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Sequence analysis of LSU rDNA of *Alexandrium tamarense/catenella* complex from Korean coastal waters

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Introduction

A great deal of effort has been put into the identification of *Alexandrium tamarense/fundyense/catenella* complex by understanding correlation between morphological and subcellular characteristics. To date, the most promising tool for the study of these species is sequence analyses of rRNA genes that have been useful for various organisms' taxonomy and phylogeny, and its application such as *in situ* hybridization. LSU rDNA sequences of *Alexandrium tamarense* and *A. catenella* regional populations occurring in various parts of Korean coasts were analyzed in order to gather a precise knowledge concerning their biogeography and dispersal in Korea.

Material and Methods

After harvesting 50mL of an exponentially growing culture, total genomic DNA was extracted by LiCl extraction method. PCR reaction was carried out according to Scholine et al. (1994). Fresh PCR products were ligated in vector and transformed using Original TA Cloning Kit (Invitrogen).

Sequencing of plasmid DNA was performed using a PRISM Dye Dideoxy Terminator Cycle Sequencing Kit (Perkin Elmer) following the manufacture's protocol. The cycle sequencing reaction was run on an ABI 377 Sequencer (Perkin Elmer). Analyzed sequences of all isolates were aligned using Clustal W. The phylogenetic tree was constructed by DNAmlk DNA Maximum Likelihood program with molecular clock of BioEdit.

Results and Discussion

Only about 700 bp PCR band was amplified at *A. tamarense* Type I isolates, wheres a lower molecular weight band in addition to a band were detected in *A. tamarense* Type II. The former exactly corresponded to North American ribotype, and the later to Temperate Asian of Scholin *et al.* (1994). The consistent presence of the extra band results from unusually large deletion (80 bp) of LSU rDNA D1/D2 regions. Such deletion of rRNA gene was also reported from *A. catenella* in China (Yeung *et al.* 1996) and probably in Korea (Lee *et al.* 1998). The validity of the band was probed as an outstanding genetic marker to discriminate the two ribotypes.

Korean isolates divided into two divergent ribotypes based on aligned sequences regardless of their morphotaxonomy. Fifteen isolates collected from three major coasts along Korean peninsula (i.e. the East Sea, the South Sea and the Yellow Sea) matched three subribotypes within North American ribotype. It is hard to say that they were translocated or moved into Korean coastal waters by recent human activities. Thus, North American ribotype is pandemic rather than distinct regional populations mainly restricted at North American regions (Scholin *et al.* 1994). Ten isolates corresponding to Temperate Asian ribotype were distributed off the coasts of the South Sea, Yeosu, Jinhae Bay and Dadaepo nearby Busan. Homogeneous populations were previously reported from Korea (Lee *et al.* 1998), Japan (Scholin *et al.* 1994) and southeastern China (Yeung *et al.* 1996). At least, the regional populations are considered to be evolved from the same ancestrial stock because of homogeneous LSU rDNA and distinct large deletion within the gene.

The existence of non-functional genes (or B genes) in "the tamarensis complex" even within a clonal culture was used as a useful genetic marker to discriminate regional populations (Scholin *et al.* 1994). At least, all examined isolates of Korean *A. tamarense* contained several classes of genes including 80 bp deletion at LSU rDNA D1 region. However, few sequence conflicts were detected between the positions at which B genes were reported and our sequences. The sequence substitutions and insertions/deletions are much more than previously considered.

References

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