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Isolation and Characterization of a Mesophilic *Arthrospira maxima* Strain Capable of Producing Docosahexaenoic Acid

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A strain of the cyanobacterium *Arthrospira* was isolated from Lake Chahannaoer in northern China and was characterized according to microscopic morphology, photosynthetic oxygen-evolving activity, growth rate, and nutritional profile. Compared with thermophilic *Arthrospira* species occurring naturally in tropical and subtropical lakes, this isolate is mesophilic and grows optimally at ~20°C. The total protein, fatty acid, phycocyanin, carotenoid, and chlorophyll *a* contents were 67.6, 6.1, 4.32, 0.29, and 0.76 grams per 100 grams of dry weight, respectively. The strain is rich in polyunsaturated fatty acids (PUFAs). An essential omega-3 fatty acid, docosahexaenoic acid (DHA), was detected, and γ-linolenic acid (GLA) and DHA accounted for 28.3% of the total fatty acid content. These features of this newly isolated strain make it potentially useful in commercial mass culture in local areas or as a biofuel feedstock. It is also an alternative resource for studying the metabolic PUFA pathways and mechanisms of cold stress tolerance in cyanobacteria.

Keywaords: Polyunsaturated fatty acids (PUFAs), docosahexaenoic acid (DHA), γ-linolenic acid (GLA), mesophilic, *Arthrospira maxima*

During the past three decades, increasing attention has been paid to microalgal biotechnology owing to the potential to produce foodstuffs, industrial chemicals, compounds with therapeutic applications, and bioremediation solutions [2, 25]. Recently, there has been renewed interested in the utilization of microalgae as an alternative biodiesel feedstock because of their high oil content and rapid production of biomass [1, 11, 22]. Microalgal oil includes some critical long-chain polyunsaturated fatty acids (LC-PUFAs) such as eicosapentaenoic acid (EPA), γ-linolenic acid (GLA), and docosahexaenoic acid (DHA) [3, 9].

DHA, an omega-3 fatty acid, is essential for brain function, heart health, and infant development. DHA deficiency causes a variety of human diseases, such as brain disorders, infertility, Alzheimer's disease, and cancer [32]. Adequate amounts of DHA must be obtained through the diet, as humans lack the ability to synthesize it *de novo* [2, 32]. For most people, the main dietary source of DHA is fatty fish; however, most of the DHA from cold-water fish originates in photosynthetic microalgae *via* the food chain, and is typically not suitable for use in infant formulas. The presence of EPA in fish oil significantly lowers growth rates and causes other developmental difficulties [2]. Moreover, the commercial production of LC-PUFAs derived from fish oil may also have some negative aspects, such as an unpleasant odor, the presence of cholesterol, and some safety issues related to the contaminant levels of various toxins accumulated in fish and further concentrated in their oils [2, 14]. In contrast, microalgae-derived PUFAs have no such drawbacks [14]. Recently, some microalgae, including diatoms, chrysophytes, cryptophytes, and dinoflagellates, have been shown to produce high levels of DHA [3, 9]. The DHA-enriched products derived from microalgae have recently become available for commercial use as animal feeds and health foods [2]. Nonetheless, high-DHA microalgae supplements are still in short supply [3, 9, 11]. Moreover, the DHA-enriched products derived from both fish oil and microalgae depend heavily on a series of complicated downstream processes. Hence, there is an urgent demand for the development of inexpensive and environmentally friendly systems to produce high-value DHA.

Arthrospira (*Spirulina*) is an edible cyanobacterium that performs prokaryotic oxygen-evolving photosynthesis, converting CO_2 into organic compounds using the energy from sunlight [7, 28]. It often dominates the plankton of warm lakes that have high carbonate/bicarbonate and pH

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levels [4, 7, 28]. It is characterized by multicellular cylindrical trichomes in an open helix along the entire length of the filaments. *Arthrospira* morphology varies in response to environmental changes [7, 28]. It is cultivated around the world, and two main *Arthrospira* species (*A. maxima* and *A. platensis*) have been widely cultivated for commercial biomass production. *A. platensis* occurs in Africa, Asia, and South America, whereas *A. maxima* was first found in warm salty ponds in California, USA, and then later in many tropical and subtropical areas [16, 28]. *Arthrospira* contains high levels of various pigments (*e.g.*, chlorophyll *a*, phycocyanin, and carotenoids), proteins (about 60–70% of dry weight), vitamins, minerals, and some essential fatty acids (*e.g.*, γ-linolenic and eicosapentaenoic acids) [2, 6, 8, 12, 25, 28]. Recently, *A. maxima* has been found to exhibit a faster water-splitting rate, a larger plastoquinon pool, and higher photosystem II turnover efficiency than other algae and plants. All of these features would be beneficial to biomass production in the commercial application of *Arthrospira* [1]. However, cyanobacterial species capable of producing DHA have rarely been described.

The objective of this work was to describe and characterize a newly isolated cyanobacterium, *Arthrospira maxima* strain NS-LC001, from Lake Chahannaoer, Inner Mongolia, an internal region of China with mild continental climate, abundant solar radiation, and many alkaline lakes favorable to *Arthrospira* [21, 34]. This filamentous cyanobacterium contains high levels of PUFAs, among which DHA has been detected. This new strain has been successfully cultivated under controlled conditions in the laboratory. Its microscopic morphology, photosynthetic oxygen-evolving activity, growth rate, and nutrient requirements were also identified and evaluated. Its potential application to commercial mass culture and basic research fields has also been discussed.

Materials and Methods

Sampling Sites and Isolation of *Arthrospira* Strain

Microalgal samples were collected from Lake Chahannaoer, an inland salt lake in Inner Mongolia, China. This lake lies at the coordinates 108°04' E latitude and 39°14' N longitude, and at an elevation of 1,274 m. The area of the lake is 6.5 km^2 and the depth of the water is 0.1–0.3 m [21, 34].

Two main approaches have been employed for the isolation and purification of this strain, as described by Mutanda *et al*. [22]. The sample suspension was first centrifuged at 500 ×*g* for 1 min and then rinsed three times with autoclaved Zarrouk's medium [33]. The isolation procedure was performed using each of the following methods. (1) Micropipette isolation: single trichomes were taken under an inverted microscope (Olympus IX71) and then each was transferred to a 10 ml test tube containing 1 ml of autoclaved Zarrouk's medium. The same procedure was repeated five times. (2) Sequential dilution method: 0.9 ml of autoclaved Zarrouk's medium was added into 10 tubes. The dense sample (0.1 ml) was taken by pipette, added to the first tube, and then stirred to homogenize. A further 0.1 ml from the first tube was added into the second tube. The same procedure was then repeated eight more times. A 10-fold serial dilution of the samples was thus obtained. Several lots of samples were produced using each method. The isolation process was performed until a unialgal culture was obtained.

Observation of Morphological Features

The original samples collected from Lake Chahannaoer were filtered through 0.45 μm membranes and washed with fresh Zarrouk's medium three times. The cell suspension was observed under an Olympus BX51 microscope with an Olympus DP70 camera attachment (Olympus, Japan).

Measurement of Net Photosynthetic Oxygen-Evolving Rate and Assessment of Growth

Samples of the purified culture suspension were diluted with fresh Zarrouk's medium to a final chlorophyll concentration of 2.5 μg/ml. Initially, the dark-adapted (~15 min) cells were exposed to a modulated measuring beam of 150 μE m^{-2} s^{-1}. The net photosynthetic oxygen-evolving rate was measured using an RSS5100 portable dissolved oxygen meter (REX, Shanghai, China) [31]. The temperature was controlled by an LKB 2219 Multi Temp II thermostatic circulator (Pharmacia, Sweden).

The growth pattern of *A. maxima* NS-LC001 was plotted based on the cell density (optical density measured at 580 nm) at different temperatures in Zarrouk's medium. The cells were inoculated into a 250 ml Erlenmeyer flask containing 50 ml of medium. The cultures were grown at 20°C under continuous irradiation provided by a fluorescent lamp (150 μE m^{-2} s^{-1}). Each experiment was repeated three times. A 1 ml sample was removed from the culture flasks each day for measurement of the optical density at 580 nm.

Determination of Biochemical Composition

Cultures grown to the logarithmic phase were harvested to assay biochemical composition. A 200 ml aliquot of culture was collected and then washed five times with 0.2 M Tris-HCl buffer (pH 7.5). After drying, the resulting powder was weighed. To estimate pigment content (chlorophyll and carotenoid), one-tenth of the powder was resuspended in 10 ml of ice-cold methanol. The mixture was maintained for 2 h at 4°C with agitation in the dark. Chlorophyll *a* and total carotenoid content was spectrophotometrically measured as previously described [13]. To determine the phycocyanin content, one-tenth of the powder was resuspended in 0.2 M Tris-HCl buffer (pH 7.5) and incubated with 1.5 mg of lysozyme overnight at 30°C with constant shaking. The phycocyanin content of the supernatant after centrifugation was then analyzed with a UV–VIS 752C spectrophotometer (No. 3 Analytical Instrument Factory, Shanghai, China). The amount of phycocyanin was calculated as described by Götz *et al*. [13]. Total protein content as a percentage of the dry weight was determined following the Lowry method as modified by Herbert *et al*. [15]. Total cell lipids were extracted with chloroform/methanol [2:1 (v/v)] and determined gravimetrically after removal of the chloroform phase and evaporation of the chloroform under vacuum. The lipid residue was transmethylated with sodium methoxide plus the fatty acid methyl esters and then analyzed by gas chromatography [5, 24, 30].

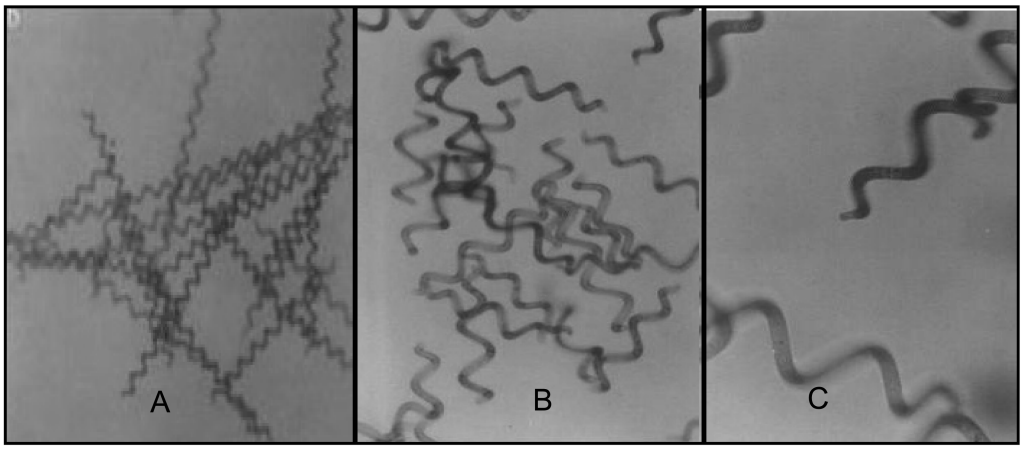

Fig. 1. Microphotographs of *Arthrospira maxima* NS-LC001 culture after washing with fresh Zarrouk's medium.
Panels **A**, **B**, and **C**: long trichomes, short trichomes, and trichomes with a cap-like cellular end, respectively.

Statistical Analysis

Data were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range test. A value of $p < 0.05$ was considered significant. Each value was based on three repetitions, and the corresponding data are presented as mean ± standard deviation ($n = 3$).

RESULTS AND DISCUSSION

Isolation, Purification, and Morphological Study of *Arthrospira* Strain NS-LC001

Arthrospira maxima NS-LC001 was isolated from Lake Chahannaoer in Inner Mongolia, China. To rapidly purify this filamentous strain, isolation of the cyanobacterial trichomes exploited either the micropipetting or serial dilution method (Fig. 1A). Eventually, a unialgal culture was obtained by each method for further evaluation of growth and assay of nutrient components.

Microscopic observation showed that this newly isolated strain is a spiral-shaped cyanobacterium having unbranched trichomes with constrictions between cells (Fig. 1). This alga is also free-floating, which is probably due to the presence of irregular gas vacuoles inside the cells. The size of the trichomes varied with different growth phases (Fig. 1A and 1B, long and short filaments, respectively). No heterocysts or akinetes were present on the filament. The typical *A. maxima* morphological feature of a trichome with a cap-like cellular end was observed (Fig. 1C). Further molecular identification was carried out and will be described elsewhere.

Growth Evaluation of *A. maxima* NS-LC001

To determine the optimal temperature for culturing *A. maxima* NS-LC001, we first measured its net photosynthetic oxygen-evolving rate at different temperatures from 10 to 40°C as previously described [31]. As shown in Fig. 2, the net photosynthetic oxygen-evolving rate of the cells was high at 15–25°C, with the highest rate at 20°C (up to 278 μmol O_2 h^{-1} mg^{-1} Chl *a*).

The growth of *A. maxima* NS-LC001 was evaluated at different temperatures. The growth patterns were studied under controlled conditions over a period of 10 days and are depicted in Fig. 3. The growth rate followed the common patterns of many other microorganisms that undergo a simple cell division without any sexual or differentiation step. There were significant variations at different temperatures. This strain was able to grow at 10–40°C, indicating its potential photosynthetic response and adaptation to a wide range of temperatures. By taking advantage of this feature, this isolate could be cultivated on a large scale in a local

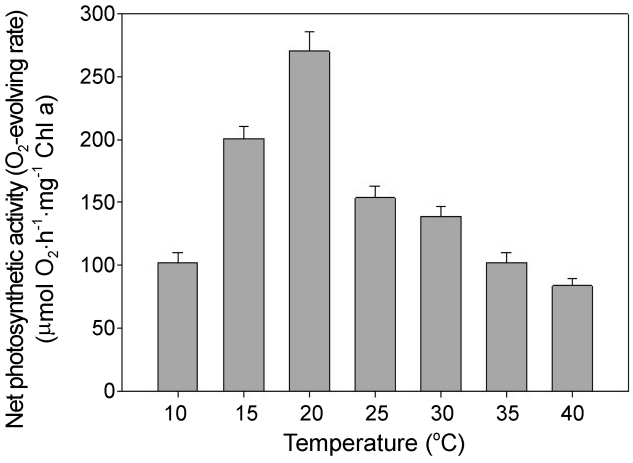

Fig. 2. Effect of temperature on the net photosynthetic O_2-evolving activity of *Arthrospira maxima* NS-LC001.
The cells were inoculated in Zarrouk's medium (adjusted to pH 9.0) at a concentration of 2.5 μg Chl *a* per milliliter and exposed to 150 μmol photons m^{-2} s^{-1} at different temperatures.

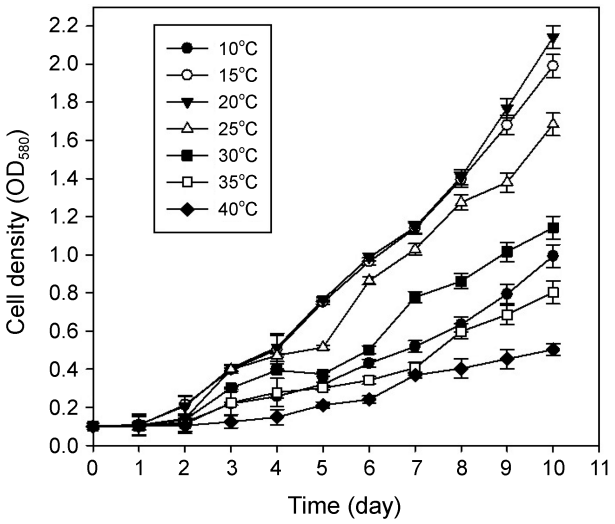

Fig. 3. Growth pattern of *Arthrospira maxima* NS-LC001 in Zarrouk's medium.
Cultures were inoculated at 10 (●), 15 (○), 20 (▼), 25 (△), 30 (■), 35 (□), and 40°C (◆).

area. The growth period of this *Arthrospira* strain could be prolonged considerably compared with other strains, from ~120 to ~165 days in Inner Mongolia, thereby decreasing the cost of production associated with limiting regional temperatures by 25–30% [21].

The preference of *A. maxima* NS-LC001 for moderate temperatures was evident since the growth rate was high at 15–25°C, with the highest growth rate occurring at ~20°C. This result agreed with the measurement of net photosynthetic oxygen-evolving rates at different temperatures (Fig. 2). Likewise, the optimal growth temperature of this strain in the laboratory is consistent with that in its natural habitat, where the average temperature is −9–13°C in January and 20–26°C in July [34]. This suggests that the growth of this mesophilic strain is stable in the laboratory, making it a suitable candidate for use as an algal species for mass culture.

To the best of our knowledge, the mesophilic *Arthrospira* strain we present here is the first to be isolated from China. Its unique optimal growth temperature (OPG, ~20°C) is much lower than the typical OPG (~30–35°C) of thermophilic *Arthrospira* strains [28]. Compared with the thermophilic *Arthrospira* species found in Lake Chad (Mid-Africa) and Lake Texcoco (Mexico) [7], this mesophilic strain isolated from Lake Chahannaoer (Inner Mongolia, China) grows at a higher elevation (1,274 m), higher latitude (108°04' E), and lower annual average temperature (6.6°C) [34]. Thus, we propose that the adaptation of this isolate to such a geographical and ecological environment is compatible with its mesophilic growth characteristic. Moreover, this isolate can be successfully grown in an open pond (~830 m^2) at 10°C (data not shown), implying that it can tolerant low temperatures.

Major Nutritional Profile of *A. maxima* NS-LC001

Owing to its mesophilic nature, we grew *A. maxima* NS-LC001 at 20–22°C in the laboratory for analysis of biochemical composition. We assayed the macronutrients and some phytonutrients of this strain. The total protein, fatty acid, phycocyanin, carotenoid, and chlorophyll *a* contents were 67.6, 6.1, 4.32, 0.29, and 0.76 grams per 100 grams of dry weight, respectively (Table 1). The total protein and fatty acid contents were equal to or higher than the 63 g and 4.3 g per 100 g dry weight, respectively, reported in the literature for *Arthrospira* [4].

Of particular interest to us was the fatty acid (FA) profile of the new isolate. As shown in Table 1, the saturated, polyunsaturated, and monounsaturated FA contents were 2.7, 2.6, and 0.8 grams per 100 grams of dry weight, respectively. Table 2 lists the contents of the major FA components (milligrams per gram of dry weight) and their relative contents (% of total FAs). The five unsaturated FAs [16:1, 18:1, 18:2(ω-6), 18:3(ω-6), and 22:6(ω-3)] and saturated FAs detected accounted for 57.8% and 41.1% of the total FA content, respectively. There were four dominant FAs, (*i.e.*, GLA, palmitic, palmitoleic, and linoleic acids), which accounted for 90% of the total FAs (Table 2). The GLA content was up to 16.94 mg/g (dry weight) (26.07% of total fatty acids). This GLA content is higher than that of the wild-type and mutagenized thermophilic *Arthrospira* species reported in the literature, ~8–10 and 15.6 mg/g (dry weight), respectively [28, 30]. Interestingly, DHA, which has rarely been found in cyanobacteria, was stably detected at 2.2% of total FAs, and GLA and DHA comprised up to 28.3% of the total FAs. No EPA was detected in this strain under the same assay conditions (Table 2).

Nutrient composition and content including fatty acids are likely to vary with the age of the culture, cultivation temperature, light and medium components, and even cell harvesting time, and so on [10, 23, 26, 27, 30]. However,

Table 1. Nutritional profile of *Arthrospira maxima* NS-LC001 powder.

Composition	per 100 g
1. *Macronutrients*	
Total proteins	67.6 ± 0.05 g
Total fatty acids	6.1 ± 0.03 g
Saturated fatty acids	2.7 ± 0.01 g
Polyunsaturated fatty acids	2.6 ± 0.01 g
Monounsaturated fatty acids	0.8 ± 0.006 g
Cholesterol	<0.1 mg
2. *Phytonutrients*	
Phycocyanin (mean)	4.32 ± 0.02 g
Total carotenoids (mean)	0.29 ± 0.05 g
Chlorophyll *a* (mean)	0.76 ± 0.005 g

Data presented as mean ± standard deviation (n = 3), $p < 0.05$.

Table 2. Major fatty acid composition of *Arthrospira maxima* NS-LC001.

Component	Name	Content (mg/g dry weight)	Relative content (% of total fatty acids)
16:0	Palmitic acid	24.88 ± 0.03	36.9
16:1	Palmitoleic acid	6.43 ± 0.01	9.8
18:0	Stearic acid	0.77 ± 0.003	1.2
18:1	Oleic acid	1.81 ± 0.004	2.6
18:2(ω-6)	Linoleic acid (LA)	1.11 ± 0.004	17.1
18:3(ω-6)	γ-Linolenic acid(GLA)	16.94 ± 0.06	26.1
20:0	Arachidic acid (AA)	1.45 ± 0.003	3.0
22:6(ω-3)	Docosahexaenoic acid (DHA)	7.88 ± 0.01	2.2
Others		0.74 ± 0.003	1.1

Data presented as mean ± standard deviation (n = 3), $p < 0.05$.

our experiments performed under defined conditions in the laboratory led to stable protein content and fatty acid profile (data shown as means with no significant differences). The stability of nutrient composition and the FA profile may result from adaptation of *A. maxima* NS-LC001 to natural habitats. This mesophilic strain is capable of stably producing DHA, which will allow us to produce high-value, large-scale commercial mass cultures. So far, we have successfully exploited natural waters for mass culture of *Arthrospira* at 10–30°C (data not shown). This will not only extend yearly production cycles, but will also reduce the production cost associated with medium preparation for mass culture.

Since this mesophilic strain is capable of producing DHA, its PUFA metabolic pathway is different from that of other cyanobacterial strains. Moreover, DHA accumulates mainly in the form of triacylglycerols, which are commonly recognized as important feedstocks for biofuel production [17]. Thus, this new DHA-producing isolate is a valuable resource not only for the investigation of the cyanobacterial PUFA metabolic pathway, but also for use as a biofuel feedstock [17]. Considering that this is the first DHA-producing *Arthrospira* strain to be found in China, we believe that it will also provide us with deep insights into some interesting evolutionary clues regarding fatty acid metabolism. DHA, the longest and most unsaturated fatty acid chain commonly found in biological membranes, is related to membrane fluidity [18] and plays a critical role in low-temperature stress tolerance [19], factors which are likely related to the mesophilic nature of *A. maxima* NS-LC001. Likewise, this strain has the potential to become an alternative resource to study mechanisms of low-temperature stress tolerance [19, 29].

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References

1. Ananyev, G., D. Carrieri, and G. C. Dismukes. 2008. Optimization of metabolic capacity and flux through environmental cues to maximize hydrogen production by the cyanobacterium *Arthrospira* (*Spirulina*) *maxima*. *Appl. Environ. Microbiol.* **74:** 6102–6113.
2. Apt, K. E. and P. W. Behrens. 1999. Commercial developments in microalgal biotechnology. *J. Phycol.* **35:** 215–226.
3. Behrens, P. W. and D. J. Kyle. 1996. Microalgae as a source of fatty acids. *J. Food Lipids* **3:** 259–272.
4. Belay, A. 2008. *Spirulina* (*Arthrospira*): Production and quality assurance, pp. 1–25. *In* M. E. Gershwin and A. Belay (eds.). *Spirulina in Human Nutrition and Health*. CRC Press, London/ New York.
5. Carreau, J. P. and J. P. Dubacq. 1978. Adaptation of a macro-scale method to the micro-scale for fatty acid methyl transesterification of biological lipid extracts. *J. Chromatogr.* **151:** 384–390.
6. Choi, G.-G., M.-S. Bae, C.-Y. Ahn, and H.-M. Oh. 2008. Enhanced biomass and γ-linolenic acid production of mutant strain *Arthrospira platensis*. *J. Microbiol. Biotechnol.* **18:** 539–544.
7. Ciferri, O. 1983. *Spirulina*, the edible microorganism. *Microbiol. Rev.* **47:** 551–578.
8. Ciferri, O. and O. Tiboni. 1985. The biochemistry and industrial potential of *Spirulina*. *Annu. Rev. Microbiol.* **89:** 503–526.
9. Cohen, Z., H. A. Norman, and Y. M. Heimer. 1995. Microalgae as a source of omega-3 fatty acids. *World Rev. Nutr. Diet* **77:** 1–31.
10. Colla, L. M., C. O. Reinehr, C. Reichert, and J. A. V. Costa. 2007. Production of biomass and nutraceutical compounds by *Spirulina platensis* under different temperature and nitrogen regimes. *Bioresour. Technol.* **98:** 1489–1493.
11. Ethier, S., K. Woisard, D. Vaughan, and Z. Wen. 2011. Continuous culture of the microalgae *Schizochytrium limacinum* on biodiesel-derived crude glycerol for producing docosahexaenoic acid. *Bioresour. Technol.* **102:** 88–93.
12. Franke, H., M. Springer, O. Pulz, U. Tietz, and U. Mueller. 1994. Polyunsaturated fatty acids from microalgae. *Int. Food Ingred.* **4:** 41–45.

13. Götz, T., U. Windhövel, P. Böger, and G. Sandmann. 1999. Protection of photosynthesis against ultraviolet-B radiation by carotenoids in transformants of the cyanobacterium *Synechococcus* PCC7942. *Plant Physiol*. **120:** 599–604.
14. Grima, E. M., R. A. Medina, and G. A. Gimenez. 1999. Recovery of algal PUFAs, pp. 108–144. *In* Z. Cohen (ed.). *Chemicals from Microalgae*. Taylor & Francis, London.
15. Herbert, D., P. J. Phipps, and R. E. Strange. 1971. Chemical analysis of microbial cells, pp. 209–344. *In* J. R. Norris and D. W. Ribbons (eds.). *Method in Microbiology*. Academic Press, London/New York.
16. Hu, H. 2003. *The Biology and Biotechnology of Spirulina (Arthrospira)* [in Chinese]. Science Press (co-published with Springer-Verlag GmbH), Beijing.
17. Hu, Q., M. Sommerfeld, E. Jarvis, M. Ghirardi, M. Posewitz, M. Seibert, and A. Darzins. 2008. Microalgal triacylglycerols as feed stocks for biofuel production: Perspectives and advances. *Plant J.* **54:** 621–639.
18. Jenski, L. J. and W. Stillwell. 2001. Role of docosahexaenoic acid in determing membrane structure and function, pp. 41–62. *In* D. Mostofsky, S. Yehuda, and N. Jr. Salem (eds.). *Fatty Acids: Physiological and Behavioral Functions*. Humana Press Inc., Totowa, NJ.
19. Kanazawa, A. 1997. Effects of docosahexaenoic acid and phospholipids on stress tolerance of fish. *Aquaculture* **155:** 129–134.
20. Li, D. M. and Y. Z. Qi. 1997. *Spirulina* industry in China: Present status and future prospects. *J. Appl. Phycol.* **9:** 25–28.
21. Lu, Y. M., W. Z. Xiang, and Y. H. Wen. 2010. *Spirulina* (*Arthrospira*) industry in Inner Mongolia of China: Current status and prospects. *J. Appl. Phycol*. **23:** 265–269.
22. Mutanda T., D. Ramesh, S. Karthikeyan, S. Kumari, A. Anandraj, and F. Bux. 2011. Bioprospecting for hyper-lipid producing microalgal strains for sustainable biofuel production. *Bioresour. Technol.* **102:** 57–70.
23. Mühling, M., A. Belay, and B. A. Whitton. 2005. Variation in fatty acid composition of *Arthrospira* (*Spirulina*) strains. *J. Appl. Phycol.* **17:** 137–146.
24. Rupp, H., P. T. Rupp, D. Wagner, P. Alter, and B. Maisch. 2006. Microdetermination of fatty acids by gas chromatography and cardiovascular risk stratification by the “EPA+DHA level”, pp. 47–79. *In* B. Maisch and R. Oelze (eds.). *Cardiovascular Benefits of Omega-3 Polyunsaturated Fatty Acids*. IOS Press, Amsterdam.
25. Sanghvi, A. M. and Y. M. Lo. 2010. Present and potential industrial applications of macro- and microalgae. *Recent Pat. Food Nutr. Agric.* **2:** 187–194.
26. Tanticharoen, M., M. Reungjitchachawali, B. Boonag, P. Vonktaveesuk, A. Vonshak, and Z. Cohen. 1994. Optimization of gamma-linolenic acid (GLA) production in *Spirulina platensis*. *J. Appl. Phycol.* **6:** 295–300.
27. Tomaselli, L., L. Giovannetti, A. Sacchi, and F. Bocci. 1988. Effects of temperature on growth and biochemical composition in *Spirulina platensis* strain M2, pp. 303–314. *In* T. Stadler, J. Mellion, M. C. Verdus, Y. Karamanos, H. Morvan, and D. Christiaen (eds.). *Algal Biotechnology*. Elsevier Applied Science, London.
28. Vonshak, A. 1997. *Spirulina*: Growth, physiology and biochemistry, pp. 175–204. *In* A. Vonshak (ed.). *Spirulina platensis* (*Arthrospira*): *Physiology*, *Cell Biology and Biotechnology*. Taylor & Francis Ltd., London.
29. Wada, H., Z. Gombos, and N. Murata. 1994. Contribution of membrane lipids to the ability of the photosynthetic machinery to tolerate temperature stress. *Proc. Natl. Acad. Sci. USA* **91:** 4273–4277.
30. Yin, C., H. Hu, X. Gong, and Y. Geng. 1997. Effects of cultural conditions on fatty acid composition and content in *Spirulina platensis*. *J. Wuhan Bot. Res.* **15:** 59–65.
31. Yin, C., H. Hu, Y. Li, X. Gong, and D. Shi. 1997. Studies on middle temperature strains selection of *Spirulina platensis*. *J. Wuhan Bot. Res*. **15:** 250–254.
32. Yurko-Mauro, K. 2010. Cognitive and cardiovascular benefits of docosahexaenoic acid in aging and cognitive decline. *Curr. Alzheimer Res.* **7:** 190–196.
33. Zarrouk, C. 1966. Contribution à l’étude d’une cyanophycée. Influènce de divers facteurs physiques et chimiques sur la croissance et la photosynthèse de *Spirulina maxima* (Setch. et Gardner) Geitl. Thèse de Doctorat d’Etat, Université de Paris, France.
34. Zheng, X., M. Zhang, J. Dong, Z. Gao, C. Xu, Z. Han, B. Zhang, D. Sun, and K. Wang. 1992. *The Salt Lakes in Inner Mongolia, China* [in Chinese], pp. 248–250. Science Press, Beijing, China.