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Evaluation of nuclear ribosomal RNA and chloroplast gene markers for the DNA taxonomy of centric diatoms

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ABSTRACT

Diatoms are highly diversified microeukaryotes with a taxonomy that has been under perpetual revision, particularly by means of molecular analyses. In this study, we evaluated typical genes to find the best molecular marker for the DNA taxonomy of centric diatoms, including *Chaetoceros* Ehrenberg, *Cyclotella* Kützing ex de Brébisson, *Discostella* Houk & Klee, *Stephanodiscus* Ehrenberg, and *Thalassiosira* Cleve. Our test genes included nuclear ribosomal RNA (e.g. small subunit, 5.8S, and large subunit [LSU]), and chloroplast genes (e.g. ribulose-1, 5-biphoshate carboxylase oxygenase and D1 protein of the photosystem II reaction centre core complex [*psb*A]). Calculated genetic divergence was highest for LSU ribosomal RNA D1-D5 (*p*-distance of 12.3), followed by 5.8S (7.7), ribulose-1, 5-biphoshate carboxylase oxygenase (7.4), small subunit (6.6), and *psb*A (3.7). The phylogenetic trees for individual genes effectively separated taxonomically tested centric diatoms with different phylogenetic resolutions; however, *psb*A was incongruent with others. These taxonomic resolving powers were in agreement with genetic divergences. Parsimony analysis showed that LSU evolved 1.97 times more rapidly than *psb*A and 1.07 times more rapidly than 5.8S. These results suggest that all of the tested genes except *psb*A can be used as taxonomic markers for centric diatoms and that LSU is the best molecular marker.

1. Introduction

Diatoms are the most speciose unicellular heterokont algae. They are ecologically important and contribute about 20% of the global primary production (Falkowski and Raven, 1997). They are a key to global carbon and silica cycling (Tréguer et al., 1995; Mann, 1999) because they perform biogenesis of their siliceous cell walls called frustules (Scala and Bowler, 2001). The siliceous cell wall is the main informative characteristic used to identify and classify diatoms (Round et al., 1990) possibly because it has discriminative potential, which is exceptional among other unicellular eukaryotes. Classical systematics based on the morphological characteristics of frustules has placed species into 1 of 2 groups (centric [radial and non-radial] and pennate [araphid or raphid]) (Simonsen, 1972, 1979; Round et al., 1990) or 3 classes (Coscinodiscophyceae [centric diatoms], Fragillariophyceae [araphid diatoms], and Bacillariophyceae [raphid diatoms]) (Round et al., 1990). However, morphology alone has by no means proven adequate to discern species boundaries in diatoms. Furthermore, accurate morphological characterization tends to be difficult, mainly owing to small cells with numerous similarities among close relatives and

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various species within the same genus, such as *Cyclotella* sensu lato from fresh waters. Additionally, the heterogeneity of some characters in certain groups is sometimes better observed using light microscopy than scanning electron microscopy—e.g. those with a flat or transversely undulate valve centre (Houk and Klee, 2004).

The phylogeny and taxonomy of diatoms were based almost exclusively on morphological characteristics of the siliceous frustules until the advent of molecular phylogenies. The use of molecular data for diatom phylogeny began with the work of Medlin et al. (1993). Since then, diatomists have used small subunit (SSU) ribosomal RNA (rRNA) from a broad range of diatom species and elucidated basic diatom phylogeny (Medlin et al., 1995, 1996a, 1997a, 1997b; Kooistra and Medlin, 1996) that generally supports traditional morphological taxonomies (Simonsen, 1979; Round et al., 1990; Medlin et al., 1993, 1996a, 1996b, 2000; Sörhannus, 2004, 2007). These studies have identified SSU sequences from representatives of most major diatom lineages. Additional research has attempted to reconstruct diatom phylogeny using the increased SSU data and taxon samplings (Medlin et al., 1993, 1997a, 2000; Medlin and Kaczmarska, 2004; Theriot et al., 2011). SSU, however, has been replaced by other genes, such as large subunit rRNA (LSU) (Sörhannus et al., 1995; Jung et al., 2010; Souffreau et al., 2011) and internal transcribed spacers (ITSs) in ribosomal DNA (rDNA) (Sörhannus et al., 2010), mainly owing to low resolution in SSU phylogeny. For the resolution of diatom phylogeny, several protein coding genes (PCGs)—e.g. ribulose-1, 5-biphoshate carboxylase oxygenase (*rbc*L) (Tamura et al., 2005; Theriot et al., 2010; Souffreau et al., 2011), D1 protein of the photosystem II reaction centre core complex (*psb*A) (Chafee, 2008; Souffreau et al., 2011), photosystem II CP43 chlorophyll apoprotein (Theriot et al., 2010), mitochondrial cytochrome *c* oxidase I, RNA polymerase α subunit (Ehara et al., 2000; Fox and Sörhannus, 2003, 2012; Imanian et al., 2007; Ravin et al., 2010), and nuclear β-tubulin (Sörhannus et al., 2010)—have been studied.

Recent progress in molecular technologies has challenged our understanding of diatom diversity, molecular systematics, and evolution. Molecular approaches using nuclear rRNA and PCGs (e.g. nuclear, chloroplast, and mitochondrial genes) have been applied to reconstruct diatom phylogeny; however, the limited availability of gene sequence data prevents the use of PCGs in molecular comparisons. Therefore, the use of nuclear rRNA molecules is advantageous because significant sequence data is available in public databases such as GenBank. In addition, the coding (e.g. SSU, 5.8S, LSU) and non-coding (e.g. ITS) regions of the rRNA have different degrees of sequence variability (or genetic divergence) and varying suitably for comparison in constructing phylogenetic relationships among taxa of different phylogenetic ranks (e.g. species, genus, family, order, and class).

Molecular genetic divergences are quite variable among PCGs, and these values may significantly affect branch patterns and resolutions of phylogenetic trees (Ki and Han, 2008). To date, rRNA and PCGs have been widely used to reconstruct diatom phylogeny; however, only a few studies have placed emphasis on comparisons of molecular divergences between selective genera of diatoms, especially those belonging to Mediophyceae Jousé and Proshkina-Lavrenko in Medlin and Kaczmarska (2004) (Adl et al., 2005)—*Stephanodiscus*, *Cyclotella*, and *Discostella* SSU, LSU D1-D5 region (Ki, 2009); *Cyclotella* and *Discostella* SSU, LSU D1-D2 region (Jung et al., 2010); and *Chaetoceros* SSU, LSU D1-D3 region (Oh et al., 2010)—with modest taxon sampling. Thus, the evaluation of other loci of the genomic rDNA in addition to PCG markers is necessary with emphasis on larger taxon sampling.

The main objective of this study was to compare molecular divergences of SSU through the LSU D5 domain along with 2 chloroplast gene markers, *rbc*L and *psb*A, among representative genera of centric diatoms, including *Chaetoceros*, *Cyclotella*, *Discostella*, *Stephanodiscus*, and *Thalassiosira*. In addition, the usefulness of these genes in the determination of molecular taxonomy of these centric diatoms was evaluated, and an effective molecular marker was selected.

2. Materials and methods

2.1. Centric diatom cultures

Eight representative centric diatom strains were obtained from the Korean Marine Microalgae Culture Center (Pukyong National University, Busan, Korea) along with 1 strain (UTCC 267) from the University of Toronto Culture Collection of Algae and Cyanobacteria. Strain KHR001 was isolated from Paldang Reservoir, a reservoir of the Han River (Ki, 2009), whereas strains HYK0210-A1 and HYK0210-A2 were isolated from the Han River in spring (Jung et al., 2010). All cultured cells were maintained in DM medium (Beakes et al., 1988) and incubated at 15 °C under 50–65 μmol photons m^{-2} s^{-1} with a 12:12 light:dark cycle. Exponential phase cells were harvested via centrifugation (3000 rpm), reconstituted with 1 × TE buffer, and stored at −20 °C until further analysis.

2.2. DNA extraction, polymerase chain reaction (PCR) amplification, and sequencing

Genomic DNA was extracted from the pellets stored at −20 °C using the Core-One™ Plant/Tissue genomic DNA isolation kit (CoreBio, Seoul, Korea). The SSU-LSU region of the genomic rDNA was amplified via PCR using universal primers for eukaryotes (Jung et al., 2010) as well as a set of specific primers for chloroplast *rbc*L and *psb*A of centric diatoms (Table 1). These primers were designed with consideration to certain conserved regions of rRNA, *rbc*L, and *psb*A sequences. The PCR reaction was performed with 20 μL of reaction mixtures containing 12.8 μL of sterile distilled water, 2 μL of 10 × Ex Taq buffer (TaKaRa, Kyoto, Japan), 2 μL of dNTP mixture (4 mM), 1 μL of each primer (10 pM), 0.2 μL of Ex Taq polymerase (2.5 U), and 1 μL of template. PCR cycling was performed in an iCycler (Bio-Rad, Hercules, CA, USA) at 94 °C for 5 min, followed by 35 cycles at