Photoautotrophic Cultivation of the Green Alga *Chlamydomonas reinhardtii* as a Method for Carbon Dioxide Fixation and α-Linolenic Acid Production

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ABSTRACT: Focusing on CO_2 fixation and α-linolenic acid (ALA) production, photoautotrophic cultivation of the green alga *Chlamydomonas reinhardtii* was investigated by using a culture medium of pH 6.8 under a 5 vol% CO_2-enriched atmosphere. The optimum cultivation temperature and light intensity for growth were 25°C and 12 klux, respectively. The cellular ALA content nearly doubled to 11.9 mg/g of dry cells when the concentration of culture medium was doubled. Simulation of chemostat cultivation showed that the rate of CO_2 fixation and ALA productivity per unit volume of culture medium could reach 1.01 kg $CO_2/(m^3 \cdot d)$ and 7.46 g ALA/$(m^3 \cdot d)$, respectively, at a cell concentration of 0.57 kg cells/m^3.
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KEY WORDS: Alga, α-linolenic acid, anti-tumor agent, carbon dioxide, *Chlamydomonas reinhardtii*, unsaturated fatty acid.

Because large-scale cultivation of photoautotrophic microalgae is relatively simple and cheap, it is an adequate method for the fixation of CO_2. Recently, this kind of microalgae has been examined as a source of valuable fine chemicals as well (1). The green alga *Chlamydomonas reinhardtii* has a high growth rate and accumulates a high level of α-linolenic acid (18:3ω–3, ALA) (2,3). This unsaturated fatty acid is essential for mammals and has antitumor activities and antiallergic activities as well (4,5). In the present paper, we determined the activity for CO_2 fixation and ALA productivity for *C. reinhardtii* (strain C-9) under optimum cultivation conditions.

MATERIALS AND METHODS

Chlamydomonas reinhardtii C-9 was supplied from the Microbial and Microalgal Research Center, Institute of Applied Microbiology, University of Tokyo (Tokyo, Japan). The organism was originally isolated in a potato field in Amherst, Massachusetts in 1945, by G.M. Smith and is equivalent to the Sager strain (6). Cells were grown axenically for 7 d at 15–35°C in a 1-L shaking flask (500 mL of culture medium) with reciprocal shaking at 110 strokes/min. The flask was aerated with 5 vol% CO_2-enriched air (100 mL/min) and illuminated by cool-white fluorescent lamps at 3–12 klux (1 klux = 92.9 ft-c). Light intensity was measured at the upper surface of the shaking flask. Growth was monitored turbidimetrically at 750 nm ($o.d._{750}$) (2). A buffered culture medium of pH 6.8, based on Beijerinck's four-salt solution, was used (2). The alga was inoculated at the fixed concentration of $o.d._{750} = 0.10$ after being precultured at 25°C and 4 klux. Harvesting and drying of cells were carried out as described previously (7). Dried cells were heated at 95°C for 3 h in methanolic HCl, and fatty acid methyl esters formed were extracted with *n*-hexane. Fatty acid content was then determined by the analysis of these methyl esters by gas–liquid chromatography (7).

RESULTS AND DISCUSSION

Cultivation temperature. *Chlamydomonas reinhardtii* accumulated exclusively C_{16}- and C_{18}-fatty acids. Palmitic acid $C_{16:0}$, oleic acid $C_{18:1}$, linoleic acid $C_{18:2}$, and ALA $C_{18:3}$ were the main cellular fatty acids. The growth rate constant μ in the first one day of cultivation at 6 klux was 0.79 d^{-1} at 15°C, which was calculated by use of Equation 1:

$$dX/dt = \mu X \qquad [1]$$

where X and t are cell concentration (kg cells/m^3) and cultivation time (d), respectively. With rising cultivation temperature, however, the growth rate constant increased ($\mu = 1.31\ d^{-1}$ at 20°C) and became maximum at 25°C ($\mu = 1.50\ d^{-1}$). With further rise in cultivation temperature, the growth rate constant rather decreased ($\mu = 1.39\ d^{-1}$ at 30°C, $1.23\ d^{-1}$ at 35°C). Table 1 summarizes the results of cultivation at 6 klux at these different temperatures. Cell yield was maximum (1.11 g/L) at 25°C, although no big difference in cell yield was observed at these cultivation temperatures. Cellular α-linolenic acid (ALA) content was greatest at the lowest temperature studied (15°C) and decreased with rising cultivation temperature. For example, cellular ALA content was as high as 26.7 mg/g at 15°C, but it was 6.0 mg/g at 35°C.

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Table 1
ALA Yields and Cellular Fatty Acid Compositions of *Chlamydomonas reinhardtii* Grown at Different Temperatures[a]

Cultivation temperature (°C)	Cell yield (g/L)[b]	Total fatty acid content (mg/g)[c]	ALA content (mg/g)[c]	ALA yield (mg/L)[b]	Cellular fatty acid composition (wt%)				
					16:0	18:1	18:2	ALA	Others[d]
15	0.90	72.0	26.7	24.0	16.8	25.5	2.4	37.1	17.2
20	0.96	72.1	21.8	20.9	20.1	26.9	3.3	30.3	19.4
25	1.11	77.4	16.1	17.9	18.4	19.0	13.9	20.8	28.0
30	1.06	81.1	9.8	10.4	16.9	20.1	16.9	12.0	34.4
35	0.97	84.1	6.0	5.8	18.0	26.1	10.1	7.2	38.6

[a]Cultivated at 6 klux for 7 d; ALA, α-linolenic acid.
[b]Per one liter of culture medium.
[c]Per one gram of dry cells.
[d]14:0 + 16:1 + 16:2 + 16:3 + 16:4 + 18:0 + 18:3ω-6 + 18:4.

Light intensity. Figure 1 shows growth curves for *C. reinhardtii* at 25°C at different light intensities. Judging from these growth curves, the growth rate increased drastically with rising light intensity from 3 to 9 klux. When it was further raised from 9 to 12 klux, however, the growth rate scarcely increased, especially in the first 3 d of cultivation. When cultivation was carried out at 12 klux in a culture medium in which the concentration was doubled, a growth curve identical to that at 9 klux in the single-strength culture medium was obtained.

Cell yield increased with increasing light intensity and reached 2.64 g/L at 12 klux (Table 2). Cellular ALA content decreased to as low as 6.3 mg/g at 12 klux. However, the ALA content doubled at 12 klux in the double-strength culture medium (11.9 mg/g, Table 2). Separate experiments, in which the concentrations of trace metals, $ZnSO_4$, NH_4NO_3 or all salts other than trace metals that constitute the culture medium were doubled, revealed that the rise in cellular ALA content was caused by the increase in NH_4NO_3 concentration. ALA yield was maximum (24.8 mg/L) at 12 klux in the double-strength culture medium.

Calculation of CO_2 fixation rate and ALA productivity. These two algal activities under optimum cultivation conditions (25°C, 12 klux and double-strength culture medium) were evaluated by assuming a chemostat cultivation at $o.d._{750}$ = 1 (0.57 kg cells/m^3). The following two equations were used for calculations:

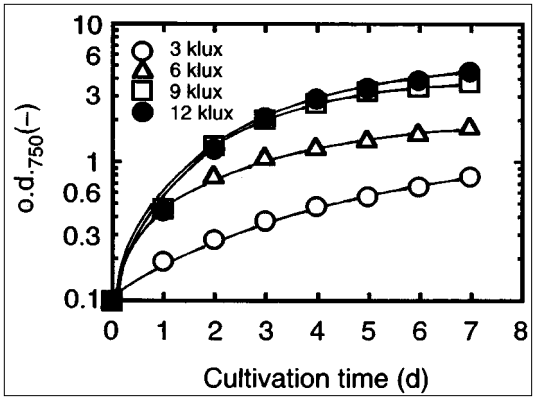

FIG. 1. Growth curves for *Chlamydomonas reinhardtii* at 25°C at different light intensities.

$$\text{rate of algal cell production [kg cells/(m}^3 \cdot d)] = dX/dt = \mu X \quad [2]$$

$$\text{rate of } CO_2 \text{ fixation[kg- } CO_2/(m^3 \cdot d)] = (44/12) \times (C_c/100) \times \mu X \quad [3]$$

where μ and C_c are growth rate constant [d^{-1}] and carbon content of dry cells (43.9 wt%), respectively. The growth rate constant μ at $o.d._{750}$ = 1 was determined to be 1.10 d^{-1} by graphical differentiation of the growth curve that was obtained under optimum cultivation conditions. Thus, the rates

Table 2
ALA Yields and Cellular Fatty Acid Compositions of *C. reinhardtii* Grown at Different Light Intensities[a]

Light intensity (klux)	Cell yield (g/L)	Total fatty acid content (mg/g)	ALA content (mg/g)	ALA yield (mg/L)	Cellular fatty acid composition (wt%)				
					16:0	18:1	18:2	ALA	Others
3	0.38	57.0	14.2	5.4	17.9	26.4	4.4	25.0	26.4
6	1.11	77.4	16.1	17.9	18.4	19.0	13.9	20.8	28.0
9	2.23	76.4	9.3	20.7	28.9	21.6	16.4	12.2	21.1
12	2.64	61.9	6.3	16.6	31.4	21.8	18.2	10.1	18.6
12[b]	2.08	57.6	11.9	24.8	18.3	19.8	13.0	20.7	27.3

[a]Cultivated at 25°C for 7 d. See Table 1 for abbreviations.
[b]Culture medium concentration was doubled.

of algal cell production and of CO_2 fixation were calculated to be 0.63 kg cells/($m^3 \cdot d$) and 1.01 kg CO_2/($m^3 \cdot d$), respectively. Because cellular ALA content was 11.9 mg/g, ALA productivity was 7.46 g ALA/($m^3 \cdot d$). This rate of CO_2 fixation for *C. reinhardtii* is greater than that for the blue-green alga *Anacystis nidulans* [0.96 kg CO_2/($m^3 \cdot d$)] and the hot-spring alga *Cyanidium caldarium* [0.72 kg CO_2/($m^3 \cdot d$)] (7,8). The ALA productivity of *C. reinhardtii* is greater than that of *C. caldarium* [5.22 g ALA/($m^3 \cdot d$)] (7) as well.

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