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EFFECT OF SALINITY ON FATTY ACID COMPOSITION OF A GREEN MICROALGA FROM AN ANTARCTIC HYPERSALINE LAKE

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Abstract—The major fatty acids in a *Dunaliella* sp. isolated from an Antarctic hypersaline lake were 18:3ω3, 16:0 and 16:4ω3, which together accounted for 72–75% of the total fatty acids in the cells. The fatty acid composition was modified by varying the salinity in the growth medium. With salinity increasing from 0.4 M to 4 M NaCl in the cultures, the proportion of total saturated and monounsaturated fatty acids increased, while total polyunsaturated fatty acids decreased. All the ω3 fatty acids showed negative trends to salinity. Total polyunsaturated fatty acid content was *ca* 10% higher in the low salinity culture. The results obtained suggest that increasing salinity in the growth medium increases the degree of fatty acid saturation and, hence, reduces the fluidity and permeability of the microalgal membranes. ©1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Lipids are vital to cell function as structural components of cell membranes and storage products. The lipid content and composition of microalgae have been shown to change in response to environmental variables, such as temperature, light and salinity [1–4]. However, detailed analyses of the effects of salinity on microalgal lipid production and composition have only been carried out with a few, temperate, species. Furthermore, individual species show different responses to salinity stress, perhaps as a result of different means of adaptation [5]. Green unicellular microalgae of the genus *Dunaliella* are well known for their capability to grow in a wide range of salinities from near fresh water to salt-saturated water. Although the fatty acid composition of lipids in other *Dunaliella* species is well documented, little is known about the fatty acid composition of organisms that normally grow under combined extremes of cold and salinity. To this end, we have examined the fatty acid composition of a species of *Dunaliella* isolated from a Antarctic hypersaline lake. The organism used in this study originated from an environment with a salinity of 200‰ and appears different from other known *Dunaliella* species in its biochemical and physiological responses to environmental variables [4].

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RESULTS AND DISCUSSION

The relationship between the cellular fatty acid composition of the total lipids in the *Dunaliella* sp. in exponential growth stage and salinity (sodium chloride concentration) is shown in Table 1. This microalga is characterised by a high proportion of C_{16} and C_{18} polyunsaturated fatty acids (PUFAs) and, in particular, by a high content of ω3-PUFAs in low salinity cultures. The composition of the fatty acids is typical of most green algae, with C_{16} and C_{18} PUFAs being the most abundant. The fatty acid pattern of this *Dunaliella* sp. is similar to those reported for *D. tertiolecta* and *D. parva* [6–8] and six *Dunaliella* sp. from the Dead Sea [9], but is somewhat different from that of the extremely halophilic species, *D. salina* [10–12] and *D. bardawil* [13].

The proportions of individual fatty acids in *Dunaliella* sp. were influenced by salinity. Within the salinity range from seawater (*ca* 0.4 M NaCl) to 4 M NaCl concentrations, the most abundant fatty acids present in this species were 18:3ω3, which represented 36–41% of the total fatty acids, together with 16:0 and 16:4ω3 (Table 1). These three major fatty acids accounted for 72–75% of the total fatty acids. Despite the large proportion of these three major fatty acids, there were 25 fatty acid species detected in this *Dunaliella* sp.; 20:0, 20:1ω9, 20:2ω6, 20:3ω3, 20:4ω6, and 20:5ω3 fatty acids were present in trace amounts and together accounted for less than 2% of the total.

Table 1. Fatty acid composition of *Dunaliella* sp. grown at different NaCl concentrations

Fatty acid	NaCl 0.4 M	1.0 M	1.6 M	2.2 M	2.8 M	3.4 M	4.0 M
14:0	1.30 (0.11)	1.77 (0.52)	1.65 (0.56)	1.59 (0.16)	1.99 (1.01)	2.26 (1.05)	1.80 (1.08)
14:1ω5	1.24 (0.21)	1.34 (0.25)	1.37 (0.20)	1.33 (0.07)	1.68 (0.55)	1.68 (0.16)	1.39 (0.25)
15:0	0.32 (0.01)	0.26 (0.1)	0.19 (0.11)	0.15 (0.06)	0.14 (0.08)	0.14 (0.01)	0.08 (0.02)
16:0	22.07 (1.97	24.34 (1.17)	22.20 (3.30)	21.44 (3.46)	23.63 (0.76)	29.88 (2.40)	26.69 (3.27)
16:1ω9	0.17 (1.01)	0.26 (0.17)	0.25 (0.13)	0.23 (0.11)	0.24 (0.16)	0.25 (0.12)	0.17 (0.11)
16:1ω7	3.67 (0.35)	3.15 (1.49)	3.14 (1.04)	3.46 (0.62)	3.13 (0.35)	2.29 (1.23)	3.15 (0.42)
16:2ω9	0.43 (0.18)	0.65 (0.41)	0.64 (0.54)	0.57 (0.21)	0.44 (0.45)	0.36 (0.16)	0.25 (0.17)
16:2ω6	0.59 (0.04)	0.48 (0.21)	0.53 (0.01)	0.52 (0.13)	0.39 (0.06)	0.31 (0.03)	0.26 (0.01)
17:0	0.50 (0.05)	0.53 (0.11)	0.55 (0.06)	0.44 (0.16)	0.59 (0.11)	0.87 (0.47)	0.55 (0.04)
13:3ω3	1.54 (0.18)	1.35 (0.11)	1.36 (0.55)	1.25 (0.52)	0.91 (0.35)	0.32 (0.08)	0.20 (0.10)
16:4ω3	12.50 (0.33)	10.32 (1.61)	11.59 (0.62)	12.27 (0.63)	10.74 (3.69)	7.91 (1.44)	7.54 (0.52)
18:0	0.51 (0.21)	0.55 (0.26)	0.55 (0.26)	0.50 (0.01)	0.59 (0.28)	0.80 (0.12)	0.60 (0.38)
18:1ω9	2.60 (0.16)	3.67 (1.21)	4.14 (0.88)	3.61 (0.31)	5.09 (1.88)	5.92 (1.15)	7.06 (0.99)
18:1ω7	1.6C (0.11)	2.11 (0.60)	1.84 (0.23)	1.73 (0.46)	1.56 (0.45)	0.79 (0.47)	0.89 (0.17)
18:2ω9	1.69 (0.17)	1.67 (0.99)	1.87 (0.69)	1.88 (0.47)	1.91 (0.59)	1.79 (0.85)	1.85 (0.06)
18:2ω6	1.92 (0.55)	2.41 (0.21)	2.85 (0.13)	2.63 (0.11)	3.13 (0.03)	3.13 (0.11)	3.76 (1.27)
18:3ω6	2.89 (0.13)	3.09 (0.22)	3.56 (0.30)	3.76 (0.55)	3.45 (0.66)	2.85 (0.13)	2.58 (0.42)
18:3ω3	40.87 (2.40)	38.42 (1.63)	38.29 (3.92)	39.63 (3.48)	37.60 (2.01)	35.83 (4.29)	39.27 (3.87)
18:4ω3	2.06 (0.14)	1.90 (0.48)	1.99 (0.45)	1.78 (0.20)	1.26 (0.21)	0.78 (0.04)	0.58 (0.08)
Others*	1.53 (0.23)	1.75 (0.71)	1.44 (0.87)	1.23 (0.06)	1.53 (0.36)	1.83 (0.55)	1.32 (0.42)
SAFA	24.69 (2.33)	27.44 (2.16)	25.14 (4.29)	24.11 (3.86)	26.94 (2.25)	33.95 (4.06)	29.72 (4.79)
MOFA	9.29 (0.84)	10.52 (3.71)	10.74 2.47)	10.36 (1.57)	11.70 (3.39)	10.93 (3.12)	12.66 (1.94)
PUFA	64.50 (4.14)	60.28 (5.86)	62.68 (7.21)	64.31 6.30)	59.83 (8.06)	53.30 (7.13)	56.30 (6.51)
ω3PUFA	56.98 (3.05)	51.98 (3.83)	53.23 (5.54)	54.93 (4.83)	50.51 6.26)	44.85(5.85)	47.60(4.58)

Means of two experiments; standard deviations given in parentheses.
* Includes 20:0, 20:1ω9, 20:2ω6, 20:ω3, 20:4ω6 and 20:5ω3.

With increasing NaCl concentrations, fatty acids of the *Dunaliella* sp. exhibited certain trends. Correlation analysis of the percentage of individual fatty acid against NaCl concentrations showed that 16:0 was positively correlated with NaCl ($r = 0.673$; 12 d.f.) and 18:3ω3 and 16:4ω3 had negative correlations with salinity ($r = -0.512$; 12 d.f. and $r = -0.796$; 12 d.f., respectively). Octadecenoic acid (18:1ω9) was positively correlated with NaCl ($r = 0.948$; 12 d.f.) with its contribution rising from 2.6% in cells cultured in 0.4 M NaCl to 7.1% in cells cultured in 4 M NaCl, 18:2ω6 also showed the same trend ($r = 0.947$; 12 d.f.). Hexadecatrienoic acid (16:3ω3) and 18:4ω3 had negative correlations with salinity ($r = -0.941$ and $r = -0.942$; 12 d.f., respectively). Thus, overall, the total saturated fatty acids (SAFA) and total monounsaturated fatty acids (MOFA) exhibited positive correlations with NaCl ($r = 0.667$ and $r = 0.861$; 12 d.f., respectively), while total PUFA and total ω3-PUFA were negatively correlated with salinity ($r = -0.766$ and $r = -0.83$; 12 d.f., respectively) in the cultures (Fig. 1). The trends in this *Dunaliella* sp. are different from those reported for *D. salina* [11], which showed the opposite trend for cells grown in the presence of 2.5% NaCl and 20% NaCl.

The main roles of fatty acids in algae are related to cell membrane functions and to metabolic processes. The degree of fatty acid unsaturation is important in maintaining the fluidity of the membrane and in providing the appropriate environment for membrane functions. The degree of unsaturation of membrane fatty acids is also important in the process of plant adaptation to growth environment. With increasing salinity in culture, the cell internal osmotic pressure will increase with the external salinity, which involves the accumulation of high concentrations of glycerol (about 80% of the external osmotic pressure) and ions [14–16]. This is especially important to species, such as *Dunaliella*, which lack a cell wall. Reducing PUFA and increasing SAFA with rising salinity suggest a reduction in membrane fluidity and permeability. Such changes would improve the performance of the alga at high salinity by preventing leakage of the compatible solute, glycerol, out of the cell and diffusion of potentially harmful ions into the cell [17]. Both short-term and long-term effects of salinity changes on the organisation of plasma membrane lipids have been investigated. Fontana and Hang [18] observed a decrease in fluidity of the plasma membrane of *D. primolecta* cells adapted to high NaCl concentration, whereas Curtain *et al.* [19] reported a similar decrease in motion of the 16-nitroxide-stearate probe in *D. salina* plasma membranes after 18 h exposure to high NaCl concentration. Our results of variations in total SAFA and PUFA contents support the above findings.

It must be recognised that the results presented here relate to the total lipid content of the cells and further

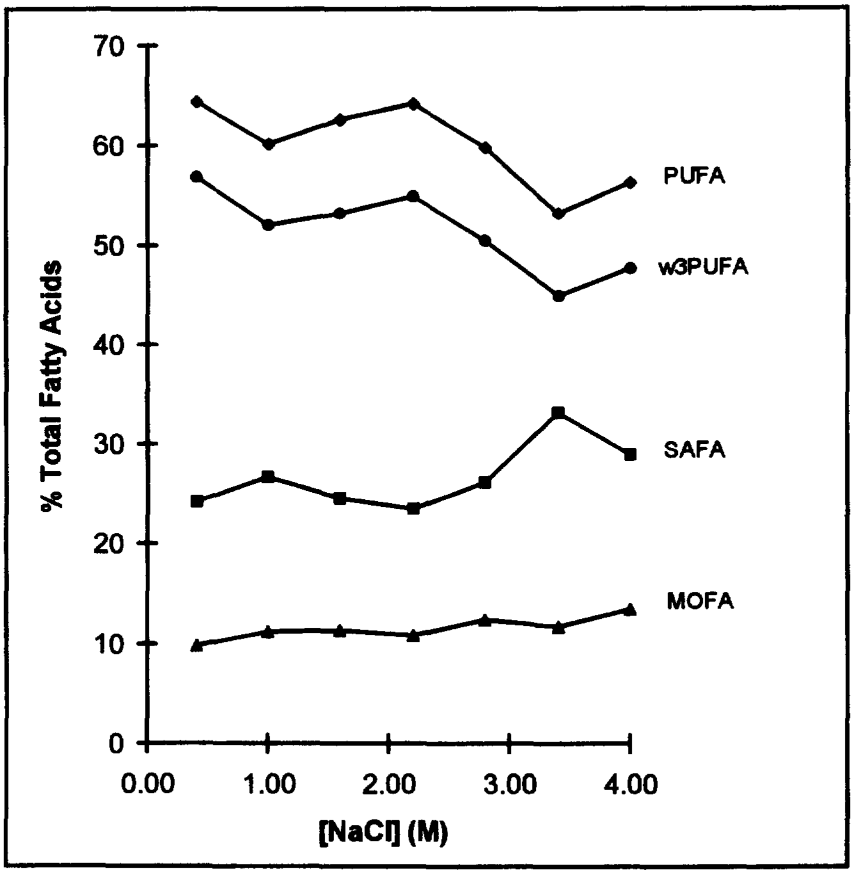

Fig. 1. Total polyunsaturated (PUFA), total ω3 polyunsaturated (ω3PUFA), total monounsaturated fatty acids (MOFA) and total saturated fatty acids (SAFA) in *Dunaliella* sp. cells grown at different salinities. Values given are the means of two replicates.

experimentation is required to examine in more detail the changes in fatty acid composition of specific membrane components, such as phospholipids or galactolipids. Changes in fatty acid composition of membrane lipids but not triacylglycerols have been noted in the green filamentous alga, *Cladophora vagabunda*, exposed to different salinities [20]. In addition, sterols are known to play an important role in membrane properties [20, 21] and investigations into changes in sterol content of membranes of our *Dunaliella* isolates under different environmental conditions are necessary.

EXPERIMENTAL

Species, medium and growth conditions. The species of green unicellular microalga, provisionally identified as *Dunaliella* sp. (Hallegraeff G., pers. comm.), used in this study was isolated from Lake Stinear in the Vestfold Hills, Australian Antarctic territory. At the time of isolation, salinity in Lake Stinear was 200‰. Cultures were grown in PHK medium, a modified 'D' medium [22 and Kelly G., pers. comm.]. Salinity was adjusted to the appropriate value by changing the concn of NaCl in PHK medium. Cultures were inoculated to *ca* 5×10^4 cells ml^{-1} using those cells grown in 1.6 M NaCl PHK medium at 5°. Cultures were grown at 10° under continuous illumination provided from cool white fluorescent lamps and given a photon flux at the culture surface of 45 $\mu E\ m^{-2}\ s^{-1}$. Cell density was determined periodically using an improved Neubauer Haemocytometer.

Extraction of lipids for fatty acid analysis. Lipids were extracted by a method modified from that described by Bligh and Dyer [23]. Cells in exponential growth phase (200 ml) were filtered onto 47 mm diameter GF/C Whatman Glass Microfibre Filters. Filtered cells were then extracted immediately in a 100 ml beaker with a mixt. of 8 ml distilled H_2O, 10 ml $CHCl_3$ and 20 ml MeOH, and sonicated for 10 min using a microtip of a B-30 Branson Sonifier Cell Disruptor. $CHCl_3$ (10 ml) and distilled H_2O (10 ml) were added sequentially to the extract and sonicated for 10 min. The resulting soln was filtered under vacuum through a 25 mm diameter GF/C Whatman Microfibre filter and the filtrate obtained sepd by centrifugation. The lower layer of $CHCl_3$ was transferred and evapd under red. pres.

Fatty acid methylation. Fatty acids were converted to their Me esters prior to analysis by capillary GC. A method modified from ref. [24] was used for fatty acid methylation. Total lipids were methylated in 4 ml of 1 M KOH in HPLC-grade MeOH for 1 hr at room temp. The fatty acid Me esters were extracted with hexane and analysed by GC immediately.

Fatty acid analysis. Analysis of fatty acid Me esters was carried out using a fused silica capillary column (J&W Scientific, DB-23, 0.25 μm film thickness, 30 m $\times$ 0.25 mm i.d.) and a FID. N_2 was the carrier gas at 30 ± 1 ml min^{-1}. Initial column temp. was 100° and was raised to 160° at 10° min^{-1}, then programmed to 220° at 2.5° min^{-1} and finally heated to 240° at 10° min^{-1}. Injector and FID detector temps were 250° and 280°C, respectively. Samples of fatty acid Me esters (1 μl) were injected through a JADE valve. Peak areas were measured by electronic integration. Me esters were identified by chromatographic comparison with authentic standards (Sigma).

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