MARINE BIOTECHNOLOGY

Development of Molecular Probes for *Dinophysis* (Dinophyceae) Plastid: A Tool to Predict Blooming and Explore Plastid Origin

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Abstract

Dinophysis are species of dinoflagellates that cause diarrhetic shellfish poisoning. We have previously reported that they probably acquire plastids from cryptophytes in the environment, after which they bloom. Thus monitoring the intracellular plastid density in *Dinophysis* and the source cryptophytes occurring in the field should allow prediction of *Dinophysis* blooming. In this study the nucleotide sequences of the plastid-encoded small subunit ribosomal RNA gene and *rbcL* (encoding the large subunit of RuBisCO) from *Dinophysis* spp. were compared with those of cryptophytes, and genetic probes specific for the *Dinophysis* plastid were designed. Fluorescent in situ hybridization (FISH) showed that the probes bound specifically to *Dinophysis* plastids. Also, FISH on collected nanoplankton showed the presence of probe-hybridized eukaryotes, possibly cryptophytes with plastids identical to those of *Dinophysis*. These probes are useful not only as markers for plastid density and activity of *Dinophysis*, but also as tools for monitoring cryptophytes that may be sources of *Dinophysis* plastids.

Key words: *Dinophysis* — fluorescent in situ hybridization (FISH) — shellfish poisoning — cryptophyte — plastid

Introduction

Some phytoplankton species are known to produce toxins that accumulate in plankton feeders. In particular, toxin accumulation in bivalves causes food poisoning in humans, and often leads to severe economic damage to the shellfish industry.

Diarrhetic shellfish poisoning (DSP) is a gastrointestinal syndrome caused by phytoplankton toxins, including okadaic acid, and several analogues of dinophysistoxin (Yasumoto et al., 1985). These toxins are derived from several species of dinoflagellates belonging to the genus *Dinophysis* (Yasumoto et al, 1980; Lee et al., 1989). Despite extensive studies in the last 2 decades, little is known about the ecophysiology and blooming mechanisms of *Dinophysis* species because they are difficult to grow in culture.

Dinophysis species are divided into 2 groups, photosynthetic and nonphotosynthetic (heterotrophic) species, which are determined by the presence or absence of plastids, respectively (Lessard and Swift, 1986). The majority of the DSP-inducing species belong to the former group. Even in the photosynthetic species, food vacuoles are occasionally seen in the cells (Jacobson and Andersen, 1994; Koike et al., 2000), and heterotrophy is one mode of nutrition. Because plastid density in *Dinophysis* cells increases prior to blooming, photosynthesis is thought to be essential for the blooming process (Koike, 2002). Thus, observation of the plastid density and understanding of the environmental conditions that cause increases in plastid density are necessary to predict blooming and subsequent outbreaks of DSP.

The plastid of *Dinophysis* is unique in dinoflagellates. It contains phycobilin-proteins as accessory pigments (Lessard and Swift, 1986; Hallegraeff and

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Lucas, 1988; Schnepf and Elbrächter, 1988; Geider and Gunter 1989; Vesk et al., 1996; Hewes et al., 1998) and a double thylakoid system with an electron-dense lumen (Schnepf and Elbrächter, 1988). Because these are characteristics of cryptophyte plastids, not of dinoflagellates, *Dinophysis* plastids are thought to be obtained through endosymbiosis with a cryptophyte. In addition, the plastid is considered a permanent organelle because there are no other remnants of a cryptophyte within the *Dinophysis* cell other than the plastids (Lucas and Vesk, 1990; Schnepf and Elbrächter, 1999).

We previously reported that 3 species of photosynthetic *Dinophysis* share a type of plastid containing identical plastid-encoded small subunit ribosomal DNA (pSSU rDNA) sequences, whereas their nuclear-encoded SSU rDNA sequences have species-specific base substitutions (Takishita et al., 2002). In general, the sequences from the fully established dinoflagellate plastids (containing–peridinin and fucoxanthin derivatives) have diverged substantially from the nuclear genes (Zhang et al., 1999, 2000; Barbrook and Howe, 2000; Tengs et al., 2000). We therefore suspect that the *Dinophysis* plastid is derived from the temporary acquisition of cryptophytes from the environment. This idea is supported by previous observations that the pigment concentrations and plastid morphologies of *Dinophysis* are extremely variable (Fukuyo, 1997; Koike, 2002) and that *Dinophysis fortii* can take up cryptophyte cells and maintain their plastids (Ishimaru et al., 1988). Hence, cryptophytes with a plastid identical to that of *Dinophysis* should be crucial for plastid acquisition and blooming.

In this study we developed suitable genetic probes for pSSU rRNA and *rbcL* (encoding the large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase) messenger RNA in photosynthetic *Dinophysis* plastids. We describe the ability of the probes to bind to various cryptophytes and *Dinophysis* cells. We also describe the results of a trial for detecting environmentally occurring cryptophyte cells that are possible sources of *Dinophysis* plastids. These probes, along with fluorescent in situ hybridization (FISH), should be useful for (1) microscopic counting of DSP-inducing *Dinophysis*, (2) estimation of plastid density and photosynthetic activity, and (3) detection and enumeration of cryptophyte cells that could be the source of *Dinophysis* plastids.

Materials and Methods

Plastid-Encoded SSU rDNA and* rbcL *Gene Sequencing from* Dinophysis *and Cryptophyte Plastids. All of the DNA sequences used in this study and their GenBank accession numbers are listed in Table 1. Sequences of pSSU rDNA for 3 *Dinophysis* species (*D. fortii* Pavillard, *D. acuminata* Claparède and Lachmann, and *D. norvegica* Claparède and Lachmann) have been reported previously (Takishita et al., 2002). In addition, the *rbcL* gene from *D. tripos* Gourret and *D. fortii* and pSSU rDNA from *D. tripos* were sequenced for the first time in this study. The *D. fortii* and *D. tripos* cells were collected at Okkirai Bay, Iwate, Japan, on May 14 and 21, 2002, respectively. Two cryptophyte isolates collected from Tokyo Bay on May 15, 2003, tentatively identified as *Plagioselmis* sp. and *Teleaulax* sp. (University of Tsukuba culture collections) on the basis of their nuclear SSU rDNA sequences, were used for pSSU rDNA and *rbcL* sequencing. Also, the *rbcL* gene sequences from *Geminigera cryophila* Hill (Marine Biotechnology Institute culture collection; MBIC10567) and *Proteomonas sulcata* Hill and Wetherbee (Provasoli-Guillard National Center for Culture of Marine Phytoplankton; CCMP 765) were determined.

DNA extraction, polymerase chain reaction (PCR) amplification of pSSU rDNA, cloning, and sequencing were performed according to Takishita et

Table 1. GenBank Accession Numbers Used in This Study

Gene and species name	*Accession number*
Plastid SSU rDNA	
Chilomonas paramecium	AB073108
Chroomonas placoidea	AB073110
Cryptomonas ovata	AB073109
Dinophysis acuminata	AB073114
D. fortii	AB073115
D. norvegica	AB073116
D. tripos	AB164405
Geminigera cryophila	AB073111
Guillardia theta	AF041468
Hemiselmis virescens	AB073112
Palmaria palmata	Z18289
Plagioselmis sp. (TUC-1)	AB164406
Porphyra purpurea	U38804
Proteomonas sulcata	AB073113
Pyrenomonas salina	X55015
Teleaulax sp. (TUC-2)	AB164407
rbcL	
Chilomonas paramecium	AY119780
Chroomonas sp. (SAG 980-1)	AY119781
Dinophysis fortii	AB164412
D. tripos	AB164413
Geminigera cryophila	AB164411
Guillardia theta	AF041468
Palmaria palmata	U28421
Plagioselmis sp. (TUC-1)	AB164409
Proteomonas sulcata	AB164410
Pyrenomonas helgolandii	AY119782
Teleaulax sp. (TUC-2)	AB164408

Table 2. Probes for Dinophysis Plastid SSU (pSSU) rRNA and *rbcL* mRNA and Their Sequences

Probe name	*Target*	*Sequence*
D16P-1	*Dinophysis* spp., pSSU rRNA	5′-CCCTTTCAGGAAGATTTGTGAC-3′
DrbcL-1	*Dinophysis* spp., plastid *rbcL* mRNA	5′-GAAGTATTGGTCTTGTGCAC-3′
G16P-1[a]	*Geminigera cryophila*, pSSU rRNA	5′-TTCTTTCAAAAAGATTTGTGAC-3′

[a]A probe for *Geminigera cryophila* pSSU rRNA used for optimizing hybridization and as a negative control.

al. (2002). The *rbcL* gene was PCR-amplified with the following set of primers: GMRUBISCO1 and GMRUBISCO2 (Takishita et al., 2000).

Phylogenetic Analysis. The pSSU rDNA sequences from 4 species of *Dinophysis* (*D. acuminata*, *D. fortii*, *D. norvegica*, and *D. tripos*) were aligned with those from 10 species of cryptophytes and 2 species of rhodophytes (Table 1) by CLUSTAL W Version 1.8 (Thompson et al., 1994). Also using CLUSTAL W, we aligned the *rbcL* gene sequences from 2 species of *Dinophysis* (*D. fortii* and *D. tripos*), 8 cryptophytes, and one rhodophyte (Table 1). The pSSU rDNA sequences of *D. acuminata*, *D. fortii*, *D. norvegica*, *G. cryophila*, *Chilomonas paramecium* Ehrenberg, *Cryptomonas ovata* Ehrenberg, *Guillardia theta* Hill and Wetherbee, *P. sulcata*, *Hemiselmis virescens* Droop, *Chroomonas placoides* Butcher, *Pyrenomonas salina* (Wislouch) Santore, *Porphyra purpurea* (Roth) C. Agardh, and *Palamaria palmata* (Linnaeus) Kuntze, and the *rbcL* sequences of *G. theta*, *P. salina*, *Chroomonas* sp., *C. paramecium*, and *P. purpurea* were obtained from the DNA Data Bank of Japan (DDBJ). The generated alignments were visually inspected and manually edited. All ambiguous sites of the alignments were removed. The alignment data for pSSU rDNA and *rbcL* are available on request from the corresponding author.

The data sets of pSSU rDNA (16 taxa/1227 sites) and *rbcL* (11 taxa/996 sites) were tested for their optimal fit to various models of nucleotide evolution using MODELTEST Version 3.06 (Posada and Crandall, 1998). The proportion of invariable sites, a discrete γ distribution (4 categories), and base frequencies were estimated from the data set. Each maximum-likelihood (ML) tree was constructed under an optimal model. The data sets of pSSU DNA and *rbcL* were also subjected to analyses by the neighbor-joining (NJ) (Saitou and Nei, 1987) and maximum parsimony (MP) methods. The NJ tree was constructed using Kimura's 2-parameter model (Kimura, 1980). Support for NJ branches was tested by bootstrap analysis of 1000 replicates. The MP tree was based on the tree-bisection-reconnection (TBR) branch-swapping algorithm with stepwise addition (the closest option) of taxa under the heuristic search method (50% confidence level). We conducted bootstrap analysis of 1000 replicates using the heuristic search method (50% confidence level) to assess the confidence of the branches in the MP tree.

For all phylogenetic analyses in this study, PAUP* Version 4.0 was used.

Genetic Probe Design. Probes for *Dinophysis* pSSU rRNA and *rbcL* mRNA were designed according to their specific regions of the sequences (Table 2). In addition to *Dinophysis* plastid-specific probes, we designed probes for *Geminigera cryophila* plastid (SSU rRNA and *rbcL* mRNA) to optimize the hybridization conditions. These oligonucleotides were synthesized with fluorescein isothiocyanate (FITC) conjugated to their 5′ ends (Espec Oligo).

Fluorescent In Situ Hybridization. *Dinophysis* cells and cryptophyte cultures used for FISH are listed in Table 3. Fixation and hybridization were performed essentially as described by Miller and Scholin (2000). Capillary-isolated *Dinophysis* cells in 20 μl of seawater or 500 μl of cryptophyte cultures

Table 3. Dinophysis Cells (with collection sites and dates) or Cryptophyte Cultures (with strain names and culture sources) Used for FISH

Species name	*Source (collection site and date, or strain name)*
Dinophysis acuminata	Isolated from Kesennuma Bay, August 8, 2002
D. fortii	Isolated from Okkirai Bay, May 21, 2002
D. norvegica	Isolated from Okkirai Bay, May 6, 2003
D. tripos	Isolated from Okkirai Bay, July 31, 2002
Geminigera cryophila	MBIC 10567 (Marine Biotechnology Institute Culture Collection)
Guillardia theta	CCMP 327 (Provasoli-Guillard National Center for Culture of Marine Phytoplankton)
Proteomonas sulcata	CCMP 327 (Provasoli-Guillard National Center for Culture of Marine Phytoplankton)
Plagioselmis sp	TUC-1 (University of Tsukuba Culture Collection)
Teleaulax sp.	TUC-2 (University of Tsukuba Culture Collection)

in mid-logarithmic growth phase were fixed in 9 volumes of the standard saline ethanol fixative (a mixture of 25 ml 90% ethanol, 2 ml H_2O, and 3 ml 25× SET buffer [3.75 M NaCl, 25 mM EDTA, 0.5 M Tris-HCl, pH 7.8]), after which the cells were stored at −20°C. To eliminate auto fluorescence from phycoerythrin pigment, which can overlap with FITC emission, fixed cells in a polystyrene tube were irradiated under a UV lamp (GL-15, Toshiba) for 1 hour. The UV-A intensity applied to the sample was less than 30 $\mu W/cm^2$. Next, the cells were vacuum-filtered and trapped onto a 13-mm ø polycarbonate filter with a 0.8-μm pore size (K080A013A, Advantec). The filter was incubated for 30 minutes at room temperature in hybridization buffer (0.1% IGEPAL CA-630 [Sigma] and 25 μg/ml Poly-A [Sigma] in 5× SET). After removal of the buffer by vacuum filtration, the filter was incubated for 3 hours at 45°C in hybridization buffer containing 500 ng/ml of the FITC-labeled probe. After being washed 2 times with 5× SET, the filter was mounted onto a nonfluorescent slide-glass, immersed in nonfluorescent immersion oil (Olympus), and observed by fluorescence microscopy (BH2-RFC, Olympus). FITC fluorescence emitted from the bound probe was observed under narrow-blue light excitation (455–490 nm) with a band-pass emission guard filter (520–530 nm). The fluorescent micrographs were taken using a cooled digital camera (Penguin 600CL, Pixera)

FISH Trial of Field-Collected Plankton. On June 19 and July 17, 2002, water samples were collected at depths of 10 and 20 m from a permanent station in Okkirai Bay, Iwate, Japan (see Koike et al., 2001) using a Van Dorn bottle. The water samples were immediately sieved through a nylon net with a 20-μm mesh size. A 5-ml portion of the filtrates was fixed with 45 ml of fixative and then stored at −20°C. Of this fixed sample, 25 ml was subjected to FISH using the D16P-1 probe. In addition, 4′,6-diamidino-2-phenylindole (DAPI; 0.5 μg/ml in 5× SET) was added to the filter after the final wash of the FISH procedure, then washed 2 times with 5× SET, and observed by fluorescence microscopy. Probe-hybridized particles on the filter were confirmed to be eukaryotes by the presence of a DAPI-labeled nucleus.

To determine the probe specificity, FISH using the D16P-1 probe was also conducted on *Dinophysis* cells within heterogeneous plankton. A plankton sample collected from the bay using a net (larger than 20 μm) was fixed and processed for FISH and DAPI staining. In this case the sample was centrifuged (1500 *g*, 10 minutes) at each of the washing steps and mounted on a slide glass for observation.

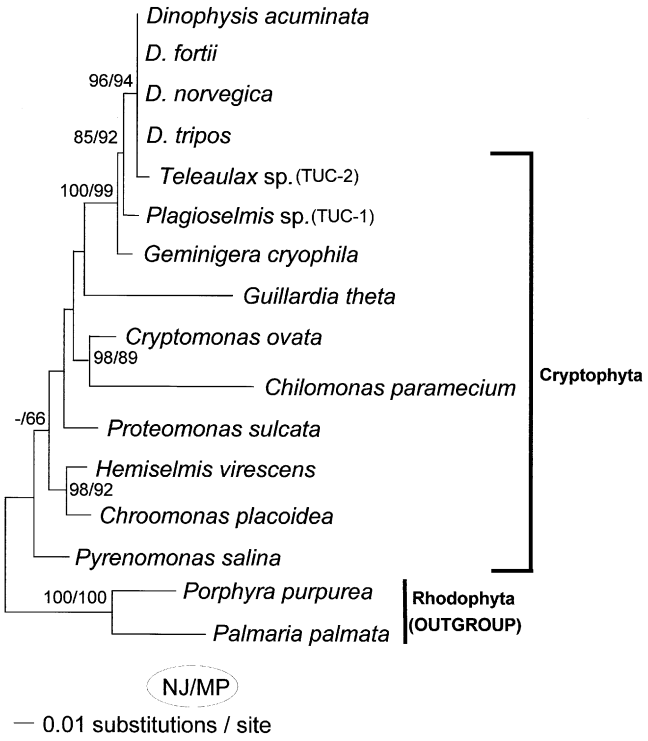

Fig. 1. ML phylogeny of pSSU rDNA from *Dinophysis* spp. and cryptophytes. The rhodophytes *Porphyra purpurea* and *Palmaria palmata* were used to root the tree. Numbers at the nodes refer to the percentage (50% or more) of bootstrap support in NJ and MP analyses.

Results

The obtained sequences of pSSU rDNA from *D. tripos* and 2 cryptophytes (*Plagioselmis* sp. and *Teleaulax* sp.), as well as of *rdcL* from *D. fortii*, *D. tripos*, and 4 cryptophytes (*Plagioselmis* sp., *Teleaulax* sp., *G. cryophila*, and *P. sulcata*), were deposited in GenBank with the accession numbers listed in Table 1. The sequence of pSSU rDNA of *D. tripos* was identical to those of 3 previously reported *Dinophysis* (*D. fortii*, *D. acuminata*, and *D. norvegica*) (Takishita et al., 2002). The sequences of the *rbcL* gene were also identical in *D. fortii* and *D. tripos*.

Phylogenic trees based on pSSU rDNA and *rbcL* sequences are shown in Figures 1 and 2, respectively. In both trees *Dinophysis* spp. were positioned within the lineage comprising *Plagioselmis* sp., *Teleaulax* sp., and *G. cryophyra* with high bootstrap support (98%–100%). Specifically, *Dinophysis* spp. were closely related to *Teleaulax* sp. (94%–100% bootstrap supports). Alignments of partial pSSU rDNA and *rbcL* sequences from *Dinophysis* spp., *Teleaulax* sp., *Plagioselmis* sp., *G. cryophila*, *G. theta*, and *P. sulcata* are shown in Figure 3. The boxed regions in Figure 3 indicate the probe recognition sites. Compared with the sequences of D16P-1 (22 bp) and

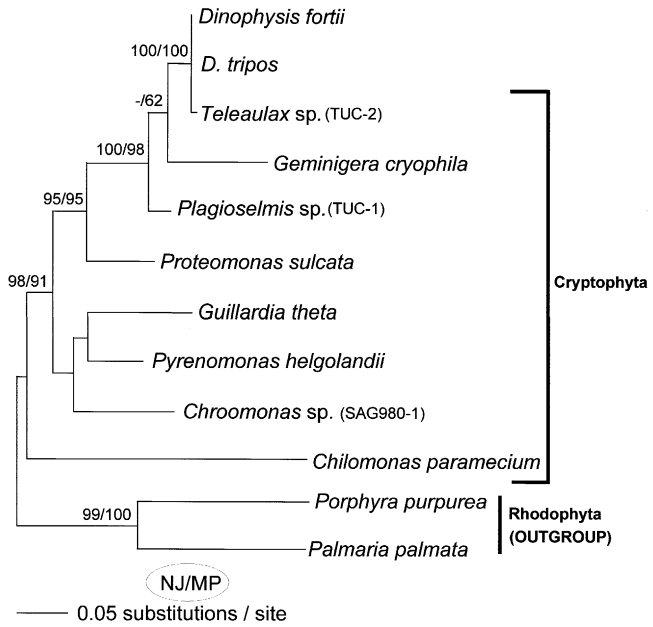

Fig. 2. ML phylogeny of *rbcL* from *Dinophysis* spp. and cryptophytes. The rhodophytes *Porphyra purpurea* and *Palmaria palmata* were used to root the tree. Numbers at the nodes refer to the percentage (50% or more) of bootstrap support in NJ and MP analyses.

DrbcL-1 (20 bp), there were 2 to 5 and 5 to 7 substitutions, respectively, within the corresponding regions of cryptophytes other than *Teleaulax* sp.

The designed FITC-labeled genetic probes (Table 2; D16P-1 for pSSU rRNA and DrbcL-1 for *rbcL* mRNA) were used for FISH with 4 species of *Dinophysis* and 5 species of cryptophytes. FISH and observation protocols were optimized using the *G. cryophila* versus G16P-1 probe (Table 2), which was designed to specifically recognize the pSSU rRNA. This probe was also used as a negative control for *Dinophysis*. Fluorescent micrographs of *Dinophysis* spp. and cryptophytes treated with these 3 genetic probes are shown in Figure 4. Probes D16P-1 and DrbcL-1 specifically hybridized only to *Dinophysis* plastids, and G16P-1 hybridized only to *G. cryophila* plastids. Positive FITC signals of both the D16P-1 and DrbcL-1 probes were not observed for *Teleaulax* sp., which has corresponding gene sequences identical to those of *Dinophysis*. UV irradiation to reduce autofluorescence from phycobilin did not affect the fluorescent signal when using the *G. cryophila* versus G16P-1 probe or the *Dinophysis* spp. versus D16P-1 or DrbcL-1 probes. Thus the negative reaction of the probes to *Teleaulax* sp. may be due to the poor physiologic state (e.g., low ribosome density and low mRNA level) of this slow-growing culture.

FISH trials with field-collected samples revealed the occurrence of D16P-1 probe-hybridized nanoplankters. These plankters were eukaryotic microalgae with nuclear fluorescence due to DAPI staining, and appeared to be cryptophytes by their cell shapes and sizes (Figure 5 a, b). They could be differentiated from other DAPI-stained prokaryotic or eukaryotic nanoplankters by their obvious FITC fluorescence (Figure 5, b). Also, *Dinophysis* cells in the heterogeneous sample were clearly discerniable from other plankters by probe binding (Figure 5, c, d).

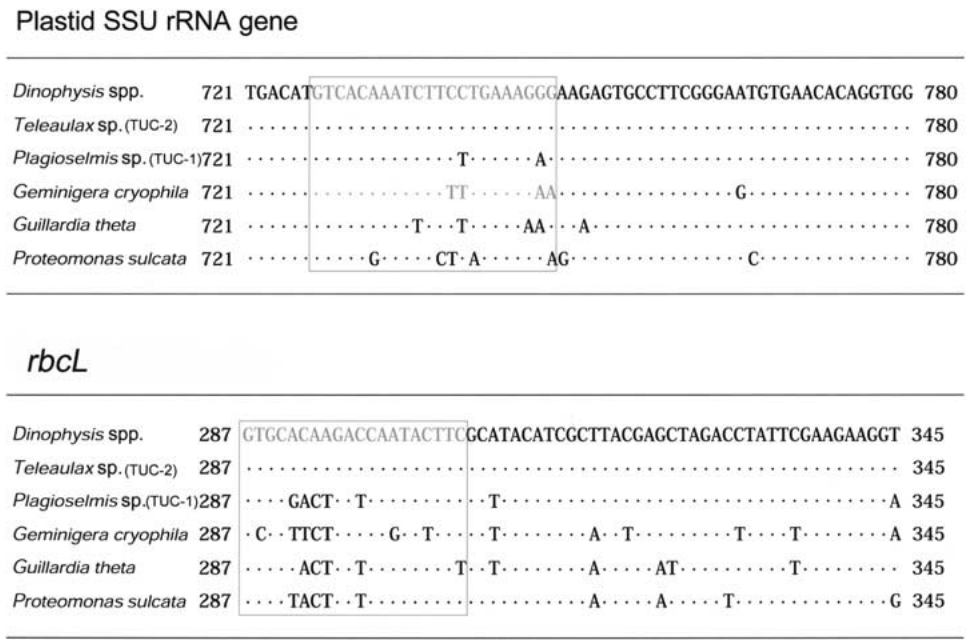

Fig. 3. Aligned nucleotide sequences of partial pSSU rDNA and *rbcL* for *Dinophysis* spp., *Teleaulax* sp., *Plagioselmis* sp., *Geminigera cryophila*, *Guillardia theta*, and *Proteomonas sulcata*. Boxed regions indicate the probe recognition sites of D16P-1 and DrbcL-1 (see Table 2).

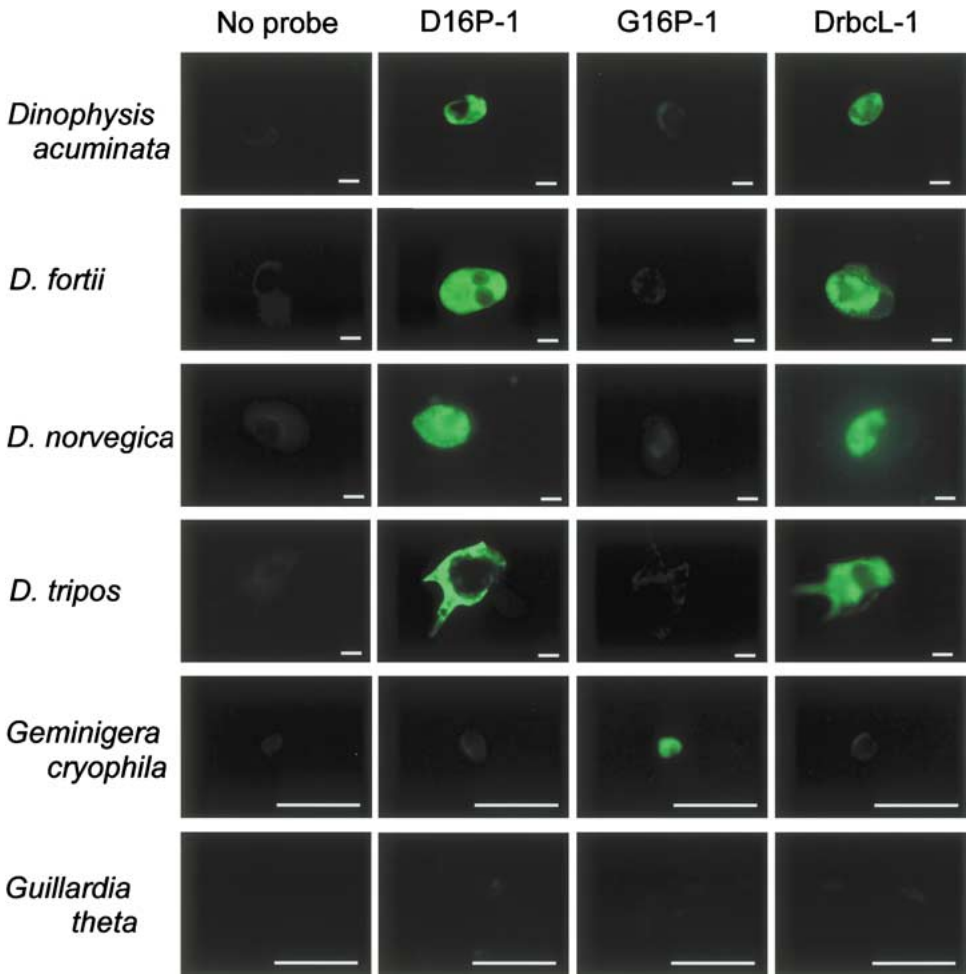

Fig. 4. Fluorescent micrographs from FISH of *Dinophysis* spp. and cryptophytes. The probe names are shown at the top. Results for *Teleaulax* sp., *Plagioselmis* sp., and *Proteomonas sulcata* were the same as those for *Guillardia theta*, and are therefore omitted. Bars = 20 μm.

Discussion

We have previously demonstrated that, on the basis of the phylogeny of pSSU rDNA, the plastids of *Dinophysis* have phylogenetic affinity with those of the cryptophyte *G. cryophyra* (Takishita et al., 2002). Recently, phylogenetic analyses of nuclear and nucleomorph SSU rDNA from cryptophytes have revealed that *G. cryophyra* constitutes a robust monophyletic group with 2 genera, *Teleaulax* and *Plagioselmis* (Deane et al., 2002; Hoef-Emden et al., 2002). Therefore, we investigated the evolutionary relationship among the plastids in *Dinophysis*, *Geminigera*, *Teleaulax*, and *Plagioselmis* by using phylogenetic analyses of pSSU rDNA and *rbcL*. We found that plastids of *Dinophysis* are more closely related to those of *Teleaulax* than those of *Geminigera* and *Plagioselmis*.

Although phototrophic *Dinophysis* species are now recognized as mixotrophic, it appears that photosynthesis contributes a great deal to their active growth because they contain numerous plastids and few food vacuoles during the blooming period (Koike, 2002). Thus monitoring plastid density and photosynthetic activity should allow prediction of *Dinophysis* blooming and subsequent outbreak of DSP. For this reason, in the present study we developed molecular probes that specifically recognize pSSU rRNA and *rbcL* mRNA of the *Dinophysis* plastid. Using in situ hybridization (Miller and Scholin, 2000), we specifically identified plastids of toxic *Dinophysis*. Furthermore, the intensities of the hybridization signal for pSSU rDNA and *rbcL* can be used to estimate their transcriptional and photosynthetic activities, respectively.

These probes should help to answer the question of whether the *Dinophysis* plastid is actually a genuine organelle of *Dinophysis* or one that is temporarily acquired from the environment. Despite the high evolutionary rate of sequences from fully established dinoflagellate plastids (Zhang et al., 1999, 2000; Barbrook and Howe, 2000; Tengs et al., 2000), the current results and our previous study (Takishita et al., 2002) show that 4 species of photosynthetic *Dinophysis* share a plastid with identical pSSU rDNA sequences. Moreover, the pSSU rDNA

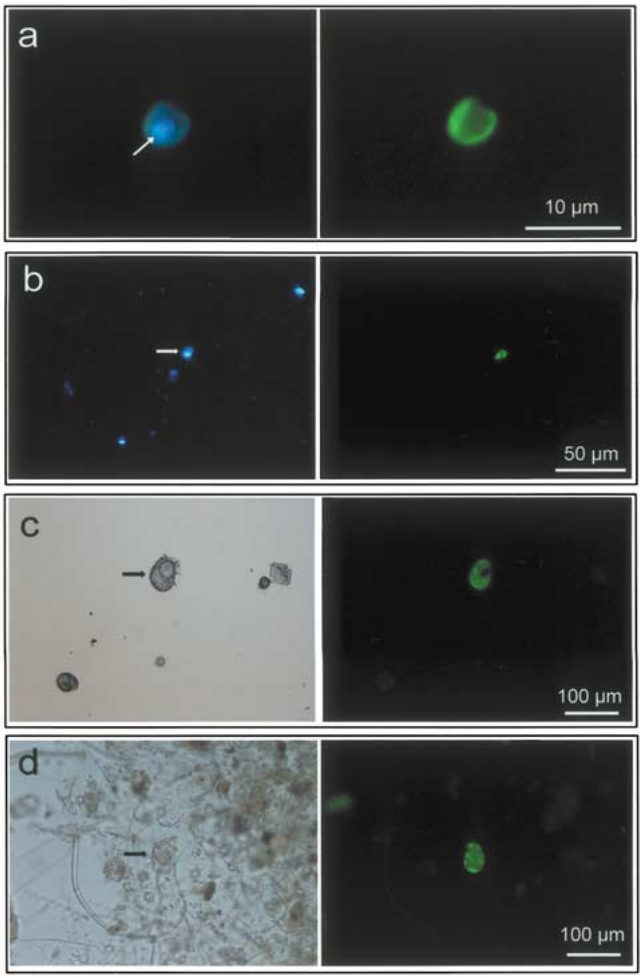

Fig. 5. Fluorescent micrographs of FISH (D16P-1) for field-collected plankton. The left column shows DAPI fluorescence under UV excitation (a, b) or transmitted light micrographs (c, d), and the right shows probe hybridized cells, indicated by FITC fluorescence. **a**: Probe-hybridized eukaryotic cells that appear to be cryptophytes. DAPI fluorescence shows the presence of a nucleus (arrow), and the probe binds to the plastidal area. **b**: Probe-hybridized cryptophytes can be distinguished from other DAPI-stained plankters. **c**: The probe specifically recognizes *Dinophysis fortii* cells (black arrows) from other dinoflagellate or protist cells. **d**: Recognition occurs even in the heterogeneous samples.

sequence was 99.8% identical with that of an environmental clone (OCS20) derived from ultra-sized (less than 10 μm in diameter) microalgae collected in the Pacific Ocean (Rappè et al., 1998). Because all known photosynthetic species of *Dinophysis* are more than 30 μm in length, it is unlikely that the OCS20 clone is from a *Dinophysis* species. Rather, OCS20 is more likely to be from a cryptophyte, many of which are less than 10 μm in size. This cryptophyte appears to be a likely source for *Dinophysis* plastids.

These molecular data support the hypothesis that the *Dinophysis* plastids are derived from temporarily acquired cryptophytes by a process termed *kleptoplastidy* (Larsen, 1992; Laval-Peuto, 1992; Schnepf and Elbrächter, 1992). Nonetheless, there have been many opposing opinions based on the differences in plastid morphologies. The possibility of kleptoplastidy is supported by other recent genetic information on the *Dinophysis* plastids. Hackett et al. (2003) reported that pSSU rDNA sequences from *Dinophysis* collected at different times of the year and from different locations were monophyletic but also showed significant variation. They claimed that these data support the contention that *Dinophysis* plastids are permanent. However, as they also observed, we cannot exclude the possibility that the polymorphism of pSSU rDNA indicates prey cryptophyte diversity.

Using FISH with the molecular probes developed here, we were able to distinguish plastids from *Dinophysis* and closely related cryptophytes. This technique should help clarify the biology of *Dinophysis* and the mechanism of DSP. In addition, we found probe-hybridized cryptophyte cells in field-collected plankters, which are likely the source of the *Dinophysis* plastid. To obtain more direct evidence of kleptoplastidy, investigations into the occurrence of source cryptophyte cells and the simultaneous increase of *Dinophysis* plastid density are needed. Ultimately, the isolation and establishment of the source cryptophyte culture and a feeding experiment for *Dinophysis* should be performed. Our probes should facilitate these explorations.

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