J. Life Science. (2001), 11(2), 74~80

JOURNAL OF
Life Science

Phylogenetic Relationship among Several Korean Coastal Red Tide Dinoflagellates Based on their rDNA Internal Transcribed Spacer Sequences

Eun-Seob Cho*, Gi-Yong Kim, Hyung-Sik Park, Byung-Hyouk Nam and Jae-Dong Lee†

Department of Microbiology, Pusan National University, Pusan 609-735, Korea
**Harmful Algae Biology Division, National Fisheries Research and Development Institute, Pusan 619-900, Korea*

Received: September 10, 2001

Abstract The nucleotide sequences of the internal transcribed spacer regions (ITS1 and ITS2) of ribosomal DNA (rDNA), and the 5.8S rRNA gene, have been determined for 13 strains of dinoflagellates in order to analyze the phylogenetic relationship. The DNA sequences contained considerable variation in the ITS regions, but little in the 5.8S rDNA. In addition, the ITS1 was more variable than the ITS2 in all species examined. The nucleotide length of this region varied from 519 bp to 596 bp depending on the taxa. The investigated taxa were divided into three large groups based on the ITS length, *i. e.*, a group with short ITS region (*A. fraterculus* and *Alexandrium* sp.), a with ITS region group (*P. micans*, *P. minimum* and *P. triestinum*) and a with ITS region group (*G. impudicum*, *C. polykrikoides*, *G. sanguineum*, *G. catenatum* and *H. triquetra*). The relationship between nucleotide length of ITS1 and that of ITS2 was negative, whereas G+C content and nucleotide length showed positive correlation. In phylogenetic analyses producing NJ trees, the topology was similar cluster and clearly divided the taxa into three groups based on 5.8S rDNA that were similar to those based on morphological characteristics. In particular, *G. impudicum* was more closely related to *G. catenatum* than to *C. polykrikoides* using phylogenetic analysis. From this study, we show that the length of ITS region contributes to discriminate Korean harmful algal species and ITS analysis is a useful method for resolving the systematic relationships of dinoflagellates.

Key words: phylogenetic relationship, red tide dinoflagellates, ITS, *Cochlodinium Polykrikoides*, G+C content

Introduction

Recent advances in DNA amplification and sequencing, new approaches in harmful algal blooms (HABs) are based on the information of genotypic properties and nucleic acid sequences of each HABs, instead of using morphological phenotypes which are widely used for classification. Due to the morphological features have a capability of great variation depending on environmental conditions 4,19. For example, the marine dinoflagellate *Alexandrium* consists of more than 20 species 2, which are morphologically similar and are difficult to identify under a light microscope. HABs taxonomists have been regarded their identification as the arrangement and fine feature of their thecal plates 12, but phenotypic characters were able to change according to environmental conditions and growth stage 19. To resolve taxonomic confusion, some researchers have tested the sequence analysis on two distinct small-subunit (SSU) ribosomal RNA and large-subunit (LSU) for several *Alexandrium* 21, 22, 24. In addition, the pennate diatom *Pseudo-nitzschia* species are difficult to identify, requiring electron microscope and expert taxonomic skills 25. Some researchers have determined the sequence analysis targeted SSU rDNA 17 and suggested that the V4 domain of the SSU rDNA region was desirable to determine the phylogenetic placement of the Raphidophyceae 30.

So far, the nuclear ribosomal RNA genes, the more rapidly evolving internal transcribed spacer regions (ITS-1 and ITS-2) surrounding 5.8S, are canditates for the clarification of taxonomic levels and phylogentic comparisons. Because ITS contains a few bp (approximately 500bp), it is easy to determine the sequence and it is not very time-consuming. Regarding of some characteristics, ITS has been a useful tool in the phylogentic relationship and in designing universal primers for the amplification of the spacer regions of a wide variety of organisms. Interspecific and intraspecific ITS sequence variation has been examined in harmful dinoflagellates *Alexandrium* and *Gymnodinium* species 1, 3, 5. In a toward to molecular phylogeny, we reported the molecular identification of *Gyrodinium impudicum* and *Gymnodinium sanguineum* isolated from Korean coastal waters by ITS regions 16. Therefore, we reported to examine the phylo-

†Corresponding author
Phone: 82-51-510-2269, Fax: 82-51-510-2269
E-mail: leejd@hyowon.cc.pusan.ac.kr

genetic relationship and align the gene library of some Korean coastal red tide microalgae targeted ITS regions.

Materials and Methods

Microalgae

Thirteen clonal isolates were tested in this study (Table 1). Microalgal samples were taken from red tide waters in Korea and then collected by picking them out individually with a micropipette or by serial dilution under a light microscope. After a clonal culture was established, it was maintained and cultured in an f/2-Si medium 14containing an antibiotic mixture 15. The culture was grown in a customized culture cabinet (BOD incubator) at 20°C under a light intensity of 100 mol m^{-2} s^{-1} from white fluorescent tubes, 12 L : 12D of light : dark cycle. An isolate of *Alexandrium fraterculus* was obtained from Pukyong University, Pusan and *Gymnodinium catenatum* (GC19V) was donated by Dr. Donald Anderson, Woods Hole Oceanographic Institution, Massachusetts, USA. Cells of microalgae were harvested from the liquid media by filtering onto Whatman No. 1 filter paper and rinsed with distilled deionized water. Before the extraction of DNA, pellets were stored in a freezer at -20°C.

DNA extraction

Algal DNA was extracted from each sample according to the procedure adapted from the benzyl chloride method 31. Approximately 0.05 g of algal pellets were suspended in 500 $\mu\ell$ of an extraction buffer (100 mM Tris-HCl, pH 8.0, 40 mM EDTA), 150 $\mu\ell$ of 10 % (w/v) sodium dodecyl sulfate (SDS), and 300 $\mu\ell$ of benzyl chloride, then incubated at 55℃ for 30 min. Phenol: chloroform: isoamylalchol (25 : 24 : 1) was treated twice, then RNase (1 mg mL^{-1}) was added. The algal cells were pelleted and the supernatant mixed with 40 $\mu\ell$ of 3 M sodium acetate and incubated on ice for at least 1 h. The DNA was precipitated from the tube at room temperature for 10 min by adding 2.5 volumes of 100% ice-cold ethanol. The pellet was washed with 2 volumes of 70% ethanol and resuspended in distilled water. The DNA was kept at -20℃.

PCR amplification and DNA sequencing

The nuclear rDNA region spanning the ITS1, ITS2 and 5.8S rRNA gene from each strain in Table 1 was amplified by PCR. Primers ITS 1 (5'-CCAAGCTTCTAGATCGTAAC AAGGTCCGTAGGT-3') and ITS 2 (5'-CCTGCAGTCGA CAATGCTTAATTCAGCGG-3') were used and derived from the conserved regions of SSU and LSU rDNA, respectively 16. PCR was performed with a Perkin-Elmer Model 480 thermocycler by using the following program : initial denaturation for 3 min at 95℃, 30 cycles of amplification (denaturation for 30 sec at 95℃, annealing for 30 sec at 50℃, and extention for 1min at 72℃) and final extention of 5min at 72℃. The PCR product from the amplification was subjected to preparative electrophoresis in a 1.6% agarose gel in a TBE buffer. All PCR products yield only a single visible band. The PCR product was excised from an ethidium bromide stained gel, then purified by using a QIAGEN Gel Elution Kit (Qiagen, Wartworth CA). Direct sequencing of PCR products was done in a Perkin-Elmer Applied Biosystems ABI 377A sequencer by using a PRISM Dye Dideoxy Terminator Cycle Sequencing kit (Perkin Elmer) following the manufacturer's protocol. Two primers, ITS 1 and ITS 2, were used for sequencing in both directions. DNA sequences were edited and assembled with the program CLONE MANAGER version 4.0 (Scientific and Educational Software, Stateline, PA).

Data analysis

The determined ribosomal DNA sequences were deposited

Table 1. The list of species and Genbank accession number of microalgae used in this study

Species	Strains No.	Geographical origin	Accession No.[a]
Alexandrium fraterculus	AF-1	Chinhae, Korea	AF 208242
Alexandrium sp.	AS-1	Chinhae, Korea	AF 208243
Cochlodinium polykrikoides	CP-1	Chungmu, Korea	AF 208248
C. polykrikoides	CP-2	Gohoung, Korea	-
Gyrodinium impudicum*	GA3-1	Chungmu, Korea	AF 131074
G. impudicum*	GA3-2	Gohoung, Korea	-
G. impudicum*	GA3-cyst	Yousu, Korea	-
Gymnodinium sanguineum*	GS-1	Masan, Korea	AF 131075
G. catenatum**	GC19V	Vigo, Spain	AF 208247
Heterocapsa triquetra	HT-1	Chinhae, Korea	AF 208249
Prorocentrum micans	PMC-1	Chinhae, Korea	AF 208245
P. minimum	PMN-1	Chungmu, Korea	AF 208244
P. triestinum	PT-1	Namhae, Korea	AF 208246

*Species was reported in Kim et al., 1999 as Molecular identification of *Gyrodinium impudicum* and *Gymnodinium sanguineum* by comparing the sequences of the internal transcribed spacers 1, 2 and 5,8S ribosomal DNA.

**Obtained from Woods Hole Oceanographic Institution, USA.

[a]The nucleotide sequence data will appear in the GenBank Database under the respective accession number.

at the NCBI (National Center for Biotechnology Information) data library. Their accession numbers are indicated in Table 1. They were initially aligned with the sequences of the related genera from the NCBI data library by using Clustal W [28]. Phylogenetic trees were constructed using neighbor-joining (NJ) method 18 in the NEIGHBOR program of PHYLIP version 3.57c package. The strength of internal branches of the resulting trees was statistically tested by bootstrap analysis [10] from 1,000 bootstrap replications. The extracted sequences from the NCBI data library are as follows: Pseudo-nitzschia pungens, P. multiseries and P. australis,. They were aligned with the sequences of related strains such as G. catenatum HU02, PR01, JU02, DE06, DE07, GPU02 [3], A. catenella M17, TNX22 and A. tamarense OFX191-A, AT4-B, 304A-A, OFX191-B, AT4-A and 304A-B [2]. The determined sequences were as follow: G. impudicum, AF 131074; G. sanguineum, AF 131075. The strains having identical sequence were registrated as only one species.

Results

Sequence alignments

Electrophoresis and direct sequencing of each PCR reaction confirmed that single products were amplified in accordance with each PCR reaction, and the size of each product corresponded to the expected rDNA. The alignment of the DNA sequences of the internally transcribed spacers ITS1, ITS2, and the 5.8S rDNA gene is shown in Fig. 1. There was considerable sequence variation in the ITS sequences, but little in the region of the 5.8S rDNA. The internal transcribed spacers contained most of the sequence variation. In particular, the ITS1 is more variable than the ITS2 in all species used in this study.

Nucleotide length of rDNA ITS regions and G+C content

The nucleotide lengths of ITS1, ITS2 and 5.8S rDNA are shown in Table 2. The shortest size of the ITS1-5.8S-ITS2 was 519 nucleotides of A. fraterculus AF-1, and the longest size of the ITS regions was 596 nucleotides of H. triquetra HT-1. Among 13 strains sequenced in this study, the difference between the shortest and the longest size was 77 nucleotides. The size variation was derived from the variation of the ITS regions as well as that of the 5.8S rDNA gene. Most of the tested taxa could be divided into three groups, depending on their ITS length. The group with short ITS includes A. fraterculus AF-1, Alexandrium sp. AS-1 and the group with middle long ITS includes P. micans PMC-1, P. minimum PMN-1 and P. triestinum PT-1, G. impudicum GA3-1, GA3-2, GA3-cyst and C. polykrikoides CP-1, CP-2, G. sanguineum GS-1, G. catenatum GC19V and H. triquetra HT-1 have the longest nucleotide on the total ITS regions sequenced in our study. The range in length of the ITS1-5.8S-ITS2 of the shortest group was from 519 to 533 nucleotides, that of the middle long group was from 554 to 568 and that of the longest group was from 576 to 596. The relationship between nucleotide length of ITS1 and that of ITS2 was negative shown in Fig. 2. In contrast to Fig. 2, positive correlations in G+C content and nucleotide length were found between ITS1 and ITS2 (Fig. 3).

Phylogenetic analysis of 5.8S rDNA sequence comparisons

Results of NJ analysis were expressed as a dendogram (Fig. 4). The topologies of the trees generated by either method were similar clusting pattern. The census tree generated by NJ method based on Kimura-2-parameter distance showed that the genus Pseudo-nitzschia formed a second clade (Fig. 4). Two isolates of A. fraterculus and Alexandrium sp. were morphologically similar under the light microscope, but located in far genetic distance from A. catenealla and A. tamarense that they formed a completely different cluster. Among Dinophyceae, three HABs of C. polykrikoides, G. impudicum and G. catenatum were indistingable fixed, as well as similar feature under the light microscope. However, the phylogenetic relationships placed G. catenatum closer to G. impudicum than to C. polykrikoides. To determine the degree of phylogenetic signal for the rDNA sequences, the genus Prorocentrum and H. triquetra showed to be genetically adjacent.

Discussion

Sequence analyses of the 5.8S rDNA and ITS regions of 13 HABs isolates from Korean coastal waters including Spanish *G. catenatum* (GC19V) clarified that the length and sequence of their isolates was variable. The length variations of the ITS regions have been found at intra-species levels 9, 26 as well as inter-species levels in various algae 13. Likewise, the nucleotide length in the ITS regions was significantly different depending on HABs used in this study (Table 2), suggesting that the length of ITS regions might play an important role in algal taxonomy.

They found a phenomenon in the G+C content of ITS regions in a wide range of organisms and called it GC balance 29. The total G+C content of ITS1-5.8S-ITS2 ranged from 43.5% in *Alexandrium* sp. to 57.4% in *C. polykrikoides* (unpublished data). *Alexandrium frateculus* and *Alexandrium* sp. had lower G+C content in the total sequences than any other HABs. As can be seen in Fig. 2 and Fig. 3, the relationships of nucleotide length and G+C content in the ITS regions were negative and positive, respectively, in particular, *C. polykrikoides* was significantly different the correlation, compared with other HABs. Some researchers suggested that temperature was an important selection factor of GC bias in the genomes of plants and warm-blooded animals 6, 20. For example, the genomes of warm-blooded vertebrates have higher G+C content than those of cool-

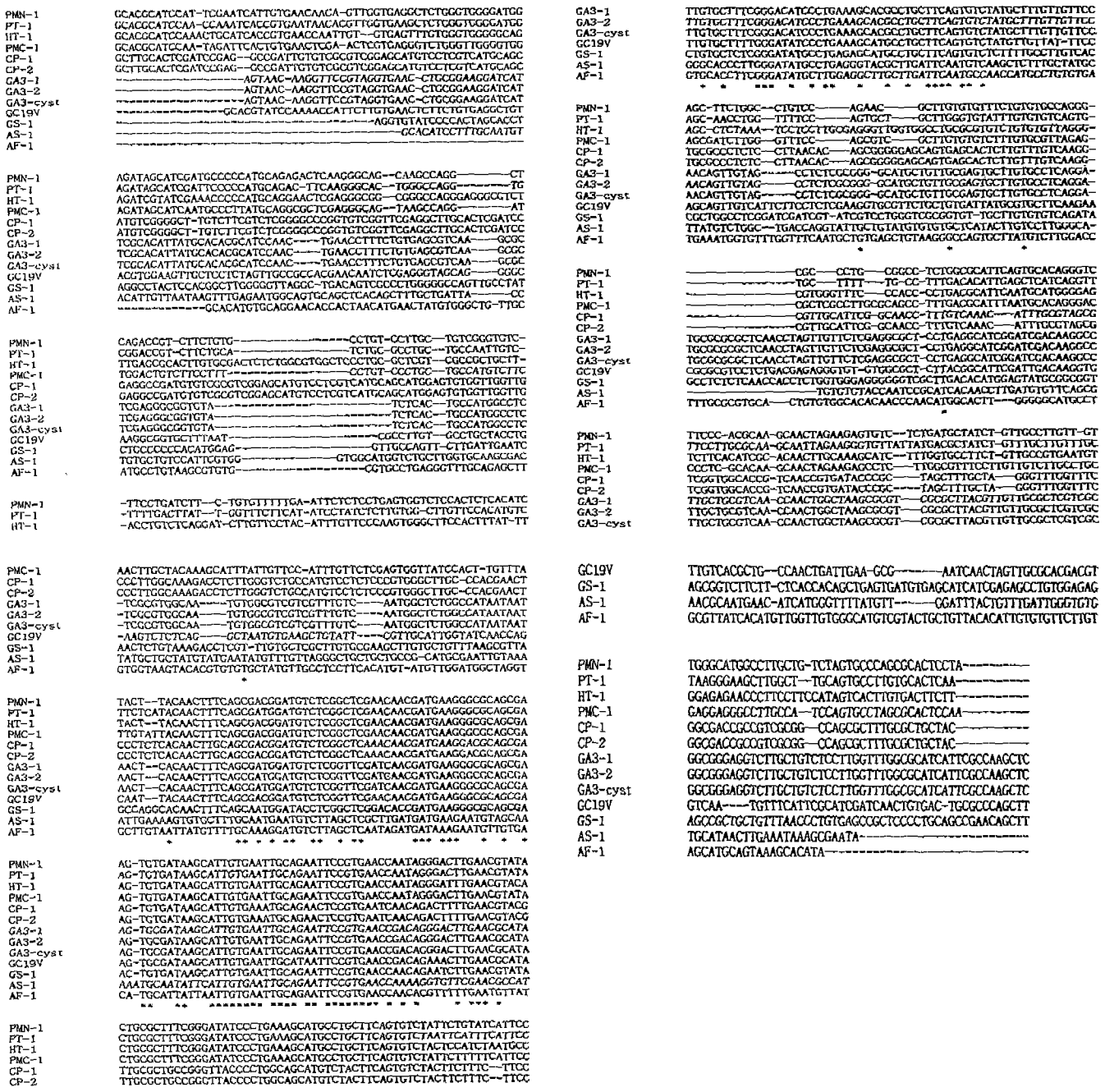

Fig. 1. The alignment of the sequences of the 5.8S rDNA with the flanking internal transcribed spacers ITS1 and ITS2. The alignment was generated by the program CLUSTAL W. ITS1 spans from 1 to 240 bp; the 5.8S coding region is from 261 to 421 bp; and ITS2 is from 421 to 700 bp. A hyphen represents a gap and a period represents a base identical to that of the top sequence. The source of each sequence are as follows: PMN-1: *Prorocentrum minimum*, PT-1: *P. triestinum*, HT-1: *Heterocapsa triquetra*, PMC-1: *P. micans*, CP-1, CP-2: *Cochlodinium polykrikoides*, GA3-1, GA3-2, GA3-cyst: *Gyrodinium impudicum*, GC19V: *Gymnodinium catenatum*, GS-1: *G. sanguineum*, AS-1: *Alexandrium* sp., AF-1: *A. fraterculus*.

blooded vertebrates. Takamatsu *et al.* have suggested that the relatively low G+C content of fungus might reflect the low optimum temperature of the fungus 27. Accordingly, *C. polykrikoides* is supposed to distribute in higher temperature regions than in cooler temperature on basis of G+C content, whereas *A. fraterculus* and *Alexndrium* sp containing lower G+C content are responsible for the distribution in cooler temperature than in higher temperature. Practically, an outbreak of *C. polykrikoides* has occurred from August to October since 1982, associated with higher G+C content. A specialist following the exhaustive description by Fraga *et al.* could consider the living cells of *G. impudicum* and

Table 2. Nucleotide length of the ITS1, ITS2 and 5.8S rDNA sequence (unit : bp)

Microalgal strain	ITS1	5.8S rDNA	ITS2	Total
AF-1	154	161	204	519
AS-1	183	162	188	533
CP-1	248	160	187	595
CP-2	248	160	187	595
GA3-1	180	161	218	559
GA3-2	180	161	218	559
GA3-cyst	180	161	218	559
GS-1	179	161	240	580
GC19V	190	161	225	576
HT-1	203	161	232	596
PMC-1	214	161	193	568
PMN-1	204	161	189	554
PT-1	206	161	190	557

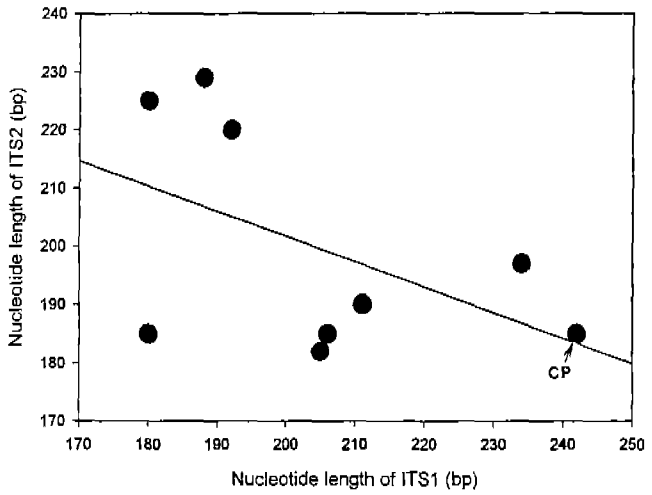

Fig. 2. The relationship of nucleotide length of ITS1 (bp) and nucleotide length of ITS2 (bp). CP for *Cochlodinium polykrikoides* isolate.

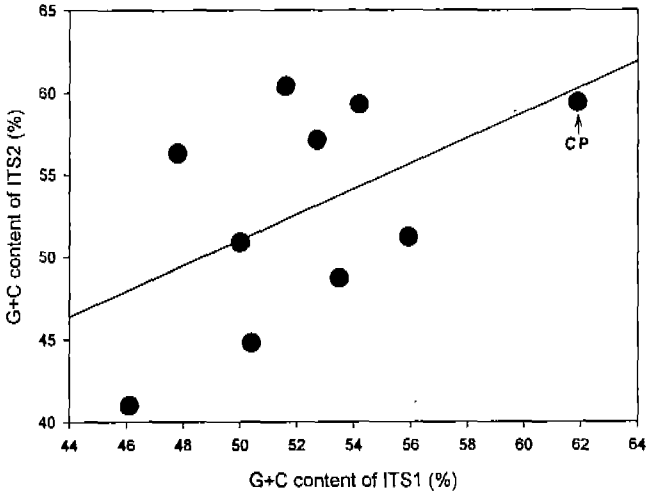

Fig. 3. Positive correlations of G+C content between ITS1 and ITS2. CP for *Cochlodinium polykrikoides* isolate.

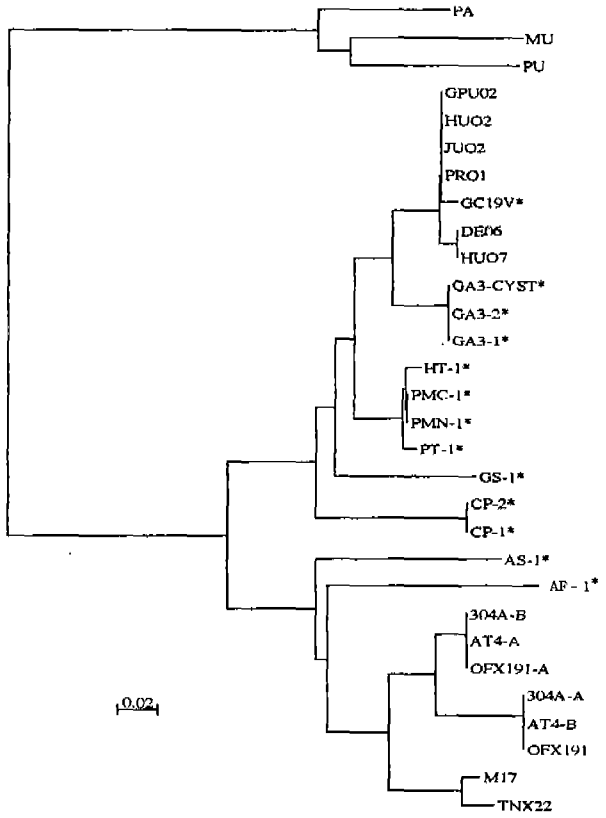

Fig. 4. Phylogenetic analysis performed on 5.8S rDNA aligned sequences using a distance method. The tree was constructed using a neighbor-joining method based on Kimura-2-parameter, The symbol of asterik represents the strain used in our study, the rest species represent the strain of *P. pungens* (PU), *P. multiseries* (MU), *P. austrarlis* (PA) extracted from the GenBank Database and HU02, PR01, JU02, DE06, DE07, GPU02 for *G. catenatum* isolate from Adachi *et al.*, 1997, M17, TNX22 for *A. catenella* isolate from Adachi et al. 1996, OFX191-B, AT4-B, 304A-B, OFX191-A, AT4-A, 304A -A for *A. tamarense* isolate from Adachi *et al.*, 1996.

G. catenatum to be separate *Gymnodiniaceae* family, corresponding to the genus *Gyrodinium* and *Gymnodinium*, respectively 11. However, Kim *et al.* suggested that *G. impudicum* and *G. catenatum* were clearly different species and that *G. impudicum* may belong to the genus *Gymnodinium* 16. At present, the phylogenetic placement of *G. impudicum* formed a near cluster of closely related *G. catenatum* with small genetic distance from *G. sanguineum*, although the genus is different from each other (Fig. 4). We realized that *G. impudicum* is more likely to belong to the genus *Gymnodinium* than to the genus *Gyrodinium* by the sequence analysis of conserved 5.8S rDNA.

In previous study, we analyzed the sequences of ITS regions for 8 isolates of *G. impudicum* vegetative cells and even cyst isolated from Chungmu, which had identical sequence in all the isolates of *G. impudicum* 16. In addition, the ITS region sequences in *G. impudicum* vegetative cells from Gohoungs and cyst from Yousu (Fig. 2) were the same

as those in *G. impudicum* isolated from Chungmu. The blooms caused by *C. polykrikoides* have occurred in the South Sea, the Yellow Sea and the East Sea in 1999, and we have conducted molecular comparison of 3 different isolates of *C. polykrikoides* targeted ITS regions, thereby identical DNA sequence 8. Presumably, Korean isolates caused by red tide have no genetic variations among strains found in Korean coastal waters. However, several researchers suggested that geographically separated populations introduced to be divergent genetically, regardless of morphotype 23, 24. Interestingly, a recent study has shown that genetic variation was partitioned mainly within more populations than regions 7, contradicting Scholin *et al.* 23, 24. Further study is needed to determine the genetic relationship between of Korean isolates and geographically isolated strains whether or not other characters support the evolutionary lineage suggested above.

Recently, we isolated a dinoflagellate *Alexandrium* species, presenting a toxin profile and a morphology similar to those of *A. tamarense*, thus tentatively calling this species *Alexandrium* sp. However, the phylogenetic framework of Korean *Alexandrium* sp. was different from that of *A. catenealla* or *A. tamarense* (Fig. 4), suggesting that the phylogenetic placement of *Alexandrium* sp. is in doubt concerning the relationship between morphological taxonomy and the molecular approach to identification.

Acknowledgement

This research was supported by Pusan National University Grant, 1998.

References

1. Adachi, M., Y. Sako and Y. Ishida. 1994. Restriction fragment length polymorphism of ribosomal DNA internal transcribed spacer and 5.8S regions in Japanese *Alexandrium* species (Dinophyceae). *J. Phycol.* **30**, 857-863.
2. Adachi, M., Y. Sako and Y. Ishida. 1996. Analysis of *Alexandrium* (Dinophyceae) species using sequences of the 5.8S ribosomal DNA and internal transcribed spacer regions. *J. Phycol.* **32**, 424-432.
3. Adachi, M., Y. Sako and Y. Ishida. 1997. Analysis of *Gymnodinium catenatum* Dinophyceae using sequences of the 5.8S rDNA-ITS regions and random amplified polymorphic DNA. *Fish. Sci.* **63**, 701-707.
4. Adachi, M., Y. Sako, Y. Ishida, D. M. Anderson and B. Reguera. 1993. Cross-reactivity of five monoclonal antibodies to various isolates of *Alexandrium* as determined by an indirect immunofluorescence method. *Nipp. Sui. Gakk.* **59**, 1807.
5. Adachi, M., Y. Sako, A. Uchia and Y. Ishida. 1995. Ribosomal DNA internal transcribed spacer regions (ITS) define species of the genus *Alexandrium*. In : Lassus, P., G. Arzul, E. Erard, P. Gentien and C. Marcailloul (eds), *Harmful Marine Algal Blooms*, Intercept Ltd, Andover, pp.15-20.
6. Bernardi, G., B. Olofsson, J. Filipsik, M. Zerial, J. Salinas, G. Cuny, M. Meunier-Rotival. and F. Rodier. 1985. The mosaic genome of warm-bloomed vertebrates. *Science.* **228**, 953-958.
7. Bolch, C. J. S., S. I. Blackburn, G. M. Hallegraeff and R. E. Vaillancourt. 1999. Genetic variation among strains of the toxic dinoflagellate *Gymnodinium catenatum* (Dinophyceae). *J. Phycol.* **35**, 356-367.
8. Cho, E. S., H. G. Kim and Y. C. Cho. 2000. Sequence analysis of *Cochlodinium polykrikoides* isolated from Korean coastal waters using sequences of Internal Transcribed Spacers and 5.8S rDNA. *J. Kor. Oceano. Soc.* **35**, 158-160.
9. Coleman, A. W., A. Suaarez.and L. J. Goff. 1994. Molecular delinedation of species and syngens in volocacean green algae (Chlorphyta). *J. Phycol.* **30**, 80-90.
10. Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution.* **39**, 738-791.
11. Fraga, S., I. Bravo, M. Delgado, J. M. Franco and M. Zapata. 1995. *Gyrodinium impudicum* sp. nov. (Dinophyceae), a non toxic, chain-forming, red tide dinoflagellate. *Phycologia.* **34**, 514-521.
12. Fukuyo, Y. 1985. Morphology of Protogonyaulax tamarensis (Lebour) Taylor and *Protogonyaulax catenella* (Whedon and Kofoid) Taylor from Japanese coastal waters. *Bull. Mar. Sci.* **37**, 529-537.
13. Goff, L. J., D. A. Moon and A. W. Coleman. 1994. Molecular deoineation of species and species relationships in the red algal agarophytes *Gracilariopis* and *Gracilaria* (Gracilariales). *J. Phycol.* **30**, 521-537.
14. Guillard, R. R. L. and J. H. Ryther. 1962. Studies of marine planktonic diatoms 1. *Cyclotella nana* Hustedt, and *Detonula confervacea* (Cleve) Gran. *Can. J. Micro.* **8**, 229-239.
15. Hasui, M., M. Matsuda, S. Yoshimatus. and K. Okutani. 1995. Production of a lactate-associated galactan sulfate by a dinoflagellate *Gymnodinium* A3. *Fish. Sci.* **61**, 321-326.
16. Kim, G. Y., M. G. Ha, E. S. Cho, T. H. Lee, S. J. Lee and J. D. Lee. 1999. Molecular identification of *Gyrodinium impudicum* and *Gymnodinium sanguineum* by comparing the sequences of the internal transcribed spacers 1, 2 and 5.8S ribosomal DNA. *J. Fish. Sci. Tech.* **2**, 66-77.
17. Miller, P. E.and C. A. Scholin. 1996. Identification of cultured *Pseudo-nitzschia* (Bacillariophyceae) using species-specific LSU rRNA-targetted fluorescent probes. *J. Phycol.* **32**, 646-655.
18. Saitou, N.and M. Nei. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4**, 406-425.
19. Sako, Y., C. H. Kim, H. Ninomiya, M. Adachi.and Y. Ishida. 1990. Isozyme and cross analysis of mating populations in the *Alexandrium catenella/tamarense* species complex. In : Graneli, E., B. Sundstrom, L. Edler. and D. M. Anderson (eds), *Toxic Marine Phytoplankton*, Elsevier, New York, pp.320-323.
20. Salinas, J., G. Matassi, L. M. Montero and G. Bernardi. 1988. Compositional compartmentalization and compositional patterns in the nuclear genomes of plants. *Nucleic Acids Research.* **16**, 4269-4285.
21. Scholin, C. A. and D. M. Anderson. 1994a. Identification of group- and strain specific genetic markers for globally distributed *Alexandrium* (Dinophyceae). I. RFLP analysis of SSU rRNA genes. *J. Phycol.* **30**, 744-754.
22. Scholin, C. A., D. M. Anderson. and M. L. Sogin. 1993. Two distinct small-subunit ribosomal RNA genes in the North American toxic dinoflagellate *Alexandrium fundyense* (Dino-

phyceae). *J. Phycol.* **29**, 209-216.
23. Scholin, C. A., G. M. Hallegraeff and D. M. Anderson. 1995. Molecular evolution of the *Alexandrium tamarense* species complex (Dinophyceae) : Dispersal in the North American and West Pacific regions. *Phycologia.* **34**, 472-485.
24. Scholin, C. A., M. Herzog, M. L. Sogin and D. M. Anderson. 1994b. Identificationof group-and strain-specific genetic markers for globally distributed *Alexandrium* (Dinophyceae). II. Sequence anlaysis of a fragment of the LSU rRNA gene. *J. Phycol.* **30**, 999-1011.
25. Scholin, C. A., M. C. Villac, K. R. Buck, J. M. Krupp, D. A. Powers, G. A. Fryxell.and E. P. Chavex. 1994c. Ribsomal DNA sequences discriminate among toxic and non-toxic *Pseudonitzschia* species. *Natural Toxins.* **2**, 152-165.
26. Steane, D. A., B. A. McClure, A. E. Clarke and G. T. Kraft. 1991. Amplication of the polymorphic 5.8S rRNA gene from selected Australian *Gigartinalean* species (Rhodophyta) by polymerase chain reaction. *J. Phycol.* **27**, 758-762.
27. Takamatsu, S., T. Hirata and Y. Sato. 1988. Phylogenetic analysis and predicted secondary structures of the rDNA internal transcribed spacers of the powdery mildew fungi (Erysiphaceae). *Mycoscience.* **39**, 441-453.
28. Thomson, J. D., D. G. Higgins, and T. J. Gibson. 1994. Clustal W : Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucl. Acids Res.* **22** : 4673-4680
29. Torres, R. A., M. Ganal and V. Hemleben. 1990. GC balance in the internal transcribed spacers ITS1 and ITS2 of nuclear ribosomal RNA genes. *J. Mol. Evol.* **30**, 171-181.
30. Tyrrell, J. V., B. R. Bergquist, R. D. Gray, L. Mackenzie and P. L. Bergquist. 1996. Phylogeny of the Raphiophtes *Heterosigma carterae* and *Chattonella antiqua* using V4 domain SSU rDNA sequence. *Bio. Syst. Ecol.* **24**, 221-235.
31. Zhu, H., F. Qu. and H. Zhu. 1993. Isolation of genomic DNAs from plants, fungi and bacteria using benzyl chloride. *Nucl. Acid. Res.* **21**, 5279-5280.