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CHEMICAL COMPOSITION AND SELENIUM DISTRIBUTION IN SELENIUM ENRICHED *Spirulina platensis* BIOMASS

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Conditions for cultivating selenium enriched Spirulina platensis *were developed. The protein, carbohydrate, lipids, and pigments were determined. The organic selenium and its distribution were analyzed.*

Key words: *Spirulina platensis*, selenium, trace elements, protein, polysaccharide, carbohydrate, lipids, fatty acid, pigments.

Selenium (Se) is an essential trace element for animals. The requirement of Se for human health has been well documented [1]. Selenium function in body has been primarily shown in glutathione peroxidase (GPx) [2] and selenoproteins, which are involved in removal of reactive oxygen species (ROS) produced during oxidative stress in cells [3]. Due to excessive farming of soils, dietary Se intake is generally low in many countries. This is responsible for some Se deficiency disorders, including Kesan diseases. In the past decades, Se supplementation has been performed in China and succeeded in preventing endemic diseases of Se deficiency [4]. Selenium enriched plants such as high Se *broccoli*, Se rich tea, and Se enriched garlic may be potential bioresources of Se because the bio-availability of organic selenium in plant materials appears to be good [5, 6].

S. platensis (SP) is a type of cyanobacterium that has been widely used as a dietary supplement and cultivated to produce biologically active food additives [7, 8]. Its therapeutic values have also been very well documented, including against virus infection of herpes, influenza, and human immunodeficiency virus (HIV), as well as beneficial effects on hypertension, cancer, and weak immune systems. Moreover, the water-soluble pigment has been determined to have significant antioxidant and radical scavenging properties [9].

However, the Se content in natural *S. platensis* biomass is low. By way of aquatic medium and mass production in practice, *S. platensis* may be an easy Se supplementable microalga. The use of *S. platensis* as a matrix for the production of Se containing pharmaceuticals has been shown experimentally [10]. Cases et al [11] reported that Se in the retentate (>30 kDa) separated from Se-enriched *S. platensis* (SeSP) is highly bioavailable and represents an interesting source of Se for food supplementation. Li et al [12] demonstrated that inorganic sodium selenite could be transformed into organic forms by *S. platensis* cultivated by adding sodium selenite in media, through binding with protein, lipids, polysaccharides, and other cell components. Based on these studies, it is concluded that *S. platensis* behaves as an excellent active Se carrier.

Both the nutritional and therapeutic values of *S. platensis* are due to an increased content of protein and other biologically active substances.The goal of our investigations was to develop the cultivation conditions of Se enriched *S. platensis* and to study the protein, carbohydrate, lipids, pigments, water soluble vitamins, as well as trace elements in Se enriched *S. platensis* biomass. We disscussed the Se distribution in the biomolecule of SeSP also.

According to previous investigation [12], although the growth of *S. platensis* could be stimulated by sodium selenite below 400 mg/L in the medium, growth enhancement did not exhibit a positive reaction with sodium selenite concentration. A peak increase was noted in the range of 0.5–40 mg/L, and the maximum cell biomass was obtained at 10 mg/L of sodium selenite. So, the effects of 10 mg/L Se addition in the medium on biomass and Se transformation were explored under different cultivation conditions. The results are shown in Table 1. According to the growth rate, final biomass (dry weight, DW), and

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TABLE 1. Effects of Se Additive Conditions on Biomass and Se Transformation in *S. platensis*

Se addition and culture conditions	Growth rate (μ/h)	Final biomass: DW (g/L)	Se in algal cell (μg/g)
10 mg/L Se with different temperature (°C)			
25±1.0	0.022±0.0014	2.33±0.11	22.6±2.5
30±1.0	0.030±0.0022	2.89±0.19	28.1±3.0
35±1.0	0.031±0.0017	3.02±0.20	33.7±2.6
40±1.0	0.023±0.0028	2.47±0.16	25.1±3.9
10 mg/L Se with different contents of S in medium (mg/L)			
50	0.026±0.0027	2.29±0.21	28.5±2.3
100	0.030±0.0019	2.87±0.25	29.9±1.9
200	0.032±0.0031	3.14±0.32	31.4±3.1
400	0.031±0.0031	3.07±0.32	30.4±3.1
10 mg/L Se with different illumination (lux)			
2000±200	0.019±0.0015	1.96±0.12	32.2±2.1
5000±200	0.034±0.0024	3.25±0.35	30.7±3.3
8000±200	0.032±0.0030	3.07±0.28	28.5±3.0

Se content in algal cell, the cultivating conditions for SeSP were optimal if we used 10 mg/L Se additive in medium with 200 mg/L of sulfate (S) at temperature 30~35°C under an illumination of 5000 lux. The final biomass obtained after 10 days of cultivation was above 3 g/L and the total Se content in algal cell dry weight was above 30 μg/g. SeSP used in the following experiments was prepared under these cultivation conditions. The optimal cultivating conditions with no sodium selenite added were used for cultivating non-Se enriched *S. platensis* (SP) as control. Growth rate, final biomass, and Se content in algal cells of SeSP and SP under these conditions are summaried in Fig. 1. Growth rate and final biomass were enhanced slightly with Se content in algal cells increasing 850 fold in SeSP, compared with that of SP.

The chemical compositions of SeSP are listed in Table 2 and compared with that of SP. *Spirulina* is rich in protein, especially antioxidant active phycobiliproteins. Total protein (TP) and water-soluble protein (WSP) were measured by the Lowry method [13] with some midification. Phycobiliproteins of Se enriched *S. platensis* were extracted by sodium phosphate buffer (0.1 M, pH 7.0, containing 1 mM sodium azide) and determined by a spectrophotometric method. Compared with SP, the contents of TP and WSP were slightly increased (6 and 4%, respectively), whereas both C-phycocyanin (C-PC) and allophycocyanin (APC) were increased (12 and 15%, respectively) in SeSP.

As for carbohydrate, it was sub-fractionated into alcohol-soluble sugars (ASS) extracted by boiling in ethanol (85%), water-soluble polysaccharides (WSPS), hot-watersoluble polysaccharides (HWSPS), pectinic substances (PS), and hemicellulose (HC) based on method previously described [8]. Their contents in SeSP were enhanced to different degrees (6~26%) except for the HWSPS.

Although total lipids and fatty acid in SeSP were slightly decreased (12 and 9%, respectively), it contained a lower content of palmitic acid (below 17 %) and a higer content of olenic acid (OA), linolenic acid (LA), and gamma-linolenic acid (GLA), increasing 12, 26 and 14%, respectively, compared with that of SP.

Pigments of chlorophyll a (Chl a) and total carotenoids (Caro) were ditermined by a spectrophotometric method based on measurement of the optical density of the acetone (90%) extract from algal biomass. Chl a was slightly decreased (5%) but Caro was obviously increased (25%) in SeSP.

Spirulina has been shown to supply pigments (C-PC, APC, β-carotene) and poly-unsaturated fatty acids (LA, GLA), which have therapeutic effects on humans. The effect of certain environmental factors has promoted the production of these compounds [14]. In this study, results of chemical compositions in SeSP have shown that secondary metabolites such as WSPS, LA, GLA, and Caro were enhanced significantly ($P<0.05$), and photosynthesis of related pigments and phycobiliproteins were increased moderately, whereas total lipid was decreased to some extent, with Se enriched cultivating of *S. platensis*. This indicates that some metabolic pathway may be affected by Se incorporation in algal cell and some biological active moleculars are enhanced in SeSP.

TABLE 2. Chemical Composition of SeSP and SP Biomass, %

Chemical composition	SP	SeSP
TP	62.40±3.40	66.20±2.90
WSP	42.75±1.74	44.3±0.95
C-PC	1045±0.64	11.68±0.58
APC	4.04±0.54	4.65±0.47
ASS	5.43±1.25	6.81±1.81[a]
WSPS	1.85±0.17	2.26±0.22[a]
HWSPS	2.33±0.29	2.07±0.14
PS	1.16±0.16	1.35±0.11
HC	3.47±0.40	3.68±0.27
Lipids	17.3±2.4	16.2±2.2
FA	12.71±1.55	11.58±1.27
16:0*	44.62±5.12	37.14±6.03[a]
18:1*	7.55±1.47	8.47±0.89
18:2*	10.91±1.06	13.78±1.24[a]
18:3*	26.15±3.30	29.85±1.87
Chl a	1.46±0.09	1.39±0.08
Caro	0.78±0.05	0.97±0.09[a]

*% fatty acids.
[a]P<0.05 *vs* SP.

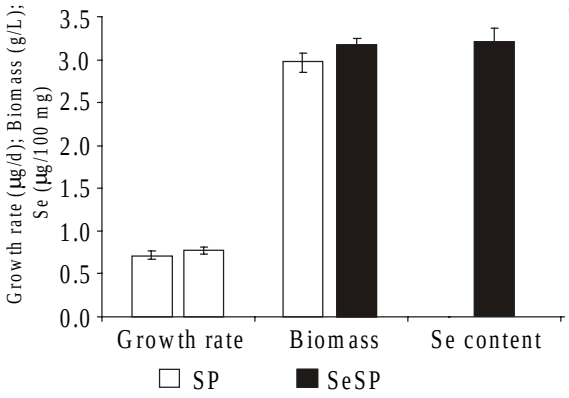

Fig. 1

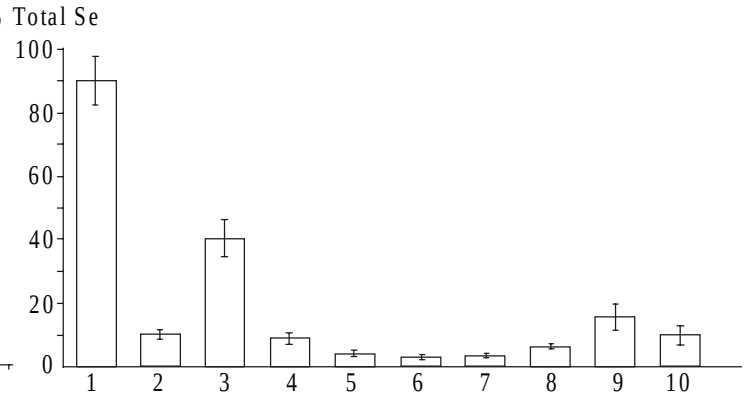

Fig. 2

Fig. 1. Growth comparison of SeSP and SP.
Fig. 2. Distribution of Se in SeSP. 1 - Organic Se, 2 - Inorganic Se, 3 - WSP, 4 - Polysaccharides, 5 - WSPS, 6 - HWSPS, 7 - PS, 8 - HC, 9 - Lipids, 10 - Others.

Generally, organic Se has higher bioavailability and lower toxicity than inorganic Se. Therefore, the highly active Se species of organic Se is used as a nutritional and potentially therapeutic additive. Under optimal cultivating conditions for biotransformation of sodium selenite with 10 mg/L Se additive in medium, the contents of total Se, organic Se, and inorganic Se in Se enriched *S. platensis* are shown in Fig. 2 where the organic Se was 90.15% of total Se in SeSP algal biomass. The organic Se distribution in different fractions of WSP, polysaccharides, and lipids were analyzed and are shown in Fig. 2. The total Se distribution was 36.35% in WSP, 20.9% in carohydrates (for polysaccharides, 8.66%; ASS, 2.85%; PS, 3.14%; HC, 6.25%), 15.56% in lipids, and 19.78% in others, maybe including free seleno-amino acid and structural proteins.

Although selenocysteine as well as Se containing tRNA can be detected in model plant cell systems of *Chlamydomonas reinhardtii*, indicating that Se might be incorporated into plant proteins by genetic control [15], more common selenocompounds including Se-methyl-selenocysteine, Se-methylselenocysteine selenoxide, Se-methylmethionine, methylselenol, dimethylselenide, and trimethylselenonium were found in plants and most were incorporated randomly as selenomethionine (SeMet) into plant proteins [5, 6]. We have detected about 17% of total Se in SeSP distribution in the form of SeMet using GC-MS analysis of the SeSP biomass digest (detailed data not shown here).

Thus, we conclude that under optimal cultivation conditions SeSP has not only an 850-fold higher content of Se in the dominant form of organic Se but also higher contents of poly-unsaturated fatty acids, pigments, and phycobiliproteins than SP. It can be used for Se supplemention and as food additive as well as therapeutic medicaments.

EXPERIMENTAL

Spirulina platensis was obtained from Aquatic Biology Institute of Jinan University (Guangzhou, China). *Zarrouks* medium [16] was used for growing *S. platensis* in 5 a L airlift fermentor under different temperatures of 25, 30, 35 and 40°C or different illuminations of 2000, 5000 and 8000 lux, and/or different sulfate (S) contents of 50, 100, 200, and 400 mg/L in the medium. Selenium was supplemented with 10 mg/mL Se stock solution prepared from sodium selenite. Different S content was supplemented with potassium sulfate (K_2SO_4). Conditions of light intensity were provided by cool-white fluorescent tubes with a dark/light cycle of 12/12 h and temperatures were maintained in a special algae culture room. Growth was monitored by measuring biomass (dry weight, DW) in triplicate using 25 mL samples according to the previous method [17]. Samples were taken for analysis at regular intervals and growth rate was calculated by the previous method [18]. Each treatment was conducted in triplicate.

Algal cell was harvested by filtration after 12 days of growth, washed with distilled water, and freeze-dried. For protein assay, TP was extracted by 0.5 M NaOH and heated to 70°C for 20 min, and WSP was extracted by sodium phosphate buffer (PB, 0.1 M, pH 7.0, containing 1 mM sodium azide), disrupted by sonication for 120 s at 150 W (JY99-III Ultrasonic Disrupter), and centrifuged (12.000 g, 5 min). Protein in the supernatant was measured by the Lowry method [13] using a *DC* Protein Assay Kit (BIO-RAD) with bovine serum albumin as standard. Phycobiliproteins in WSP were determined by a spectrophotometric method [19] on a SHIMADZU UV2450 spectrophotometer (Japan) to measure absorbance at wavelengths 620 and 652 nm for calculating the concentrations of C-PC and APC using the following equation (Bennett, 1973): C-PC (mg/mL) = [A620 – 0.474(A652)]/5.34; APC (mg/mL) = [A652 – 0. 208(A620)]/5.09. Carbohydrates were extracted and differentiated into fractions of ASS, WSPS, HWAPS, PS, and HC, and each fraction was estimated quantitatively according to the previous method [9]. Total lipids were extracted with a reagent of chloroform and methanol (2:1) and were quantified spectrophotometrically at 628 nm by the reaction with sulfophosphovanillin as previously described [20]. For fatty acid (FA) determination, lipids were extracted and FA was transmethylated by treatment with methanolic boron trifluoride ($CH_3OH–BF_3$) according to the previous method [21]. The fatty acid methyl esters (FAME) were analyzed by gas chromatography-mass spectrometry (HEWLETT PACKARD 6890, MSD-5973), using a Carbowax 20M capillary column. Analysis conditions [17] were: temperature from 120°C to 210°C at 8°C min^{-1} and held isothermally; temperature of injector and detector (FID) was 240°C; helium was utilized as the carrier gas. Identification of FAME was accomplished by comparison between retention times of experimental samples and of known standards (SIGMA).

Pigments of Chl a and Caro were measured by the previous method using the following equations [22]: Chl a (μg/mL) = 11.93 A664 – 1.93 A647; Caro (μg/mL) = 7.6 (A480 – 1.49 A510).

Selenium content was determined by a modified 2,3-diaminonaphthalene (DAN) fluorocence method [12] with a SHIMADZU RF-540 fluorimeter (Japan) followed by Se (VI) reduction to Se (IV) with 15% hydrochloric acid; total Se was determined after sample digestion with perchloric acid and nitric acid at 180°C for 2 hours, and inorganic Se was determined directly without the treatment as above. Organic Se was calculated as total Se minus inorganic Se. All measurements were done in triplicate.

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