Journal of the Korean Society of Oceanography
Vol. 35, No. 3, pp. 158—160 September 2000

[Note]

Sequence Analysis of *Cochlodinium polykrikoides* Isolated from Korean Coastal Waters Using Sequences of Internal Transcribed Spacers and 5.8S rDNA

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The relativity of four isolates of *C. polykrikoides* was determined by comparative sequence analysis based on direct sequencing of PCR amplified ribosomal DNA (the internal transcribed spacer region and the 5.8S rDNA). Sequence comparisons indicated that four isolates had the same nucleotide sites in the ITS regions, as well as a total of 585 nucleotide length and 100% homology. The molecular data revealed that *C. polykrikoides* in Korean coastal waters show no genetical difference.

Recent advances in DNA amplification and sequencing based on the information of genotypic properties and nucleic acid sequences instead of conventional morphological classification is a promising tool for the identification of harmful algae (HA). A variety of molecular techniques based on genomic variations, such as DNA fingerprinting, restriction fragment length polymorphism or random amplified polymorphic DNA and DNA sequence analysis of various ribosomal spacer regions (Scholin *et al.*, 1994; Costas *et al.*, 1995; Adachi *et al.*, 1996), have all been used for species identification.

The nuclear ribosomal RNA genes, and in particular the more rapidly evolving internal spacer regions (ITS-1 and ITS-2) included 5.8S, are promising for the clarification of taxonomic levels and for phylogenetic comparisions.

Furthermore, *C. polykrikoides* has been assocaited with massive fish mortalities and is considered as the most ichthyotoxic dinoflagellate in Korea, occurring annually in Korean coastal waters. In 1998 and 1999, blooms of *C. polykrioides* were observed in Kunsan coastal waters where they had been previously rare. *C. polykrikoides* ocurred two months later in Kunsan than that in the South Sea, where it has occurred in annually in September since 1995. We present the use of DNA sequences targeted ITS region to determine the relatedness of four *C. polykrikoides* strains isolated from Korean coastal waters.

Algal DNA was extracted from each sample according to the procedure adapted from the benzyl chloride method (Zhu *et al.*, 1993). Primers ITS1 (5'-CCAAGCTTCTAGATCGTAACAAGGTCCGTAGGT-3') and ITS2 (5'-CCTGCAGTCGACAATGCTTAATTCAGCGG-3') were derived from the conserved regions of 3' end of the 18S and the 5' of the 28S rDNA, respectively. The procedure was as follow: initial denaturation for 3 min. at 95°C, 30 cycles of amplification (denaturation for 30 sec. at 95°C, annealing for 30 sec. at 50°C, and extention for 1 min. at 72°C) and final extention of 5 min. at 72°C. The PCR (Perkin-Elmer #480) product from the amplification was subjected to preparative electrophoresis in a 1.6% agarose gel in TBE buffer. All PCR products yield only a single visible band. The PCR product was excised from the ethidium bromide stained gel and purified using a QIAGEN gel elution kit (Qiagen, Wartworth CA). Direct sequencing of PCR products was conducted in an Perkin-Elmer Applied Biosystems ABI 377A sequencer using a PRISM Dye Dideoxy Terminator Cycle Sequencing kit (Perkin Elmer) following the manufacture's protocol. Two primers, ITSA and ITSB, were used for sequencing in both directions. DNA sequences were edited and assembled with the program CLONE MANAGER version 4.0 (Scientific Educational Software, Stateline, PA). The degree of sequence similarity was examined by calculating the nucleoetide substitution rates for transitions and transversions using the CLUSTAL W (Thomson *et al.*, 1994).

Electrophoresis and direct sequencing of each PCR reaction confirmed that single product was amplified in accordance with each PCR reaction and the size of each product corresponded to the expected rDNA.

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CP-1 GCTTGCACTCGATCCGAGGCCGATTGTGTCGCGTCGGAGCATGTCCTCGTCATGCAG CAT   60
CP-2 GCTTGCACTCGATCCGAGGCCGATTGTGTCGCGTCGGAGCATGTCCTCGTCATGCAG CAT
CP-3 GCTTGCACTCGATCCGAGGCCGATTGTGTCGCGTCGGAGCATGTCCTCGTCATGCAG CAT
CP-4 GCTTGCACTCGATCCGAGGCCGATTGTGTCGCGTCGGAGCATGTCCTCGTCATGCAG CAT

CP-1 GTCGGGGCTTGTCTTCGTCTCGGGGCCCGGTGTCGGTTCGAGGCTTGCACTCGATCCGAG  120
CP-2 GTCGGGGCTTGTCTTCGTCTCGGGGCCCGGTGTCGGTTCGAGGCTTGCACTCGATCCGAG
CP-3 GTCGGGGCTTGTCTTCGTCTCGGGGCCCGGTGTCGGTTCGAGGCTTGCACTCGATCCGAG
CP-4 GTCGGGGCTTGTCTTCGTCTCGGGGCCCGGTGTCGGTTCGAGGCTTGCACTCGATCCGAG

CP-1 GCCGATGTGTCGCGTCGGAGCATGTCCTCGTCATGCAGCATGGAGTGTGGTTGGTTGCCC  180
CP-2 GCCGATGTGTCGCGTCGGAGCATGTCCTCGTCATGCAGCATGGAGTGTGGTTGGTTGCCC
CP-3 GCCGATGTGTCGCGTCGGAGCATGTCCTCGTCATGCAGCATGGAGTGTGGTTGGTTGCCC
CP-4 GCCGATGTGTCGCGTCGGAGCATGTCCTCGTCATGCAGCATGGAGTGTGGTTGGTTGCCC

CP-1 TTGGCAAAGACCTCTTGGGTCTGCCATGTCCTCTCCCGTGGGCTTGCCCACGAACTCCCT  240
CP-2 TTGGCAAAGACCTCTTGGGTCTGCCATGTCCTCTCCCGTGGGCTTGCCCACGAACTCCCT
CP-3 TTGGCAAAGACCTCTTGGGTCTGCCATGTCCTCTCCCGTGGGCTTGCCCACGAACTCCCT
CP-4 TTGGCAAAGACCTCTTGGGTCTGCCATGTCCTCTCCCGTGGGCTTGCCCACGAACTCCCT

CP-1 CTCACAACTTGCAGCGACGGATGTCTCGGCTCAAACAACGATGAAGGACGCAGCGAAGTG 300
CP-2 CTCACAACTTGCAGCGACGGATGTCTCGGCTCAAACAACGATGAAGGACGCAGCGAAGTG
CP-3 CTCACAACTTGCAGCGACGGATGTCTCGGCTCAAACAACGATGAAGGACGCAGCGAAGTG
CP-4 CTCACAACTTGCAGCGACGGATGTCTCGGCTCAAACAACGATGAAGGACGCAGCGAAGTG

CP-1 TGATAAGCATTGTGAAATGCAGAACTCCGTGAATCAACAGACTTTTGAACGTACGTTGCG  360
CP-2 TGATAAGCATTGTGAAATGCAGAACTCCGTGAATCAACAGACTTTTGAACGTACGTTGCG
CP-3 TGATAAGCATTGTGAAATGCAGAACTCCGTGAATCAACAGACTTTTGAACGTACGTTGCG
CP-4 TGATAAGCATTGTGAAATGCAGAACTCCGTGAATCAACAGACTTTTGAACGTACGTTGCG

CP-1 CTGCCGGGTTACCCCTGGCAGCATGTCTACTTCAGTGTCTACTTCTTTCTTCCTGCGCCC  420
CP-2 CTGCCGGGTTACCCCTGGCAGCATGTCTACTTCAGTGTCTACTTCTTTCTTCCTGCGCCC
CP-3 CTGCCGGGTTACCCCTGGCAGCATGTCTACTTCAGTGTCTACTTCTTTCTTCCTGCGCCC
CP-4 CTGCCGGGTTACCCCTGGCAGCATGTCTACTTCAGTGTCTACTTCTTTCTTCCTGCGCCC

CP-1 TCTCCTTAACACAGCGGGGGAGCAGTGAGCACTCTTGTTTGTCAAGGCGTTGCATTCGGC  480
CP-2 TCTCCTTAACACAGCGGGGGAGCAGTGAGCACTCTTGTTTGTCAAGGCGTTGCATTCGGC
CP-3 TCTCCTTAACACAGCGGGGGAGCAGTGAGCACTCTTGTTTGTCAAGGCGTTGCATTCGGC
CP-4 TCTCCTTAACACAGCGGGGGAGCAGTGAGCACTCTTGTTTGTCAAGGCGTTGCATTCGGC

CP-1 AACCTTTGTCAAACATTTGCGTAGCGTCGGTGGCACCGTCAACCGTGATACCCGCTAGCT  540
CP-2 AACCTTTGTCAAACATTTGCGTAGCGTCGGTGGCACCGTCAACCGTGATACCCGCTAGCT
CP-3 AACCTTTGTCAAACATTTGCGTAGCGTCGGTGGCACCGTCAACCGTGATACCCGCTAGCT
CP-4 AACCTTTGTCAAACATTTGCGTAGCGTCGGTGGCACCGTCAACCGTGATACCCGCTAGCT

CP-1 TTGCTAGGGTTTGGTTTCGGCGACCGCCGTCGCGGCCAGCGCTTT                  585
CP-2 TTGCTAGGGTTTGGTTTCGGCGACCGCCGTCGCGGCCAGCGCTTT
CP-3 TTGCTAGGGTTTGGTTTCGGCGACCGCCGTCGCGGCCAGCGCTTT
CP-4 TTGCTAGGGTTTGGTTTCGGCGACCGCCGTCGCGGCCAGCGCTTT
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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 multiple alignment program CLUSTAL W using a gap weight of 3.0 and a gap length weight of 0.1. ITS1 spans from 1 to 240 bp; the 5.8S coding region is from 261 to 421 bp; and ITS2 is from 421 to 595 bp. The source of each sequence are as follows: CP-1 for *C. polykrikoides* isolate from Yeongil Bay, CP-2 for *C. polykrikoides* isolate from Kunsan, CP-3 for *C. polykrikoides* isolate from Tongyong and CP-4 for *C. polykrikoides* isolate from Chodo.

The alignment of the DNA sequences of the internal transcribed spacers ITS1, ITS2 and 5.8S rDNA is shown in Fig. 1. By comparison the four isolates displayed the same nucleotide sites in the ITS region. The aligned, sequenced data spanned a total of 585 sites, with having 100% homology. In a previous study (Kim *et al.*, 1999), the sequences of ITS regions for 8 isolates of *Gyrodinium impudicum* were analyzed. Vegetative cells and even cysts isolated from Tongyong, all had identical sequences. In addition, ITS regions sequences of *G. impudicum* from Gohoung and cyst from Yousu (Kim *et al.*, 2000) were the same as *G. impudicum* isolated from Tongyong. Several researchers suggested that geographically separated populations introduced to be divergent genetically, regardless of morphotype (Scholin *et al.*, 1994, 1995). Interestingly, recent studies have shown that a genetic variation was partitioned mainly within more populations than regions (Bolch *et al.*, 1999). Further study is needed to determine the genetic relatedness of Korean isolates of *C. polykrikoides* to geographically isolated strains and whether other characters support the evolutionary lineage suggested as above.

ACKNOWLEDGEMENTS

The authors were appreciated Gi Young Kim, Pusan National University, for skillful helpness and valuable suggestions. I am grateful to Dr. Lesley Rhodes, Cawthron Institute, New Zealand, for her sincere manuscription in English. An anomymous reviewer made valuable comments on the manuscript.

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Manuscript received February 7, 2000
Revision accepted April 11, 2000
Handling Author: Jae-Sang Hong