0273-2289/95/5301—0091$05.60

The Studies About Some Characteristics of *Spirulina subsalsa var.* from Laboratory to Outdoors

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Received April 4, 1994; Accepted August 2, 1994

ABSTRACT

Some important characteristics of *Spirulina subsalsa var. crassior virieux* have been investigated in this article. At the 36°C, the growth rate K of this alga is 0.18 and the yield as high as 48.4 $g/m^2/d$ (dry wt). In practice, a yield with 17.7 $g/m^2/d$ was reached by mass culture outdoors in the natural conditions of Hangzhou, China. The protein content of this alga was over 50.30% in its dry matter. The composition and content of its amino acids and superoxide dismutase were analyzed. The results indicate that the *Spirulina subsalsa var.* is a promising thermophilic species for algal protein production.

Index Entries: *Spirulina subsalsa var.*; growth rate; protein; amino acids; superoxide dismutase.

INTRODUCTION

Exploring new sources of protein has recently become an important aim because of the food crisis that is threatening to humans, the number of whom is increasing at a high rate. During the last decade, the mass culture of *Spirulina* was developed by more than 20 countries, including China (*1–3*). The vast results demonstrate that the biomass yield of *Spirulina* may have reached 40 $g/m^2/d$ and the protein content of this alga ranges from 45 to 70% under the controllable conditions (*4*).

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So far, two species of *Spirulina*, *S. platensis* and *S. maxima*, have been widely produced as a native healthful diet for humans. They were separately discovered in Chad and Mexico 50 years ago. In this article, a rare species, *Spirulina subsalsa var. crassior virieux*, was further studied by us. This alga has never been declared in mass culture by anyone else since the Kaixian and Sato's publication appeared in the USA in 1984 (*5*).

The morphological character of *Spirulina subsalsa var.* is very different from *S. platensis* and *S. maxima*. Its filament looks like a straight string under the microscope. In fact, the filament twines along the longitudinal axis into a very tight helix with equal width. Under the scanning electron microscope, the filament appears to be a tight anticlockwise spiral structure.

In this article, the growth rate and some compositions of *Spirulina subsalsa var.* under laboratory conditions or mass culture outdoors are reported.

MATERIALS AND METHODS

Materials

About 12 filamental cells of *Spirulina subsalsa var.* were discovered and isolated by the author (Kaixian, Q) from a hot spring (about 32°C) situated at the southern desert of California, in 1983. This *Spirulina* was then discovered by us from a hot spring at Nanjing in China in 1986.

Scanning Electron Microscopy

Algal filaments were dispersed on 0.22-μm nitrocellulose filters. The filaments were rinsed several times with buffer solution of 0.1*M* sodium cacodylate + 0.05% $CaCl_2 \cdot 2H_2O$ at pH 7.4 and fixed with 2.5% glutaraldehyde in the same buffer for 4 h at 4°C, postfixed with 1% aqueous osmium tetroxide for 2 h at room temperature, and then dehydrated through a graded ethanol series (*6*). The filaments were critical-point-dried using 100% ethanol as the intermediate fluid and carbon dioxide as the transitional fluid. The algae were then gold-coated in a Desk-1 sputter coater and viewed in an AMRAY 1200 microscope at an acceleration voltage of 15 kV.

Protein Assay

The method of Lowry et al. was used (*7*).

Analysis of Amino Acids

To 15 mg of dry algae were added 500 mL 6*N* HCl, which were digested for 24 h at 110°C. It took 1 mL of sample to determine the composition and content of amino acids in the Backman System 6300 Analyzer.

Determination of Superoxide Dismutase (SOD)

Five hundred grams of fresh algae were subjected to ultrasound to break cells in phosphate buffer, pH 7.8. The solution was centrifuged, then the protein was precipitated from supernatant by means of salting out in 85% saturated ammonium sulfate. The precipitates were separated and eluted stepwise by a chromatographic column of DEAE-23, CM-52, DEAE-52, and Sephadex G-75 (*8*). The purified SOD fraction was collected finally and the enzymic content was measured by Bradford's method (*9*), whereas the enzymic activity was tested according to NBT procedure (*10*).

Algal Experimental Conditions

The algal mass was collected by filtering and homogenizing into a dense suspension as inoculum for experiments. The inoculating density was 2 mL homogenized algal suspension/100 mL medium. A 1-L glass flask was used as experimental vessel and the inoculum were incubated in water baths at 36 or 26°C, which were controlled by WMZK-01 thermostats. The light intensity for the experiment was 72 $\mu E \cdot M^{-2} \cdot s^{-1}$, which illuminate from cool white fluorescent tubes.

The growth medium consisted of $NaNO_3$ (2.5 g/L), K_2HPO_4 (0.5 g/L), $NaHCO_3$ (10 g/L), NaCl (1 g/L), $MgSO_4 \cdot 7H_2O$ (0.2 g/L), $CaCl_2 \cdot 2H_2O$ (0.02 g/L), $FeSO_4 \cdot 7H_2O$ (0.01 g/L). The pH was adjusted to 9 with NaOH.

In the outdoors, the culture of *Spirulina subsalsa var.* was expanded to a large scale with 30-m^2 cement pond (3 × 10 × 0.5 m) and three 200-m^2 cement ponds (5 × 40 × 0.5 m). The ponds were located in a place where sunshine exposure was abundant. They were covered with transparent plastic sheets to protect from contamination and conserve heat (Fig. 1). The ponds were constructed by the Orient Blue-Algae Company, which is situated in the north of Hangzhou, Zhejiang Province. The algal biomass was harvested by dredging it up through a gauze screen.

RESULTS

Growth Rate

At the beginning of the test, the density of inoculated algae was 0.1280 ± 0.002 g/L in 1-L glass flasks. After having grown for 9 d at 26°C, the weight of the algae went up to 1.0640 ± 0.0050 g/L, an increase of about 8.3-fold totally or 0.1040 ± 0.0035 g/L/d. The growth rate K_1 (at 26°C) was 0.04 according to calculation by the equation: $K = \text{Log}\,(N / N_0)$. After having grown for 9 d at 36°C, the weight of the algae rose to 1.4360 ± 0.0040 g/L. That is an increase of 13.5-fold, which means an increase to 0.1453 ± 0.0030 g/L/d. The growth rate K_2 (at 36°C) was 0.18 (Fig. 2).

Fig. 1. Photograph of cement pond with transparent plastic sheets for mass culture of *Spirulina subsalsa var.* in outdoor at Hangzhou, China.

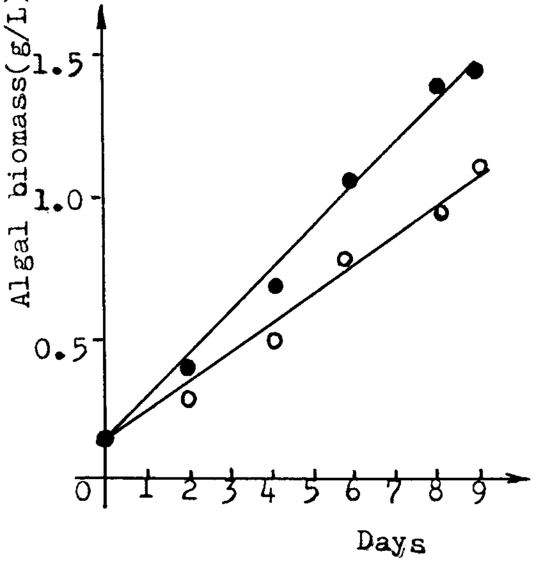

Fig. 2. The growth curves of *Spirulina subsalsa var.* vs the different temperature. ● 36°C, O 26°C.

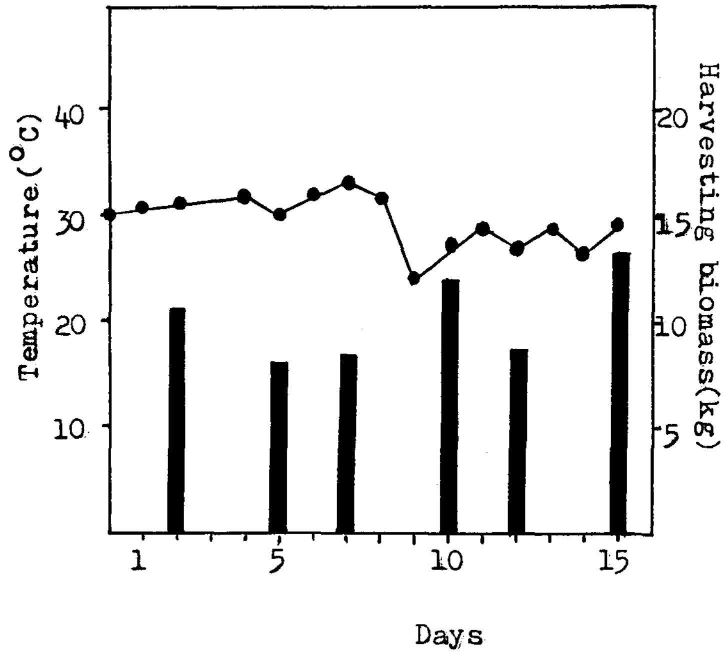

Fig. 3. The temperature curve and harvesting biomass of *Spirulina subsalsa var.* cultured in a 30-m³ pond July 30 to August 14, 1993. ● Temperature (°C), ——— harvesting biomass (kg).

Theoretically, according to the calculation of the growth rate, the yield of *Spirulina subsalsa var.* was 34.7 g/m²/d at 26°C. At 36°C, the yield of algae could reach 48.4 g/m²/d which is much more than the yield at 26°C. In other words, the theoretical yield of this alga is as high as 50,506 kg/acre/yr (dry wt).

The practical yield of *Spirulina subsalsa var.* was obtained from the results of successive production in a 30-m² outdoor pond from July 30 to August 13, 1993. The temperature was measured at 9 AM every day (Fig. 3). One kilogram of fresh algal mass was inoculated into the pond on July 30, and total fresh biomass of 79.5 kg was harvested separately on August 1, 4, 6, 9, 11, 13, and 14 (Fig. 3). The fresh algal mass was dried instantly by heat process into dry powder weighing 7.96 kg. The calculated annual yield of *Spirulina subsalsa var.* was approx 25,740 kg/acre (dry wt) in the outdoor mass culture under the experimental conditions.

Protein Content

Under the experimental conditions, the protein content of *Spirulina subsalsa var.* was in range of 50.64 ± 0.40% to 50.30 ± 0.30% when this alga grew at 26 and 36°C, respectively. In fact, the rates of protein synthesis and accumulation in the algal cells were almost same in the range of experimental temperatures (Table 1). The protein yield of *Spirulina subsalsa var.* reach a value one-half of algal biomass yield in the artificial production.

Table 1
The Protein Content of *Spirulina subsalsa var.* Grown at Different Temperatures

	At 26°C			At 36°C		
	NO. 1	NO. 2	NO 3	NO. 1	NO. 2	NO. 3
Protein content (%)	49.31	50.64	51.99	50.89	51.05	48.96
Average value		50.64 ± 0.40			50.30 ± 0.30	

Table 2
The Composition and Content of Amino Acids
of *Spirulina subsalsa var.* Under the Different Growth Temperatures

	Content of amino acids, %	
Amino acids	At 36°C	At 26°C
Arginine	3.266	3.174
Aspartic acid	4.364	4.164
Threonine	2.550	2.491
Serine	2.528	2.421
Glutamic acid	6.147	5.736
Proline	2.473	2.430
Glycine	2.738	2.685
Alanine	3.928	3.701
Valine	2.873	2.859
Methionine	1.508	1.593
Isoleucine	2.669	2.628
Leucine	3.988	3.917
Tyrosine	2.278	2.327
Cystine	0.988	0.902
Phenylalanine	2.402	2.487
Histidine	1.904	1.842
Lysine	2.424	2.338

Amino Acids

The composition and content of amino acids have been analyzed by amino acid auto-analyzer from algal dry powder. There are 17 kinds of amino acids listed in Table 2, in which the tryptophane was damaged by the procedure of acidic hydrolysis on samples. It is evident that the difference is slight among the contents of these amino acids when the algae grew at 26 or 36°C. Among all amino acids, the content of glutamine is the highest, followed by asparagine, leucine, alanine, and arginine, whereas the contents of histidine, methionine, and cystine successively decrease in comparison with relative major amino acids as above (Table 2).

Table 3
The Activity Value of Fe-Superoxide Dismutase
of *Spirulina subsalsa var.* in the Progress of Enzymic Isolation and Purification

Procedures	Activity value, U/mg	Purified folds
Crude extract	144.7	1
Precipitation of saturated ammonium sulfate	312.4	2
Elute from DE-23 and CM-52 column	1165.3	10.2
Elute from DE-52 column	4698.0	32.5
Elute from Sephadex G-75 column	5577.7	38.6

Superoxide Dismutase

The superoxide dismutase (EC 1.15.1.1) has been isolated and purified from the extract of *Spirulina subsalsa var.* The electrophoretic belts on SDS-polyacrylamide gel showed that this enzyme consists of two equal subunits with the mol wt of about 35.000.

Each molecule of superoxide dismutase contains one atom of iron, which was analyzed in an atomic absorption spectrophotometer. The amount of Fe-superoxide dismutase of this alga appears to be a considerable value, possibly as much as 0.09% of algal dry wt. In practice, 25.05 mg Fe-superoxide dismutase were collected from 500 g of fresh algal mass in this experiment according to the procedures described in this article.

The activity value is an important parameter for superoxide dismutase, which progressively increases with each step of the purificated procedure and the achieved maximum value is as much as 5577.7 U/mg at termination (Table 3).

DISCUSSION

The characteristics of growth and composition of *Spirulina subsalsa var.* make it evident that this alga is a promising species for mass culture for the propose of protein production. The optimal temperature for *Spirulina subsalsa var.* (36°C) suggests the possibility of culturing this alga. Richmond and Preiss pointed out that algal growth increases exponentially with temperature until an optimal temperature is reached (*11*). Daytime air temperature in the summer in Hangzhou approaches 32°C on average. The water in ponds would usually be a few degrees warmer. Hence, normal temperature in daytime in the summer would fall in a range that is suitable for this algal production at a maximal yield. Our outdoor algal agriculture under the solar irradiation of 0.30 kcal/m^2/d in August gives a yield of 17.7 g/m^2/d (dry matter) in practice, which is much less than its theoretical yield of 48 g/m^2/d at 36°C in laboratory conditions.

Increasing the algal yield in mass culture under natural conditions is realizable if the technological design is improved, including the avariable heat conservation, favorable irradiation, nutrient supplement, successive harvest, and so forth. For example, the problem of heat conservation of the culture could be solved by the utilization of waste heat water of industrial enterprise, which can be pumped into a metal pipe immersed in the pond and used to adjust the culture temperature up to the optimal range.

Why was the superoxide dismutase of this alga measured independently here? This is an unexpected discovery. In practice, the superoxide dismutase was abundantly present in the fraction of this algal protein extract when it was studied by chromatographic column at our laboratory. Surprisingly, the cellular protein of *Spirulina subsalsa var.* is probably a promising new source of superoxide dismutase for enzyme preparation.

ACKNOWLEDGMENT

This project was supported by grant from Zhejiang province Natural Science foundation.

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