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Genetic evolution and characteristics of ichthyotoxic *Cochlodinium polykrikoides* (Gymnodiniales, Dinophyceae)

Eun-Seob Cho* and Chang-Su Jeong

South Sea Fisheries Research Institute, NFRDI

1. Introduction

HABs (Harmful Algal Blooms) are increasing in frequency or severity in many coastal environments and worldwide. Most HAB species belong tothe class Dinophyceae, which can be resolved to five types (Gmnodinioids, Gonyaulacoids, Dinophysoids, Prorocentroids, and Peridinoids) on the morphological features paradigm as described by Taylor (1985). Next, Zardoya et al. (1995) reported that molecular analysis firmly supported the morphological phylogenesis. Among the section of protests, *Cochlodinium polykrikoides* Margalef is unarmoured and chain-forming, and containing eyespots which are located under the anterior portion of the sulcus (Lee et al., 1993; Park and Park, 1999 Cho et al., 2001a). This species is found to be the most ichthyotoxic genera in Korea, which is associated with suffocation due to oxygen depletion by the production of mucus during massive blooms (Cho et al., 1999; Kim et al., 2000). Recently, we compared and determined the nucleotide sequences of C. polykrikoideswith Korean HABs targeted SSU (Small Subunit, Kim et al., 2004), LSU (Large Subunit, Park and Park, 1999), and ITS (Internal Transcribed Spacer, Cho et al., 2001b) in order to analyze their phylogenetic relationship. Most of our current understanding suggests that this species should be close to the genus Gymnodinium on the basis of morphological features, but appear to be not genetically adjacent. *Cochlodinium polykrikoides* has been regarded as a photosynthetic dinoflagellate. More recently, C. polykrikoides could feed on small phytoplankton species and had a considerable grazing impact on the populations of cryptophtes described by Jeong et al. (2004). Understanding the nuclear gene sequence-based studies on the evolutionary process of C. polykrikoides has been limited. For the host cell, the basal lineage is ambiguous, and the evolutionary relationship among different taxa is controversial for several lineages. SSU has proven to be useful for the study of intra and inter-specific genetic variation in Dinophyceae. Here, we sequenced SSU to investigate phylogenetic relationships, and the evolutionary process by undertaking a detailed examination of the mixotrophic *C. polykrikoides* to establish its association with related species. Wealso obtained other sequences through GenBank search to form a combined data set.

2. Materials and Methods

Sequence data were aligned using the multiple alignment program Clustal W (Thomson et al., 1994) and determined by parsimony and distances methods incorporated in PHYLIP (Phylogeny Inference Package) ver. 3.5c (Felsenstein, 1993) as a subprogram NEIGHBOR. PHYLIP was used dinoflagellates such as *Protoperidinium thulesense* AB261522, Noctiluca scintillans DQ388461, *Gyrodinium aureolum* D-97, *Dinophysis acuminate* AJ506972, *D. fortii* AB073118, *D. norvegica* AY260470, *Prorocentrum lima* AB189780, *P. minimum* AJ415520, *P. triestinum* AB183673, *Gambierdiscus toxicus* DQ388463, *Alexnadrium tamarense* AJ415510, *A. ostenfeldii* AJ535384, *Coolia monotis* AJ415509, *Ostreopsis ovata* AF244939, *Ceratium furca* AJ276699, *C. fusus* AF022153, *Gonyalulax polygramma* AJ833631, *Gymnodinium mikimotoi* AF022195, *Karena brevis* AF352822, *Pfiesteria shumwaye* AF080093, *P. piscicida* AY033488, *Gymnodinium fuscum* AF022194, *G. galatheanum* AF272049, *Gyrodinium spirale* AB120001, *Scrippsiella trochoidea* AJ415515, *Takayama pulchellum* AY800130, *Akashiwo sanguinea* AJ415513. This search for parsimony analysis was repeated several times from differentrandom starting points using the stepwise addition option to make certain the most parsimonious tree was found. For distance analysis, subprogram DNADIST in PHYLIP was used to obtain a matrix of Kimura's two-parameter distance (Kimura, 1980). Distance matrix was analyzed by subprogram NEIGHBOR in PHYLIP with algorithms based on Saitou and Nei's NJ method (Saitou and Nei, 1987). All nucleotide substituions were equally wighted and unordered alignment gaps were treated as missing information. Reliability of the tree was constructed using subprogram CONSENSE in PHYLIP after pairwise sequence distances were estimated by Kimura's two-parameter method, which attempts to correct observed dissimilarities for multiple substitutions in sequences evolving with a transition bias.

4. Results and Discussion

The PHYLIP NJ and parsimony analyses produced a tree that was similar to the substitution rate calibraton tree and all groups were recovered. The aspectsof topology were retained. Tree members of each clade by NJ and parsimony analyses in PHYLIP included their own groups, forming monophyletic groups. The essential aspects of topology were that the class Dinophyceae (DI) clade formed a strong monophyletic relationship (bootstrap >70% based on NJ and parsimony analyses in PHYLIP) with *C. polykrikoides* and several taxa (*Amphidinium*, *Gymnodinium*, *Prorocentrum*, *Heterocapsa*, and *Gyrodinium*). *Prorocentrum*, *Heterocapsa*, and *Gyrodinium* showed the most basal section. That was nested to *G. catenatum* and *C. polykrikoides*, which were supported by a strong bootstrap value of >85% in NJ and parsimony analyses. Although *G. aureolum* was considered to be a member of Dinophyceae on the basis of morphological

taxonomy, the phylogenetic relationship placed *G. aureolum* in the class Chlorophyceae rather than Dinophyceae.
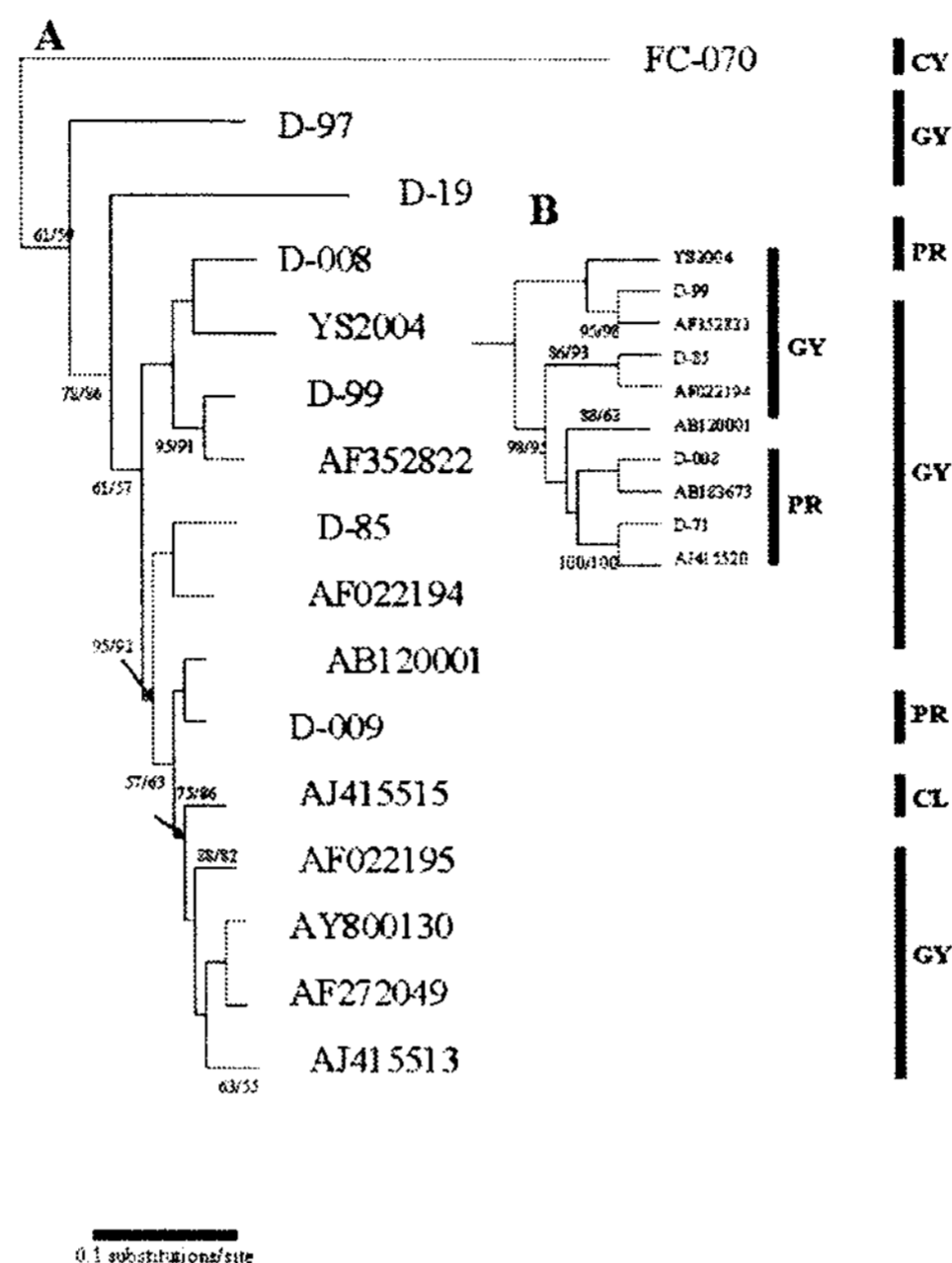

Fig. 1. PHYLIP analysis of harmful dinoflagellates obtained from GenBank database. A, phylogenetic analysis of 16 species within the family Gymnodiniaceae and Claciodiniellaceae and Prorocentrum, B, phylogenetic analysis of 10 species within the family Gymnodiniaceae and Prorocentrum. Phylogram was constructed by inferring from nucleotide sequences of partial SSU region. The tree was obtained using subprogram NEIGBOR in PHYLIP with the option of Kimura's two-parameter. The topology represents the consensus tree from a heuristic search yielding two equally most parsimonious. The tree was rooted using Microcystis aeruginosaFC-070. The numbers shown on branches, which represent bootstrap values for 100 replications, were obtained from using the subprogram CONSENSE. Numbers at the nodes refer to the percentage (>50%) of bootstrap support in NJ and parsimony analyses. Bootstrap of <50% represents a hypen on node. CY, cyanobacteria, GY, gymnodiniaceae, PR, prorocentrum, CL, claciodiniellaceae, FC-070, *Microcystis aeruginosa*, D-97, *Gyrodinium aureolum*, D-19, *Amphidinium caterae*, D-008, *Prorocentrum micans*, YS2004, *Cochlodinium polykrikoides*, D-99, *Gymnodinium catenatum*, AF352822, *Karenia brevis*, D-85, *Gyrodinium impudicum*, AF022194, *Gymnodinium fuscum*, AB120001, *Gyrodnium spirale*, D-009, *Heteroscaps triquetra*, AJ415515, *Scrippsiella trochoidea*, AF022195, Gymnodinium mikimotoi, AY800130, Takayama pulchellum, AF272049, *Gymnodinium galatheanum*, AJ415513, *Akashiwo sanguinea*, AB183673, *Prorocentrum triestinum*, D-71, *Prorocentrum balticum*, AJ415520, *Prorocentrum minimum*.

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