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INTRASPECIFIC VARIATIONS IN THE FATTY ACIDS OF THE DIATOM *SKELETONEMA COSTATUM*

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Key Word Index—*Skeletonema costatum*; diatom; unicellular alga; intraspecific variations; fatty acids.

Abstract—The fatty acid composition of triplicate cultures of four clones of the marine diatom *Skeletonema costatum* are reported. Variations in the percentage of individual components and in total acid concentrations are significantly different between clones from coastal, estuarine and oceanic locations. The percentage abundance of individual fatty acids showed the greatest diversity between clones, with all clone pairs except the two isolated from coastal environments being significantly different. Growth curves and cell size were also different for individual clones, providing further evidence of intraspecific diversity.

INTRODUCTION

Intraspecific variations in a range of physiological processes have been observed in marine algae and these appear to be widespread among the major phytoplankton groups [1 and references therein]. Carpenter and Guillard [2] demonstrated that nitrate half-saturation constants exhibit clonal variation for three algal species, with major differences between clones isolated from estuarine and shelf regimes. They suggested that physiological races of marine phytoplankton exist, which have adapted to high or low nutrient concentrations. Later research measuring uptake of nitrate and phosphate supports these findings and is consistent with the hypothesis that algae from eutrophic waters are more productive, but less nutrient efficient than those from oligotrophic waters [3]. Similar variations are observed in response to chemical stress [4, 5], with estuarine clones less sensitive to organic pollutants than those from the open ocean.

Intraspecific diversity is well known in phytoplankton [1, 6] and may have important chemotaxonomic implications. Lipid composition, using distribution of compound classes and individual components, has been used as a taxonomic feature in the classification of algae [7–10] and in the identification of algal contribution to sedimented organic material [11, 12]. Although variations in the chemical composition of various algal species has been studied extensively [13–17], as have the effects of growth stage, culture medium and illumination [10, 13, 14], little is known of intraspecific lipid variability in algae.

We report here the fatty acid composition of four clones of the diatom *Skeletonema costatum* isolated from different environments (Fig. 1) and grown concurrently under identical conditions. Comparison of fatty acid distributions is used to determine statistically significant differences between clones isolated from oceanic, coastal and estuarine environments. *Skeletonema costatum* was chosen for this study because of its ubiquitous global distribution [1] and popularity as a laboratory test organism.

RESULTS

The relative percentage abundances of individual acid components are presented in Table 1 as the means of triplicate cultures. Other components were present in the acid fractions, but only those greater than 0.2% have been reported due to difficulty in the accurate quantification of minor components.

The present analysis of *S. costatum* acids with 16:1Δ9,* 16:4Δ6, 20:5Δ5, 16:3Δ6 and 14:0 as the major components (Table 1) is consistent with those of previous workers [15, 18–20]. Acids from a two week culture analysed by Chuecas and Riley [15] are quantitatively similar to the two day rather than the ten day culture of Ackman *et al.* [18]. This is likely to have resulted from differences in culture conditions, the clone studied, extraction procedure and analysis techniques.

Differences in fatty acid composition of *S. costatum* clones in the present study are unlikely to be due to variations in growth stage as cells were harvested after 10–13 days log phase growth (Fig. 2) when cell counts were comparable to those reported by Ackman *et al.* [18]. The acid distribution of *S. costatum* stabilizes after five days [18] and the similar distribution of major acids to that reported [18] indicates that cells were not harvested at an early growth stage when component acids were undergoing rapid changes.

For comparisons with our results we use mainly the fatty acid composition reported by Ackman *et al.* [18] as their three separate experiments quoting variability of acids with culture age represents the most thorough

*Unless specified, all double bonds are *Z*-isomers. Double bonds are numbered from the carboxyl end of the fatty acid, with all subsequent unsaturations methylene interrupted.

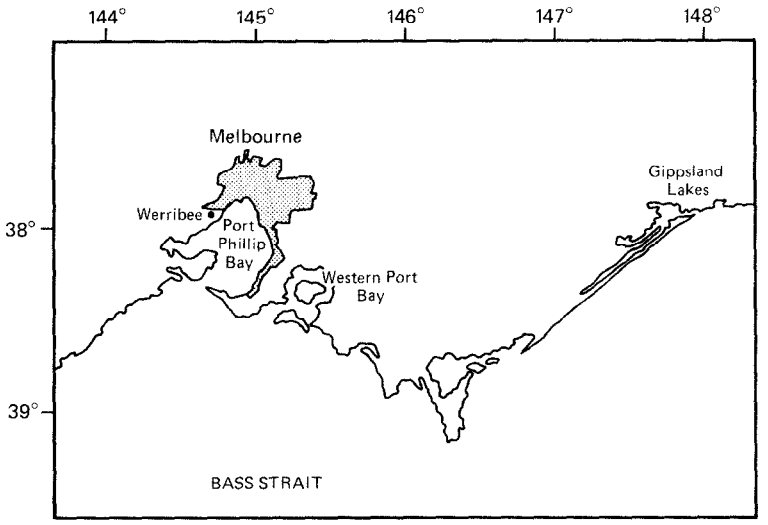

Fig. 1. Map showing areas from which clones of *S. costatum* were isolated.

Table 1. Means ($\bar{x}$) and standard deviations (s) of relative percentage fatty acid composition for triplicate cultures of *S. costatum*

Strain no.	N4-4 Coastal/eutrophic		N6-2 Coastal		3057 Estuarine		B2 Oceanic	
Acid	$\bar{x}$	(s)	$\bar{x}$	(s)	$\bar{x}$	(s)	$\bar{x}$	(s)
14:0	13.09	(0.80)	12.76	(0.91)	15.92	(0.73)	16.77	(0.28)
15:0	0.48	(0.08)	0.54	(0.24)	0.74	(0.05)	0.55	(0.06)
16:0	6.11	(0.55)	4.75	(1.50)	8.94	(0.14)	4.65	(0.15)
18:0	0.33	(0.02)	0.35	(0.03)	0.56	(0.06)	0.44	(0.06)
E-16:1Δ3	0.41	(0.02)	0.72	(0.49)	0.90	(0.08)	1.62	(0.18)
16:1Δ9	22.70	(1.27)	21.27	(1.95)	20.40	(0.22)	14.98	(1.44)
16:1Δ11	1.33	(0.11)	1.31	(0.05)	0.72	(0.06)	1.94	(0.16)
18:1Δ9	0.73	(0.10)	1.35	(1.31)	1.04	(0.07)	0.80	(0.07)
18:1Δ11	0.51	(0.24)	0.53	(0.16)	0.69	(0.11)	0.60	(0.14)
16:3Δ6	14.88	(0.47)	14.20	(1.77)	13.71	(0.22)	12.22	(0.43)
16:4Δ6	13.13	(1.15)	14.07	(1.56)	17.42	(0.56)	22.94	(0.86)
18:2Δ9	1.18	(0.11)	1.09	(0.07)	0.97	(0.08)	1.35	(0.16)
18:3Δ9	0.33	(0.07)	0.32	(0.05)	0.27	(0.05)	1.15	(0.08)
18:4Δ6	4.02	(0.19)	5.11	(1.48)	4.82	(0.19)	6.44	(0.22)
20:5Δ5	18.57	(0.54)	18.18	(1.57)	10.59	(0.34)	10.52	(1.31)
20:6Δ4	2.23	(0.10)	3.46	(1.46)	2.31	(0.10)	3.05	(1.02)
SFA	20.01	(0.45)	18.40	(1.02)	26.16	(0.90)	22.55	(0.24)
MUFA	25.65	(1.39)	25.18	(0.51)	23.75	(0.08)	19.79	(1.76)
PUFA	54.34	(1.18)	56.42	(1.49)	50.09	(0.95)	57.66	(1.71)

analysis of the fatty acids of *S. costatum*. It is interesting that *E*-16:1Δ3 has not been reported previously in *S. costatum*, particularly since it is commonly found in the chloroplasts of photosynthetic organisms [21–23]. Ackman *et al.* [18] stated that their techniques would not detect this component, but in subsequent work report its absence [19]. Concentrations of 14:0 are lower, and 16:1Δ9 are higher, than those reported by Ackman's group [18–20], but they are similar to those reported by Chuecas and Riley [15]. The acid 16:2Δ9 was not detected in our study due to coelution with 16:4Δ6 under the conditions used, and this also accounts for the apparently higher abundance of the latter component than in previous reports.

Selected individual fatty acid components, and the relative composition of acid groups comprising saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) components, can be used to distinguish clone pairs from all environments other than the coastal/coastal-eutrophic pair to significance levels of $P<0.001$ and $P<0.01$, respectively (Table 2). The coastal and the coastal-eutrophic clones have similar individual acid dis-

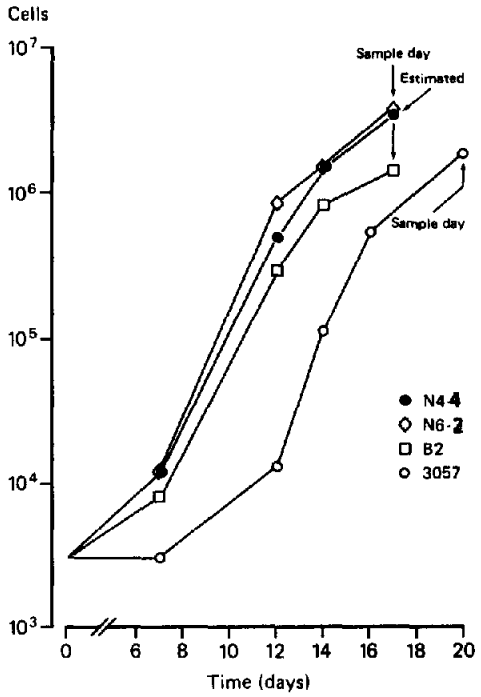

Fig. 2. Growth curves for the four *S. costatum* clones studied in cells/ml.

tributions, with only total saturated acids exhibiting a significant difference. Total fatty acid content on a dry weight basis is not a good measure of clonal diversity, with significant variation between only the estuarine-oceanic pair ($P<0.05$).

Cell growth rate measurements (Table 3) are subject to maximum relative errors of $\pm 10\%$. Cell size varied among clones, with the estuarine and oceanic cells being larger and heavier than the coastal and coastal-eutrophic clones, which were similar in size and mass (Table 3). The estuarine clone showed fastest growth and largest cell mass, indicating substantially faster biomass production than by the other clones. The extended lag phase observed in this study (Fig. 2) accounts for the long culturing time required.

DISCUSSION

Fatty acids of the diatom *S. costatum* are significantly different ($P<0.01$) among clones isolated from coastal, estuarine and oceanic environments, although the two clones from coastal locations were indistinguishable using fatty acid distribution or absolute abundance. This is consistent with cells of a particular genetic composition being selected by a favorable fatty acid composition. Study of a greater number of clones would be required to test this hypothesis.

The abundance of total saturated acids can be used to distinguish clone pairs to the same level of significance as

Table 2. Probability of significant difference in acid composition of clone pairs of *S. costatum*

Acid	Clone pairs C/CE†	C/OE	C/O	CE/E	CE/O	E/O
14:0	—	**	***	**	***	—
15:0	—	—	—	—	—	—
16:0	—	**	—	**	—	**
18:0	—	**	*	**	*	*
E-16:1Δ3	—	—	*	—	**	*
16:1Δ9	—	—	**	—	***	**
16:1Δ11	—	***	***	***	***	***
18:1Δ9	—	—	—	—	—	—
18:1Δ11	—	—	—	—	—	—
16:3Δ6	—	—	—	—	*	—
16:4Δ6	—	**	***	**	***	***
18:2Δ9	—	—	*	—	—	*
18:3Δ9	—	—	***	—	***	***
18:4Δ6	—	—	—	—	*	—
20:5Δ5	—	***	***	***	***	—
20:6Δ4	—	—	—	—	—	—
SFA	*	***	***	***	**	***
MUFA	—	—	**	—	**	**
PUFA	—	*	—	*	*	***
FA‡	—	—	—	—	—	*

—: $P>0.05$, *: $P<0.05$, **: $P<0.01$, ***: $P<0.001$.

† C: Coastal, CE: Coastal eutrophic, O: Oceanic, E: Estuarine.

‡ Fatty acid concentration (mg/g dry wt), See Table 3 for absolute wts.

Table 3. Growth rate, cell density, cell mass and acid concentration of clones of *S. costatum*

Strain no.	Clone	Specific growth rate (doublings per day)	Cell density* (cells/ml)	Cell mass (pg/cell)	Fatty acids (mg/g dry wt) $\bar{x}$	(s)
N4-4	Coastal/eutrophic	0.69	3.40×10^6	10.9	34.51	(5.56)
N6-2	Coastal	0.69	3.76×10^6	11.3	32.12	(3.72)
3057	Estuarine	0.66	1.83×10^6	17.9	38.10	(1.51)
B2	Oceanic	0.92	1.44×10^6	22.0	32.92	(0.84)

*Measured at harvesting, during late log phase.

selected individual acids [except for coastal-eutrophic/oceanic], and was the only variable to differentiate the coastal and coastal-eutrophic clones. The effectiveness of this acid group in distinguishing clones from different environments may be related to the importance of the proportion of saturated acids in controlling membrane fluidity.

The presence of 18:1Δ11 in *S. costatum* is of interest. It has been commonly associated with bacterial acid synthesis [25], but has also been identified in a variety of marine algae [7, 26–29]. This study extends the range of organisms other than bacteria in which 18:1Δ11 occurs. The total absence of branched chain fatty acids common in bacteria [30], with the absence of bacteria on peptone agar plates innoculated with algal culture media, precludes a bacterial source of 18:1Δ11 in our cultures.

The long culturing time of all clones is interesting as Sakshaug and Holm-Hansen [31] report an initial lag phase of less than two days for *S. costatum* and Ballantine *et al.* [14] report that cells have entered stationary phase after ten days—much sooner than in this study. Gallagher [32] found that some clones from Narraganset Bay grew slowly in artificial seawater and required preadaption to culture media. The slow growth was not due to an extension of lag phase as longer acclimation times in artificial seawater did not lead to significantly increased growth rates. The extended lag phase encountered in the present study is, therefore, unlikely to be the reason for the observed clonal variation in fatty acid composition.

Cell size of *S. costatum* in this experiment differed among clones from different environments (Table 3). Size variations have been observed in cells of *S. costatum* in the form of vegetative cell enlargement morphologically similar to auxospore formation [32], with enlarged cells being two to three times the diameter of normal cells. Periods of synchronous cell enlargement are common in Atlantic populations of *S. costatum* [32], however, the cells were consistent in their dimensions, throughout these experiments, confirming that cell size is genetically controlled by the clones.

Phospholipids comprise the major fatty acid fraction in log phase cells [33] and component fatty acids important in determining clonal variability, such as 14:0, 16:0, 16:1Δ11, 16:4Δ6 and 20:5Δ5 are likely to be found in the cellular phospholipids. These acids have previously been found as major components in triacylglycerols [20], but as this class is usually a minor fraction of the lipids [34], such a source is unlikely here.

Acid composition of algae is controlled enzymatically by chain elongase and desaturase enzymes, a range of which occur in diatoms [24]. Activity of these enzymes is variable, with the chain elongase enzyme showing no trend between clones and only the Δ6 and ω3 desaturases showing major differences between clones. Specific fatty acids are important in controlling membrane functions, with Na^+/K^+ ATPase activity being affected by membrane lipid composition [35] and high ATPase activity in phospholipid vesicles requiring that they contain polyunsaturated fatty acids [36]. Fluidity is also known to be influenced by the level of polyunsaturated components, with greater unsaturation leading to greater membrane fluidity.

The effectiveness of total saturated fatty acids in differentiating clones indicates that membrane fluidity may be directly affected by source environment in *S. costatum*, even though the ratio of unsaturated to saturated fatty acids shows no trend between the estuarine, coastal and oceanic clones used in this study. This ratio was unaffected by decreasing temperature in an alga isolated from low temperature waters [37], although changes in the ratios of individual components indicated that specific desaturase enzymes were affected. Heterozygote genotypes have become homozygous in cultured clones [38], however *S. costatum* can maintain itself indefinitely by purely asexual means [32] and clonal stability for more than seven years has been reported [32]. Varying culture parameters such as temperature and salinity during culturing may demonstrate that the same algal species isolated from more extreme environments are capable of changing their lipids, and thus membrane properties, but under the culture conditions employed here this effect is not seen.

The observed differences in fatty acid composition may help to explain the previously observed intraspecific variation in membrane-related functions, including cellular nutrient uptake [3] and the effects of chemical stress [4, 5].

EXPERIMENTAL

S. costatum (clonal designations N4-4, N6-2, 3057 and B2, Marine Science Laboratories, Queenscliff, Victoria, Australia) were aseptically maintained in f/2 medium [39] at 18° under 14/10 hr light/dark cyclic illumination, provided by cool, white fluorescent light. Clone N6-2 was isolated from Western Port Bay, N4-4 from Port Phillip Bay offshore from a major sewerage outlet, 3057 from the Gippsland Lakes and B2 from Bass Strait (Fig. 1). Clones are referred to in terms of their isolation environment, these being coastal (N4-4), coastal eutrophic (N6-2), estuarine (3057) and oceanic (B2).

Cells harvested in late log phase were filtered onto $CHCl_3$ washed glass fibre filters (Whatman, GF/C) and lipids extd using 3×10 ml $CHCl_3$–MeOH (2:1) (+0.05% pyridine). Blanks (sterile medium incubated concurrently with cultures) were treated identically to cell cultures. Cell density determined microscopically using an Improved Neubauer Haemacytometer and dry wt were obtained as described in ref. [7]. The absence of bacteria in the experimental cultures was confirmed by plating aliquots of culture onto seawater peptone agar. The three 10 ml extracts were combined to give the total solvent extractables, from which were obtained total neutral lipids and fatty acids as their Me esters (FAMEs) as described in refs [8, 39]. FAMEs were analysed by GC on an SE30 fused silica capillary column (25 m $\times$ 0.2 mm i.d.), temp. prog. from 170 to 270° at 4°/min. H_2 was carrier gas (linear flow, 20 ml/min). FAMEs were identified by co-chromatography with standards, comparison of R_fs with those of FAMEs extracted from cod liver oil [40, 41] and ECL measurements [40, 42–44]. GC-MS was used to confirm the carbon number and number of unsaturations of acid components, using conditions reported in ref. [45]. Each component was quantified from the FID response calibrated with a range of satd and unsatd acid Me ester stds and tetracosane as int. std. Individual components are subject to maximum relative errors of $\pm 5\%$.

The significance of variance between clones was determined using the Neuman–Keuls test for multiple ranges [46, 47]. The data were arcsine transformed to arcsine transformation ($X' = \text{arcsine}\sqrt{X}$) prior to statistical analysis because of deviations from normality encountered using percentage values [6].

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