

Fatty acid composition profiles of 235 strains of three microalgal divisions within the NIES Microbial Culture Collection

Eri Mitani¹⁾, Fumiyo Nakayama¹⁾, Izumi Matsuwaki¹⁾, Ikuyo Ichi^{1, 2)},
Atsushi Kawabata³⁾, Masanobu Kawachi^{3)*} and Misako Kato^{1, 2)}

¹⁾Graduate School of Humanities and Sciences, ²⁾Institute for Human Life Innovation,
Ochanomizu University, 2-1-1 Otsuka, Bunkyo-ku, Tokyo 112-8610, Japan

³⁾National Institute for Environmental Studies, Onogawa 16-2, Tsukuba, Ibaraki 305-8506, Japan

To evaluate the occurrence of polyunsaturated fatty acids as phenotypic markers, we analyzed the fatty acid compositions of 235 strains of microalgae within the divisions Heterokontophyta, Cryptophyta, and Haptophyta held in the Microbial Culture Collection at the National Institute for Environmental Studies. Eicosapentaenoic acid (EPA, 20:5) was present at >10% of the total fatty acid composition in 119 strains. Docosahexaenoic acid (DHA, 22:6) DHA was present at >10% of the total in 16 strains, most in the Haptophyta, in addition to two unidentified strains in the Pelagophyceae (Heterokontophyta). Strains of the Heterokontophyta other than the Chrysophyceae and the Pelagophyceae and the strains of Cryptophyta typically contained more EPA than DHA. The Heterokontophyta, with 11 classes, including the newly recognized Aurearenophyceae, Pelagophyceae, and Schizocladiophyceae, showed diverse fatty acid profiles, although clear phenotypic results defining each taxonomic group were not confirmed.

Key words: Cryptophyta, docosahexaenoic acid, eicosapentaenoic acid, fatty acid, Haptophyta, Heterokontophyta, microalgae

INTRODUCTION

The fatty acid compositions of a large number of microalgae have been reported. Of particular interest from a chemotaxonomic viewpoint is the occurrence of polyunsaturated fatty acids (PUFAs). Culture strains of microalgae are maintained in the Microbial Culture Collection at the National Institute for Environmental Studies, Tsukuba, Japan (MCC-NIES). An exhaustive analysis of fatty acid composition using this culture collection will provide useful information for investigators. Differential segregation of microalgae by their fatty acid profiles indicates the potential utility of fatty acids as biomarkers (Sahu *et al.*, 2013). We hope to establish the fatty acid compositions of algae in the MCC-NIES in order to distinguish them. Microalgae are a potential source of bioactive compounds with pharmaceutical, biomedical, and nutraceutical prospects. In particular, their production of PUFAs, such as eicosapentaenoic acid (EPA, 20:5) and docosahexaenoic acid (DHA, 22:6), is the subject of intensive research and increas-

ing commercial attention (Guschina & Harwood, 2006). PUFAs have important nutritional value for human health (Abedi & Sahari, 2014). Selection and screening of PUFA-producing species are considered important to increasing the commercial use of algae. Here we show the fatty acid composition of 235 strains within the divisions Heterokontophyta, Cryptophyta, and Haptophyta held in the MCC-NIES.

MATERIALS AND METHODS

Microalgae were supplied by MCC-NIES and were grown under light at 20°C in the culture media designated by MCC-NIES. Stationary growing cells were harvested by centrifugation and then lyophilized.

Lipids were extracted by the method of Bligh & Dyer (1959). The dried lipid fraction was mixed with 2 ml of 5% (w/w) HCl-methanol and heated at 90°C for 2 h to obtain fatty acid methyl esters. The solution was extracted twice with 2 ml *n*-hexane. The *n*-hexane fraction was concentrated for use in gas chromatographic analysis. Gas chromatography was performed with a fused-silica capillary column (0.25 mm internal diameter × 50 m; Chrompack Cp-Sil 88, Agilent Technologies Inc., USA), with the oven tem-

*Corresponding author

E-mail: kawachi.masanobu@nies.go.jp

perature programmed to increase from 150 to 210°C at 5°C min⁻¹. The fatty acid composition was calculated by a Chromatopac C-R8A data processor (Shimadzu Corp., Kyoto, Japan). To identify the fatty acid methyl esters, we performed gas chromatographic – mass spectrometric analysis with a GCMS-QP2010 Ultra (Shimadzu) equipped with a Varian capillary column (0.25 mm internal diameter × 60 m; Agilent Technologies) at an ionization energy of 70 eV. The oven temperature was initiated at 120°C for 1 min and then raised to 175°C at 25°C min⁻¹, then to 235°C at 5°C min⁻¹, and then held for 5 min. The injection and interface temperatures were 250°C, and the ion source temperature was 200°C.

RESULTS AND DISCUSSION

Fatty acid compositions are shown in Table 1.

Heterokontophyta

Through the analysis of nuclear-encoded SSU *rRNA* and plastid-encoded *rbcL*, *psaA*, *psbB*, and *psbC*, Yang *et al.* (2012) reported three major clades of the Heterokontophyta. SI clade comprised the Schizocladiphyceae, Phaeophyceae, Aurearenophyceae, Chrysomeroephyceae, Phaeophyceae, Raphidophyceae, and Xanthophyceae. In particular, Schizocladiphyceae and the Phaeophyceae formed a subclade within SI clade. The Chrysophyceae, Eustigmatophyceae, Pinguiphyceae, Synchronophyceae and Synurophyceae formed the SII clade. The Bacillariophyceae, Bolidophyceae, Dictyochophyceae and Pelagophyceae formed the SIII clade.

The major fatty acids in the Heterokontophyta were 14:0, 16:0, and 16:1, and most Bacillariophyceae species contained 20:5. The highest content of 20:5 was found in *Asterionellopsis glacialis* (NIES-265, 417), in which it accounted for 40% of the total fatty acids. One of the most well-known marine diatoms that produce 20:5 is *Phaeodactylum tricornutum*, with 20:5 constituting up to 40% of its total (Yongmanitchai & Ward, 1991; Fernández *et al.*, 2000; Tonon *et al.*, 2002; Liang *et al.*, 2006). The yield of 20:5 in *A. glacialis* was equivalent to that in *P. tricornutum*. The content of 22:6 was 0% to 4% in the Bacillariophyceae. The fatty acid composition of *Chaetoceros* spp., which are food for the larvae of crustaceans and fish, has been reported (Liang *et al.*, 2006; Chen, 2012; Boelen *et al.*, 2013; Wang *et al.*, 2014). Although trace amounts of 22:6 have been reported in *Chaetoceros* spp. (Liang *et al.*, 2006; Chen,

2012; Boelen *et al.*, 2013; Wang *et al.*, 2014), we did not detect any in three strains (NIES-377, 553, 586). Sixteen strains in the genus *Skeletonema* (NIES-17, 223, 323, 324, 2533, 2534, 2535, 2536, 2537, 2837, 2839, 2840, 2841, 2842, 2843, 2844) had similar fatty acid compositions to those of *S. costatum* (Berge *et al.*, 1995; Chen, 2012) and *S. marinoi* (Chandrasekaran *et al.*, 2014).

In addition to fatty acid production, biodiesel production by several genera of diatoms has been reported (Graham *et al.*, 2012). Some, such as *Cyclotella*, are capable of heterotrophic growth, so a high yield of biomass is expected (Glaude & Maxey, 1994; Pahl *et al.*, 2010). The content of 20:5 in four strains of *Cyclotella* was 8.3% to 15.4%. *Fistulifera saprophila* (NIES-2722) had a very high content of 20:5 (25.7%). The closely related *F. solaris* was shown to be a powerful candidate for production of 20:5 and biodiesel because of its high lipid productivity (Liang *et al.*, 2014).

In the Chrysophyceae, *Ochromonas danica* is one of the most studied species with respect to fatty acid and lipid biosynthesis (Vogel & Eichenberger, 1992). The structure of two algal betaine lipids —1,2-diacylglyceryl-*O*-4'-(*N,N,N*-trimethyl)homoserine and 1,2-diacylglyceryl-*O*-2'-hydroxymethyl-(*N,N,N*-trimethyl)β-alanine— was first determined from their production in *O. danica* (Nichols & Appleby, 1969; Vogel *et al.*, 1990). The algae in the genus *Synura*, sometimes classified in the class Synurophyceae (Siver *et al.*, 2015), are characterized by distinctive siliceous surface scales. In *Synura*, 14:0, 16:0, and 18:4 were abundant, and 22:6 was more abundant than 20:5.

Nannochloropsis oculata (NIES-2145) is the most studied alga in the Eustigmatophyceae (Tonon *et al.*, 2002; Olofsson *et al.*, 2012). A high 20:5 content appears to be a characteristic feature of *Nannochloropsis* (Huerlimann *et al.*, 2010; Lim *et al.*, 2012). Contents of 16:0, 16:1, 18:0, and 20:5 were >10% in *N. oculata*, and the high contents of 16:0 and 16:1 were similar to those in *Vischeria* sp. (NIES-2147, 2148).

The picoplankton *Pelagomonas calceolata* was described as a new species, in a new genus, family, order, and class, the Pelagophyceae, by Andersen *et al.* (1993). We detected 12:0, 14:0, 16:0, 16:1, 18:0, 18:1, 18:2, 18:3, 18:4, and 22:6 in all Pelagophyceae strains, but detected 20:5 in only five strains. The fatty acid and polar lipid compositions in the Pelagophyceae have not previously been reported.

The major fatty acids in 24 strains in the Raphidophyceae were 14:0, 16:0, and 20:5. *Chattonella marina*

Table 1 Relative proportions (%) of fatty acid in crude lipids from microalgal strains in the MCC-NIES

Species	Strain	120	140	160	16:1	16:2	16:3	16:4	18:0	18:1	18:2	18:3	18:4	18:5	20:4	20:5	22:5	22:6	24:1	Others	
Heterokontophyta	<i>Aurearena cruciata</i>	NIES-1863	5.1	3.3	43.7	1.3	0.0	0.0	0.0	11.7	6.5	2.7	9.3	8.8	0.0	1.3	4.4	0.0	0.0	1.9	
	<i>Aurearena cruciata</i>	NIES-1864	2.1	1.5	26.7	1.0	0.6	0.0	0.0	0.8	11.1	5.6	16.4	17.0	0.0	6.2	10.3	0.0	0.0	0.0	0.7
	<i>Aurearena cruciata</i>	NIES-1865	2.0	1.2	24.4	1.1	0.6	0.0	0.0	0.9	8.1	5.3	16.9	20.9	0.0	4.5	13.8	0.0	0.0	0.0	0.3
Bacillariophyceae	<i>Achnanthes kuwaitensis</i>	NIES-1349	1.4	5.0	32.9	22.2	7.8	4.7	0.8	2.5	3.7	1.7	0.9	0.4	0.0	0.4	13.8	0.0	0.9	0.6	0.3
	<i>Achnanthes subconstricta</i>	NIES-330	1.3	1.3	14.1	31.0	2.5	1.0	5.1	0.5	13.4	1.1	0.4	1.4	0.0	4.3	21.0	0.0	1.6	0.0	0.0
	<i>Achnanthydium convergens</i>	NIES-2717	3.3	4.7	21.3	33.5	0.0	2.7	0.0	4.5	10.6	3.0	0.0	0.0	0.0	0.0	6.5	0.0	0.0	5.5	4.4
	<i>Achnanthydium minutissimum</i>	NIES-71	0.4	2.5	17.0	54.6	0.8	1.9	0.7	0.6	1.1	2.2	0.9	0.6	0.2	2.1	10.3	0.0	0.0	2.9	1.2
	<i>Achnanthydium minutissimum</i>	NIES-372	3.0	4.9	16.3	53.2	2.5	2.3	0.3	0.7	2.9	0.6	0.5	0.4	0.0	3.1	7.4	0.0	0.0	0.3	1.6
	<i>Achnanthydium minutissimum</i>	NIES-407	3.7	9.5	25.1	21.3	4.5	5.9	0.0	10.8	5.2	2.2	0.0	0.0	0.0	1.0	4.3	0.0	0.0	0.0	6.5
	<i>Achnanthydium minutissimum</i>	NIES-408	1.5	3.0	13.8	50.5	2.3	3.6	1.4	1.1	3.1	1.8	1.1	0.2	0.0	1.7	11.7	0.0	0.0	0.0	3.2
	<i>Achnanthydium minutissimum</i>	NIES-409	0.6	1.8	14.2	58.7	2.0	1.6	0.6	0.5	1.3	2.5	0.6	0.5	0.0	1.7	11.2	0.0	0.0	0.7	1.5
	<i>Achnanthydium minutissimum</i>	NIES-413	1.6	2.5	14.2	48.3	2.1	3.0	0.0	0.8	2.3	3.4	0.8	1.2	0.0	2.7	14.4	0.0	0.0	0.5	2.2
	<i>Achnanthydium minutissimum</i>	NIES-414	1.6	4.7	18.1	40.5	1.7	3.6	0.0	1.8	3.8	4.1	0.9	0.0	0.0	5.0	12.7	0.0	0.0	0.3	1.2
	<i>Asterionellopsis glacialis</i>	NIES-265	1.9	13.4	8.1	21.2	4.5	0.0	2.6	1.6	0.0	1.4	0.0	1.5	0.0	0.0	41.5	0.0	2.3	0.0	0.0
	<i>Asterionellopsis glacialis</i>	NIES-417	3.0	14.6	3.6	11.4	3.0	1.0	9.4	0.5	0.6	0.0	0.4	1.1	0.0	0.0	46.1	0.0	1.3	1.2	2.8
	<i>Alucocoeira granulata</i>	NIES-333	21.4	26.0	24.6	0.0	0.0	0.0	0.0	18.1	2.8	7.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	<i>Chaetoceros sociale</i>	NIES-377	4.3	27.4	22.3	8.6	6.9	1.6	4.2	13.3	0.0	1.4	0.0	0.0	0.0	0.0	1.2	8.8	0.0	0.0	0.0
	<i>Chaetoceros sociale</i>	NIES-553	4.0	21.4	7.7	12.7	6.1	0.9	10.5	2.4	4.0	0.0	0.4	0.0	0.0	0.0	0.0	28.1	0.0	0.0	1.8
	<i>Cyrtocula molestiformis</i>	NIES-2718	3.3	5.1	21.2	38.3	0.0	3.9	0.0	5.0	8.4	2.1	0.0	0.0	0.0	0.0	0.0	9.6	0.0	0.0	3.1
	<i>Cyclotella meneghiniana</i>	NIES-805	5.7	9.4	17.5	19.7	4.3	13.4	0.6	9.6	2.4	2.2	0.0	0.5	0.0	0.0	12.4	0.0	1.4	0.9	0.0
	<i>Cyclotella meneghiniana</i>	NIES-2363	1.4	11.9	20.4	28.0	3.8	10.4	0.5	0.8	2.5	2.2	0.4	0.0	0.0	0.0	8.3	0.6	1.6	1.2	6.0
	<i>Cyclotella meneghiniana</i>	NIES-2364	1.8	6.1	15.1	34.8	4.8	12.6	0.5	0.7	2.2	0.8	0.0	0.3	0.0	0.0	14.7	1.0	0.7	1.1	2.8
	<i>Cyclotella meneghiniana</i>	NIES-2365	2.7	5.1	12.0	15.5	7.8	21.6	1.5	2.1	0.6	1.5	1.1	0.0	0.0	0.0	15.4	1.2	3.1	2.0	6.8
<i>Cylindrotheca closterium</i>	NIES-1045	16.0	22.2	15.2	14.9	1.8	3.7	1.6	6.0	3.1	2.3	0.0	1.2	0.0	1.3	9.2	0.9	0.6	0.0	0.0	
<i>Cylindrotheca fusiformis</i>	NIES-1046	2.9	6.3	15.8	23.2	1.6	3.3	0.7	0.8	6.7	3.8	2.3	1.4	1.0	10.5	17.4	0.8	0.7	0.0	0.8	
<i>Cylindrotheca sp.</i>	NIES-1047	5.5	16.9	17.3	22.0	4.4	4.7	0.7	2.3	6.5	1.5	0.0	2.7	0.0	3.4	10.4	0.7	1.0	0.0	0.0	
<i>Ditytium brightwellii</i>	NIES-350	2.4	7.9	24.2	19.4	7.6	2.4	0.0	6.1	2.7	11.5	0.0	0.0	0.0	14.8	0.0	0.0	0.6	0.0	0.4	
<i>Eolimna minima</i>	NIES-2720	0.9	5.5	10.7	53.2	7.5	3.7	0.0	0.6	2.0	0.0	0.7	0.0	0.0	5.2	9.2	0.0	0.0	0.3	0.5	
<i>Ecampia sp.</i>	NIES-2668	2.8	21.1	17.4	20.1	3.7	1.7	2.9	9.3	2.3	4.7	2.2	2.4	0.0	0.0	3.4	2.8	0.0	3.2	0.0	
<i>Eumotia pectinatis</i>	NIES-461	0.5	7.8	54.0	1.3	0.4	0.0	0.0	2.4	2.2	28.2	0.0	1.4	0.0	0.5	0.0	0.0	0.0	0.0	1.3	
<i>Fistularia saprophila</i>	NIES-2722	3.0	4.5	13.8	23.5	8.4	5.2	5.3	0.9	2.2	0.0	0.4	0.0	0.2	1.4	25.7	0.0	1.2	1.3	3.0	
<i>Fragilaria capucina</i>	NIES-391	0.8	6.6	21.6	35.5	4.0	2.6	1.4	0.6	3.4	3.4	2.0	0.2	0.2	1.0	14.6	0.0	1.0	0.0	1.1	
<i>Gomphonema parvulum</i>	NIES-466	1.4	5.1	25.3	44.7	2.8	2.2	2.9	1.1	4.1	0.5	0.0	0.0	0.0	1.6	5.0	0.0	0.0	0.0	3.3	
<i>Gomphonema parvulum</i>	NIES-2723	1.1	5.6	21.5	39.0	5.1	2.0	3.7	1.6	1.6	4.9	0.5	1.0	0.0	1.9	6.8	0.0	0.0	3.0	0.7	
<i>Hantzschia amphioxys</i>	NIES-587	0.6	0.9	17.9	51.1	1.7	1.4	0.0	0.8	4.1	4.9	0.9	1.0	0.3	2.3	10.1	0.0	0.7	0.0	1.3	
<i>Lithodessium variabile</i>	NIES-588	4.4	26.8	20.4	7.0	4.9	2.9	0.0	7.0	2.9	5.9	0.0	1.2	0.0	0.0	14.9	0.0	1.7	0.0	0.0	
<i>Meyaneca atomus</i>	NIES-2725	2.3	4.8	9.9	40.9	7.2	6.1	0.6	0.4	0.2	0.6	0.6	0.0	0.3	1.2	9.2	0.0	0.0	0.1	4.6	
<i>Nitzschia closterium</i>	NIES-2351	0.6	15.8	30.5	19.6	0.9	3.4	0.9	1.1	5.3	3.9	0.9	0.4	1.0	7.0	7.4	0.0	0.4	0.0	0.9	
<i>Nitzschia palea</i>	NIES-487	1.6	5.3	15.9	43.5	7.0	2.2	3.3	0.0	0.4	0.0	0.5	0.4	0.0	1.6	13.5	0.0	1.7	0.5	2.6	
<i>Nitzschia palea</i>	NIES-2726	1.7	20.6	11.5	38.4	2.7	1.0	3.2	0.6	0.9	0.0	1.1	1.2	0.0	0.9	14.2	0.0	1.3	0.4	0.3	
<i>Nitzschia palea</i>	NIES-2729	1.7	5.2	23.0	49.6	3.3	1.0	1.0	2.1	4.7	1.4	0.5	1.0	0.0	0.9	0.0	0.0	0.9	2.7	1.0	
<i>Nitzschia sp.</i>	NIES-1339	2.2	1.8	28.4	22.7	3.4	4.1	0.4	0.7	1.9	1.2	2.0	0.0	0.0	1.8	26.6	0.0	1.0	0.0	1.8	
<i>Nitzschia sp.</i>	NIES-1340	1.5	1.8	32.4	24.0	3.5	2.1	1.4	0.5	2.0	0.8	2.3	0.2	0.0	1.6	21.3	0.0	1.1	0.0	3.5	
<i>Odontella aurita</i>	NIES-589	2.1	10.5	25.1	14.9	3.9	3.1	5.5	8.4	2.0	0.4	0.0	0.4	0.0	1.0	19.8	0.0	2.9	0.0	0.0	
<i>Odontella longicirris</i>	NIES-590	3.3	18.7	6.6	7.6	3.8	3.0	8.0	0.9	1.7	0.0	0.5	0.0	0.0	12.0	30.6	0.0	3.3	0.0	0.0	
<i>Planolithidium frequentissimum</i>	NIES-2730	9.4	25.0	32.6	0.6	1.3	0.0	0.0	20.4	0.0	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	7.7	
<i>Planolithidium lanceolatum</i>	NIES-2731	1.9	4.3	17.3	45.3	3.4	4.5	0.0	1.1	1.4	2.2	0.0	0.0	0.0	2.6	16.0	0.0	0.0	0.0	0.0	
<i>Pseudonitzschia sp.</i>	NIES-1383	0.3	3.8	28.0	52.8	1.1	0.0	0.7	0.4	1.2	0.8	0.4	0.0	0.2	3.4	5.0	0.0	0.6	0.4	0.9	

Table 1 Continued

	Species	Strain	120	140	160	16:1	16:2	16:3	16:4	18:0	18:1	18:2	18:3	18:4	18:5	20:4	20:5	22:5	22:6	24:1	Others			
Heterokontophyta	Bacillariophyceae	<i>Sellaphora seminulum</i>	0.7	2.6	22.1	42.1	3.6	3.9	0.0	0.6	2.0	0.5	0.7	0.0	0.0	7.0	11.3	0.0	0.0	1.9	1.0			
		<i>Sellaphora seminulum</i>	1.5	3.5	14.4	42.8	5.0	6.8	0.0	0.9	3.7	1.4	1.3	0.0	0.5	8.4	8.5	0.0	0.0	0.0	1.3	2.0		
		<i>Skeletonema ardens</i>	1.8	18.9	15.6	25.5	5.7	3.5	1.0	1.8	7.9	3.6	0.0	0.6	0.0	0.5	8.2	0.0	1.9	1.5	1.5	2.0		
		<i>Skeletonema dohrnii</i>	3.8	18.5	12.3	22.2	9.8	5.7	6.9	3.5	2.9	0.6	0.0	1.8	0.0	0.0	7.7	0.0	1.2	1.0	2.4	0.0		
		<i>Skeletonema dohrnii</i>	2.7	14.7	6.0	22.7	9.8	6.4	9.3	2.2	2.8	0.0	0.9	2.4	0.0	0.0	18.5	0.0	1.6	0.0	0.0	0.0		
		<i>Skeletonema dohrnii</i>	2.4	13.0	13.8	24.7	6.4	2.7	3.0	2.9	14.1	3.0	0.0	2.2	0.0	0.4	6.7	0.0	1.3	0.5	2.9	3.4		
		<i>Skeletonema grevillei</i>	4.2	29.9	12.5	16.0	6.3	5.6	0.0	3.4	9.3	1.7	0.0	0.0	0.0	0.0	5.3	0.0	0.7	1.7	3.4	0.6		
		<i>Skeletonema japonicum</i>	3.7	14.8	6.1	21.0	10.4	7.7	8.5	1.3	0.8	0.0	2.5	0.0	0.0	19.2	0.0	2.8	0.0	0.0	1.2	1.9		
		<i>Skeletonema japonicum</i>	9.9	22.1	19.6	14.9	4.1	1.7	0.0	10.0	7.1	4.0	0.0	0.7	0.0	0.0	2.8	0.0	0.0	0.0	1.2	1.9		
		<i>Skeletonema japonicum</i>	3.8	17.2	10.1	17.7	5.0	7.4	1.0	4.5	6.7	2.1	1.4	2.4	0.0	0.0	17.7	0.0	1.8	0.0	1.2	1.9		
		<i>Skeletonema marinoi-dohrnii complex</i>	2.1	14.2	9.8	17.6	7.1	5.2	8.9	0.8	0.0	9.3	0.6	2.0	0.0	0.5	16.6	0.9	2.0	0.4	2.0	2.5		
		<i>Skeletonema marinoi-dohrnii complex</i>	2.0	18.5	6.7	12.0	6.8	10.2	10.7	1.1	2.0	0.3	0.6	3.6	0.0	0.7	18.2	1.0	2.3	0.8	2.5	3.5		
		<i>Skeletonema marinoi-dohrnii complex</i>	6.1	9.1	9.0	13.3	9.3	20.2	8.1	1.1	0.0	0.5	0.5	2.5	0.0	0.0	11.8	1.7	2.4	0.9	3.5	3.4		
		<i>Skeletonema marinoi-dohrnii complex</i>	4.9	18.8	9.7	18.9	9.8	14.0	6.6	0.5	0.0	0.4	0.4	1.6	0.0	0.0	8.2	1.1	1.1	0.6	3.4	2.3		
		<i>Skeletonema menzelii</i>	1.8	11.2	16.9	20.9	6.0	9.7	0.9	2.1	8.7	0.9	0.0	1.8	0.0	0.3	12.3	0.0	2.1	2.1	2.3	0.0		
		<i>Skeletonema pseudocostatum</i>	5.0	19.2	12.0	16.9	8.2	4.6	4.7	3.7	9.1	2.2	0.0	1.9	0.0	0.0	9.8	0.0	1.8	0.9	0.0	0.0		
		<i>Skeletonema tropicum</i>	7.7	9.9	25.2	5.5	0.0	1.1	0.0	24.4	19.1	1.6	0.0	0.0	1.0	0.0	1.0	0.0	0.0	1.9	2.6	3.8		
		<i>Skeletonema tropicum</i>	2.2	27.8	14.8	17.7	3.6	2.1	0.0	3.1	14.4	0.0	0.0	0.0	0.0	0.0	10.5	0.0	0.0	0.0	0.0	0.0		
		<i>Tabellaria flocculosa</i>	2.0	6.0	14.9	37.8	8.0	5.1	2.5	2.9	2.1	2.0	0.0	0.3	0.0	0.3	10.1	0.0	1.1	2.5	2.4	0.0		
		<i>Thalassionema nitzschoitoides</i>	2.4	22.7	15.3	17.1	6.8	4.8	4.1	4.9	1.9	0.0	0.4	0.4	0.0	0.0	16.3	0.4	2.5	0.0	0.0	0.0		
		<i>Triceratium dubium</i>	0.8	10.5	26.8	34.7	4.7	1.5	0.5	2.4	2.7	1.2	0.3	0.0	0.0	1.7	6.9	0.0	0.2	3.6	1.5	0.0		
		Chrysomerothyceae	Chrysophyceae	<i>Chryduopsis</i> sp.	5.0	11.9	26.1	3.4	0.9	0.0	0.0	9.3	5.7	5.6	7.0	7.0	0.2	5.4	12.5	0.0	0.0	0.0	0.0	
				<i>Chrysamoeba radians</i>	6.4	23.7	24.1	0.0	0.0	0.0	0.0	16.4	0.0	0.6	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0	0.0	28.1
				<i>Dinobryon divergens</i>	39.5	22.4	19.8	0.0	0.0	0.0	0.0	15.2	2.3	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
				<i>Epiphyxis glabra</i>	5.7	23.2	29.9	4.4	1.5	0.0	0.0	9.7	4.6	2.5	6.4	7.9	0.0	0.0	0.0	1.8	2.4	0.0	0.0	0.0
<i>Lagynion subglobosum</i>	2.8			23.2	25.2	1.7	0.6	0.0	0.0	6.7	4.1	6.3	12.0	7.5	0.0	0.8	0.3	5.1	0.7	0.8	2.2	0.0		
<i>Mallomonas</i> sp.	1.8			30.5	43.7	1.4	1.5	0.0	0.0	19.5	0.0	0.8	0.0	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.8		
<i>Mallomonas</i> sp.	2.6			15.9	9.4	0.0	0.0	0.0	0.0	2.5	6.1	7.5	15.4	15.6	0.6	3.1	8.6	4.1	7.3	0.0	1.3	0.0		
<i>Ochromonas danica</i>	4.6			19.4	9.7	0.0	0.0	0.0	0.0	4.3	5.6	9.5	21.7	0.0	0.0	7.0	6.9	8.6	2.7	0.0	0.0	0.0		
<i>Ochromonas minuta</i>	6.9			14.5	14.0	0.0	0.0	0.0	0.0	13.9	4.6	8.9	5.7	18.9	0.0	3.0	0.0	7.6	2.0	0.0	0.0	0.0		
<i>Ochromonas</i> sp.	3.5			14.4	7.0	0.0	0.0	0.0	0.0	4.6	3.9	11.8	34.8	0.0	1.6	5.4	4.2	6.6	1.3	0.0	0.9	0.0		
<i>Peteriochromonas malhamensis</i>	13.8			11.7	17.2	0.0	0.0	0.0	0.0	10.3	7.9	6.0	13.3	2.1	0.0	2.9	3.1	6.2	2.4	3.1	0.0	0.0		
<i>Synura petersenii</i>	4.0			27.7	20.7	1.2	1.6	0.0	0.0	12.1	4.0	2.4	5.3	14.3	0.0	0.0	0.7	3.4	2.6	0.0	0.0	0.0		
<i>Synura petersenii</i>	3.6			20.2	5.8	2.4	3.1	0.0	0.0	2.1	2.7	5.9	10.2	29.3	0.8	1.2	1.4	7.6	3.7	0.0	0.0	0.0		
<i>Synura sphagnicola</i>	7.2			16.2	10.1	0.0	0.0	0.0	0.0	5.8	5.5	11.1	16.6	14.8	0.0	0.0	4.0	3.5	5.2	0.0	0.0	0.0		
<i>Synura sphagnicola</i>	4.9			20.1	11.6	0.0	0.0	0.0	0.0	3.5	7.7	9.4	13.1	11.9	0.0	0.0	5.9	5.5	6.4	0.0	0.0	0.0		
<i>Synura spinosa</i>	4.2			19.4	9.8	2.4	2.5	0.0	0.0	1.9	3.4	4.8	10.7	29.1	0.0	2.9	1.0	3.1	4.8	0.0	0.0	0.0		
<i>Uroglena americana</i>	16.7			22.7	20.9	4.1	0.0	0.0	0.0	15.5	4.4	1.6	1.8	0.0	0.8	2.0	1.6	2.3	0.0	0.0	5.6	0.0		
Eustigmatophyceae				<i>Nannochloropsis oculata</i>	0.9	3.9	27.5	21.5	0.7	0.0	0.0	18.0	7.9	2.0	0.3	0.1	0.5	3.4	13.1	0.0	0.0	0.0	0.2	
				<i>Vischeria punctata</i>	1.2	5.1	18.1	47.4	0.7	0.0	0.0	0.7	14.4	3.4	0.5	0.3	0.3	1.4	4.8	0.0	0.0	1.5	0.2	
				<i>Vischeria stellata</i>	2.4	1.4	22.2	32.9	1.6	0.0	0.0	0.9	13.9	4.3	3.8	0.2	0.3	2.1	12.4	0.0	0.0	0.6	1.0	
				<i>Chrysophaeum taylorii</i>	5.2	10.3	49.6	1.7	0.0	1.2	1.5	11.0	7.9	1.4	0.9	1.6	1.2	0.0	0.0	1.9	2.9	1.1	0.6	0.0
				<i>Chrysophaeum taylorii</i>	5.4	10.9	40.0	0.0	0.0	0.0	0.0	9.5	2.9	3.7	4.1	2.3	0.0	0.0	14.0	7.2	0.0	0.0	0.0	
Pelagophyceae				<i>Pelagomonas calceolata</i>	6.1	12.1	16.1	9.0	0.0	0.0	7.2	5.5	5.9	10.5	16.7	5.5	0.0	0.0	1.6	3.8	0.0	0.0	0.0	
				<i>Pelagomonas calceolata</i>	1.5	8.9	17.9	14.0	0.0	0.0	0.0	1.9	18.1	4.5	6.9	12.4	3.9	0.0	1.0	2.0	7.0	0.0	0.0	
				<i>Pelagomonas calceolata</i>	16.1	18.5	18.1	5.0	0.0	0.0	6.5	12.2	3.2	3.6	9.2	2.8	0.0	0.4	0.8	3.6	0.0	0.0	0.0	
		<i>Pelagomonas calceolata</i>	2.7	14.1	15.4	9.0	1.9	0.0	0.0	3.5	1.7	1.6	9.7	21.4	9.8	0.0	0.3	7.7	1.2	0.0	0.0			
		<i>Pelagomonas calceolata</i>	3.5	9.7	16.6	8.7	0.0	0.0	0.0	6.5	8.7	3.1	6.3	21.6	5.0	0.0	2.3	8.0	0.0	0.0	0.0			

Table 1 Continued

	Strain	120	140	160	161	162	163	164	180	181	182	183	184	185	204	205	225	226	241	Others
Heterokontophyta																				
Pelagophyceae																				
	NIES-3689	9.8	15.0	19.6	3.9	0.0	0.0	0.0	12.8	6.8	2.5	3.8	13.9	6.0	0.0	0.0	0.0	5.9	0.0	0.0
	NIES-3690	11.6	13.1	29.8	3.2	0.0	0.0	0.0	19.6	4.7	2.4	0.0	8.7	2.1	0.0	0.0	0.0	3.0	1.8	0.0
	NIES-3691	14.0	15.8	25.0	0.0	0.0	0.0	0.0	17.8	3.1	1.4	2.4	8.5	6.0	0.0	0.0	3.1	2.9	0.0	0.0
	NIES-2770	3.6	9.9	15.8	10.5	0.0	0.0	0.0	3.7	6.8	2.9	5.9	23.0	5.3	0.0	0.6	2.8	8.0	0.0	1.2
	NIES-1386	4.2	22.0	15.4	1.5	0.0	0.0	0.0	4.5	23.4	3.9	4.4	0.0	2.3	0.0	2.8	4.0	11.6	0.0	0.0
	NIES-1387	2.8	29.0	23.7	12.6	0.4	0.0	0.0	3.2	5.3	2.3	7.0	7.3	1.0	0.0	0.0	2.8	1.3	0.0	1.3
	NIES-2692	6.3	13.5	15.6	4.5	0.0	0.0	0.0	14.6	4.8	2.7	5.8	11.0	2.9	0.0	0.0	0.0	11.9	0.0	6.4
	NIES-2771	5.1	18.0	19.5	9.1	0.0	0.0	0.0	7.9	4.5	2.6	6.6	17.3	3.3	0.0	0.0	1.7	9.3	0.0	0.0
	NIES-2772	5.7	6.6	33.1	0.0	0.0	0.0	0.0	7.0	12.7	9.7	9.7	13.3	0.6	0.0	0.0	0.0	3.7	0.0	0.0
	NIES-548	3.2	5.3	28.7	1.9	0.9	0.0	0.0	3.5	11.7	10.2	9.8	10.1	0.2	4.5	6.9	0.0	0.0	0.0	3.1
Phaeophyceae																				
	NIES-1002	1.5	37.3	25.6	5.5	0.0	0.0	0.0	4.2	12.0	1.8	0.0	3.7	0.0	2.9	3.4	0.0	0.0	0.0	2.1
	NIES-1302	1.1	19.8	29.1	5.0	0.2	0.0	0.0	2.2	14.6	4.3	0.5	0.0	1.9	6.3	11.5	1.1	0.8	0.0	1.6
	NIES-2693	8.2	43.4	12.2	3.5	0.7	0.3	0.0	6.8	3.0	3.0	0.0	0.0	0.0	0.7	14.8	0.0	2.6	0.0	0.8
Raphidophyceae																				
	NIES-1	7.7	9.4	22.0	6.0	1.2	0.2	0.0	4.1	4.0	3.3	4.3	13.6	0.4	3.7	14.5	1.5	1.3	0.2	2.6
	NIES-14	2.0	7.4	18.7	8.3	2.2	0.2	0.1	1.2	1.4	4.4	5.0	11.5	1.2	9.2	21.8	1.9	0.5	0.0	3.0
	NIES-115	4.0	8.2	19.7	8.0	1.5	0.2	0.0	1.2	2.0	2.9	4.2	9.2	0.2	3.2	24.7	2.7	3.0	0.1	5.0
	NIES-557	2.0	8.4	16.9	4.7	0.5	0.0	0.0	1.4	2.3	3.0	5.0	5.4	0.3	3.4	38.9	0.9	2.1	0.2	4.6
	NIES-558	2.2	12.1	15.9	7.1	1.5	0.0	0.0	1.4	2.0	2.0	4.9	12.8	0.0	2.6	27.3	2.0	2.5	0.0	3.7
	NIES-559	3.5	9.3	17.9	5.8	0.5	0.0	0.0	1.2	2.2	6.6	7.6	11.9	0.0	6.3	20.9	2.1	1.6	0.0	2.6
	NIES-603	2.7	8.5	28.5	11.7	2.6	0.4	0.0	3.1	1.2	1.9	2.9	14.4	0.0	3.2	13.3	1.8	0.9	0.0	2.9
	NIES-1974	2.8	11.1	21.7	8.2	2.5	0.6	0.0	1.8	3.0	4.7	1.6	6.4	0.0	4.8	21.8	2.4	2.0	0.0	4.6
	NIES-1975	2.6	12.9	22.6	9.1	3.3	1.0	0.2	1.4	2.5	3.3	3.4	8.4	0.3	3.2	17.4	1.3	1.2	0.0	5.9
	NIES-1976	2.5	8.7	29.6	7.1	1.2	0.2	0.0	1.6	5.0	4.0	2.4	7.0	0.3	2.7	16.1	2.7	3.8	0.0	4.2
	NIES-2633	2.3	4.4	20.4	4.3	0.9	0.0	0.0	2.1	7.0	4.8	5.0	11.0	0.9	4.4	20.9	0.9	3.6	0.0	7.1
	NIES-136	4.4	22.4	15.6	3.8	1.4	0.0	0.0	2.6	8.4	2.9	2.7	10.8	0.0	6.0	19.0	0.0	0.0	0.0	0.0
	NIES-462	5.1	23.3	15.6	4.7	0.9	0.0	0.0	2.4	5.1	4.9	2.4	13.2	0.0	5.0	16.8	0.0	0.0	0.0	0.6
	NIES-560	2.9	14.1	10.3	3.2	1.2	0.0	0.0	0.5	4.5	3.1	4.9	12.3	0.0	7.1	34.7	0.0	0.0	0.0	1.2
	NIES-605	3.1	23.0	14.6	4.9	1.1	0.1	0.0	0.5	9.2	4.0	5.4	10.6	0.3	4.9	16.8	0.0	0.0	0.1	1.4
	NIES-1303	3.0	18.8	22.2	7.4	2.3	0.4	0.0	0.5	7.4	2.2	4.5	11.6	0.0	1.6	16.1	0.0	0.0	0.0	2.0
	NIES-1009	6.8	7.7	39.0	7.1	1.6	0.0	0.0	12.5	7.9	2.6	3.4	6.0	0.0	0.0	5.4	0.0	0.0	0.0	0.0
	NIES-716	1.7	10.6	54.9	4.9	0.0	0.0	0.0	7.7	4.0	3.1	3.8	2.5	0.0	1.1	1.5	0.0	0.4	0.0	3.8
	NIES-9	1.2	6.3	53.4	0.0	1.5	0.0	0.0	2.1	1.0	2.6	5.6	6.6	2.0	1.4	6.2	0.0	0.7	4.9	4.5
	NIES-145	1.9	4.0	22.2	13.8	5.2	0.4	0.0	0.5	0.5	1.2	6.7	9.4	1.3	2.3	25.1	0.0	0.5	0.0	5.0
	NIES-293	1.3	3.7	25.4	11.7	1.5	0.2	0.1	0.5	0.7	1.0	3.2	15.4	2.1	2.5	21.1	0.3	2.1	0.0	7.2
	NIES-15	1.0	14.8	27.0	15.1	0.0	0.0	0.0	0.9	7.6	6.3	2.7	0.2	0.5	7.6	16.3	0.0	0.0	0.0	0.0
	NIES-1379	5.0	5.5	17.3	11.1	2.0	0.0	0.0	0.6	5.3	3.7	1.2	0.0	0.2	11.7	35.1	0.0	0.0	0.0	1.3
	NIES-1831	1.8	5.7	28.0	14.7	1.1	0.6	0.0	3.6	12.1	4.3	1.5	0.3	0.8	8.7	14.0	0.1	0.0	0.0	2.7
Schizocladiophyceae																				
	NIES-1044	1.4	7.0	37.8	11.6	0.5	0.0	0.2	2.3	11.6	5.5	2.6	5.6	0.3	4.9	7.8	0.0	0.0	0.0	0.9
Xanthophyceae																				
	NIES-621	1.3	1.0	17.4	2.4	1.7	0.0	0.0	1.3	21.7	38.0	13.9	0.5	0.0	0.0	0.0	0.0	0.0	0.3	0.5
	NIES-2899	8.1	26.4	34.0	0.0	0.0	0.0	0.0	4.4	8.1	7.3	11.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	NIES-622	3.1	3.2	21.0	35.8	8.3	7.2	0.0	3.7	0.0	1.5	0.0	0.0	0.0	0.0	3.5	12.7	0.0	0.0	0.0
	NIES-3333	0.9	6.8	23.5	38.8	1.9	1.0	0.0	1.9	4.7	2.5	0.0	0.0	0.0	0.2	3.7	9.3	0.0	0.4	3.4
	NIES-1963	3.3	12.7	34.2	20.6	11.4	0.0	0.0	7.4	0.0	1.2	0.0	0.0	0.0	0.0	2.7	6.5	0.0	0.0	0.0
	NIES-1011	5.1	5.9	19.8	17.3	6.8	5.7	0.0	5.0	3.8	1.2	0.0	0.0	0.0	0.0	9.7	15.9	0.0	0.0	1.6
	NIES-1384	4.0	3.6	17.9	24.7	7.0	3.3	0.0	3.1	3.1	1.0	1.5	0.0	0.0	6.2	23.6	0.0	0.0	0.0	1.0
	NIES-1385	1.2	3.2	22.2	31.4	4.3	2.3	0.0	1.2	3.1	1.9	0.6	0.4	0.4	11.8	11.1	0.4	0.0	3.5	1.0
	NIES-2859	3.4	2.9	24.4	41.7	1.3	0.0	0.0	1.9	1.7	5.3	0.0	0.0	0.0	9.6	7.8	0.0	0.0	0.0	0.0

Table 1 Continued

Cryptophyta	Species	Strain	120	140	160	16:1	16:2	16:3	16:4	180	18:1	18:2	18:3	18:4	18:5	20:4	20:5	22:5	22:6	24:1	Others
Cryptophyceae	<i>Chroomonas caudata</i>	NIES-712	121	152	196	2.5	0.0	0.0	0.0	94	10.3	1.7	2.0	13.1	0.0	0.8	9.4	0.0	0.0	0.0	3.9
	<i>Chroomonas coerulea</i>	NIES-713	4.8	2.6	22.8	3.2	0.0	0.0	0.0	7.8	6.1	0.0	11.9	17.6	3.5	0.0	14.6	5.1	0.0	0.0	0.0
	<i>Chroomonas coerulea</i>	NIES-714	5.0	4.6	13.3	2.9	0.0	0.0	0.0	4.9	8.2	1.7	13.8	28.9	0.0	0.0	10.5	6.2	0.0	0.0	0.0
	<i>Chroomonas coerulea</i>	NIES-1004	2.3	0.8	18.4	10.3	0.6	0.0	0.0	1.8	3.9	3.5	14.3	22.8	0.0	0.5	14.7	5.3	0.0	0.0	0.8
	<i>Chroomonas collegionis</i>	NIES-703	2.6	3.8	24.0	0.0	0.0	0.0	0.0	3.3	4.8	1.9	12.4	23.0	4.9	0.0	15.0	4.3	0.0	0.0	0.0
	<i>Chroomonas dispersa</i>	NIES-704	1.9	1.9	24.5	3.6	0.0	0.0	0.0	3.9	7.9	2.1	13.5	20.4	4.9	0.0	10.3	4.3	0.8	0.0	0.0
	<i>Chroomonas mesostigmatica</i>	NIES-1370	8.9	5.2	16.1	2.8	0.0	0.0	0.0	4.1	4.4	2.4	16.3	20.0	0.0	0.0	13.6	4.0	2.2	0.0	0.0
	<i>Chroomonas nordstedtii</i>	NIES-706	3.6	4.5	12.9	1.2	0.0	0.0	0.0	2.0	5.1	3.1	16.1	27.7	0.0	1.1	16.5	6.2	0.0	0.0	0.0
	<i>Chroomonas nordstedtii</i>	NIES-707	3.4	4.4	18.1	3.5	0.0	1.8	0.0	5.2	7.0	4.3	16.5	18.2	0.0	0.7	11.6	4.4	0.5	0.0	0.0
	<i>Chroomonas nordstedtii</i>	NIES-708	4.7	3.2	12.3	2.1	0.0	0.0	0.0	1.8	1.8	4.6	11.5	35.3	0.0	0.0	14.3	7.1	1.3	0.0	0.0
	<i>Chroomonas nordstedtii</i>	NIES-709	3.0	1.6	18.9	3.0	0.0	0.0	0.0	2.5	3.4	5.8	0.0	30.4	0.0	0.6	21.7	8.3	0.8	0.0	0.0
	<i>Chroomonas nordstedtii</i>	NIES-710	2.6	0.0	13.9	3.4	0.0	0.0	0.0	1.9	1.4	2.3	15.6	33.8	0.0	0.0	16.8	7.3	1.0	0.0	0.0
	<i>Chroomonas nordstedtii</i>	NIES-711	4.6	5.2	13.9	3.2	0.0	0.0	0.0	8.9	10.3	7.3	12.2	13.7	0.0	0.0	13.4	7.3	0.0	0.0	0.0
	<i>Chroomonas nordstedtii</i>	NIES-705	0.7	4.4	24.0	3.8	0.0	0.0	0.0	1.7	20.1	9.2	17.3	9.8	0.0	0.7	5.4	1.4	0.0	0.0	1.5
	<i>Chroomonas placoides</i>	NIES-2331	3.8	4.1	14.2	2.3	0.0	0.0	0.0	1.7	4.4	3.9	20.1	25.2	0.0	0.0	13.7	0.0	0.7	0.0	5.9
	<i>Chroomonas sp.</i>	NIES-697	6.0	6.5	14.4	0.0	0.0	0.0	0.0	7.9	13.4	4.1	13.9	24.5	0.0	0.0	5.6	0.0	3.7	0.0	0.0
	<i>Cryptomonas acuta</i>	NIES-698	3.4	4.5	10.1	2.7	0.0	0.0	0.0	6.7	11.7	4.9	14.7	25.6	0.0	0.0	10.4	0.0	5.3	0.0	0.0
	<i>Cryptomonas irregularis</i>	NIES-274	2.4	2.7	16.3	0.0	0.0	0.0	0.0	2.1	0.0	4.0	25.5	21.4	0.0	2.3	16.4	1.3	5.6	0.0	0.0
	<i>Cryptomonas ovata</i>	NIES-275	3.5	2.1	16.0	0.0	0.0	0.0	0.0	3.5	0.0	3.5	24.3	21.3	0.0	3.3	15.0	2.3	5.2	0.0	0.0
	<i>Cryptomonas paramacium</i>	NIES-715	11.3	10.9	17.3	0.8	0.0	0.0	0.0	4.2	5.9	4.3	24.0	0.4	0.0	0.3	11.9	7.1	0.6	0.0	1.0
	<i>Cryptomonas paramacium</i>	NIES-766	1.4	4.2	18.0	2.3	0.0	0.0	0.0	5.3	9.6	6.1	27.6	0.0	0.0	1.0	12.6	7.9	1.8	0.0	2.2
	<i>Cryptomonas platyuris</i>	NIES-276	5.0	4.7	19.0	0.0	0.0	0.0	0.0	3.7	7.5	4.5	21.2	0.0	0.0	0.0	17.9	13.6	1.8	0.0	1.1
	<i>Cryptomonas platyuris</i>	NIES-276	2.6	2.6	21.9	0.0	0.0	0.0	0.0	2.8	3.0	3.5	32.1	12.1	0.0	0.0	12.4	3.1	3.0	0.0	0.0
	<i>Cryptomonas rostratiformis</i>	NIES-344	3.3	1.4	11.5	1.7	0.0	0.0	0.0	0.9	1.5	5.0	34.4	19.0	0.0	0.7	14.1	2.5	4.0	0.0	0.0
	<i>Cryptomonas rostratiformis</i>	NIES-277	2.7	4.9	27.9	0.0	0.0	0.0	0.0	4.1	0.0	2.3	16.4	21.9	0.0	0.0	14.2	2.8	2.8	0.0	0.0
	<i>Cryptomonas rostratiformis</i>	NIES-278	6.2	5.8	12.8	0.0	0.0	0.0	0.0	2.1	1.1	0.7	19.2	21.0	0.0	1.8	21.0	4.2	4.1	0.0	0.0
	<i>Cryptomonas rostratiformis</i>	NIES-345	3.2	1.9	13.8	0.0	0.0	0.0	0.0	1.4	4.8	3.2	19.6	23.8	0.0	2.4	18.1	3.8	4.0	0.0	0.0
	<i>Cryptomonas rostratiformis</i>	NIES-1327	7.9	5.0	17.7	0.0	0.0	0.0	0.0	5.5	4.0	5.5	13.5	21.5	0.0	1.1	13.3	3.4	1.6	0.0	0.0
	<i>Cryptomonas tetrapyrenoidosa</i>	NIES-279	4.6	3.9	16.8	0.0	0.0	0.0	0.0	4.0	3.1	8.6	17.6	20.5	0.0	0.0	14.1	3.4	3.4	0.0	0.0
	<i>Cryptomonas tetrapyrenoidosa</i>	NIES-280	5.9	6.1	12.5	0.0	0.0	0.0	0.0	2.1	1.1	1.1	19.1	21.1	0.0	1.4	21.2	4.3	4.1	0.0	0.0
	<i>Cryptomonas tetrapyrenoidosa</i>	NIES-281	3.9	3.7	29.1	0.0	0.0	0.0	0.0	8.4	1.4	1.2	9.1	22.3	1.5	1.3	13.6	3.1	1.4	0.0	0.0
	<i>Cryptomonas tetrapyrenoidosa</i>	NIES-282	3.1	3.1	19.1	0.0	0.0	0.0	0.0	4.3	2.5	2.1	25.8	19.3	0.0	0.0	15.8	2.4	2.5	0.0	0.0
	<i>Cryptomonas tetrapyrenoidosa</i>	NIES-346	3.5	5.1	15.3	0.0	0.0	0.0	0.0	1.3	0.3	0.6	14.9	30.3	0.0	1.2	19.7	3.5	4.3	0.0	0.0
	<i>Cryptomonas tetrapyrenoidosa</i>	NIES-347	5.4	1.5	16.0	0.0	1.8	0.0	0.0	1.8	0.0	1.6	11.5	28.6	0.0	1.2	22.7	4.2	3.7	0.0	0.0
	<i>Cryptomonas tetrapyrenoidosa</i>	NIES-348	2.4	4.6	19.1	2.0	0.0	0.0	0.0	1.5	2.5	2.1	17.4	18.6	0.0	1.8	19.4	3.2	5.4	0.0	0.0
	<i>Cryptomonas sp.</i>	NIES-2964	4.1	7.0	12.5	5.2	0.0	0.0	0.0	1.5	2.0	0.6	15.4	28.1	0.0	3.1	13.9	3.3	2.8	0.0	0.5
	<i>Hemiselmis anderseni</i>	NIES-2716	5.0	6.2	22.3	0.0	0.0	0.0	0.0	4.6	2.6	0.0	12.2	24.3	0.0	0.0	15.9	2.8	4.1	0.0	0.0
	<i>Rhodomonas atrovirens</i>	NIES-699	4.3	4.2	9.3	1.9	0.4	0.0	0.0	2.9	12.4	1.8	10.6	30.8	0.0	2.1	11.8	0.0	7.5	0.0	0.0
	<i>Rhodomonas baltica</i>	NIES-700	16.0	11.8	13.6	10.6	0.0	0.0	0.0	5.6	8.1	2.3	5.9	15.5	0.0	0.0	7.2	0.0	3.4	0.0	0.0
	<i>Rhodomonas chrysoidea</i>	NIES-701	4.0	9.4	12.0	3.2	0.0	0.0	0.0	3.3	7.9	2.6	12.3	21.6	0.0	1.3	13.3	0.0	9.1	0.0	0.0
	<i>Rhodomonas duplex</i>	NIES-765	5.9	8.3	14.2	1.5	0.0	0.0	0.0	8.7	5.1	1.6	17.7	22.7	0.0	0.0	6.9	0.3	7.1	0.0	0.0
	<i>Rhodomonas falcata</i>	NIES-702	6.0	6.4	10.4	2.3	0.0	0.0	0.0	3.4	8.5	6.8	11.7	24.5	0.0	0.6	10.3	0.0	8.2	0.0	0.9
	<i>Rhodomonas salina</i>	NIES-1006	3.6	12.4	12.8	3.8	0.0	0.0	0.0	7.1	8.2	4.0	9.4	24.3	0.0	0.0	10.0	0.0	4.4	0.0	0.0
<i>Rhodomonas salina</i>	NIES-1375	9.3	5.5	16.3	2.2	0.4	0.0	0.0	4.8	3.0	0.6	14.1	21.1	0.0	0.5	12.3	1.9	7.4	0.0	0.6	
<i>Rhodomonas salina</i>	NIES-3015	5.5	7.4	9.0	2.5	0.0	0.0	0.0	2.9	9.0	3.1	7.5	30.9	0.0	0.0	14.3	0.0	7.9	0.0	0.0	
<i>Rhodomonas sp.</i>	NIES-1005	5.9	6.1	19.4	2.4	0.0	0.0	0.0	3.0	8.0	0.7	17.8	23.1	0.0	0.0	7.1	0.9	5.6	0.0	0.0	
<i>Rhodomonas sp.</i>	NIES-1730	2.3	4.3	17.9	5.9	0.0	1.8	0.0	3.0	5.5	1.8	13.2	14.9	0.0	9.5	13.4	0.0	6.5	0.0	0.0	
<i>Rhodomonas sp.</i>	NIES-2332	2.1	5.8	15.2	2.5	0.0	0.0	0.0	1.8	7.2	1.6	14.2	29.1	0.0	0.6	11.2	0.0	8.7	0.0	0.0	

Table 1 Continued

Haptophyta	Species	Strain	120	140	160	161	162	163	164	180	181	182	183	184	185	204	205	225	226	241	Others	
Pavlovophyceae	<i>Pavlova gyans</i>	NIES-623	1.0	22.8	12.3	9.3	2.4	0.4	0.0	0.7	0.3	0.5	0.9	11.5	0.0	0.3	24.8	4.2	7.4	0.0	1.2	
	<i>Pavlova pinguis</i>	NIES-1398	1.4	21.0	12.0	13.8	5.7	2.6	0.0	0.6	0.0	0.0	0.0	3.8	0.0	1.5	25.0	8.5	3.4	0.0	0.7	
	<i>Pavlova</i> sp.	NIES-1399	1.5	16.8	19.7	25.3	0.0	0.0	0.0	2.9	2.9	1.4	0.3	3.2	0.0	1.2	11.9	6.9	4.0	0.0	2.0	
	<i>Pavlova</i> sp.	NIES-1400	3.4	20.3	12.5	13.4	0.0	0.0	0.0	1.7	3.0	2.0	0.9	9.3	0.0	0.0	16.9	7.1	8.6	0.0	0.9	
	<i>Pavlova</i> sp.	NIES-1401	1.1	19.5	12.2	13.8	0.6	0.0	0.0	0.5	0.4	2.5	0.5	9.6	0.0	1.0	20.0	8.6	8.9	0.0	0.8	
	<i>Pavlova</i> sp.	NIES-1815	2.8	19.1	10.7	9.8	3.4	2.7	0.0	1.5	0.0	1.3	1.0	4.6	0.0	1.3	26.2	2.1	9.0	3.4	1.1	
	<i>Pavlova</i> sp.	NIES-1816	2.2	17.9	14.7	9.0	0.0	0.0	0.0	1.0	1.1	2.4	1.0	7.3	0.0	1.3	22.7	8.0	11.4	0.0	0.0	
	<i>Pavlova</i> sp.	NIES-1965	3.5	20.9	12.8	9.5	1.4	0.0	0.0	2.1	1.6	1.2	1.2	4.5	0.0	1.1	24.7	8.8	5.4	0.0	1.3	
	<i>Pavlova</i> sp.	NIES-2773	7.6	14.5	24.3	9.8	2.7	1.3	0.0	6.2	9.9	1.3	0.0	2.7	0.0	0.0	8.0	5.2	1.3	0.0	5.2	
	Prymnesiophyceae	<i>Calcidiscus leptoporus</i>	NIES-2694	4.5	6.3	28.5	0.0	0.0	0.0	0.0	5.4	6.1	5.7	4.0	12.1	9.4	0.0	4.7	0.0	12.6	0.0	0.7
		<i>Calyptrosphaera oblonga</i>	NIES-2872	2.9	12.7	34.4	3.5	0.0	0.0	0.0	16.2	12.1	2.6	2.4	2.5	3.8	0.0	0.0	0.6	5.0	0.8	0.5
		<i>Calyptrosphaera sphaeroide</i>	NIES-997	0.7	6.3	33.3	1.3	1.1	0.0	0.0	0.6	28.2	2.6	3.1	14.9	0.8	0.0	1.1	0.0	6.0	0.0	0.0
		<i>Chrysochromulina brevifilum</i>	NIES-2904	9.0	43.2	33.2	0.0	0.0	0.0	0.0	14.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		<i>Chrysochromulina hirta</i>	NIES-741	8.4	23.7	12.7	0.0	0.0	0.0	0.0	6.3	0.0	2.9	6.0	5.8	13.8	0.0	3.0	0.0	17.4	0.0	0.0
		<i>Chrysochromulina parva</i>	NIES-562	3.6	24.1	18.9	2.1	0.0	0.0	0.0	7.1	4.9	10.8	2.1	5.0	8.0	0.0	3.0	5.6	3.5	0.0	1.3
		<i>Chrysochromulina simplex</i>	NIES-1392	2.9	32.7	12.0	4.5	0.0	0.0	0.0	4.3	11.7	6.5	2.4	5.5	5.2	0.0	2.7	0.5	7.2	0.0	1.9
		<i>Chrysochromulina</i> sp.	NIES-1391	3.9	23.7	11.6	0.9	0.0	0.0	0.0	5.3	7.2	4.7	3.1	5.4	15.6	0.0	0.0	15.5	0.0	3.1	0.0
		<i>Chrysochromulina</i> sp.	NIES-2506	7.0	20.5	11.7	0.0	0.0	0.0	0.0	6.0	10.0	2.4	6.2	8.5	10.1	0.0	3.8	1.2	12.6	0.0	0.0
<i>Chrysoculler rhomboides</i>		NIES-1874	2.8	8.7	28.2	6.6	2.5	2.1	0.0	8.8	8.6	4.5	1.4	5.0	5.4	0.0	4.0	0.0	11.4	0.0	0.0	
<i>Coccolithus braarudii</i>		NIES-2696	2.0	14.1	18.3	6.5	2.6	0.0	0.0	1.6	10.1	3.2	4.7	10.5	10.2	0.0	6.3	0.0	9.9	0.0	0.0	
<i>Corisphaera gracilis</i>		NIES-2369	0.5	1.7	38.9	1.4	0.7	0.2	0.0	0.8	23.9	3.5	1.8	4.2	1.6	0.5	1.9	0.8	16.6	0.0	1.0	
<i>Emiliana huxleyi</i>		NIES-837	1.1	20.3	8.9	1.2	0.0	0.0	0.0	1.2	13.9	1.3	3.2	6.5	8.3	0.0	1.4	0.0	22.2	2.0	8.5	
<i>Emiliana huxleyi</i>		NIES-2370	3.9	18.2	23.3	3.8	0.0	0.0	0.0	11.8	20.8	2.4	0.7	2.0	1.3	0.0	0.0	0.0	5.1	0.4	6.3	
<i>Emiliana huxleyi</i>		NIES-2697	6.9	16.3	14.1	0.0	0.0	0.0	0.0	12.2	7.9	0.0	5.7	11.6	6.8	0.0	0.0	18.5	0.0	0.0	0.0	
<i>Emiliana huxleyi</i>		NIES-2698	7.8	18.9	12.0	0.0	0.0	0.0	0.0	11.5	10.5	1.5	2.8	11.6	1.8	0.0	0.0	16.0	2.6	3.0	0.0	
<i>Gephyrocapsa ericsonii</i>		NIES-3121	1.7	20.2	12.7	0.0	0.0	0.0	0.0	3.2	21.1	3.4	3.3	5.1	4.5	0.0	0.8	0.0	13.4	4.7	5.9	
<i>Gephyrocapsa oceanica</i>		NIES-353	4.5	23.9	12.1	0.0	0.0	0.0	0.0	3.4	10.6	0.0	3.0	5.4	5.0	0.0	0.0	19.0	4.4	8.7	0.0	
<i>Helicosphaera wallichii</i>		NIES-2376	3.2	11.9	37.6	0.9	1.9	0.0	0.0	6.3	12.3	2.8	3.5	4.7	4.7	1.3	1.0	0.0	7.9	0.0	0.0	
<i>Hyalolithus neolepis</i>		NIES-1393	1.2	33.6	16.8	0.0	0.0	0.0	0.0	2.6	15.4	3.9	2.9	4.8	8.2	0.0	1.0	0.6	9.0	0.0	0.0	
<i>Hymenomonas coronata</i>		NIES-1016	1.6	0.5	20.2	0.0	0.0	0.0	0.0	1.8	18.9	4.0	11.6	25.8	0.9	0.8	4.6	0.0	8.7	0.0	0.6	
<i>Imantonia rotunda</i>		NIES-1001	6.6	29.1	18.3	4.7	0.0	0.0	0.0	7.1	14.4	5.7	1.4	3.5	2.6	0.0	1.2	1.1	1.5	0.0	2.8	
<i>Isochrysis galbana</i>		NIES-2590	1.5	12.2	16.6	4.0	0.0	0.0	0.0	1.5	23.8	2.8	8.6	18.3	4.7	0.0	0.0	1.5	2.3	2.2	0.0	
<i>Ochrosphaera neapolitana</i>		NIES-1395	0.5	0.9	28.9	1.3	0.0	0.0	0.0	1.3	34.4	8.7	6.8	10.4	0.0	0.2	3.4	0.0	3.2	0.0	0.0	
<i>Ochrosphaera neapolitana</i>		NIES-1964	0.7	0.4	26.5	0.6	0.0	0.0	0.0	3.3	30.0	6.9	13.4	7.6	0.8	0.9	3.0	0.7	5.2	0.0	0.0	
<i>Phaeocystis globosa</i>		NIES-388	2.1	15.1	9.7	5.4	0.0	0.0	0.0	3.2	10.7	0.0	5.4	11.2	9.2	0.0	11.0	0.0	14.2	0.0	2.8	
<i>Platychrysis</i> sp.		NIES-2703	3.9	32.5	25.5	0.8	0.3	0.0	0.0	7.0	12.0	1.6	2.5	9.7	0.0	0.0	0.9	2.1	0.5	0.7	0.0	
<i>Platychrysis haptonemafera</i>		NIES-1813	2.2	0.7	24.3	1.5	4.6	0.0	0.0	1.9	11.4	6.8	13.1	19.7	4.0	0.0	1.2	0.0	8.6	0.0	0.0	
<i>Platychrysis roscoffensis</i>		NIES-8	3.4	1.2	20.2	3.3	0.0	0.0	0.0	2.8	13.7	19.1	10.1	12.5	2.5	0.0	2.3	0.0	8.9	0.0	0.0	
<i>Prymnesium calathiferum</i>		NIES-1330	1.9	45.8	20.6	1.0	0.0	0.0	0.0	2.8	8.2	0.4	0.7	8.8	3.7	0.0	0.0	1.4	1.1	0.0	3.6	
<i>Prymnesium parvum</i>		NIES-1017	1.9	17.9	8.8	2.6	1.0	0.2	0.0	0.5	6.0	1.2	6.7	15.2	22.5	0.0	0.5	6.1	8.9	0.0	0.0	
<i>Syracosphaera pulchra</i>		NIES-2705	7.3	15.2	26.9	0.0	0.0	0.0	0.0	23.3	9.8	2.2	2.1	3.0	5.0	0.0	0.0	0.0	5.2	0.0	0.0	
<i>Umbilicosphaera foliosa</i>		NIES-2878	4.7	29.3	51.2	0.0	0.0	0.0	0.0	9.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.5	2.2	
<i>Umbilicosphaera hulburtiana</i>		NIES-2707	2.7	8.7	27.7	1.9	0.0	0.0	0.0	4.7	7.3	9.0	2.3	9.4	8.0	0.0	4.2	0.0	12.5	0.0	1.6	
<i>Umbilicosphaera sibogae</i>		NIES-1324	1.93	16.0	36.2	0.0	0.0	0.0	0.0	20.6	4.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.7	0.0	2.0	

16:1 is mixture of isomers 16:1 (9) and 16:1 (30); 18:1 (11); 18:3 is a mixture of isomers 18:3 (6, 9, 12) and 18:3 (9,12,15); 20:4 is a mixture of isomers 20:4 (5, 8, 11, 14) and 20:4 (8, 11, 14, 17).

(NIES-1, 14, 115, 557, 558, 559, 603, 1974, 1975, 1976), *Fibrocapsa japonica* (NIES-136, 462, 560, 605, 1303), and *Heterosigma akashiwo* (NIES-9, 145, 293) kill fish in Japanese aquaculture through algal blooms (Giner *et al.*, 2008). These strains also contained a higher proportion of 18:4 than other raphidophytes. Other studies of the content of 18:4 in the Raphidophyceae support our results (Mostaert *et al.*, 1998; Nichols *et al.*, 1987; Marshall *et al.*, 2002; Giner *et al.*, 2008; Dorantes-Aranda *et al.*, 2015). 16:0 and 16:1 were abundant in the Xanthophyceae, and 20:5 was detected in all strains except those of *Botrydiopsis* (NIES-621, 2899).

The Pinguiphyceae and the Aurearenophyceae were identified as new classes in 2002 and 2008, respectively (Kawachi *et al.*, 2002; Kai *et al.*, 2008). High EPA content (23.5% to 56.0% of total fatty acids) is a characteristic feature of five genera in the Pinguiphyceae (Kawachi *et al.*, 2002). We found 20:5 in three strains at 3% to 15% of the total. *Aurearena cruciata* (NIES-1863, 1864, 1865) had >20% of 16:0 and 4% to 14% of 20:5.

Most of the Phaeophyceae are macroalgae, with only one filamentous unialgal strain in the MCC-NIES (NIES-548). The major fatty acids in *Acinetospora crinita* (NIES-548) were 16:0, 18:1, 18:2, 18:3, 18:4, and 20:5.

The Schizocladiphyceae were classified in 2003 (Kawai *et al.*, 2003). The major fatty acids in the filamentous marine alga *Schizocladia ischiensis* (NIES-1044) were 16:0, 16:1, 18:1, and 20:5. The major fatty acid composition of *Giraudyopsis* sp. (NIES-1862), in the Chrysomerophyceae, was similar to that of *A. crinita* (NIES-548), in the Phaeophyceae.

Cryptophyta

The Cryptophyceae, the only class in the Cryptophyta, are marine or freshwater flagellated unicells, and most are photosynthetic. The major fatty acids in most strains were 16:0, 18:3, 18:4, and 20:5. In particular, most strains contained high proportions of 18:3+18:4 (15.1%–53.4%). Furthermore, 22:6 was found in all strains of *Cryptomonas*, *Rhodomonas*, and *Hemiselmis* except for NIES-3185. The results in *Cryptomonas* and *Rhodomonas* support previous reports (Dunstan *et al.*, 2005; Wacker *et al.*, 2016).

Haptophyta

The Haptophyta are divided into two classes, the Pavlovophyceae and the Prymnesiophyceae, which

contain well-known species of the Prymnesiales and coccolithophorids. The major fatty acids in the Haptophyta were 14:0, 16:0, 16:1, 18:4, and 22:6. The content of 20:5 also was high in the Pavlovophyceae. In addition, 18:5 was detected in some strains in the Prymnesiophyceae. *Emiliania huxleyi* is a model coccolithophorid, and its fatty acid composition has been reported (Bell & Pond, 1996; Boelen *et al.*, 2013; Kotajima *et al.*, 2014). Four *Emiliania* strains and the closely related species *Gephyrocapsa oceanica* had similar fatty acid compositions to those previously reported (Bendif *et al.*, 2016). In the Pavlovophyceae, PUFA production has been investigated most intensively in *Pavlova lutheri* (Kato *et al.*, 1995; Meireles *et al.*, 2003; Guihéneuf & Stengel, 2013). This species is widely used in aquaculture as live feed for marine invertebrates (Rico-Villa *et al.*, 2006; Ponis *et al.*, 2008). Fatty acid compositions of MCC-NIES strains in the Pavlovophyceae were similar to that of *P. lutheri* (Kato *et al.*, 1995). In addition, two unidentified strains of *Pavlova*, NIES-1815 and 1816, had good quantities of both 20:5 and 22:6, suggesting advantages in aquaculture nutrition.

CONCLUSION

The fatty acid profiles presented here provide useful information in the search for microalgae as sources of valuable compounds. Microalgae as sources of PUFAs such as 20:5 and 22:6, with beneficial effects on human health, are attracting increasing attention. Microalgae offer sources of many other useful compounds, such as antioxidants, pigments, biofuel, and bioplastics. We hope to enrich the information available to microalga researchers through the MCC-NIES.

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NIES 藻類コレクションに属する 3 植物門 235 株の脂肪酸組成プロファイル

三谷英李¹⁾, 中山史葉¹⁾, 松脇いずみ¹⁾, 市 育代^{1,2)}, 川端篤志³⁾, 河地正伸³⁾, 加藤美砂子^{1,2)}¹⁾お茶の水女子大学大学院人間文化創成科学研究科,²⁾お茶の水女子大学ヒューマンライフイノベーション研究所, ³⁾国立環境研究所

表現型的マーカーとしての高度不飽和脂肪酸の有無について調査するために、国立環境研究所（NIES）の藻類コレクションから、不等毛植物門、クリプト植物門、ハプト植物門について、合計 235 株の脂肪酸分析を行った。エイコサペンタエン酸（EPA, 20:5）は分析した 119 株において、総脂肪酸の 10% 以上を占めていた。ドコサヘキサエン酸（DHA, 22:6）は 16 株のハプト植物と未同定の 2 株のペラゴ藻綱（不等毛植物門）において、総脂肪酸の 10% 以上を占めていた。黄金色藻綱やペラゴ藻綱以外の不等毛植物の株やクリプト植物の株では、一般に DHA よりも EPA のほうが、多く含まれていた。近年新しく設立され、本研究で初めて脂肪酸分析が行われたアウレアレナ藻綱、ペラゴ藻綱、シゾクラディア藻綱を含む 11 綱の不等毛植物を調査した結果、多様な脂肪酸組成プロファイルが明らかになったものの、表現型として分類群を特徴づけるような明瞭な結果は確認されなかった。