Enhanced Biomass and γ-Linolenic Acid Production of Mutant Strain *Arthrospira platensis*

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A mutant of *Arthrospira platensis* PCC 9108, strain M9108, obtained by mutagenesis with UV treatment, was able to mixotrophically grow in an SOT medium containing 40 g of glucose/l. The biomass and specific growth rate of strain M9108 (4.10 g/l and 0.70/d) were 1.9-fold and 1.4-fold higher, respectively, than those of the wild type (2.21 g/l and 0.58/d) under mixotrophic culture condition. In addition, when compared with the wild type, the content of γ-linolenic acid (GLA) in the mutant was increased when glucose concentration was increased. Compared with the wild type, the GLA content of the mutant was 2-fold higher in autotrophic culture and about 3-fold higher in mixotrophic culture. Thus, the mutant appears to possess more efficient facility to assimilate and metabolize glucose and to produce more GLA than its wild-type strain.

Keywords: *Arthrospira platensis*, biomass, γ-linolenic acid, GLA, mixotrophic cultivation, UV

Cyanobacteria belong to a group of ubiquitous photosynthetic prokaryotes possessing the ability to synthesize chlorophyll *a*, essential amino acids, and polyunsaturated fatty acids (n3 and n6) [14]. *Arthrospira platensis* (*Spirulina*) is a commercially important cyanobacterium. This strain is a rich source of proteins, minerals, vitamin B_{12}, β-carotene, and essential fatty acids, such as γ-linolenic acid (GLA, 18:3ω6). Indeed, it can be used worldwide as feed for fish, poultry, and farm animals and as an additive food for humans [12, 13].

GLA has been shown to have potential pharmaceutical value to alleviate the symptoms of premenstrual syndrome [10], treat atopic eczema [1], and lower low-density

lipoproteins in hypocholesterolemic patients [11]. GLA is found in evening primrose oil, black current, and borage. It is also found in some fungi, although large-scale cultivation is not yet available. In plants, GLA constitutes only 8–12% of total fatty acids and is accompanied by other fatty acids (*e.g.*, 18:4) with undesired properties. The separation of GLA from other fatty acids is difficult, and thus, costly in a large scale [6]. In comparison, the content of GLA in *A. platensis* is very high, as 25% of the total fatty acids [20]. Therefore, the simple and effective production of highly pure GLA from the cyanobacteria would appear to be highly promising. GLA in *Arthrospira* plays a similar role to α-linolenic acid (ALA, 18:3ω3) in algae and higher plants [16]. Furthermore, the occurrence of GLA, which is a precursor in animals, has led to the idea that certain cyanobacteria are the origin of both red and green algae. According to a previous study, which dealt with 18 different *Arthrospira* strains [4], the kind of fatty acids in *Arthrospira* is very similar, but their composition varies significantly among different strains.

In the present study, mutants of *A. platensis* were obtained by exposure to UV and screened for the capability of growing on SOT medium, containing a high concentration of glucose (40 g/l). The growth and GLA content of the mutant were studied and compared with those of the wild-type strain. The culture experiments were conducted with varying concentrations of glucose and maltose as carbon sources under light or dark conditions, in order to optimize culture conditions for the production of biomass and GLA with *A. platensis*.

MATERIALS AND METHODS

Strains and Culture Conditions
Arthrospira platensis PCC 9108 obtained from the Pasteur Culture Collection of cyanobacterial strains (Paris, France) was used in this study. The *A. platensis* PCC 9108 was maintained in SOT liquid

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medium [13], containing 16.8 g of NaHCO₃, 0.5 g of K₂HPO₄, 2.5 g of NaNO₃, 1 g of K₂SO₄, 1 g of NaCl, 0.2 g of MgSO₄·7H₂O, 0.04 g of CaCl₂·2H₂O, 0.01 g of FeSO₄·7H₂O, 0.08 g of Na₂EDTA, and 1 ml of A5 solution per liter, where one liter of A5 solution contained 2.86 g of H₃BO₃, 2.5 g of MnSO₄·7H₂O, 0.222 g of ZnSO₄·7H₂O, 0.079 g of CuSO₄·5H₂O, and 0.021 g of Na₂MoO₄·2H₂O. The culture was carried out in 125-ml Erlenmeyer flasks containing 30 ml of the SOT medium under continuous light conditions, with an illumination of 120 μmol photons/m²/s provided by cool white fluorescent lamps at 30±1°C and 150 rpm. All the cultures were grown exponentially under appropriate conditions for 7 days prior to the experiments. The photoautotrophic culture contained no glucose in the growth medium with illumination, whereas the heterotrophic and mixotrophic cultures contained glucose or maltose at 1.0, 2.5, 5.0, and 10.0 g/l without illumination and with illumination, respectively. Sampling was performed over a period of 8 days. Biomass was determined by culture turbidity at 680 nm using a spectrophotometer (Optizen UV-2450; Mecacy Ltd., Korea). The conversion factor for dry cell weight was Y=0.542·X+0.005 (r²= 0.994; X: Optical density; Y: Dry cell weight).

Mutant Induction

UV treatment was a modified version of the method of Tripathi *et al.* [21]. Seven-day-old *A. platensis* PCC 9108 cells were centrifuged, and the pellet was washed with 10 mM sodium phosphate buffer saline (PBS), pH 7.2. The cell suspension (1 ml) was then subjected to UV irradiation for 0, 10, 20, and 30 min in a sterile open Petri dish using a UV lamp (UV-C 254 nm, 15 W, Philips) at a height of 20 cm in a clean bench. Thereafter, the irradiated cultures were plated on SOT medium containing 40 g of glucose/l and incubated under dim light (20 μmol photons/m²/s) at 30°C until colonies were formed.

Fatty Acid Determination

The total lipids were extracted using a modified method of Bligh and Dyer [2]. The lipid fraction was extracted with chloroform-methanol (2:1, v/v), and then isolated in the chloroform phase after adjusting the solvent ratio to 2:2:1 (chloroform-methanol-water, v/v/v). The chloroform phase was then removed by evaporation under a vacuum, and the weight was determined gravimetrically.

The fatty acid analysis followed the method of Lepage and Toy [15]. Sample aliquots (10 mg) were placed in capped test tubes, and then saponified with 1 ml of saturated KOH-CH₃OH solution at 75°C for 10 min, followed by methanolysis with 5% HCl in methanol at 75°C for another 10 min. Finally, 2 ml of distilled water was used to extract the fatty acids.

All the samples were analyzed using a gas chromatograph (HP5890A, U.S.A.) equipped with a flame ionization detector. The injector temperature was 250°C, and the separation was achieved on a 25 m×Φ0.32 mm fused silica capillary column. The flow of the carrier gas (N₂) was 2 ml/min, and 1 μl of methyl ester solution was injected for each analysis. The program temperature was as follows: the initial temperature of 80°C was maintained for 3 min, then raised to 250°C at a rate of 10°C/min, and then the final oven temperature of 250°C was maintained for 5 min. The components were identified by comparing their retention time and fragmentation pattern with established standards.

RESULTS AND DISCUSSION

Isolation of Mutant with Tolerance to High Glucose Concentration

Strain M9108, a mutant of *A. platensis* PCC 9108 resulting from UV light treatment, was isolated, based on its ability to grow on a SOT medium plate containing 40 g of glucose/l. *A. platensis* PCC 9108 was unable to grow on the same plate. To investigate the resistance to high glucose concentrations, the wild type (*A. platensis* PCC 9108) and mutant (*A. platensis* M9108) were cultured on the SOT medium containing 20 and 40 g of glucose/l under light condition. As shown in Fig. 1, the biomass of the mutant was decreased less than that of the wild type, when increasing the glucose concentration. The biomass of the mutant was 2.85 and 2.59 g/l after 8 days of cultivation with initial glucose concentrations of 20 and 40 g/l, respectively, whereas the specific growth rate of the mutant was 0.66 and 0.64/d in SOT medium containing 20 and 40 g glucose/l, respectively. In the wild type, the biomass was measured as 2.07 and 1.47 g/l after 8 days of cultivation with initial glucose concentrations of 20 and 40 g/l, respectively, and the specific growth rate of the wild type was 0.55 and 0.53/d in a SOT medium containing 20 and 40 g of glucose/l, respectively. Thus, the biomass and specific growth rate of the mutant were 1.4-fold and 1.2-fold higher than those of the wild type, respectively. Therefore, compared with the wild type, the mutant grew well in the SOT medium containing a high concentration of glucose and exhibited tolerance to high glucose concentration. In a previous study, the growth of *Arthrospira* was reduced when the glucose concentration was increased above 5 g/l in the culture medium [3]. However, the mutant in the
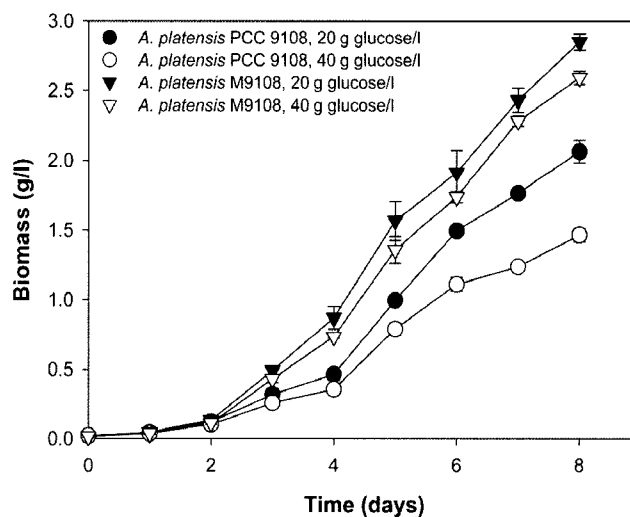

Fig. 1. Growth curves of *A. platensis* PCC 9108 and M9108 cultures in SOT medium containing 20 or 40 g of glucose/l under light condition.
Each value indicates mean±SD (n=3).

present study showed less growth inhibition with high concentration of glucose up to 40 g/l.

UV irradiation has been known to affect motility, community composition, pigmentation, and several metabolic processes in algal systems [9]. In the current study, the mutant resulting from UV treatment exhibited tolerance to high glucose concentration (40 g/l). Whereas the wild type showed growth inhibition with an increasing glucose concentration (20 g/l), the mutant showed less growth inhibition when the glucose concentration was increased. This phenomenon might be due to inhibition of chlorophyll biosynthesis or degradation of pigments or their precursors. Nonetheless, no morphological differences were found between the mutant and wild type under light microscopy.

Effect of Concentration of Carbon Substrate

We carried out a preliminary experiment in which *Arthrospira* strains were cultivated in SOT media containing one of 16 carbon sources (5 g/l). Among these carbon sources, glucose and maltose were finally selected as effective carbon sources. The wild type and mutant were grown under light and dark conditions for 8 days in a shaking incubator in SOT medium containing glucose or maltose. The glucose and maltose concentrations in the medium

varied from 1.0 to 10.0 g/l, whereas 16.8 g/l bicarbonate was used as the control. As shown in Fig. 2, the specific growth rate and biomass were significantly enhanced by the addition of glucose or maltose under light and dark conditions. For the mutant, the highest specific growth rate (0.70/d) and highest biomass (4.10 g/l) were found with an initial glucose concentration of 10.0 g/l under light condition. The highest specific growth rate (0.58/d) and highest biomass (2.21 g/l) for the wild type were found with an initial glucose concentration of 5.0 g/l under light condition. Thus, the biomass and specific growth rate of the mutant were 1.9-fold and 1.4-fold higher than those of the wild type, respectively. Both the biomass and specific growth rate of the mutant increased when glucose concentration was increased up to 10 g/l, whereas those of the wild type increased with only up to 5 g of glucose/l, and thereafter, decreased. The specific growth rate of the mutant also increased when the glucose concentration was increased under light condition, whereas that of the wild type increased less when the glucose concentration was increased. Thus, it was suggested that the mutant appears to be able to utilize more glucose. Under dark condition, the highest biomass for the mutant and wild type was 0.31 and 0.19 g/l, respectively, with an initial glucose concentration of 5.0 g/
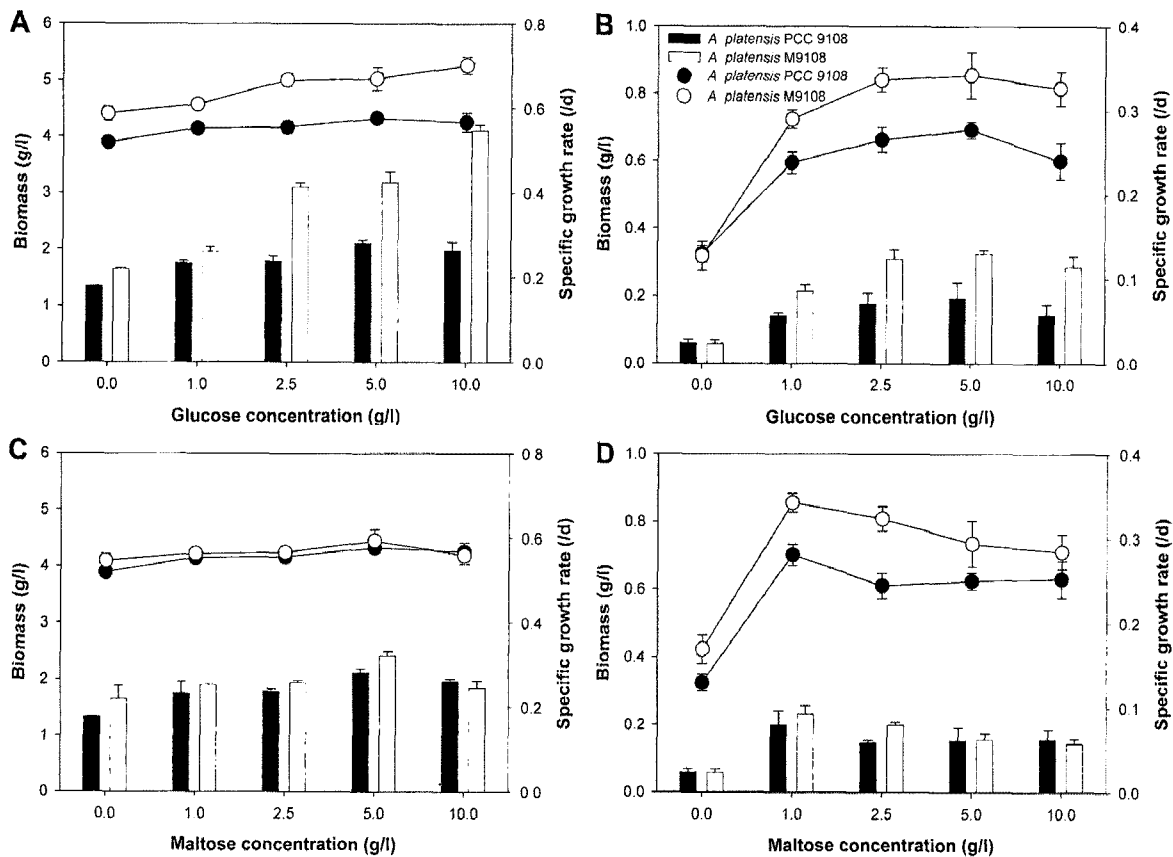

Fig. 2. Biomass (bar) and specific growth rate (circle) of *A. platensis* PCC 9108 and M9108 cultures in SOT medium containing various concentrations of glucose (A, B) and maltose (C, D) under light condition (A, C) and dark condition (B, D). Each value indicates the mean±SD (n=3).

1 (Fig. 2B). Furthermore, the mutant biomass was 1.6-fold higher than the wild-type biomass in the case of heterotrophic cultivation. The specific growth rate of the wild type was also much lower than that of the mutant with an initial glucose concentration of 10 g/l. These results show that the mutant has a stronger tolerance to high glucose concentrations and enhanced utility of glucose.

The mutant and wild type cultures in the SOT medium containing maltose exhibited the highest specific growth rate (0.59 and 0.57/d, respectively) and highest biomass (2.4 and 2.1 g/l, respectively) with an initial maltose concentration of 5.0 g/l under light condition (Fig. 2C). For the maltose-containing cultures, the specific growth rate and biomass were slightly higher than those for the control, although the difference in the specific growth rate between the mutant and wild type was negligible. Under dark condition, the highest biomass for the mutant and wild type was 0.23 and 0.20 g/l, respectively, with an initial maltose concentration of 1.0 g/l (Fig. 2D). However, both the biomass of the mutant and wild type decreased when the maltose concentration was increased.

The above results indicated that *A. platensis* was able to use glucose and maltose as its carbon source in heterotrophic growth, although maltose has not previously been reported to support the growth of *A. platensis*. It was found that glucose was superior to maltose as a carbon source. Under the experimental conditions used, glucose showed 19% higher specific growth rate and 70% higher dry cell weight than maltose. In addition, the highest biomass (4.1 g/l) obtained was higher than that previously reported for *A. platensis* UTEX 1926 on 2.5 g of glucose/l in a mixotrophic culture, where the highest cell concentration was 2.68 g/l [3].

Total Lipid Content and Fatty Acid Composition

Environmental conditions have a major effect on the fatty acid composition and content in microalgae, and it has

already been reported that the fatty acid composition and GLA content of *Arthrospira* are affected by temperature, light intensity [7], cell concentration [6], growth phase, light/dark cycle [19], nitrogen [17], and outdoor cultivation [19]. However, the effect of glucose concentrations on the fatty acid and GLA content has not yet been tested.

The fatty acid composition and content of *A. platensis* can be manipulated so as to increase both the proportion of GLA and its content in the biomass. The fatty acid composition and total fatty acid content of the wild type and mutant strains after 8 days of cultivation are shown in Table 1. The main fatty acids in the wild type and mutant were palmitic acid (16:0), linoleic acid (18:2), and GLA (18:3). When cultivated with various concentrations of glucose under light condition, the fatty acid content and GLA content of the mutant increased when the glucose concentration was increased, representing 2.80–4.89% and 0.84–1.53%, respectively. These results are similar to those previously reported for *Arthrospira* sp. strain Z19 [5]. Thus, the mutant had an improved GLA content (percent of dry weight) of 1.53% compared with 0.54% in the wild type. The GLA content in the mutant was also 2-fold higher than that in the wild type with an autotrophic culture and about 3-fold higher in a glucose (10 g/l) supplied culture. Moreover, GLA content in the mutant increased with increasing glucose concentration. In contrast, GLA content in the wild type did not differ significantly between a glucose-free and -added medium. In a previous study in which *Arthrospira* cultures were maintained in the dark for a week, preferential consumption of sugars over fatty acids resulted in a relative increase of fatty acid content, including GLA, by 50% [8]. Thus, the high glucose concentration tolerance of the mutant enhanced its utility of glucose.

Effect of Bicarbonate

To test the effect of bicarbonate as a carbon source, the wild type and mutant were cultured with and without

Table 1. Fatty acid content and distribution profile of *A. platensis* PCC 9108 and M9108 under various growth conditions. Values represent means±SD (n=3).

stain	Growth condition (glucose concentration)	Fatty acid composition (% of total fatty acids)						Fatty acid content (% of biomass)	
		16:0	16:1	18:0	18:1	18:2	GLA	TFA*	GLA
A. platensis PCC 9108	Autotrophic	32.4±0.12	5.8±0.16	5.6±0.26	0.1±0.02	16.9±0.21	24.8±0.25	1.64±0.21	0.407±0.13
	Mixotrophic (1.0 g/l)	35.7±0.18	8.2±0.25	5.3±0.21	2.1±0.18	14.9±0.25	26.6±0.26	1.81±0.26	0.482±0.17
	Mixotrophic (2.5 g/l)	36.1±0.27	9.2±0.17	2.4±0.22	1.5±0.17	16.6±0.18	29.1±0.18	1.74±0.11	0.506±0.14
	Mixotrophic (5.0 g/l)	37.9±0.19	8.6±0.14	4.0±0.19	1.5±0.20	15.7±0.21	26.5±0.26	1.84±0.16	0.488±0.11
	Mixotrophic (10.0 g/l)	39.0±0.25	8.4±0.23	1.5±0.15	1.5±0.25	15.6±0.23	27.4±0.23	1.99±0.28	0.545±0.19
A. platensis M9108	Autotrophic	30.1±0.25	5.9±0.15	5.9±0.26	5.0±0.15	15.9±0.16	30.0±0.21	2.80±0.18	0.840±0.18
	Mixotrophic (1.0 g/l)	36.1±0.22	9.2±0.29	2.4±0.17	1.5±0.23	16.6±0.26	29.1±0.28	3.96±0.21	1.152±0.16
	Mixotrophic (2.5 g/l)	30.1±0.11	5.9±0.21	5.9±0.22	5.0±0.21	15.9±0.23	30.0±0.29	4.14±0.29	1.242±0.15
	Mixotrophic (5.0 g/l)	34.6±0.28	8.4±0.21	3.0±0.25	2.0±0.18	16.8±0.15	30.6±0.24	4.04±0.13	1.236±0.19
	Mixotrophic (10.0 g/l)	31.7±0.19	7.7±0.27	2.9±0.20	4.0±0.28	15.5±0.26	31.2±0.19	4.89±0.11	1.526±0.21

*TFA, total fatty acids.

Table 2. Biomass and specific growth rate of *A. platensis* PCC 9108 and M9108 in SOT medium with or without bicarbonate. Values represent means±SD (n=3).

Bicarbonate (16.8 g/l)	Growth condition*	*A. platensis* PCC 9108		*A. platensis* M9108	
		Biomass (g/l)	Specific growth rate (/d)	Biomass (g/l)	Specific growth rate (/d)
Added	Autotrophic	1.33±0.01	0.52±0.05	1.65±0.05	0.59±0.03
	Mixotrophic	2.11±0.17	0.56±0.03	2.86±0.04	0.67±0.01
	Heterotrophic	0.19±0.04	0.28±0.03	0.32±0.05	0.34±0.02
Not added	Autotrophic	0.35±0.03	0.30±0.01	0.42±0.01	0.31±0.01
	Mixotrophic	0.37±0.01	0.29±0.01	0.51±0.03	0.33±0.02
	Heterotrophic	0.09±0.01	0.09±0.01	0.13±0.01	0.12±0.01

*Growth condition: Autotrophic, without glucose under light condition; Mixotrophic, with glucose (5 g/l) under light condition; Heterotrophic, with glucose (5 g/l) under dark condition.

bicarbonate under light and dark conditions (Table 2). In the case of no added bicarbonate, the biomass of both strains was reduced by 25.4–26.3, 17.5–17.8, and 40.6–47.4% when compared with the addition of bicarbonate under autotrophic, mixotrophic, and heterotrophic cultivation conditions, respectively. Under heterotrophic and mixotrophic culture conditions, the biomass and specific growth rate of the mutant were 1.35-fold to 1.68-fold and 1.11-fold to 1.21-fold enhanced, respectively, compared with those of the wild type in a medium with and without bicarbonate. However, under autotrophic culture condition, the biomass and specific growth rate of the mutant were only slightly (1.20-fold to 1.24-fold and 1.03-fold to 1.13-fold, respectively) increased compared with those of the wild type in a medium with and without bicarbonate. In conclusion, the biomass and specific growth rate of both strains were higher with bicarbonate than without, under all the cultivation conditions. A previous report also showed that the growth rate of *A. platensis* decreased with bicarbonate concentration lower than 8.4 g/l in the medium [18]. Therefore, the above results show that bicarbonate is essential for culture of *Arthrospira* under all cultivation conditions.

Acknowledgment

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