DPA: docosapentaenoic acid
EPA: eicosapentaenoic acid
FAMEs: Fatty acid methyl esters
FAS: fatty acid synthase
GPY medium: Glucose, Peptone and Yeast medium
ME: minimum evolution
PKS: polyketide synthase
PUFA: polyunsaturated fatty acid
SFA: saturated fatty acid
TFA: total fatty acid
UFA: unsaturated fatty acid
ω3 LC-PUFA: omega-3 long chain polyunsaturated fatty acid

1 INTRODUCTION

Docosahexaenoic acid (DHA, 22:6, n-3) is widely recognized as an important nutritional component during invertebrate and vertebrate development[1)], and it has been verified that DHA plays a key role in improving neural and retinal development in infants and lowering the incidence of certain cardiovascular diseases[2, 3)]. Moreover, DHA is an essential component of cellular membranes in some human tissues, mainly in the brain and retina[4)]. For these reasons, DHA is incorporated into infant formulae in many countries

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around the world, as well as in adult oil supplements.

Oceanic fish and fish oil products are the typical dietary sources of DHA[5]. However, due to emerging concerns over the sustainability of marine resources and of the levels of environmental contaminants present in fish, major efforts have been made to identify or create alternative sources of DHA. Two genera of microorganisms, *Crypthecodinium cohnii* and *Aurantiochytrium*, have been developed as alternative commercial sources of oils enriched in DHA[6].

Aurantiochytrium (known as *Schizochytrium* prior to 2007[7]; for clarification, *Aurantiochytrium* was thoroughly used in this paper according their position on evolution tree despite of their names in the original literatures) is a thraustochytrid, a group of heterotrophic protists commonly found in marine environments and capable of producing a number of omega-3 long chain polyunsaturated fatty acids (ω3 LC-PUFA), especially DHA[8]. *Aurantiochytrium* can produce large amounts of oil, up to 55% of the dry cell weight (DCW) where DHA comprises as much as 35% of the total fatty acid (TFA) content. *Aurantiochytrium* has been utilized for the commercial production of DHA-rich oils and the dried powders used as a source of DHA in foods, feeds, and nutritional supplements[9].

In this study, we investigated the biological characteristics of *Aurantiochytrium* sp. SD116 isolated from Shuidong Bay, Guangdong Province, China, including its morphological features, PUFA composition, and molecular phylogenetic relationships. We also investigated the optimization of the culture conditions for *Aurantiochytrium* sp. SD116 for high biomass and DHA yields in 250 mL flasks and 5 L fermenters.

2 EXPERIMENTAL

2.1 Isolation of Aurantiochytrium strains

Aurantiochytrium strains were obtained from soil and leaf samples from the mangrove ecosystem in Shuidong Bay, Guangdong Province, China, using the pine pollen technique reported by Yokochi et al.[10]. The samples were transferred into test tubes containing 5 mL of sterilized 50% seawater and pine pollen, and then incubated at 25℃ in the dark. After 24 h of incubation, the pine pollen floating on the surface of the tubes was streaked onto KMV+ medium agar plates (1.0 g L^{-1} glucose; 1.0 g L^{-1} gelatin hydrolysates; 0.1 g L^{-1} yeast extract; 0.1 g L^{-1} peptone and 12 g L^{-1} agar; in 1 L natural seawater containing 50 ppm penicillin G and 50 ppm streptomycin sulfate)[11]. The plates were incubated at 25℃ in the dark for 3-5 d during which their morphology was observed using a light microscope (Olympus CX21); the *Aurantiochytrium* cells were shown to be round and clustered around the pine pollen. The *Aurantiochytrium* colonies were transferred onto KMV+ medium several times to suppress bacterial and fungal growth. Isolated strains were maintained on KMV medium (without antibiotics) as monoclonal cultures.

2.2 Cloning and Sequencing of the 18S rDNA

Genomic DNA from selected strains was extracted using a Universal Genomic DNA extraction kit (Takara, DV811A) according to the manufacturer's protocol. DNA containing the 18S rDNA was amplified using the forward primer P1 (5′- CCAACCTGGTTGATCCTGCCAGTA-3′) and the reverse primer P2 (5′- CCTTGTTACGACTTCACCTTCCTCT-3′). Each 50 μl PCR reaction mixture contained 10 μl 5X PCR buffer, 10 mM dNTPs, 10 μM of each primer, 1.25 U Taq DNA polymerase (Takara), and 1 μg genomic DNA. The PCR protocol consisted of 0.5 min of denaturation at 94℃, followed by 30 cycles each composed of 0.5 min at 94℃, 0.5 min at 55℃, and 2 min at 68℃. The final extension step consisted of 5 min at 68℃. The PCR products were visualized using agarose gel electrophoresis and purified using the TIAN gel Midi Purification Kit (TIANGEN, DP209). The purified PCR products were then cloned into the pGEM-T Easy vector (Promega) and sequenced by Takara Co., LTD.

The nucleotide sequences of the 18S rDNA from related microorganisms were downloaded from GenBank (http://www.ncbi.nlm.nih.gov/genbank/), and a phylogenetic tree was inferred using the Minimum Evolution method of MEGA version 5.0. Tree reliability was evaluated by bootstrap analysis of 1,000 replicates.

2.3 Strains and Cultivation

Strain SD116 was cultivated on GPY medium (6% glucose, 1% peptone, and 1% yeast extract) with a salinity equivalent to 50% that of sea water in 250 ml flasks at 25℃ and 200 rpm in a horizontal rotator (QHZ-123B, Huamei Biochemistry Instrument Ltd., Jiangsu Province).

2.4 Optimization of Culture Conditions

To investigate the effects of various cultivation media, 7 carbon sources (6% of glucose, D-fructose, D-xylose, maltose, sucrose, glycerol and starch) and 6 nitrogen sources (5% of yeast extract, peptone, tryptone, urea, ammonium acetate and sodium nitrate) were tested. The pH value of each experiment was measured every 6 hours, and 1 M NaOH or 1 M HCl was used to control the pH. The effects of varying concentrations (0-60g L^{-1}) of sea salt were also studied using the optimized medium.

To examine the effects of various growth parameters, *Aurantiochytrium* sp. SD116 was cultivated at different pH values (pH 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0) and temperatures (20℃, 25℃, 28℃, 30℃, and 37℃).

Fermentation experiments were performed in a 5 L Biostat® B plus bioreactor equipped with controls for pH, temperature, agitation and dissolved oxygen concentration (DO). Batch cultures were grown in 3.5 L of production medium. The temperature was maintained at 28℃ for the

first 72h and then dropped to 20℃ for another 40h. Based on the results described in section 3.3, low temperature was determined to benefit DHA accumulation. Therefore, during the later phase of the fed-batch experiment, we changed the temperature from 28 to 20℃ for DHA accumulation. The agitation speed automatically varied from 300 to 800 rpm at a fixed airflow rate of 1.2 vvm to maintain the DO at 20% air saturation. The pH was maintained at 6.5 by adding 2N NaOH or 14% citrate automatically. To control foam formation, 1ml of antifoam was added at the beginning of the run. Samples (50 ml) for off-line determination of biomass, glucose, lipid, and DHA concentrations were withdrawn every 12 h until the end of the fermentation run (110 h).

2.5 Biomass determination

The biomass of *Aurantiochytrium* sp. SD116 was expressed in terms of the DCW. Samples (30 mL) of cell suspensions were centrifuged at 7,000 g and 4℃ for 10 min after washing twice with 0.2 M phosphate buffer solution. Cell pellets were then freeze-dried to constant weight at −50℃ for approximately 60 h.

2.6 Lipid extraction and fatty acid composition analysis

The total lipid content was calculated using a modified miniaturized Bligh-Dyer method[12]. Harvested cells were extracted into 100 mL chloroform/methanol (2:1, v/v) at room temperature. The lipid extract was dried over anhydrous Na_2SO_4 and the solvent was removed by evaporation. Afterward, the total lipid was weighed. Fatty acid methyl esters (FAMEs) were prepared according to the method described by Song et al[13]. Total lipid was suspended in 2 mL of 0.4 M methanolic KOH for 1 h at 60℃, esterified for another 1 h in 2 mL BF_3-methanol (14%, w/w) reagent at 60℃, and extracted with 2 mL n-hexane; nonadecanoic acid was used as an internal standard. FAMEs were separated by Agilent 7890A GC with He as the carrier gas using an HP-INNOWax column (30 m × 0.25 mm i.d., 0.25 μm film thickness; Agilent Technologies). The temperature of the column was raised from 100 to 240℃ at 15℃/min and then maintained at 240℃ for another 10 min. The temperature of the injection port and flame ionization port was 250℃.

3 RESULTS

3.1 Isolation and identification of *Aurantiochytrium* sp. SD116

The samples obtained from the mangrove ecosystem were screened for *Aurantiochytrium* microalgae, and almost two hundred purified colonies were isolated on solidified KMV medium and microscopically observed. Among these isolated colonies, the biomass (DCW) ranged from 4.46 to 31.30 g l^{-1}, and the lipid (percent of DCW) and DHA (percent of TFA) contents ranged from 9.4% to 49.3% DCW and 20.1% to 46.7% TFA, respectively.

From the tested strains, colony SD116 was chosen for further study because it showed the highest levels of DCW and lipid content and had an acceptably increased DHA concentration. The 18S rDNA sequence of SD116 was determined and deposited under GenBank Accession No. JX863672. Subsequently, SD116 was confirmed to be an *Aurantiochytrium* strain based on morphological observation (Fig. 1) and the Minimum Evolution (ME) tree (Fig. 2) constructed by MEGA 5.0 with a high bootstrap value of 1,000 replicates. Thus, colony SD116 was renamed as *Aurantiochytrium* sp. SD116.

As shown in Fig. 1, *Aurantiochytrium* sp. SD116 is a spherical single-celled organism. The thallus is thin-walled, globose, and orange; the ectoplasmic nets are not well developed; and the zoospores are biflagellate, heterokont, and reniform to ovoid. The vegetative cells are spherical with diameters ranging from 7 ± 0.5 μm to 20 ± 0.5 μm, and usually form a cluster in liquid medium (Fig. 1a). The cells had many oil bodies under the cell membrane (Fig. 1b). The fatty acid composition of SD116 was analyzed by GC, and the main fatty acid composition is shown in Table 1 and listed here: C14:0 (2.85%), C15:0 (7.30%), C16:0 (25.75%), C16:1n-7 (0.51%), C17:0 (2.05%), C18:0 (3.54%), C20:0 (1.26%), ARA: C20:4n-3 (0.91%), EPA: C20:5n-3 (0.81%), DPA: C22:5n-6 (9.12%), and DHA: C22:6n-3 (45.52% of TFA). The chromatogram of fatty acid composition is shown in figure 1d.

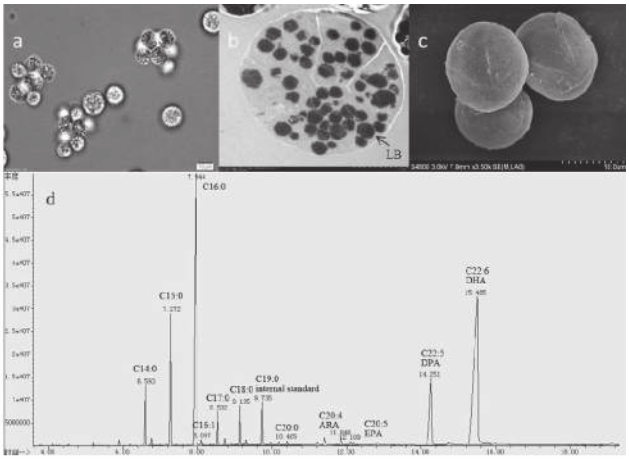

Fig. 1 Morphology of *Aurantiochytrium* sp. SD116 (a, light microscope, 400 magnification; b, transmission electron microscope, 1000 magnification, LB: lipid body; c, scanning electron microscope, 3500 magnification; d, fatty acid profiles of *Aurantiochytrium* sp. SD 116).

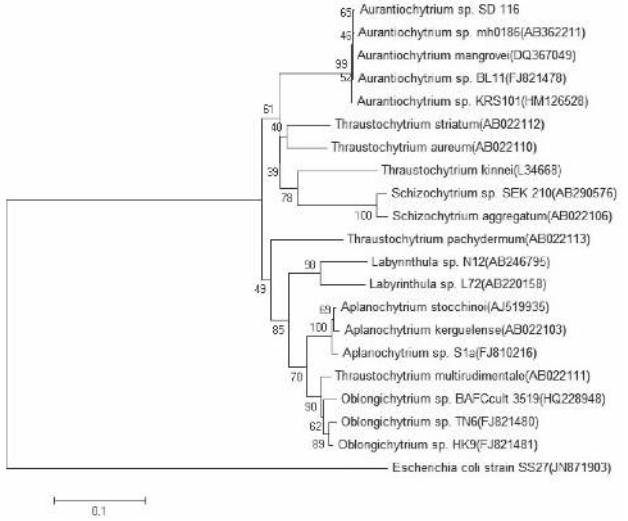

Fig. 2 Phylogenetic relationship of *Aurantiochytrium* species based on the alignment of 18S rDNA sequences. The tree was constructed using the Minimum Evolution method and 1,000 bootstrapped replicates in MEGA 5.0.

Table 1 Fatty acid profiles of *Aurantiochytrium* sp. SD116.

Fatty acids	Content (% TFA)
C14:0	2.85
C15:0	7.30
C16:0	25.75
C16:1 (n-7)	0.51
C17:0	2.05
C18:0	3.54
C20:0	1.26
C20:4 (n-6, ARA)	0.91
C20:5 (n-3, EPA)	0.81
C22:5 (n-6, DPA)	9.12
C22:6 (n-6, DHA)	45.52

3.2 Cell growth and lipid production on various carbon and nitrogen sources

Different carbon and nitrogen sources were used to investigate the cell growth and lipid yield of *Aurantiochytrium* sp. SD116.

The hexose monosaccharides, such as glucose and fructose, were found to be well utilized by *Aurantiochytrium* sp. SD116 with good growth and DHA production, but pentoses (such as xylose), disaccharides, and polysaccharides were not effective for cell growth (Fig. 3a). These results were consistent with the results obtained for *Aurantiochytrium limacinum* strains SR21 and S31[14, 15]. The polyalcohol glycerol produced a cell growth and DHA yield equivalent to that of glucose. In the case where glucose, fructose, and glycerol were used as the carbon source, the lipid and DHA contents were 54.6%, 40.2%, and 49.7% of the DCW, and 44.6%, 35.5%, and 40.9% of the TFA, respectively. The content of saturated fatty acids (SFA), mainly palmitic acid and myristic acid, varied from 30% to 48%, while the remainder was composed mostly of DHA and DPA.

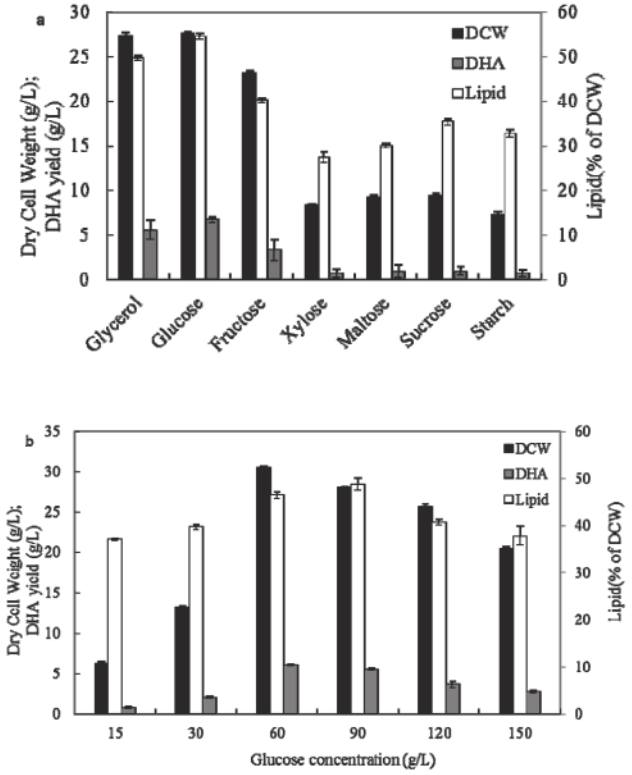

Fig. 3 Effects of carbon source (a) and glucose concentration (b) on cell growth, lipid accumulation and docosahexaenoic acid (DHA) yield in *Aurantiochytrium* sp. SD116. All data are means of three replicates; vertical bars represent error bars with the value equal to the standard error of the mean.

The effects of carbon source concentration on cell growth and DHA yield were also examined using various concentrations of glucose (Fig. 3b). The initial glucose concentration in the medium affected both cell growth and fatty acid synthesis in the broth, and increasing the glucose concentration from 1.5% to 6.0% resulted in high levels of cell growth and high DHA yields. The maximum DCW and DHA yields of 30.6 g L^{-1} and 6.07 g L^{-1}, respectively, were obtained at a glucose concentration of 6%, with a high glucose-biomass conversion ratio of 0.51 g DCW per 1 g glucose.

The utilization of various organic and inorganic nitrogen sources by *Aurantiochytrium*. sp. SD116 was also investigated (Fig. 4a). The highest biomass and DHA production (28.76 and 6.62 g L^{-1}, respectively) was obtained using

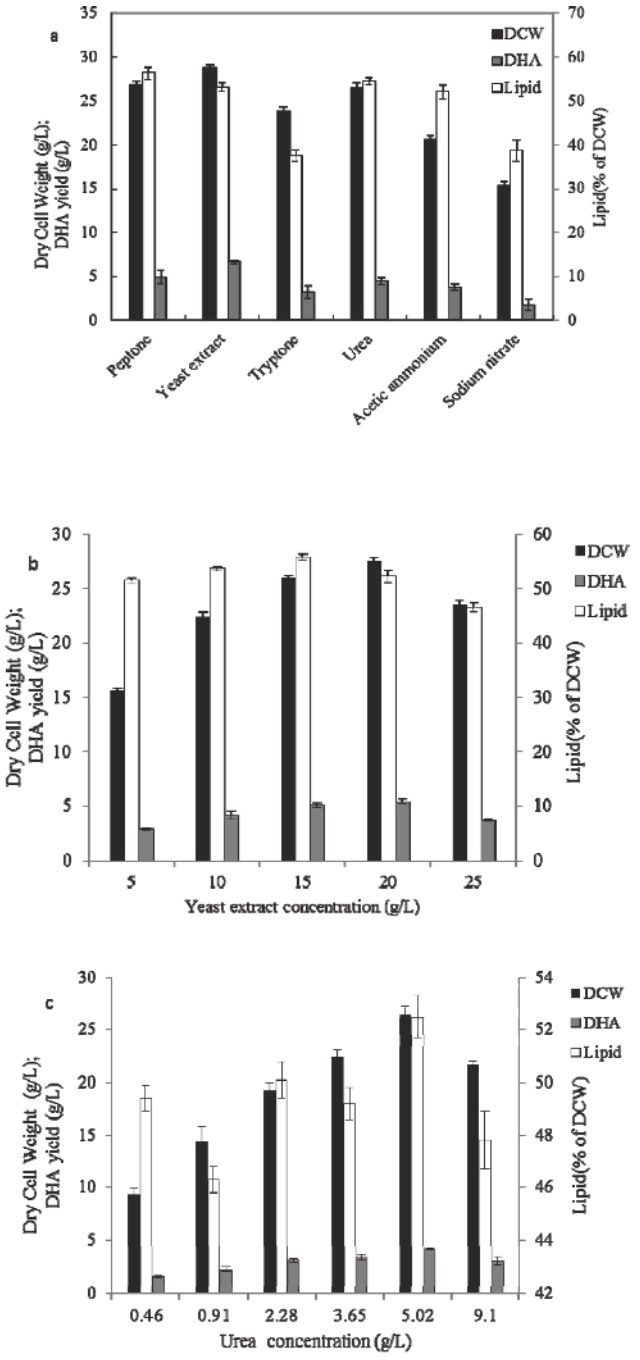

Fig. 4 Effects of nitrogen source (a) and concentration (b, c) on cell growth, lipid accumulation and docosahexaenoic acid (DHA) yield in *Aurantiochytrium* sp. SD116. All data are means of three replicates; vertical bars represent error bars with the value equal to the standard error of the mean.

yeast extract as a nitrogen source, followed by peptone, urea, tryptone, ammonium acetate and sodium nitrate. The effects of yeast extract concentration on cell growth and DHA yield for SD116 were then examined (Fig. 4b). Cell growth increased when the concentration of yeast extract was raised to 2.0%; the highest DHA yield (5.41 g L^{-1}) was also obtained at 2.0% yeast extract. The maximum lipid content (55.7% of DCW) was obtained when the yeast extract concentration was 15 g L^{-1}. Notably, relative high levels of both biomass and lipid were obtained when urea was used as a nitrogen source, and the effects of urea concentration on growth and DHA production were investigated (Fig. 4c). At an increased urea concentration of 5.02 g L^{-1}, the biomass and DHA yields reached maximum values of 26.5 g L^{-1} and 4.63 g L^{-1}, respectively. SD116 was thus able to use both ammonium and nitrate nitrogen, with ammonium nitrogen resulting in a higher biomass and lipid production than nitrate nitrogen.

3.3 Effects of salt concentration and culture temperature on cell growth

The effect of salt concentration on growth and lipid production was examined over a range of 0 to 6% salt. *Aurantiochytrium* sp. SD116 showed a wide tolerance towards salinity. As shown in Fig. 5a, its optimum salt concentration was 1.5%, at which the biomass and DHA yields were 28.2 g L^{-1} and 6.11 g L^{-1}, respectively. Little change in cell growth was observed from 1.5%-6% salt. SD116 could even grow under fresh water conditions, with growth levels and DHA yields almost half that of the maximum. The same results were also obtained for *A. limacinum* SR21 and *A. mangrovei* PQ6[14, 16].

The effects of culture temperature on biomass and lipid yield were tested from 20 to 37℃. As shown in Fig. 5b and Table 2, there were only slight fluctuations in biomass and lipid content at temperatures ranging from 20 to 28℃, and biomass production was the highest (29.56 g L^{-1}) at 28℃. However, when the temperature was raised to 37℃, biomass production decreased to 9.24 g L^{-1} and lipid content decreased significantly from 47.8% at 28℃ to 33.3% at 37℃. These changes resulted in an increase in total SFA and a decrease in total unsaturated fatty acid (UFA) when the temperature was increased from 20 to 37℃ (Table 2). Temperature also affects the degree of fatty acid unsaturation. The proportion of UFA (including DPA and DHA) in the TFA fell from 57.0% to 29.3% when the temperature was raised from 20 to 37℃. These results agreed with previous reports[14, 16–18]. Variations in the degree of fatty acid unsaturation were regarded as an alternative response to providing an appropriate degree of membrane fluidity for microbial growth.

3.4 Effects of initial pH on cell growth

The initial pH value of the medium profoundly affects cell membrane function and cell metabolism, as well as nutrient uptake and product biosynthesis[19]. For these reasons, pH values of the medium from 4.0 to 9.0 were tested. The results showed that a slightly acidic or neutral

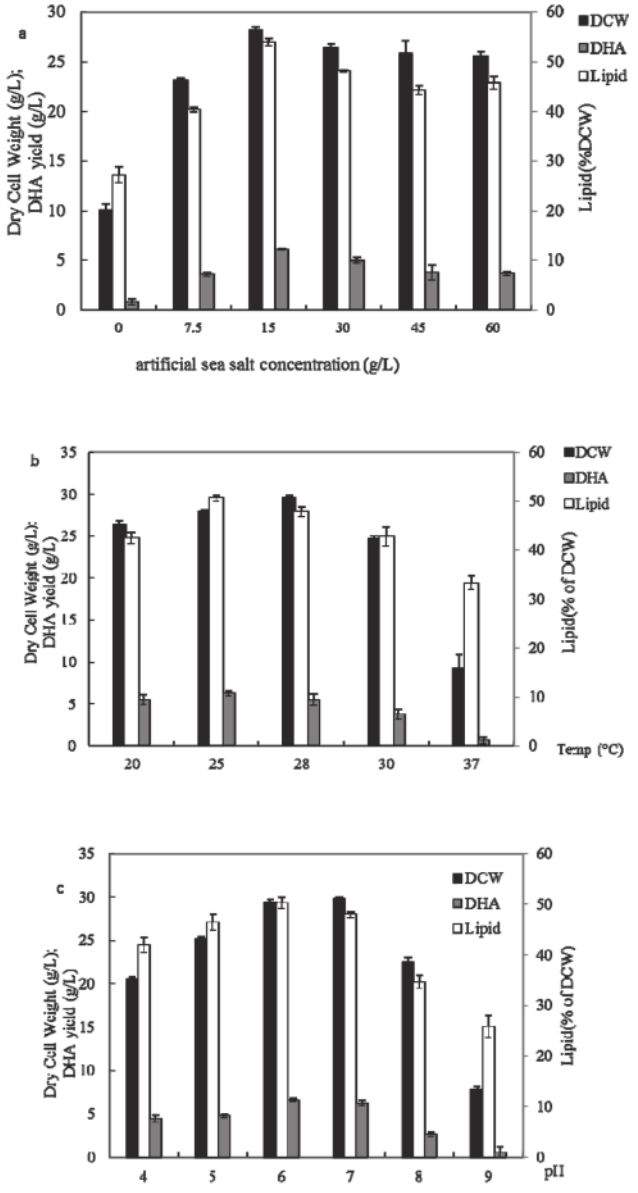

Fig. 5 Effect of artificial sea salt concentration (a), temperature (b) and initial pH (c) on cell growth, lipid accumulation and docosahexaenoic acid (DHA) yield in *Aurantiochytrium* sp. SD116. All data are means of three replicates; vertical bars represent error bars with the value equal to the standard error of the mean.

initial pH (4.0-7.0) was favorable for growth and lipid accumulation in *Aurantiochytrium* sp. SD116 (Fig. 5c). Maximum biomass was obtained at pH 7.0 (29.77g L^{-1}), while maximum lipid and DHA levels were obtained at pH 6.0 (50.2% of DCW and 6.58 g L^{-1}, respectively); these values agree well with the experimental findings for *Aurantiochytrium* sp. S31 and *Aurantiochytrium* sp. OUC88[13, 15]. Nakahara et al. (1996) reported that pH 4.0 was optimal for DHA production in *A. limacinum* SR21[8]. The same result was obtained in our experiment, with the maximum DHA content of the TFA reaching 50.9%, but the production of biomass and lipid in *Aurantiochytrium*. sp. SD116 was relatively low. These results indicate that some of the carbon source was being used to maintain physiological conditions at low pH, rather than contributing to cell growth and lipid accumulation[15].

Table 2 The fatty acid composition of *Aurantiochytrium* sp. SD116 under different temperatures.

Fatty acids	Temperature (℃)				
	20	25	28	30	37
C14:0	6.33	6.61	7.06	7.35	6.26
C15:0	1.13	1.01	1.54	4.23	10.21
C16:0	34.31	36.64	38.99	40.58	45.63
C16:1 n-7	0.31	0.25	0.33	0.29	0.29
17:0	0.25	0.36	0.75	2.93	7.58
18:0	0.55	0.53	0.67	0.93	0.12
20:0	0.47	0.5	0.62	0.73	1.02
20:4 n-6	0.39	0.43	0.51	0.39	0.88
20:5 n-3	0.52	0.52	0.78	0.59	1.44
22:5 n-6	7.04	8.31	8.33	6.36	6.22
22:6 n-3	48.77	44.32	40.69	35.63	20.51
Saturated	43.04	45.65	49.63	56.75	70.82
Unsaturated	57.03	53.83	50.64	43.26	29.34

3.5 Fermentation experiments for *Aurantiochytrium* sp. SD116

Using the optimized medium and culture conditions described above, the DCW of *Aurantiochytrium* sp. SD116, as well as glucose consumption, lipid production, and DHA

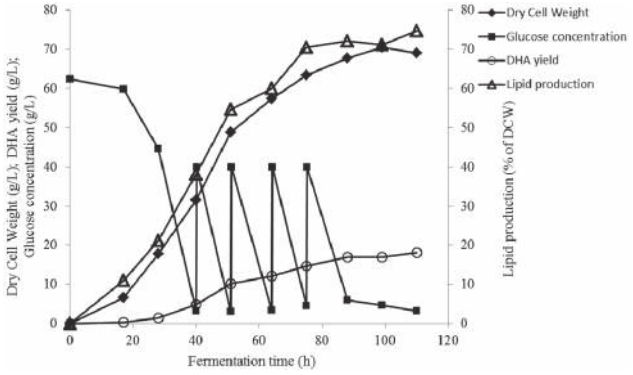

Fig. 6 Fed-batch fermentation of *Aurantiochytrium* sp. SD116. Residual glucose, closed squares; dry cell weight, closed diamonds; lipid production, open triangles; DHA content, open circles.

synthesis, were examined during fed-batch cultivation over 110 h (Fig. 6). It was found that a total glucose concentration of 220 g L^{-1} was consumed, and the maximum DCW was 70.43 g L^{-1} with a lipid accumulation of 71.09% of the DCW (50.07 g L^{-1}) at 99 h. The fatty acid profile of SD116 varied slightly throughout the fermentation period, and the DHA content reached 34.79% of the TFA.

4 DISCUSSION

It is well known that the C/N ratio of the culture medium dramatically affects the lipid content of microorganisms. A high C/N ratio is effective for increasing the lipid content of cells[20]. However, increasing the glucose level resulted in a relatively high C/N ratio, but did not result in an increase in DHA yield in *Aurantiochytrium* sp. SD116, most likely because *Aurantiochytrium* produces DHA through a polyketide synthase (PKS) synthesis pathway, rather than an elongase-desaturase system using the production of the fatty acid synthase (FAS) synthesis pathway. In future research, the FAS synthase inhibitors such as cerulenin and C75 (a synthetic compound used as a fatty acid synthase inhibitor)[21], which have been reported to bind in equimolar ratios to β-keto-acyl-ACP synthase to block the interaction of malonyl-CoA, would be investigated to reduce UFA synthesis activity, thereby increasing PUFA synthesis.

According to Ganuza[22], *Aurantiochytrium* can tolerate a high NH_4^+ concentration (5.8 g L^{-1}). We obtained similar results in our experiment, demonstrating that *Aurantiochytrium* sp. SD116 could endure a urea concentration of 9 g L^{-1}, producing 21.7 g L^{-1} of biomass and 10.3 g L^{-1} of lipid, while the highest biomass (26.5 g L^{-1}) and lipid production (13.9 g L^{-1}) were obtained at a urea concentration of 5 g L^{-1}. In contrast, *A. limacinum* SR21 only grew at a low concentration of urea (0.9g L^{-1})[14]. The high tolerance for urea or NH_4^+ may be the result of long-term acclimation to high NH_4^+ conditions because Shuidong Bay is a mudflat aquaculture area with a high nitrogen fertilizer concentration (convert to N- element, 1.773 mg L^{-1}). These results indicate that urea could be a potential nitrogen source for the industrial fermentation of *Aurantiochytrium* because of its cheap price and its ability to induce relatively high production of biomass and lipid.

Strains isolated from marine mangrove environments often possess a wide tolerance for different salt concentrations. Many strains of *Aurantiochytrium*, such as *A. limacinum* SR21, *A. mangrovei* Sk-02, *Aurantiochytrium* sp. mh0186[14, 23, 24] and *Aurantiochytrium* sp. SD116, prefer a salt concentration of 1.5%, while some *Aurantiochytrium* species produce high levels of DHA at low salinity. Strain BL10 accumulates higher levels of fatty acids when grown at lower salinity (0.5%), and strain KRS101 produces relatively high DCW, lipid content, and DHA levels at optimal sea salt concentrations of 6 g L^{-1}. The preference for low salinity may further benefit the cost saving and quality control aspects of industrial fermentation.

5 CONCLUSION

In summary, the results of this study show that *Aurantiochytrium* sp. SD116 is a promising candidate for commercial DHA production and is useful for generating biomass-related products (Table 3). The results indicate that the growth, lipid profile, and fatty-acid profile of *Aurantiochytrium* sp. SD116 can be enhanced by adjusting the cultivation conditions and medium composition during fermentation.

Table 3 Comparison of the biomass and DHA yield by the strains mentioned in this study.

Strains	Biomass (g/L)	DHA yield (g/L)	Ref.
Aurantiochytrium limacinum OUC88*	24.1	4.8	13
Aurantiochytrium limacinum SR21*	48.1	13.3	14
Aurantiochytrium sp. S31*	5.57	0.308	15
Aurantiochytrium Mangrovei PQ6*	25.34	11.55	16
Aurantiochytrium sp. BL10	59.0	16.8	17
Aurantiochytrium sp. KRS101	50.2	8.8	18
Aurantiochytrium sp. G13/2S*	63.3	2.69	22
Aurantiochytrium mangrovei Sk-02*	28.0	6.0	23
Aurantiochytrium limacinum mh0186	23.1	4.3	24
Aurantiochytrium sp. SD116	70.4	17.4	This study

*These strains were known as *Schizochytrium* prior to 2007.

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