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CHANGES IN LIPID AND FATTY ACID COMPOSITION OF *PAVLOVA LUTHERI*

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Key Word Index—*Pavlova lutheri*; Prymnesiophyceae; microalga; lipids; fatty acids; culture conditions.

Abstract—The effects of growth temperature on the lipid and fatty acid composition of *Pavlova lutheri* were examined. Major fatty acids derived from the total lipids were 14:0, 16:0, 16:1, 20:5 and 22:6. The main lipid classes found in this strain consisted of triacylglycerol, monogalactosyldiacylglycerol, sulphoquinovosyldiacylglycerol and betaine lipids. The saturated fatty acids, 14:0 and 16:0, were found in all lipid fractions; the polyunsaturated fatty acids, 20:5 and 22:6, were distributed in triacylglycerol, monogalactosyldiacylglycerol and betaine lipids. At low temperature (15°), there was a tendency for an increase in the relative percentage of betaine lipids and the relative amount of the polyunsaturated fatty acids, 20:5 and 22:6. These results suggest that, in *P. lutheri*, polyunsaturated fatty acids and betaine lipids serve as modulators of low temperature adaptation.

INTRODUCTION

Pavlova lutheri occurs widely in oceanic and coastal waters [1]. It is used as food for larval fish and prawns in mariculture hatcheries [2], because it accumulates polyunsaturated fatty acids (PUFA), such as icosapentaenoic acid (20:5) and docosahexaenoic acid (22:6), which are essential nutrients for cultured marine animals. It was considered that PUFA in polar lipids are more effective for nutrition of fish and prawns than those in neutral lipids. A large quantity of PUFA has been found in the glycolipids from the red alga, *Porphyra tenera* [3], in polar lipids from the diatom, *Phaeodactylum tricornutum* [4], and in all lipid classes from the diatom, *Navicula incerta* [5]. However, the fatty acid composition of lipid classes, and the influence of growth conditions on the production of lipids in *P. lutheri* have not been determined. We therefore carried out a study on the effects of growth temperature on the lipid and fatty acid composition of *P. lutheri*.

RESULTS AND DISCUSSION

The culture reached the stationary growth phase in 7 days at 25° and in twelve days at 15°. There was no significant difference in the final cell density of all cultures (7.8×10^6 cells ml^{-1} at 25° and 8.9×10^6 cells ml^{-1} at 15°), and in the total fatty acid content of the organism cultured at the different growth temperatures (25° 1.4 pg $cell^{-1}$ and 15° 1.2 pg $cell^{-1}$). The total fatty acid composition of *P. lutheri* is shown in Table 1. Major fatty acids ($\geq$ 10% of total fatty acids) were 14:0, 16:0, 16:1, 20:5 and 22:6. With a decrease in growth temperature, the relative percentage of 20:5 and 22:6 to total fatty acids increased (from 20 to 30%, from 8 to 17%, respectively), while those of 16:0 decreased (from 21 to 10%).

Table 1. Fatty acid composition (molar %) of total lipids from *Pavlova lutheri*

Fatty acid*	25°	15°
14:0	12.5	13.2
16:0	20.7	9.6
16:1	22.8	18.6
18:0	2.1	2.9
18:1	4.3	4.8
18:2	3.7	tr†
18:3	tr	tr
18:4	1.7	2.8
20:4	1.4	tr
20:5	20.3	30.3
22:5	2.3	tr
22:6	8.1	16.8

*Fatty acids are designated by the number of carbon atoms:number of double bonds.

†trace (< 1% of total fatty acids).

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The compositions of the individual lipid fractions in *P. lutheri* are shown in Table 2. The major lipid classes were triacylglycerol (TG), monogalactosyldiacylglycerol (MGDG), sulphoquinovosyldiacylglycerol (SQDG) and betaine lipids (BL). BL were identified from their R_f values; one was the BL diacylglycerylhydroxymethyltrimethyl-β-alanine (DGTA) [6–8], the other, diacylglyceryl-*O*-carboxyhydroxymethylcholine (DGCC) [9]. With the decrease in growth temperature, the relative percentage of SQDG and BL to the total lipid content increased (from 10 to 16%, and from 6 to 26%, respectively). In particular, the amount of BL in cells grown at 15° was four times higher than that in cells at 25°. In contrast, the amount of TG decresed at the lower temperature (from 40 to 14%).

The fatty acid compositions of the lipid classes in *P. lutheri* are shown in Table 3. The main saturated fatty acids were 14:0 and 16:0, these being found in all lipid fractions. With a decrease in growth temperature, the relative percentage of 16:0 in all lipid classes, especially in TG and MGDG, decreased (to about one-fifth and one-third, respectively) at the low temperature. The monounsaturated fatty acid, 16:1, comprised 10–30% of the total fatty acids in TG, MGDG and SQDG, but accounted for only 3% of those in BL. With a decrease in growth temperature, the relative percentage of 16:1 in MGDG increased (from 25 to 38%), whilst that of SQDG and BL remained almost unchanged. On the other hand, the relative percentage of 16:1 in TG decreased (from 25 to 15%) at the lower temperature. The PUFA, 20:5, was the major component of total fatty acids in TG, MGDG and BL (13, 20 and 31% of total fatty acids, respectively), but this accounted for only 5% of those in SQDG. With a decrease in growth temperature, the relative percentage of 20:5 in the total fatty acid content increased in all fractions. The other PUFA, 22:6, accounted for more than 10% of the total fatty acids in TG, MGDG and BL, but was not detected in SQDG. The relative percentage of 22:6 increased at the lower temperature in TG and BL (from 10 to 33%, and from 10 to 14%, respectively), although it was decreased (from 12 to 3%) in MGDG.

Table 2. Lipid composition (molar %) of total lipids from *Pavlova lutheri*

Lipid class	25°	15°
TG	40.3	14.1
MGDG	24.7	18.2
DGDG	7.6	7.6
SQDG	9.7	16.0
PG*	4.0	8.7
UN†	5.7	7.5
BL	6.3	25.8
Others	1.7	2.1

*Phosphatidylglycerol.
†Unknown choline lipid.

The major fatty acids (present at levels above 10% of total fatty acids) in *P. lutheri* were 14:0, 16:0, 16:1 and 20:5, in agreement with previous data [2]. With a decrease in growth temperature, there was no significant change in the total fatty acid content. However, fatty acid composition differed between the two conditions. At the lower temperature, the relative percentage of 20:5 and 22:6 in the total fatty acid content increased, whereas those of saturated and monosaturated fatty acids decreased. These results are different from previous data [2, 10], and suggest that higher levels of 20:5 and 22:6 are needed for growth of this alga at low temperature. The major lipids found in *P. lutheri* were TG, MGDG, SQDG and BL. With a decrease in growth temperature, it was found that the amount of TG decreased and that of BL increased. According to the fatty acid composition of lipid classes, a large amount of 20:5 was found in all lipids. On the other hand, 22:6 was found only in TG, MGDG and BL as a major component of their total fatty

Table 3. Fatty acid composition (molar %) of major lipids in *Pavlova lutheri*

	TG		MGDG		SQDG		BL	
Fatty acid	25°	15°	25°	15°	25°	15°	25°	15°
14:0	10.7	10.3	14.3	13.7	42.2	43.5	9.2	16.5
16:0	19.8	3.9	14.9	6.6	37.7	25.4	34.3	19.4
16:1	25.1	14.5	25.2	38.2	10.3	13.7	3.2	3.8
18:0	1.1	1.2	1.5	4.1	1.1	1.0	2.3	0.9
18:1	6.6	4.0	1.7	5.2	3.6	5.7	2.6	1.9
18:2	8.0	2.3	8.5	3.0	nd*	nd	nd	tr†
18:3	tr	tr	tr	tr	nd	nd	nd	nd
18:4	1.7	5.0	1.1	4.6	tr	1.2	nd	tr
20:4	1.3	tr	1.1	nd	nd	nd	5.1	nd
20:5	13.3	24.8	19.9	21.4	4.9	9.3	31.2	42.8
22:5	2.6	tr	nd	nd	nd	nd	2.4	nd
22:6	9.5	32.5	11.6	2.8	nd	tr	9.7	14.1

*Not determined.
†Trace (< 1% of total fatty acids).

acids. In BL, the relative concentrations of 20:5 and 22:6 were higher than in other lipid classes. Fatty acids were more unsaturated in MGDG and less unsaturated in SQDG in *P. lutheri*, consistent with data for higher plants [11]. At the low temperature, the relative percentage of 16:1 to total fatty acids in MGDG increased, but was almost unchanged in SQDG and BL. In addition, fatty acid composition of phosphatidylglycerol (PG) was almost unchanged with a decrease in growth temperature (data not shown). It is known that the content of 16:0 and 16:1 in PG are closely associated with the chilling-sensitivity of higher plants [12, 13]; the rapid desaturation of 16:0 to 16:1 in MGDG is considered to play a central role in the thermo-adaptation of cyanobacteria [14, 15]. It would appear that our results on *P. lutheri* are consistent with those for cyanobacteria. On the other hand, the relative percentages of 20:5 and 22:6 to total fatty acids in TG and BL increased at the lower temperature, but were decreased in MGDG. In general, in microorganisms, the fatty acid composition of membrane glycerolipids is dependent on growth temperature [16]. Increases in the level of PUFA, as well as increases in the levels of monounsaturated, branched and/or short-chain fatty acids, are considered to provide a mechanism for the thermoadaptive regulation of membrane lipid fluidity [17]. Previous reports have suggested that decreasing temperature causes a general increase in the degree of unsaturation of fatty acids in marine phytoplankton [18, 19]. We suggest that, in *P. lutheri*, the polyunsaturated fatty acids 20:5 and 22:6, and betaine lipids, serve as modulators of low-temperature adaptation. This is supported by the observation that 20:5 and 22:6 are distributed at maximum levels in betaine lipids (Table 2) and that the relative percentages of these acids and lipids are increased in cells grown at low temperature (Table 3). Further testing is required to elucidate the mechanism of lipid synthesis at low temperature.

EXPERIMENTAL

Culture conditions. Pavlova lutheri, CSIRO-182, was obtained from the CSIRO Algal Culture Collection, Australia. The expt was carried out using 1.5 l of f/2 culture medium [20] in a 2 l spherical glass container with air-exposure (1 l min^{-1}) under illumination from an incandescent electric lamp. The alga was grown at two different temperatures (15° and 25°). Growth was monitored by cell-counting using a haemacytometer.

Lipid extraction and fractionation. Algal samples at early stationary phase were collected by centrifugation and lipids extracted using the method of ref. [21]. Crude lipid extracts were evapd and dissolved in *n*-hexane. Samples containing *ca* 10 mg lipids were fractionated by silica Sep-Pak CC (2 g silica, Waters) using a modification of the method of ref. [22]. The following solvents were used: (1) hexane–Et_2O, (99:1) (25 ml, carotenes), (2) hexane–Et_2O, (4:1), 26 ml, TG, (3) $CHCl_3$ (40 ml, chlorophylls), (4) Me_2CO–$CHCl_3$, (2:1), 30 ml and Me_2CO–MeOH, (29:1), 12 ml, MGDG, (5) Me_2CO–MeOH (29:1), 12 ml, DGDG, (6) Me_2CO–MeOH, (19:1), 35 ml, SQDG, (7) Me_2CO–MeOH, (2:1), 21 ml, phosphatidylglycerols and phosphatidylinositols), (8) MeOH, 16 ml, phosphatidylethanolamines, (9) MeOH, 41 ml, phosphatidylcholines. Further fractionation of fr. 7 was done with $CHCl_3$–MeOH, (11:10), 11 ml, phosphatidylglycerols, and $CHCl_3$–MeOH (1:4), 12 ml, phosphatidylinositols. TLC was used to investigate the identity and purity of acyl lipids by placing samples on precoated silica-gel plates (Merck). The following solvents were used: (1) TG: heptane–Et_2O–HOAc (75:25:4) [23], and (2) glycolipids and phospholipids: $CHCl_3$–MeOH–HOAc–H_2O (85:15:10:3) [24]. Pure reference substances were co-chromatographed for identification. Spots were detected using cupric acetate [25] for TG, α-naphthol [26] for glycolipids, molybdenum trioxide–H_2SO_4 [27] for phospholipids and Dragendorff's reagent [28] for choline lipids.

Fatty acid analysis. Lipids were methanolized with 5% HCl in MeOH for 2 hr at 95°. The resulting Me esters were analysed FID- by using a WCOT-fused silica capillary column (0.25 mm i.d. × 50 m length; CP-Sil-88; Chrompack; film thickness: 0.20 μm). The flow rate of the carrier gas (He) was 1.3 ml min^{-1}. The column temp. was prog. to increase from 180° to 240° at 4°C min^{-1}; injector and detector temp. were 240 and 260°. Fatty acid Me esters were identified by comparing R_t values with those of authentic standards and also by GC–MS (injection and separator temp. were 240° and 250°). The ionization voltage, emission current and multiplier were 70 eV, 200 μA, and 1.1 kV, respectively. MS were obtained in both the EI and methane-CI modes. Quantitative analysis was done using a GC, comparing peak areas with those of standard reference materials.

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