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NOTE

PULSED-FIELD GEL ELECTROPHORESIS ANALYSIS OF GENOME SIZE AND STRUCTURE IN *PAVLOVA GYRANS* AND *DIACRONEMA* SP. (HAPTOPHYTA)[1]

Tetyana Nosenko, Brenda Boese, and Debashish Bhattacharya[2]

Department of Biological Sciences and the Roy J. Carver Center for Comparative Genomics, University of Iowa, 446 Biology Building, Iowa City, Iowa 52242, USA

Information regarding genome size and structure is a prerequisite for selecting model organisms and for facilitating the most efficient study of their chromosomal DNA. The goal of this study was to identify future candidates for complete-genome-sequencing projects among economically or evolutionarily important species of haptophyte algae. Using pulsed-field gel electrophoresis (PFGE), we identified relatively small genomes and chromosome sizes in two haptophyte species from the class Pavlovophyceae, *Pavlova gyrans* Butcher and *Diacronema* sp. The basal position of Pavlovophyceae in the Haptophyta; the key position of this group in the chromalveolates; and their economic and potential biomedical importance, ease of culturing, and small genome size make these taxa ideal models for complete-genome sequencing.

Key index words: ***Diacronema*; genome size and structure; haptophyte algae; *Pavlova;* Pavlovophyceae; PFGE**

***Abbreviations:* CHEF, contour-clamped homogeneous electric field; EST, expressed sequence tag; LCPUFA, long-chain polyunsaturated fatty acid; PFGE, pulsed-field gel electrophoresis**

Haptophyte algae are one of the most successful groups of marine eukaryotes. Because of their broad distribution and high abundance, haptophytes are key components of food chains in the marine environment (Moon-van der Staay et al. 2001, Tang et al. 2001). The ecology of haptophyte algae has been intensively studied because of their roles in marine calcification and global carbon and sulfur cycles as well as their potential influence on global climate (Malin et al. 1992, Westbroek et al. 1993, Tchernov et al. 2003). Several species of haptophyte algae are agents of HABs that have negative economic impacts due to toxin or foam production (James and de la Cruz 1989, Moestrup 1994). Haptophytes include ~300 species that are classified into two classes: the Pavlovophyceae, which forms an early diverged branch of haptophytes, and the more morphologically complex Prymnesiophyceae (Cavalier-Smith 1993, Edvardsen et al. 2000). Members of both these groups demonstrate an astonishing diversity of morphology, ultrastructure, biochemistry, and ecology (Jordan and Chamberlain 1997). In addition to their ecological and economic importance, haptophyte algae are of special interest for evolutionary biologists because of their affiliation with chromalveolate protists. The ancestor of this putative ''supergroup'' is believed to have contained a red algal plastid that originated through secondary endosymbiosis (Gibbs 1993, Hackett et al. 2007). Until recently, little research on chromalveolate algae was carried out using genomic approaches. This has changed because of the wide availability of genomic technology, although most studies until now have focused on expressed sequence tag (EST) methods (e.g., Hackett et al. 2004, Protist EST Program, http://megasun.bch.umontreal.ca/pepdb/pep.html). It is clear, however, that sequencing of complete genomes provides the most comprehensive data for comparative genomics. The large genome sizes that have been identified in most investigated chromalveolates (Beaton and Cavalier-Smith 1999, LaJeunesse et al. 2005) and the associated costs of sequencing to high coverage generally remain the major obstacles to overcome for genomic studies of this group. Given this consideration, we used PFGE to study several candidate algae and identified relatively small genome and chromosome sizes in two species of haptophyte algae from the class Pavlovophyceae: *Pavlova gyrans* and *Diacronema* sp.

Cultures of *P. gyrans* CCMP 607 and *Diacronema* sp. CCMP 610 were obtained from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton (CCMP) and grown on f/2 medium in a controlled environment chamber at 18°C with a 13:11 light:dark (L:D) period. To prepare agarose plugs for PFGE, approximately 10^9 cells were harvested by centrifugation at 1000*g* for 10 min at 4°C, washed with culture medium, and resuspended in 0.2 mL of 50°C preheated buffer A (450 mM EDTA, 10 mM Tris-HCl [pH 8], and 100 mM NaCl). The cell suspension was incubated for 5 min at 42°C,

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[2]Author for correspondence: e-mail debashi-bhattacharya@uiowa.edu.

mixed with 0.2 mL of 1% low-melting agarose (InCert Agarose, Cambrex Bio Science, Rockland, ME, USA) in 125 mM EDTA and 10 mM Tris-HCl (pH 8) solution containing 100 mM β-mercaptoethanol and 1 mg · mL^{-1} lysozyme at 50°C, and pipetted into plug molds. The plugs were washed for 15 min at room temperature in buffer B (500 mM EDTA [pH 8], 10 mM Tris-HCl [pH 8], and 1% sodium lauryl/sarcosinate), incubated overnight at 37°C in lysozyme-containing (1 mg · mL^{-1}) buffer B, and then incubated for 24 h at 50°C in buffer C (buffer B + 0.2 mg · mL^{-1} Proteinase K). After incubation, the plugs were washed twice in 1.5 mL of buffer A at 50°C for 4 h and stored in buffer A at 4°C. The PFGE was performed using the CHEF (contour-clamped homogeneous electric field)-DR II Pulsed Field Electrophoresis System (Bio-Rad Laboratories, Hercules, CA, USA). We used two electrophoresis modes to separate algal chromosomes. Chromosomes that ranged in size from 100 kb to 2000 kb were separated using 1% agarose gel (SeaKem Gold Agarose, Cambrex Bio Science, Rockland, ME, USA) in 0.5× TBE under the following electrophoresis conditions: Stage I—30 V, 500 s switch time, 3 h run time; Stage II—200 V, 60 s switch time, 13 h; Stage III—200 V, 120 s switch time, 10 h (12°C). Chromosomes of 2000–6000 kb in size were separated using 0.8% agarose gel in 1× TAE. The electrophoresis conditions were as follows: Stage I—40 V, 500 s switch time, 4 h; Stage II—50 V, 3600 s switch time, 92 h (9°C). Chromosomes of yeast (225–2200 kb) and fission yeast (3500–5700 kb; Bio-Rad Laboratories) were used as size standards. The number of chromosomes that were condensed in one DNA band was approximated as being one or two (minimal approximation) depending on the intensity of staining with ethidium bromide (EtBr) relative to adjacent bands. The relative DNA concentrations in bands visualized in the gel were estimated using gel analysis tools in the computer program ImageJ (http://rsb.info.nih.gov/ij/). To perform Southern blot analysis, gels were incubated in TBE buffer containing EtBr (1.5 μg · mL^{-1}) for 30 min at room temperature, washed with TBE buffer for 30 min, and irradiated with UV light using BioRad GS Gene Linker UV Chamber (Bio-Rad Laboratories) to nick the DNA fragments. The gels were simultaneously blotted onto two positively charged nylon membranes (Hybond-N, Amersham, Little Chalfont, UK) using the method described in Sambrook et al. (1989). One of these membranes was used for the hybridization reaction with probes to a nuclear encoded gene (actin), whereas the second was used for testing the presence of plastid DNA. A ~500 bp fragment of the *Guillardia theta* D. R. A. Hill et Wetherbee actin gene was amplified using PCR, purified, labeled with [α^{-32P}] dCTP (50 μCi) using the HexaLab DNA labeling kit (MBI Fermentas, York, UK), and used as a probe in the Southern hybridization. Radioactively labeled fragments of the plastid genes *psb*A, *psb*C, *psb*D, and *psa*A in the glaucophyte alga *Glaucocystis nostochinearum* Itzigs. were used to test for the presence of contaminating plastid DNA in the gels. Hybridization reactions were performed overnight at 40°C in 6× SSC (0.15 M sodium citrate); 0.5% SDS; 100 μg · mL^{-1} denatured, fragmented salmon sperm DNA; and deionized 30% formamide. After hybridization, membranes were washed twice in 2× SSC and 0.1% SDS at 40°C for 1.5 and 2 h, respectively, and once briefly in 1× SSC at room temperature.

Analysis of the *P. gyrans* and *Diacronema* sp. genomes using the two modes of the CHEF system showed that they are composed of small to midsize chromosomes ranging in size from 300 to 4000 kb and from 180 to 3500 kb, respectively. A total of 17 DNA bands were resolved in the *P. gyrans* gel run under the conditions used to separate small chromosomes (Mode I; Fig. 1A). Application of Mode II of CHEF, designed to separate midsize (2000–6000 kb) chromosomes, allowed us to determine the size of the two upper DNA bands and suggested the absence of chromosomes larger than 4000 kb in *P. gyrans* (Fig. 1D). According to these results, the *P. gyrans* chromosome sizes correspond to 300, 350, 450, 680, 720, 785, 800, 950, 980, 1125, 1240, 1350, 1500, 1700, 1900, 3700, and 4000 kb. The higher DNA concentration that we observed in bands of size 450, 785, 1240, and 3700 kb indicates the presence of several chromosomes of similar size in these bands (Fig. 1B). Assuming that each of these strongly staining bands contains two chromosomes (minimal estimate), the total size of the haploid *P. gyrans* genome is 28,705 kb. To confirm that the DNA bands visualized in the gel were of eukaryotic origin, we performed Southern blot hybridization with a probe against the actin coding region. This conserved gene probe hybridized to 15/17 *P. gyrans* DNA bands (Fig. 1C). The greatest actin cross-reaction was observed in bands of sizes 450, 785, 1240, and 3700 kb. This finding probably indicates that several chromosomes compressed in these bands contain a sequence(s) encoding an actin gene family member. A previous Southern blot analysis of *Emiliania huxleyi* (Lohmann) W. H. Hay et H. Mohler using restriction digested DNA and RT-PCR approaches and sequencing (Bhattacharya et al. 1993) also showed the presence of multiple, dispersed actin genes comprising a complex family as indicated here for *P. gyrans*. The negative result for the hybridization reaction with probes for four plastid-encoded genes—*psb*A, *psb*C, *psb*D, and *psa*A (results not shown)—confirms the absence of plastid DNA from the *P. gyrans* gel. This result is consistent with the observation that circular DNA molecules larger than 80 kb are generally trapped in the sample wells during CHEF (Wang and Lai 1995). Haptophyte plastid genomes vary in size from 105 to 165 kb (Saez et al. 2001, Sanchez-Puerta et al. 2005).

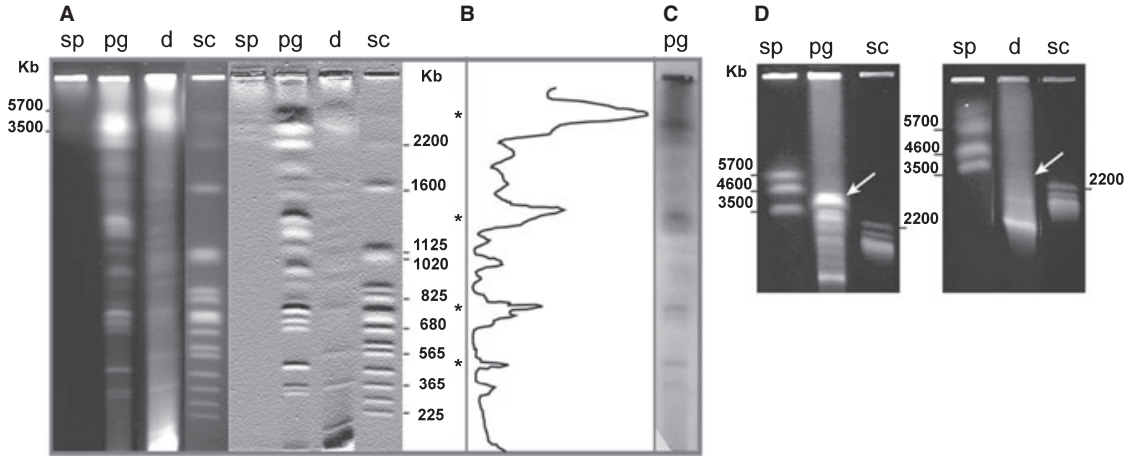

FIG. 1. Pulsed-field gel electrophoresis (PFGE) analysis of genomes from the haptophyte algae *Pavlova gyrans* and *Diacronema* sp. (A) Separation of small chromosomes under Mode I of CHEF (contour-clamped homogeneous electric field). (B) The lane profile plot of gel A. The size of each peak is proportional to the DNA concentration in the corresponding band in this gel. The asterisks (*) indicate peaks corresponding to the bands that contain more than one chromosome. (C) Southern blot of gel A lane *P. gyrans* (pg) probed with a eukaryotic actin sequence. (D) Separation of midsize chromosomes under Mode II of CHEF. The arrows indicate bands containing the largest chromosomes of the *P. gyrans* and *Diacronema* sp. genomes. d, *Diacronema* sp.; sp, *Schizosaccharomyces pombe* Linder DNA size standard; sc, *Saccharomyces cerevisiae* Meyen DNA size standard.

The genome structure of *Diacronema* sp. is similar to that of *P. gyrans* (Fig. 1). We identified 16 bands in this species corresponding to chromosomes of sizes 180, 330, 520, 750, 780, 960, 980, 1000, 1200, 1300, 1400, 1550, 1700, 2000, 2500, and 3500 kb. Therefore, the estimated total genome size in a haploid *Diacronema* sp. cell is ~20,650 kb.

Analysis of previously published data regarding protist genome sizes estimated using PFGE, when compared with actual genome sizes obtained by generating complete genome sequences, shows that PFGE provides reliable estimates for genomes composed of small and midsize chromosomes (Table 1). Therefore, genome sizes in *Ostreococcus tauri* C. Courties et Chret.-Dinet and *Cyanidioschyzon merolae* De Luca, Taddei et L. Varano that were predicted on the basis of PFGE (Takahashi et al. 1995, Courties et al. 1998) represent 81% and 86%, respectively, of the actual sizes of the complete genomes (Matsuzaki et al. 2004, Derelle et al. 2006). In both cases, the underestimation of genome size was <20%. This underestimation results from the presence of chromosomes of similar sizes that fail to segregate in a gel. The intensity of DNA staining with EtBr does not allow the identification of the exact number of chromosomes that may be compressed in one band. If we make a correction for a possible 20% underestimation, the genome sizes of *P. gyrans* and *Diacronema* sp. would correspond to ~35.88 and 25.81 Mb, respectively.

These haptophyte genome sizes are comparable with the recently sequenced genome of the diatom *Thalassiosira pseudonana* Hasle et Heimdal (34,500 kb; Armbrust et al. 2004) and are significantly smaller than two other chromalveolates, *E. huxleyi* (220 Mb; http://www.jgi.doe.gov/sequencing) and *Ectocarpus siliculosus* (Dillwyn) Lyngbye (214 Mb; http://www.cns.fr/externe/English/Projets), which are currently being sequenced. The observation that representatives of the two genera, *Pavlova* and *Diacronema*, have relatively small genomes suggests that this feature may be conserved among pavlovophyceans. Apart from a small nuclear genome size, members of this group are characterized by other features that

TABLE 1. Estimates of genome size in different algae.

Species	Chromosome no.		Chromosome size (Mb)	Genome size (Mb)	
	PFGE	CGS[a]		PFGE	CGS[a]
Pavlova gyrans	21	–	0.300–4.000	28.705	–
Diacronema sp.	16	–	0.180–3.500	20.650	–
Ostreococcus tauri	14[b]	20[c]	0.120–1.500[c]	10.200[b]	12.560[c]
Cyanidioschyzon merolae	17[d]	20[e]	0.420–1.620[e]	14.200[d]	16.500[e]

[a]Complete genome sequence. –, data not available.
[b]Courties et al. (1998).
[c]Derelle et al. (2006).
[d]Takahashi et al. (1995).
[e]Matsuzaki et al. (2004).

are important considerations for model organisms, such as their scientific and economic importance, short cell-division time [0.5–1.0 divisions · d^{-1} (Ponis et al. 2006], and ease of maintenance as unialgal or axenic cultures. Their basal position in the Haptophyta also makes Pavlovophyceae a group of high interest from an evolutionary perspective. It is conceivable that a comparative analysis of a pavlovophycean genome with that of *E. huxleyi* would reveal the molecular basis for the origins of adaptive traits that differentiate these important unicellular, free-living protists. These traits include the ability to form calcium-carbonate-containing organic external body scales (coccoliths) in different prymnesiophycean algae. Apart from the potential to elucidate haptophyte biology, a genomic study of pavlovophycean algae may also have a significant economic and biomedical impact. A high nutritional value, absence of toxic compounds, and rapid growth rates in commercial mass cultures make species of *Pavlova* and *Diacronema* important feedstock in industrial aquaculture of bivalve mollusks, crustaceans, and fish (Brown 2002, Meireles et al. 2003, and Ponis et al. 2006). The biochemistry and physiology of pavlovophycean algae have been intensively studied for their ability to synthesize long-chain polyunsaturated fatty acids (LCPUFA), such as docosahexaenoic (DHA) and eicosapentaenoic (EPA) acids (Carvalho and Malcata 2005), which are essential for the growth and survival of many marine animals and play important roles in human health (Horrocks and Yeo 1999). DHA and EPA are not synthesized in adequate amounts in humans to meet metabolic demands and are currently provided by fish oils. The presence of these fatty acids in fish oils is owing to the consumption by these taxa of DHA- and EPA-synthesizing microalgae. In this regard, pavlovophycean algae are considered potential candidates for the commercial production of DHA- and EPA-enriched oils (Carvalho and Malcata 2005). Genomic study of pavlovophycean algae could potentially help to uncover the mechanisms involved in the regulation of LCPUFA production and could enable the manipulation of these organisms to enhance DHA and EPA production. Based on these considerations, we recommend *P. gyrans* and *Diacronema* sp. as candidates for complete genome sequencing.

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