JOURNAL OF BACTERIOLOGY, Sept. 1991, p. 5924–5927
0021-9193/91/185924-04$02.00/0

Vol. 173, No. 18

Plasmids in Diatom Species

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We have discovered plasmids in 5 of 18 diatom species surveyed. In several species, more than one type of plasmid is present. Several of the plasmids show similarity by hybridization to previously characterized plasmids in *Cylindrotheca fusiformis* (J. D. Jacobs et al., unpublished data). Additionally, there is similarity between the plasmids found in *C. fusiformis* and chloroplast DNA in three diatom species. These results add to the evidence that the plasmids have features of mobile genetic elements.

We have recently discovered two small circular DNA plasmids, pCf1 and pCf2, in the marine diatom *Cylindrotheca fusiformis* (9). These plasmids are 4.27 kbp (pCf1) and 4.08 kbp (pCf2) in size. Sequence analysis (8) shows that the plasmids share a large region of significant similarity. They hybridize to each other under low-stringency conditions but not under high-stringency conditions. Under high-stringency conditions, both plasmids hybridize to high-molecular-weight (genomic) chloroplast DNA, and pCf2 also hybridizes to nuclear DNA. When coupled with the sequence information, this suggests that substantial portions of the plasmids are present in genomic DNA. The evidence to date is consistent with the hypothesis that the plasmids are mobile genetic elements (8). These intriguing results prompted us to see whether plasmids commonly occur in other diatom species and to determine whether they share similar features.

We examined a total of 18 diatom species from the orders *Pennales* and *Centrales*, with most species isolated from marine environments, but including examples of littoral and freshwater diatoms (Table 1). Total DNA was isolated from these species according to method I of Rochaix et al. (14) and was centrifuged on CsCl-Hoechst 33258 gradients, consisting of 50% (wt/vol) CsCl in TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM Na_2EDTA), with 10 μg of Hoechst 33258 dye per ml. Centrifugation was in a Beckman Ti50 rotor at 110,000 × *g* (42,000 rpm) and 18°C for 72 h. Diatom DNA separates into two bands on the basis of AT content (10, 12) on these gradients. The lower band is greatly enriched in nuclear DNA, and the upper band is greatly enriched in chloroplast DNA, mitochondrial DNA, and plasmids, when present (9). The banding position suggests that the upper band DNA is AT rich (10, 12).

Total DNA and upper-band DNA (undigested) were electrophoresed on 1.2% agarose gels in TBE buffer (100 mM Trizma base, 100 mM boric acid, 2 mM Na_2EDTA) and examined for the presence of lower-molecular-weight bands distinct from the genomic DNA. Such bands, indicative of plasmids, were identified in five species (Fig. 1 and Table 2) representing two genera (Table 1). In each case, the plasmids were enriched in the upper-band sample. In some cases, faint bands were visible in the original gel photograph but were not reproduced in Fig. 1. These bands are noted in Table 2.

In *C. fusiformis*, three clones from different habitats contained plasmids of identical size. Upon agarose gel electrophoresis, three sets of two lower-molecular-weight bands which are enriched in the upper-band DNA from the CsCl-Hoechst 33258 gradients (Fig. 1, lane 4) are visible (Fig. 1, lanes 3 and 4). We have determined (9) that these sets of bands consist of pCf1 and pCf2 in three forms: open circular (slowest-migrating set), linear (intermediately migrating set), and supercoiled (fastest-migrating set). In the other diatom species, it is possible that several of the bands represent different topological forms of the same plasmid. For reference, all DNA bands were included in the tabulation presented in Table 2; however, their sizes should be regarded only as apparent, since we have not determined the topological form of the DNA in each band.

In *Cylindrotheca closterium*, only one of three clones examined contained plasmids (Table 1). Four major bands were visible (Fig. 1, lane 6), and two fainter ones were present (Table 2), containing DNA in various quantities. In *Nitzschia angularis*, three sets of two lower-molecular-weight bands were visible (Fig. 1, lanes 7 and 8), similar to those in *C. fusiformis* but slightly smaller in size. In *Nitzschia curvilineata*, two bands of high molecular weight were consistently visible in a number of electrophoretic separations (Fig. 1, lane 10). The data suggest two possibilities. One is that the higher-molecular-weight band is chloroplast DNA and the lower-molecular-weight band is a large plasmid. Alternatively, the chloroplast DNA may consist of two different size classes of circular molecules, as occurs in the brown alga *Pylaiella littoralis* (2). In *Nitzschia* sp. strain SIO two major bands were visible (Fig. 1, lanes 11 and 12), and a number of fainter ones were present (Table 2), containing DNA in various quantities. In addition, bands of limited electrophoretic mobility are visible in lanes 4, 6, 11, and 12; at this point, we cannot comment on their nature.

To determine the extent of similarity of the *C. fusiformis* plasmids to those in other diatom species, cloned fragments representing full-length pCf1 and pCf2 were radioactively labeled and probed separately to a blot of total and upper-band DNA from plasmid-containing diatom species. From sequence analysis, we have determined that 650-bp portions of pCf1 and pCf2 share 77% nucleotide identity, including several stretches of exact identity more than 19 nucleotides in length (8). In these experiments, we have chosen hybrid-

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TABLE 1. Types and sources of diatom species tested for the presence of plasmids

Pennales
- *Navicula*
  - *N. pelliculosa* (*N. saprophila* [FW-50]); freshwater; R. A. Lewin
  - *N. incerta* (2046)[a]; saline lake shore; Salton Sea, Calif.
- *Phaeodactylum tricornutum*; marine; three morphotypes: fusiformis, oval, and triradiate
- *Cylindrotheca*
  - *C. closterium*; three clones from different habitats
    - Salton Sea,[b] Calif.; saline lake; W. H. Thomas
    - SIO; on retaining wall at the ocean aquarium seawater effluent
    - 424[c]; Vancouver, British Columbia, Canada
  - *C. fusiformis*; marine; three clones
    - R. L. L. Guillard[b]
    - Culture 417[b,c]
    - Culture 425[b,c]
- *Nitzschia*
  - *N. alba*; marine; nonphotosynthetic; Scripps Institution of Oceanography shore, on the seaweed *Macrocystis pyrifera*
  - *N. angularis* (35-M)[b]; marine; Herring Cove, Nova Scotia, Canada, on the red alga *Chondrus*; R. A. Lewin
  - *N. curvilineata* (2033)[a,b]; marine; shoreline, New Haven, Conn.
  - *N. frustulum*; marine; Muroran, Pacific Ocean, Japan; R. A. Lewin
  - *N. laevis* (2047)[a]; marine; seawater tank, Woods Hole, Mass.
  - *N. ovalis* (13-M)[a]; marine; tide pool, Nova Scotia, Canada
  - *Nitzschia* sp. strain Mono Lake, Calif.; saline carbonaceous lake; W. H. Thomas
  - *Nitzschia* sp. strain SIO; marine; on retaining wall at the ocean aquarium seawater effluent; C.-W. Li
- *Amphiprora* sp. strain SIO; marine; on retaining wall at the ocean aquarium seawater effluent
- *Amphora* sp. strain T-34; salt marsh; Greater Sippewissett, Falmouth, Mass.; J. Lee

Centrales
- *Cyclotella nana*; marine
- *Skeletonema costatum*; marine; fjord, Trondheim, Norway; S. Myklestad
- *Chaetoceros gracilis*; marine; Costa Rica, Dom, Pacific Ocean; W. H. Thomas

[a] The Culture Collection of Algae at the University of Texas, Austin, Tex.
[b] Contains plasmids.
[c] North East Pacific Culture Collection, University of British Columbia, Vancouver, British Columbia, Canada.

ization conditions under which pCf1 and pCf2 do not hybridize to each other; therefore, a positive hybridization signal indicates that a substantial portion of the plasmid sequences, in terms of nucleotide length and identity, is present in the hybridizing DNA. These results are shown in Fig. 2 and summarized in Table 2.

Upon long autoradiographic exposure (data not shown) a very faint band is visible in the *C. closterium* chloroplast DNA sample when probed with pCf1. A faint band of different molecular weight hybridizes to pCf2 (Fig. 2, lane 4B). In *N. angularis*, pCf1 hybridizes to three plasmid bands and additionally to genomic chloroplast DNA (Fig. 2, uppermost band in lane 6A). pCf2 hybridizes to two plasmid bands and very intensely to genomic chloroplast DNA (Fig. 2, lane 6B). The plasmid bands that hybridize to pCf1 are the larger of each set of two bands; pCf2 hybridizes to the smaller of each set, further substantiating the similarity of the plasmids from *N. angularis* and *C. fusiformis*. In *N. curvilineata*, there is no hybridization to either pCf1 or pCf2 (Fig. 2, lanes 7 and 8, A and B). In *Nitzschia* sp. strain SIO, pCf1 hybridizes to two plasmid bands (Fig. 2, lanes 9A and 10A), while pCf2 hybridizes very weakly to two other plasmid bands but very strongly to genomic chloroplast DNA (Fig. 2, lanes 9B and 10B). In no case did pCf1 and pCf2 hybridize to the same plasmid in another species.

We have identified plasmids in 5 of 18 tested species of diatoms representing two genera. With the exception of *N. curvilineata*, we have shown that each species contains more than one type of plasmid, as judged by hybridization of different bands to pCf1 and pCf2 or lack of hybridization of some bands to these probes. Although not quantitatively determined, the amount of plasmid DNA extracted by this procedure (14) appears quite high relative to other cellular

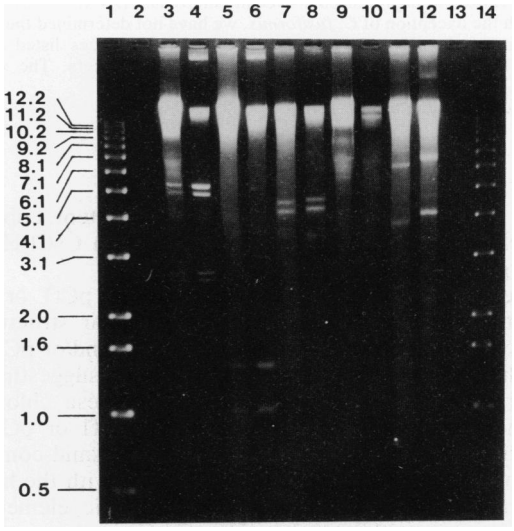

FIG. 1. Agarose gel (1.2%) electrophoretic separations of DNA from plasmid-containing diatom species. Diatom samples contain either a total-DNA extract from the cell or the upper band of a CsCl-Hoechst 33258 gradient separation of total DNA, which is enriched in plasmids and chloroplast DNA (see text). Lanes 1 and 14, 1-kb molecular weight markers (Bethesda Research Laboratories, Gaithersburg Md.). Sizes of the markers (in kilobase pairs) are indicated to the left of the photograph. Other lanes are as follows: 2 and 13, blank; 3, *C. fusiformis* total DNA; 4, *C. fusiformis* upper-band DNA; 5, *C. closterium* total DNA; 6, *C. closterium* upper-band DNA; 7, *N. angularis* total DNA; 8, *N. angularis* upper-band DNA; 9, *N. curvilineata* total DNA; 10, *N. curvilineata* upper-band DNA; 11, *Nitzschia* sp. strain SIO total DNA; 11, *Nitzschia* sp. strain SIO upper-band DNA.

TABLE 2. Apparent size and similarity of plasmids in diatoms

Organism[a]	Apparent size of plasmid (kbp)[b]	Plasmid which hybridizes to pCf1 or pCf2
C. fusiformis	5.10	pCf1
	4.86	pCf2
	4.25	pCf1
	4.02	pCf2
	2.79	pCf1
	2.58	pCf2
C. closterium (from Salton Sea)	5.09	pCf1
	4.70[c]	pCf2
	3.54[c]	
	3.00	
	1.43	
	0.98	
N. angularis	4.55	pCf1
	4.24	pCf2
	3.74	pCf1
	3.55	pCf2
	2.47	pCf1
	2.34	
N. curvilineata	11.6	
Nitzschia sp. strain SIO	7.20[c]	
	6.10	pCf1
	4.75	pCf1
	4.20	pCf2
	3.65	pCf2
	3.31	
	2.38	
	2.04[c]	
	1.79[c]	

[a] For *C. fusiformis*, pCf1 and pCf2 also hybridize to high-molecular-weight chloroplast DNA; for *N. angularis*, pCf1 and pCf2 also hybridize to high-molecular-weight chloroplast DNA; and for *Nitzschia* sp. strain SIO, pCf2 also hybridizes to high-molecular-weight chloroplast DNA.

[b] With the exception of *C. fusiformis*, we have not determined the topological form of the DNA in each band; therefore the sizes listed are only apparent, relative to linearized molecular weight markers. The sizes are averages from several electrophoretic separations.

[c] Band not visible in Fig. 1.

DNA. All of the plasmids have a high AT content, cobanding with chloroplast and mitochondrial DNA in CsCl-Hoechst 33258 gradients.

Several of the plasmids hybridize with pCf1 or pCf2, indicating that they probably contain similar structural or functional features. In three species, pCf1 and/or pCf2 also hybridizes to genomic chloroplast DNA, suggesting that similar sequences are found as well in these chloroplast genomes. We have not tested whether pCf1 or pCf2 will hybridize to chloroplast DNA from non-plasmid-containing diatom species. Our results are consistent with the hypothesis that these plasmids are mobile genetic elements (8) which can exist either as plasmids or integrated into genomic DNA (6).

Our characterization of the diatom plasmids adds to a growing number of reports of plasmids in algal species. Plasmids have been identified in the red algae (Rhodophyta [1, 4, 16]), brown algae (Phaeophyta [2]), *Acetabularia cliftonii*, *Acetabularia acetabulum*, and *Chlamydomonas moewusii* (Chlorophyta [3, 5, 11, 15]), and *Euglena gracilis* (Euglenophyta [7, 13]), in addition to the diatom species (Bacillariophyta) described in this article. If indeed the diatom plasmids do contain transposons, this could greatly facilitate the ability to genetically manipulate these ecologically important organisms.

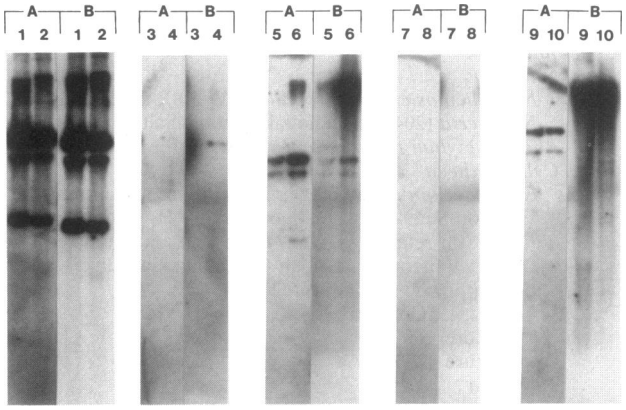

FIG. 2. Autoradiograms of blot hybridization experiments using labeled pCf1 (lanes A) and pCf2 (lanes B) as probes against separations of DNA from plasmid-containing diatom species. Lanes are as follows: 1, *C. fusiformis* total DNA; 2, *C. fusiformis* upper-band DNA; 3, *C. closterium* total DNA; 4, *C. closterium* upper-band DNA; 5, *N. angularis* total DNA; 6, *N. angularis* upper-band DNA; 7, *N. curvilineata* total DNA; 8, *N. curvilineata* upper-band DNA; 9, *Nitzschia* sp. strain SIO total DNA; 10, *Nitzschia* sp. strain SIO upper-band DNA.

We are grateful to R. L. L. Guillard, J. Lee, R. A. Lewin, C.-W. Li, S. Myklestad, and W. H. Thomas for providing diatom cultures. We thank Gina Moore for isolation of probe fragments used in the hybridization experiments. Stephen Mayfield generously provided the use of his research facilities for a portion of this work.

This work was supported by Public Health Service grant GM-08229 from the National Institute of General Medical Sciences (B.E.V.). T.-Y.F. was supported by an Advanced Abroad Research Grant from the National Science Council of the Republic of China. M.H. was supported in part by a donation from the Ethyl Corporation.

REFERENCES

1. **Bird, C. J., W. A. Nelson, E. L. Rice, K. G. Ryan, and R. Villemur.** 1990. A critical comparison of *Gracilaria chilensis* and *G. sordida* (Rhodophyta, Gracilariales). J. Appl. Phycol. **2**:375–382.
2. **Dalmon, J., S. Loiseaux, and S. Bazetoux.** 1983. Heterogeneity of plastid DNA of two species of brown algae. Plant Sci. Lett. **29**:243–253.
3. **Ebert, C., M. J. Tymms, and H.-G. Schweiger.** 1985. Homology between 4.3 μm minicircular DNA and plastomic DNA in chloroplasts of *Acetabularia cliftonii*. Mol. Gen. Genet. **200**: 187–192.
4. **Goff, L. J., and A. W. Coleman.** 1990. Red algal plasmids. Curr. Genet. **18**:557–565.
5. **Green, B. R.** 1976. Covalently closed minicircular DNA associated with *Acetabularia* chloroplasts. Biochim. Biophys. Acta **447**:156–166.
6. **Grindley, N. D., and R. R. Reed.** 1985. Transpositional recombination in prokaryotes. Annu. Rev. Biochem. **54**:863–896.
7. **Heizmann, P., P. Ravel-Chapuis, and V. Nigon.** 1982. Minicircular DNA having sequence homologies with chloroplast DNA in a bleached mutant of *Euglena gracilis*. Curr. Genet. **6**:119–122.
8. **Hildebrand, M., et al.** Unpublished data.
9. **Jacobs, J. D., et al.** Unpublished data.
10. **Manuelidis, L.** 1977. A simplified method for preparation of mouse satellite DNA. Anal. Biochem. **78**:561–568.
11. **Mazza, A., A. Casale, P. Sassone-Corsi, and S. Bonotto.** 1980. A minicircular component of *Acetabularia acetabulum* chloroplast DNA replicating by rolling circle. Biochem. Biophys. Res. Commun. **93**:668–674.

12. **Muller, W., and F. Gautier.** 1975. Interactions of heteroaromatic compounds with nucleic acids: AT-specific non-intercalating DNA ligands. Eur. J. Biochem. **54**:385–394.
13. **Nass, M. M. K., and Y. Ben-Shaul.** 1972. A novel closed circular duplex DNA in bleached mutant and green strains of *Euglena gracilis*. Biochim. Biophys. Acta **272**:130–136.
14. **Rochaix, J. D., S. Mayfield, M. Goldschmidt-Clermont, and J. Erickson.** 1988. Molecular biology of *Chlamydomonas*, p. 253–275. *In* C. H. Shaw (ed.), Plant molecular biology: a practical approach. IRL Press, Washington, D.C.
15. **Turmel, M., G. Bellemare, R. W. Lee, and C. Lemieux.** 1986. A linear DNA molecule of 5.9 kilobase-pairs is highly homologous to the chloroplast DNA in the green alga *Chlamydomonas moewusii*. Plant Mol. Biol. **6**:313–319.
16. **Villemur, R.** 1990. Circular plasmid DNAs from the red alga *Gracilaria chilensis*. Curr. Genet. **18**:251–257.