Harmful Algae 13 (2012) 105–111

Contents lists available at SciVerse ScienceDirect

Harmful Algae

journal homepage: www.elsevier.com/locate/hal

The marine dinoflagellate genus *Dinophysis* can retain plastids of multiple algal origins at the same time

Miran Kim [a,1], Sunju Kim [b,1], Wonho Yih [c], Myung Gil Park [a,*]

[a] LOHABE, Department of Oceanography, Chonnam National University, Gwangju 500-757, Republic of Korea
[b] Department of Biology, Kongju National University, Kongju 314-701, Republic of Korea
[c] Department of Oceanography, Kunsan National University, Gunsan 573-701, Republic of Korea

ARTICLE INFO

Article history:
Received 16 June 2011
Accepted 12 October 2011
Available online 22 October 2011

Keywords:
Dinophysis
Heterosigma akashiwo
Mesodinium rubrum
Plastid
PCR
psbA
Pyramimonas
RFLP

ABSTRACT

The 'phototrophic' *Dinophysis* Ehrenberg species are well known to have plastids of a cryptophyte origin, more specifically cryptophyte genus complex *Teleaulax*/*Geminigera*. However, how often the phototrophic *Dinophysis* could retain both types of plastids from this genus complex at the same time in a cell and also whether the phototrophic cells could retain plastids of the other algal origins rather than cryptophyte have not been investigated in detail. We isolated a total of 67 phototrophic *Dinophysis* spp. cells between May 2008 and September 2009 along western and southern coasts of Korea and amplified *psb*A as a tracer to investigate plastid diversity from the isolated cells. Then, the PCR products were digested with a restriction enzyme, SfaNI, to distinguish between the most common *Teleaulax amphioxeia*-type and the less common *T. acuta*-type plastids. During this study, we sometimes encountered 'green' *Dinophysis acuminata* cells, which contained varying degree of red autofluorescencing green plastids inside the cell, along together typical orange autofluorescencing reddish-brown plastids. The RFLP patterns of the PCR products digested by SfaNI revealed that a total of 66 *Dinophysis* cells analyzed in this study all contained *T. amphioxeia*-type plastid. Further, approximately two-thirds of the analyzed *Dinophysis* cells contained both *T. amphioxeia*-type and *T. acuta*-type plastids at the same time in a single cell. Interestingly, SfaNI digestion of the products amplified on *psb*A gene from 10 *Dinophysis* cells produced a different RFLP pattern: in addition to *T. amphioxeia*-type and sometimes *T. acuta*-type plastid, undigested fragments occurred. We cloned the PCR products and determined *psb*A gene sequences from the cells containing undigested fragments with SfaNI. Surprisingly, these *Dinophysis* cells contained even three (i.e. cryptophytes *T. amphioxeia* and *T. acuta* and raphidophyte *Heterosigma akashiwo*) or four (i.e. cryptophytes *T. amphioxeia* and *T. acuta*, raphidophyte *H. akashiwo*, and chlorophyte *Pyramimonas* sp.) different plastid sequences at the same time in a single cell. Our result indicates that besides the sole prey *Mesodinium rubrum* (=*Myrionecta rubra*) known until now, there may be other potential prey organisms, presumably the plastid-retaining ciliates, from which phototrophic *Dinophysis* may acquire plastids of several algal origins other than cryptophyte.

1. Introduction

The marine dinoflagellate genus *Dinophysis* Ehrenberg is an important harmful alga that causes human illness (diarrhetic shellfish poisoning). This genus includes both phototrophic and heterotrophic species (Hallegraeff and Lucas, 1988). The 'phototrophic' *Dinophysis* species are well known to have plastids of a cryptophyte origin, based on ultrastructural and pigment analyses (Schnepf and Elbrächter, 1988; Lucas and Vesk, 1990; Hewes et al., 1998; Takishita et al., 2002), and later, more specifically of the cryptophyte *Teleaulax amphioxeia* origin, based on the sequencing of several plastid genes (Hackett et al., 2003; Janson and Granéli, 2003; Janson, 2004). Interestingly, subsequent molecular studies carried out with field samples and culture strains found that in addition to the plastids of *T. amphioxeia* origin, *Dinophysis* could also retain the other cryptophyte types of plastids, more specifically, of *Geminigera cryophila* origin (Minnhagen and Janson, 2006; Nishitani et al., 2010; Park et al., 2010). Given that the ciliate prey *Mesodinium rubrum*, which is a sole prey so far known (Park et al., 2006), can ingest a variety of cryptophytes (Park et al., 2007; Nishitani et al., 2010; Myung et al., 2011), it is now not surprising that natural *Dinophysis* cells could, although temporarily, frequently retain the plastids originated from the cryptophyte genus

* Corresponding author. Tel.: +82 62 5303468; fax: +82 62 5303469.
E-mail address: mpark@chonnam.ac.kr (M.G. Park).
[1] These authors contributed equally to this work.

1568-9883/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.hal.2011.10.010

complex *Teleaulax*/*Geminigera*. However, one exception to these findings can be seen from the result by Hackett et al. (2003), who found that *Dinophysis* spp. have polymorphic plastids sequences of cryptophyte origin as well as of florideophyte origin, but they thought that it may result from the presence of florideophyte DNA within the food vacuoles of *Dinophysis* feeding on red algae. Nonetheless, previous studies raise a question as to whether *Dinophysis* really feeds on only *M. rubrum* as prey which has already fed cryptophyte genus complex *Teleaulax*/*Geminigera* and thus retain only their plastids. To investigate the plastid diversity of 'photosynthetic' *Dinophysis* would be helpful to provide the insights for exploring potential prey organisms, either the purpose of feeding is to acquire plastids or to utilize as only food source.

The goal of this study was to investigate how often *Dinophysis* species in the wild samples have the plastids of several algal lineages. To do this, we isolated a total of 67 phototrophic *Dinophysis* spp. cells from western and southern coasts of Korea and amplified *psb*A gene as a molecular marker from individually isolated cells and then analyzed its restriction fragment length polymorphism (RFLP) patterns. Our result suggests that phototrophic *Dinophysis* could at times temporarily retain plastids of diverse algal origins at the same time in the field.

2. Materials and methods

2.1. Sampling and single-cell isolation

Seawater samples for microscopic observations and single-cell PCR were collected using a 20 μm plankton net from 8 sites along the Korean coast (Fig. 1) between 2008 and 2009. A total of 67 *Dinophysis* cells (1–11 cells per sampling site) were isolated from the concentrated plankton samples using drawn Pasteur glass pipettes under an inverted microscope (Olympus model IX51, Tokyo, Japan) or a stereomicroscope (Olympus model SZX7, Tokyo, Japan). The isolated single cells were used for either microscopic observations or as templates for single-cell PCR to determine psbA gene sequence (see below).

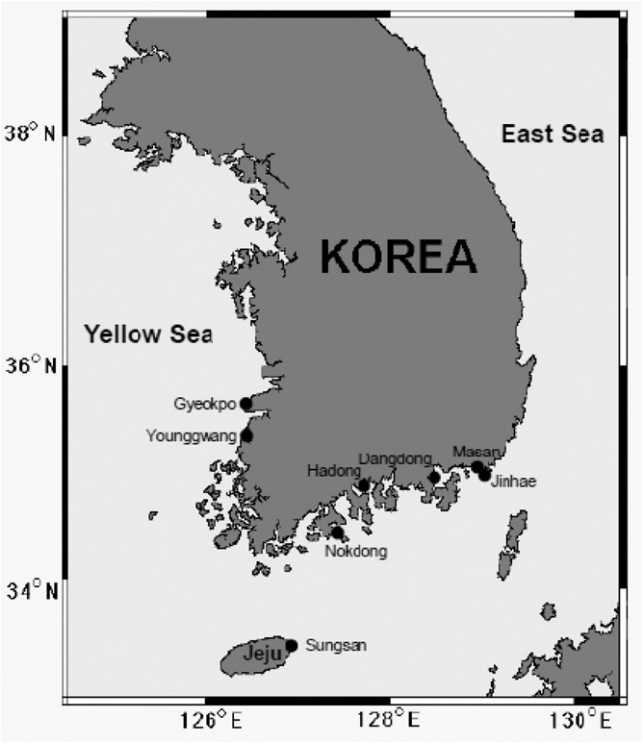

Fig. 1. Sampling sites along the Korean coasts. Closed circles indicate sites where samples for single-cell PCR and microscopic observations were collected.

2.2. Microscopy

Bright-field and epifluorescence micrographs of live *Dinophysis* cells were taken at 1000× magnification using a digital camera (PowerShot G5, Canon, Tokyo, Japan) coupled to the Olympus BX51 microscope (Olympus, Tokyo, Japan) equipped with differential interference contrast and fluorescence cube (U-MWB2, 450–480 nm excitation, 500 nm emission).

2.3. DNA extraction, PCR amplification and restriction fragment length polymorphism (RFLP) analysis

Individual *Dinophysis* cells isolated from each sampling site were put into 10 μL of Tris–EDTA (TE) buffer (pH 8.0) (Bioneer, Daejeon, Republic of Korea). The cells were then boiled at 95 °C for 10 min to lyse the cell. One microliter of each boiled specimen was used as a template to amplify the *psbA*. The *psbA* gene was amplified in the first PCR round using the primer set, pbAf3 (5′-ATCTTCGCTCCACCAGTTGAYATHGAYGG-3′) and pbAr1 (5′-GTTGTGAGCGTTACGTTCRTGCATNACYTC-3′) (Zhang et al., 2000). The PCR reactions were performed using MyGenie™ 96 Gradient Thermal Block (Bioneer, Daejeon, Republic of Korea). The PCR was run as follows: 5 min at 94 °C, followed by 35 cycles of 30 s at 94 °C, 30 s at 55 °C, 2 min at 72 °C, and a final incubation for 10 min at 72 °C. The size of the PCR products from amplified *psbA* gene fragments of each individual cell was 858 bp when analyzed by electrophoresis in a 1% agarose gel stained with ethidium bromide. When amplifying small amount of DNA from single cells, it is likely that rare template may not be detected. Thus, we ran nested PCR reactions to avoid this. In nested PCR reactions, 3 μL of the product from the first PCR round was used as a template in the second round reaction. The second pair of primers was designed by shifting 5 bases toward the 3′ direction in the first primers binding loci: pbAf3-2nd (5′-CGCTCCACCAGTTGAYATHGAYGGTATCCG-3′) and pbAr1-2nd (5′-GAGCGTTACGTTCRTGCATNACYTCCATACCT-3′). The second PCR round was run as above, except that 20 instead of 35 cycles of 30 s at 94 °C, 30 s at 55 °C, and 2 min at 72 °C were used. Restriction fragment length polymorphism (RFLP) analysis of the PCR products obtained from the second round was done by restriction digestion with a SfaNI restriction enzyme (New England Biolabs, MA, USA) for 4 h at 37 °C. The digestion of the PCR products with a restriction enzyme, SfaNI, produces distinct RFLP patterns between the cryptophytes *T. amphioxeia* (two major fragments of 712 and 112 bp and one indiscernible fragment of 34 bp) and *Teleaulax acuta* (three discernible fragments of 545, 201, and 112 bp) and thus makes it possible to discriminate between the two types of plastids (Park et al., 2010). The digested products were then analyzed by electrophoresis in 1% agarose gels and visualized by ethidium bromide staining and UV transillumination.

2.4. DNA sequencing

The amplified products were purified using a PCR purification kit (Bioneer, Daejeon, Republic of Korea) according to the manufacturer's instructions, and then ligated into the pGEM®-T Easy vector supplied with the pGEM-T Easy Vector System (Promega, Madison, USA) according to the manufacturer's protocols. Plasmid DNA from putative positive colonies was harvested using a PCR purification kit (Bioneer, Daejeon, Republic of Korea). Typically, 10–20 positive clones from each strain were partially sequenced using the T7 promoter sequencing primer (i.e. 5′-AATACGACTCACTATAG-3′) derived from the cloning vector, and subsequently, all partial sequences (approximately 700 bp) were