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A Molecular Phylogenetic Study on Korean *Alexandrium catenella* and *A. tamarense* Isolates (Dinophyceae) Based on the Partial LSU rDNA Sequence Data

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Sequences of the large subunit ribosomal (LSU) rDNA D1-D2 region of *Alexandrium catenella* (=*A.* sp. cf. *catenella*) and *A. tamarense* isolates, which were collected along the Korea coasts, were analyzed to understand their phylogenetic relationships and geographical distributions. All *A. catenella* and *A. tamarense* isolates belonged to the *A. tamarense/catenella/fundyense* complex and were grouped with the North American and temperate Asian ribotypes, respectively, regardless of the presence or absence of a ventral pore in the first apical plate. A consistent and peculiar characteristic that differentiated the *Alexandrium* isolates was amplification of a second PCR product with a lower molecular weight in addition to the predicted one; ten *A. catenella* isolates belonging to the temperate Asian ribotype yielded this additional PCR product. Sequence alignment revealed that the shorter PCR product resulted from an unusual large deletion of 87 bp in the LSU rDNA D1 domain. The North American and temperate Asian ribotypes were prevalent along the Korean coasts without geographical separation. Given the high genetic homogeneity among widely distributed *Alexandrium* populations, each ribotype appeared to be pandemic rather than to constitute a distinct regional population.

Key words: *Alexandrium catenella*, *Alexandrium tamarense*, geographical distribution, LSU rDNA, molecular phylogeny

INTRODUCTION

Taxonomy of *Alexandrium* species remains problematic because of a lack of easily recognizable morphological characteristics, with the exception of a few species that have distinct features [e.g., *Alexandrium balechii* (Steidinger) Balech, *A. concavum* (Gaarder) Balech, *A. insuetum* Balech]. Thus, species designations of *Alexandrium* seem to vary often among researchers. This situation can have serious consequences when attempting to discriminate among morphologically closely related taxa such as *A. catenella* (Whedon and Kofoid) Balech, *A. fundyense* Balech and *A. tamarense* (Lebour) Balech. The three species are distributed worldwide, and are notorious for their potent toxicity and taxonomic difficulty. Moreover, they cause paralytic shellfish poisoning (PSP) even at low cell concentrations leading to a ban on shellfish harvesting (Hallegraeff, 1993). Harmful algal bloom (HAB) monitoring programs are also seriously hampered when different morphospecies coexist (Fukuyo, 1985; Kim *et al.*, 2002), with toxic and non-toxic forms co-occurring (Yentsch *et al.*, 1978).

Over the last two decades, *A. catenella*, *A. fundyense* and *A. tamarense* (commonly called the "*A. tamarense/catenella/fundyense* complex"; Scholin *et al.*, 1994) have been scrutinized in a series of comprehensive studies to resolve taxonomic problems and further to find genetic markers that distinguish the toxic species from the non-toxic. However, their morphotaxonomy was frequently incongruent with various subcellular characters such as isozyme electrophoretic patterns, reactivity of monoclonal antibodies, or PSP toxin profiles (Adachi *et al.*, 1993; Cembella and Taylor, 1986; Cembella *et al.*, 1987; 1988; Hayhome *et al.*, 1989).

To date, the most promising tool for understanding systematics of *Alexandrium* species appears to be sequence analyses of ribosomal RNA (rRNA) or its coding region (rDNA), which has been useful in investigating evolutionary histories of many organisms (Cavalier-Smith, 1993; Olsen *et al.*, 1994), and biogeography or dispersal of marine organisms in

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particular (van Oppen *et al.*, 1993; Scholin *et al.*, 1994; Medlin *et al.*, 1998; de Vargas *et al.*, 1999; Darling *et al.*, 2000). rDNA molecules have both conserved and variable domains allowing discrimination of dinoflagellates at various levels of taxonomy (Zardoya *et al.*, 1995; Saunders *et al.*, 1997; Daugbjerg *et al.*, 2000; Saldarriaga *et al.*, 2001).

Since the first *Alexandrium* bloom in Korea in 1977 (Cho, 1978), a number of PSP incidents caused by toxic *Alexandrium* spp. have been reported, sometimes resulting in human deaths (Chang *et al.*, 1987; Lee *et al.*, 1997). Kim *et al.* (2002) reported that toxigenic *Alexandrium* species distributed at all sites of the Korean coasts. Here, we analyzed the sequences of the large subunit (LSU) rDNA D1-D2 region from the *Alexandrium* isolates to elucidate their phylogenetic relationships and geographical distributions.

MATERIAL AND METHODS

Algal cultures

All isolates used in this study were established and maintained as described in Material and Methods of Kim *et al.* (2002) and listed in Table 1.

Extraction and PCR amplification of genomic DNA

Algal cells were harvested from 50 mL of exponentially growing cultures, and genomic DNA was extracted using the slightly modified LiCl method (Hong *et al.*, 1995). PCR amplification was carried

Table 1. List of Korean *Alexandrium* isolates used in this study, with the results of PCR amplification, sequence analyses of the LSU rDNA D1-D2 region, and GenBank accession numbers

Isolate code[a]	Species designation[b]	Presence of the shorter PCR product	Ribotype[c]	GenBank accession number
BSW97	*A. tamarense*	-	North American	AY082028
CMC98a	*A. tamarense/catenella*	+	temperate Asian	AY082046, 47
CMC98b	*A.* sp. cf. *catenella*	+	temperate Asian	AY082048
DPC95a	*A.* sp. cf. *catenella*	+	temperate Asian	AY082049, 50
DPC95b	*A.* sp. cf. *catenella*	+	temperate Asian	AY082051
DPC95c	*A.* sp. cf. *catenella*	+	temperate Asian	AY082052
JDC00a	*A. tamarense*	-	North American	AY082029
JDW0004-13	*A. tamarense*	-	North American	AY082030
KJC97a	*A. tamarense*	-	North American	AY082031
KMC98a	*A.* sp. cf. *catenella*	+	temperate Asian	AY082053, 54
SJC95a	*A. tamarense*	-	North American	AY082032
SJC95b	*A. tamarense*	-	North American	AY082033
SJC00a	*A. tamarense*	-	North American	AY082034
SJW9704-3	*A. tamarense*	-	North American	AY082035
SJW9704-6	*A. tamarense*	-	North American	AY082036
SJW9704-15	*A. tamarense*	-	North American	AY082037, 38
SJW0003-11	*A. tamarense*	-	North American	AY082039
SJW0007-7	*A.* sp. cf. *catenella*	+	temperate Asian	AY082055
SJW0007-8	*A.* sp. cf. *catenella*	+	temperate Asian	AY082056
SOW0004-8	*A. tamarense/catenella*	+	temperate Asian	AY082057, 58
SSW0006-3	*A. tamarense*	-	North American	AY082040
SSW0006-7	*A. tamarense*	-	North American	AY082041
ULW9903	*A. tamarense*	-	North American	AY082042
YOC98a	*A. tamarense*	-	North American	AY082043
YOC98b	*A. tamarense/catenella*	-	North American	AY082044
YOC98c	*A. tamarense*	-	North American	AY082045
YSC98a	*A.* sp. cf. *catenella*	+	temperate Asian	AY082059, 60

[a]See Table 1 of Kim *et al.* (2002) for the sampling sites, dates and origins.
[b]See Kim *et al.* (2002) for more details.
[c]According to Scholin *et al.* (1994).

out in a 50 μL-reaction volume containing 1×*Ex Taq*™ buffer, 200 μM of each dNTP, 1 μL of template DNA (*ca.* 10 ng·μL^{-1}), 0.2 μM of forward and reverse primers, and 0.25 unit of *TaKaRa Ex Taq*™ (TaKaRa, Japan). The primers for amplification of the LSU rDNA D1-D2 region were D1R (5'-ACCCGCTGAATTTAAGCATA-3') and D2C (5'-CCTTGGTCCGTGTTTCAAGA-3') (Scholin *et al.*, 1994). PCR cycles were composed of one initial denaturation at 94°C for 3 min, followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec and elongation at 72°C for 30 sec. The reaction was completed by the final elongation at 72°C for 7 min. PCR products (5 μL) were electrophoresed in an 1% agarose gel in TAE buffer (40 mM Tris/acetate, 1 mM EDTA) and photographed under UV trans-illumination after staining with ethidium bromide (0.5 μg·mL^{-1}).

Ligation, cloning and sequencing

Fresh PCR products were ligated into the pCR®2.1 vector, which was used to transform *E. coli* with an Original TA Cloning® Kit (Invitrogen), according to the manufacturer's instructions. After the color-based selection of transformants using X-gal, one to eight white *E. coli* transformant colonies were cultured, and their plasmid DNAs were extracted using a Quantum Prep® Plasmid Miniprep Kit (Bio-Rad).

Sequencing was performed with an ABI 377 Sequencer (Applied Biosystems, Perkin Elmer) using an ABI PRISM BigDye™ Terminator v3.0 Cycle Sequencing Kit (Applied Biosystems, Perkin Elmer) following the manufacturer's protocol. Plasmid DNAs were sequenced in both directions with an M13 primer set. The sequence data analyzed in this study were deposited in GenBank under the accession numbers AY082028-AY082060 (Table 1).

Phylogenetic analyses

Analyzed sequences of all the PCR-amplified clones were aligned using Clustal W (Thomson *et al.*, 1994). The alignment result was examined, and errors were corrected manually. All phylogenetic analyses were conducted in PAUP* 4.0b8 (Swofford, 2001) with representative *Alexandrium* sequences available as follows; *A. affine* AFF37 (GenBank accession number: AB088227), *A. affine* CU1 (U44935), *A. catenella* A3 (AF200667), *A. catenella* ACBOPNZ (AF019408), *A. catenella* ATT98 X11 (AF318219), *A. catenella* Dino-6 (AF042818), *A. catenella* HK1998 (AF118547), *A. catenella* K-0270 (AF200666), *A. catenella* OF101 (U44931), *A. excavatum* Ge1V (L38632), *A. fraterculus* DPW9709 (AB088244), *A. fraterculus* SJW9709 (AB088262), *A. fundyense* AFNFA3.1 (U44926), *A. fundyense* AFNFA3.2 (U44928), *A. tamarense* ATBB01 (U44933), *A. tamarense* CU13 (U44934), *A. tamarense* G.Hope1 (U44932), *A. tamarense* K-0055 (AF200668), *A. tamarense* OF041 (U44929), *A. tamarense* Pgt183 (U44930), *A. tamarense* Plymouth (AF033534), *A. tamarense* PW06 (U44927), *A. tamiyavanichii* TAMI22012 (AB088263), *A. tamiyavanichii* TAMI2207 (AB088265). *A. minutum* AMAD06 (U44936) was used as the outgroup.

Maximum parsimony (MP) analysis was conducted using the heuristic search option with the random addition of sequences (100 replicates) and tree bisection-reconnection (TBR) branch swapping. Characters were weighted equally, and gaps were treated as missing data. Maximum likelihood (ML) analysis was conducted with the TrN+G model with the following likelihood settings, determined by ModelTest (Posada and Crandall, 1998): base frequencies A = 0.2606, C = 0.1420, G = 0.2662; base substitution rates AC = 1.0000, AG = 2.2267, AT = 1.0000, CG = 1.0000, CT = 3.8314; gamma distribution shape parameter = 0.5781. The heuristic searches with random sequence addition and TBR branch rearrangements were conducted with 10 replicates. Bootstrap analyses were conducted to determine the robustness of clades (Felsenstein, 1985) with 1,000 replicates for MP and ML analyses.

RESULTS

PCR amplification

The LSU rDNA D1-D2 region from each of 27 *Alexandrium* isolates was amplified by PCR. PCR amplification of seventeen isolates yielded only the predicted fragment of ~700 base pairs (bp). In contrast, amplification of ten isolates yielded a second, weaker fragment of ~610 bp, which represented an additional, shorter PCR product (Fig. 1). Scholin and Anderson (1996) also identified the temperate Asian ribotype as a distinctive group by the presence of additional longer and shorter PCR products than the predicted one. However, the longer product was not observed in the present study.

Sequence analyses

Sequences of the LSU rDNA D1-D2 region from

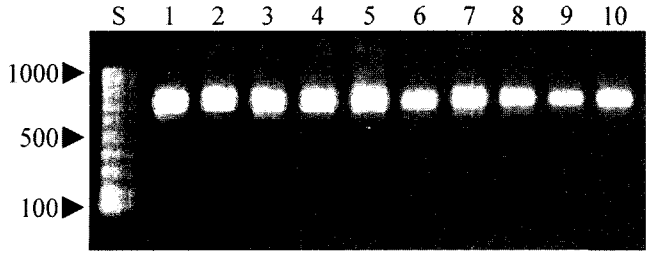

Fig. 1. The results of PCR amplification of the LSU rDNA D1-D2 region of Korean *Alexandrium* sp. cf. *catenella* isolates. Lane designations are as follows: S, 100 bp size marker; 1, CMC98a; 2, CMC98b; 3, DPC95a; 4, DPC95b; 5, DPC95c; 6, KMC98a; 7, SJW0007-7; 8, SJW0007-8; 9, SOW0004-8; 10, YSC98a. Note the shorter PCR product in addition to the predicted one.

Korean *A. tamarense* and *A.* sp. cf. *catenella* isolates were analyzed after cloning and sequencing their PCR products. The complete sequence data are presented in Fig. 2; as there were no sequence variations among KJC97a, SJC00a, SJW9704-3 and ULW9903, only one of the sequences is shown. Sequence alignment revealed that the sequence lengths of the predicted PCR products were nearly identical to those previously reported (662-665 bp) (Scholin *et al.*, 1994; Medlin *et al.*, 1998; Higman *et al.*, 2001). Sequence analyses of at least two PCR-amplified clones from five representative *A.* sp. cf. *catenella* isolates (CMC98a, DPC95a, KMC98a, SOW0004-8 and YSC98a) that yielded the shorter PCR products (Fig. 1) revealed that the additional fragment resulted from an unusual large deletion of 87 bp in the LSU rDNA D1 domain (aligned position: 134-220 bp; Fig. 2). Excluding the deletion, the predicted and shorter products were highly homogeneous (average, 99.24%).

At least four PCR-amplified clones from each of four representative isolates of *A. tamarense* (SJC95a and SJW9704-15) and *A.* sp. cf. *catenella* (DPC95a and YSC98a) were analyzed, and minor sequence substitutions and/or indels were found even within single isolates (Fig. 2). However, the positions at which these sequence polymorphisms occurred sometimes differed from those previously reported (Scholin *et al.*, 1994; Medlin *et al.*, 1998; Higman *et al.*, 2001). Such polymorphisms were also observed among the PCR clones of the shorter PCR products (i.e., DPC95a.2 and YSC98a.2) (Fig. 2).

Sequence polymorphisms identified among eight PCR clones of *A. tamarense* SJW9704-15 were significant enough to separate the clones into two groups of variants (SJW9704-15.1 and SJW9704-15.2) (Fig. 2). The former comprised two clones characterized by TGTGA at the aligned positions 102-106, one nucleotide deletion at 145 and five TG repeats at 576-585, whereas the latter comprised six clones characterized by GTGGA at positions 104-108 and four TG repeats at 576-583. The similar polymorphisms were also noticed in *A. fundyense* AFNFA3 from eastern North America (Scholin *et al.*, 1994), *A. tamarense* BAH ME 182 from Scotland (Medlin *et al.*, 1998), and *A. tamarense* UW4 and UW61 from Scotland (Higman *et al.*, 2001). However, such polymorphisms were not observed in *A. tamarense* SJC95a, although eight PCR clones were analyzed.

Phylogenetic analyses

Sequences of the LSU rDNA D1-D2 region from *A. tamarense* and *A.* sp. cf. *catenella* isolates (including two variants of SJW9704-15) were subjected to phylogenetic analyses with representative *Alexandrium* sequences available in GenBank. MP and ML analyses produced phylogenetic trees with nearly identical tree topologies (Fig. 3). All of the Korean isolates belonged to the *A. tamarense/catenella/fundyense* complex (Scholin *et al.*, 1994); *A. tamarense* and *A.* sp. cf. *catenella* isolates clustered with the North American and temperate Asian ribotypes of Scholin *et al.* (1994), respectively (Fig. 3). Three isolates designated as *A. tamarense/catenella* owing to considerable variation in their thecal plate formulae were clustered with one of the two ribotypes. Ten *A.* sp. cf. *catenella* isolates that yielded two distinct PCR products shown in Fig. 1 corresponded to the temperate Asian ribotype, and seventeen *A. tamarense* isolates that generated a single PCR product corresponded to the North American ribotype. *A. tamarense* CU13, which had been included as a member of the *A. tamarense/catenella/fundyense* species complex (Scholin *et al.*, 1994), was phylogenetically separated from the species complex; it showed a sister-group relationship to *A. tamiyavanichii* Balech supported by a high bootstrap value (Fig. 3).

DISCUSSION

PCR amplification

Generation of the shorter PCR product in addition to the predicted one upon PCR amplification of the LSU rDNA D1-D2 region was a consistent and peculiar characteristic differentiating Korean *Alexandrium* isolates. Sequence alignment revealed that the shorter

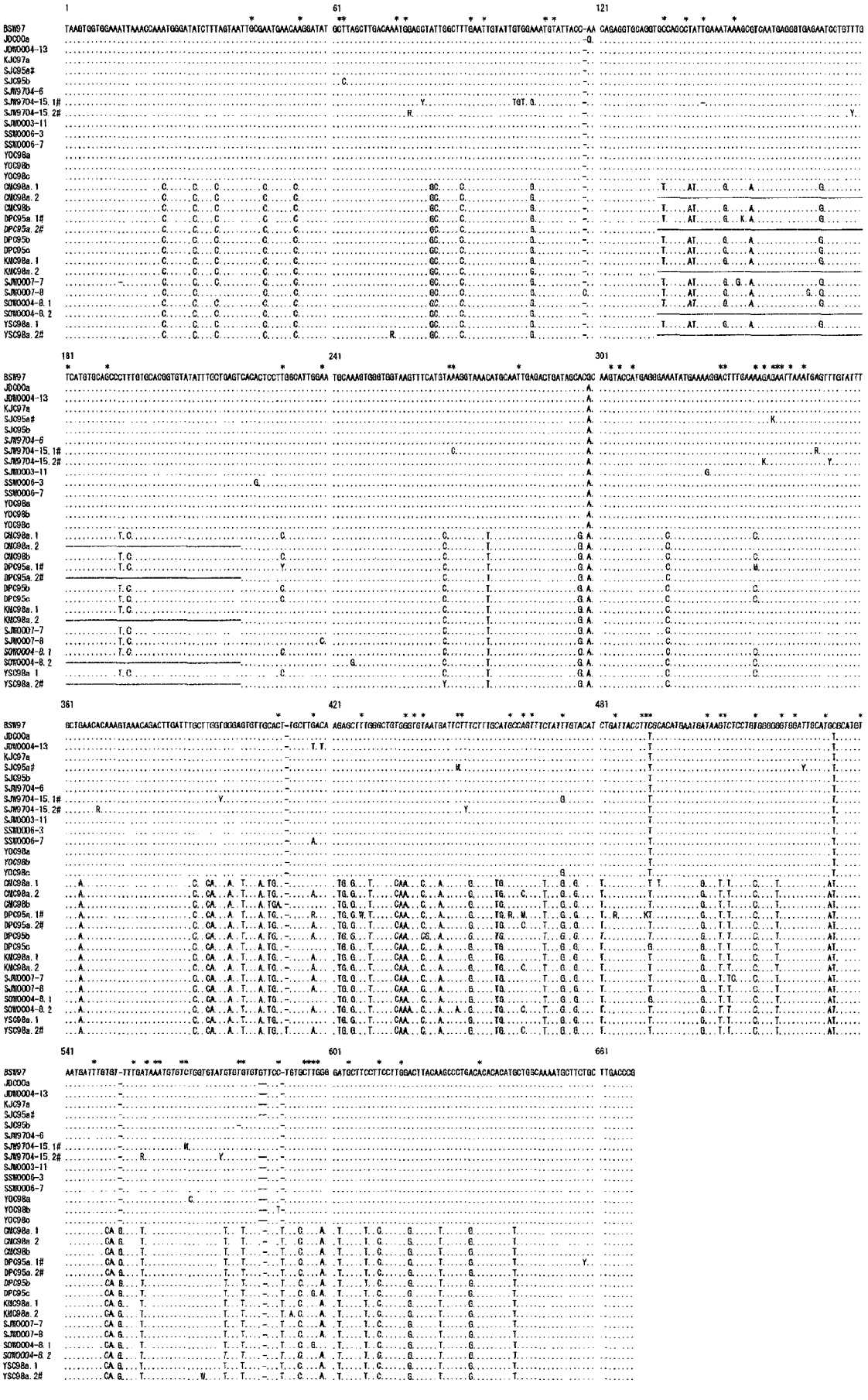

Fig. 2. Sequence alignment of the LSU rDNA D1-D2 region of Korean *Alexandrium catenella* and *A. tamarense* isolates. Periods and dashes indicate identical nucleotides and inserted alignment gaps, respectively. KJC97a, SJC00a, SJW9704-3 and ULW9903 were 100% homogeneous, so only one sequence was aligned. The sequences marked with the number sign (#) indicate that multiple PCR-amplified clones were analyzed from single isolates; note the minor sequence polymorphisms within each isolate. A pair of two sequences under the same isolate code is presented when significant sequence polymorphisms occurred. Asterisks (*) represent the reported sequence polymorphisms in single isolates of Scholin *et al.* (1994), Medlin *et al.* (1998) or Higman *et al.* (2001). Sequence ambiguities are noted with the standard IUPAC nomenclature (R=A or G; Y=C or T; M=C or A; K=G or T; W=A or T).

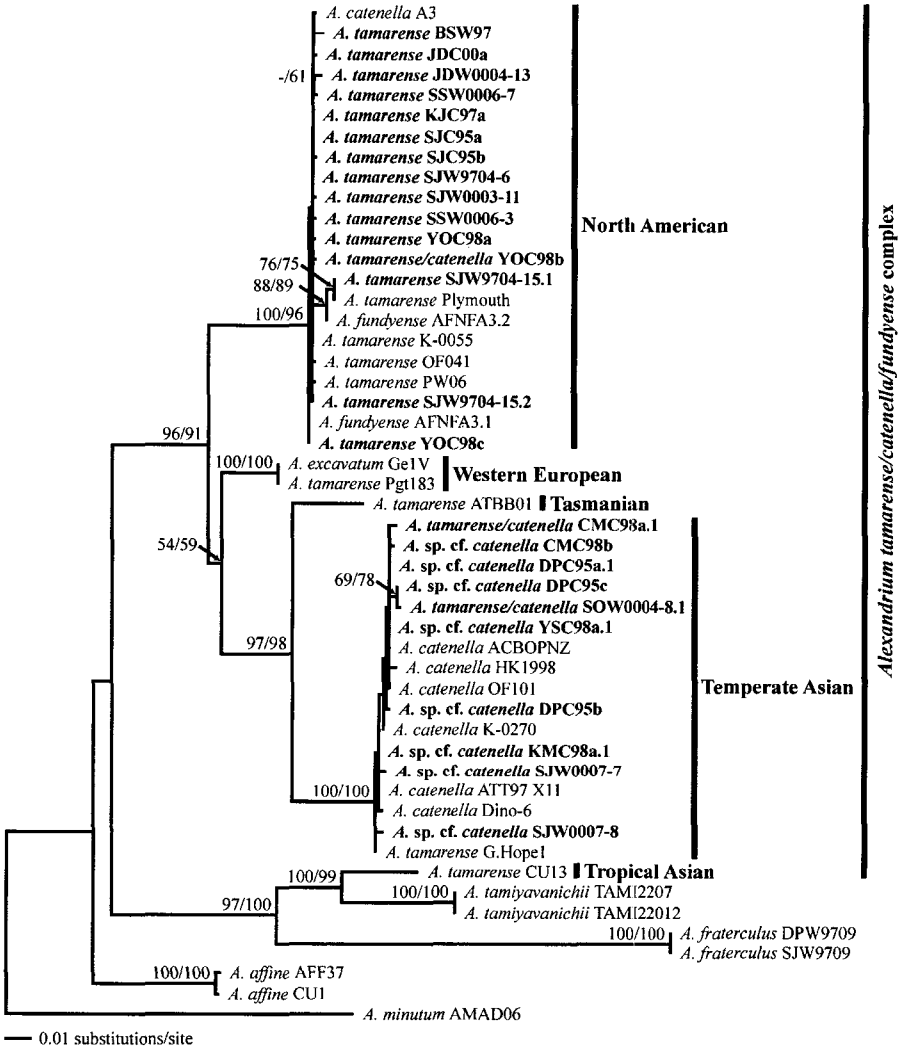

Fig. 3. The phylogenetic tree of Korean *Alexandrium catenella* and *A. tamarense* isolates inferred from sequence analyses of the LSU rDNA D1-D2 region based on the maximum likelihood method with representatives of each ribotype of Scholin *et al.* (1994). KJC97a, SJC00a, SJW9704-3 and ULW9903 had no sequence variation, so only one of the sequences was included. Five ribotypes of the *A. tamarense/catenella/fundyense* complex (Scholin *et al.*, 1994) were indicated. The sequences of isolates with bold-faced letters were determined in the present study. Bootstrap values above 50% inferred from maximum parsimony and maximum likelihood analyses are indicated at each internal branch point.

PCR product was derived from a large deletion of 87 bp in the LSU rDNA D1 domain, and the two PCR products were highly homogeneous, confirming that the shorter one was derived from a single isolate. This unusual deletion was also reported to occur in Chinese *A. catenella* CCMP 1493 (Yeung *et al.*, 1996) and French *Alexandrium* sp. X3 (GenBank accession number: AF318227). The description of two sizes of PCR products in Japanese *A. catenella* and Korean *A. tamarense* isolates (Scholin and Anderson, 1996), Korean *A. catenella* Dino-6 (Lee *et al.*, 1998), and Chilean *A. catenella* isolates (Uribe *et al.*, 1999) strongly suggests that all of them harbor the same unusual deletion. Similarly, the presence of two types of the rDNA molecule has been also reported in another dinoflagellate, *Dinophysis acuminata* Claparède and Lachmann (Rehnstam-Holm *et al.*, 2002). It is generally assumed that the shorter PCR product is a non-functional pseudogene (Yeung *et al.*, 1996; Rehnstam-Holm *et al.*, 2002). The lower yield of the shorter product upon PCR amplification suggests that this large deletion occurs only at limited copies of the LSU rDNA molecule.

In phylogenetic trees, *A.* sp. cf. *catenella* isolates

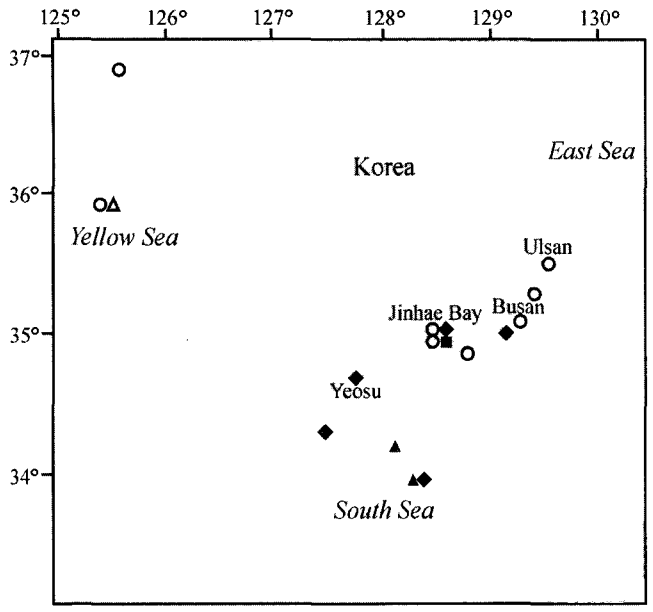

Fig. 4. Geographical distributions of Korean *Alexandrium* isolates with their species designations and ribotypes: ○, *A. tamarense* and the North American ribotype; □, *A.* sp. cf. *catenella* and the temperate Asian ribotype; ■, *A. catenella* and the temperate Asian ribotype (Lee *et al.*, 1998); □ and △, unable to identify at the species level, but typical of *A. tamarense/catenella* and the North American and temperate Asian ribotypes, respectively.

that yielded two PCR products always clustered with the temperate Asian ribotype, whereas *A. tamarense* isolates that produced only the predicted one clustered with the North American ribotype. Consequently, the high sequence homogeneity and the large deletion at limited copies of the LSU rDNA molecule of the temperate Asian isolates unequivocally reflect its independent evolution from an ancestral stock. The presence of the shorter PCR product could be used as an outstanding genetic marker to easily discriminate the temperate Asian ribotype from the other *Alexandrium* ribotypes or species.

Sequence polymorphisms

Sequence polymorphisms of rDNA molecules have been previously reported from *A. catenella, A. fundyense* and *A. tamarense* (Scholin *et al.*, 1993; 1994; Medlin *et al.*, 1998; Higman *et al.*, 2001) as well as from other microorganisms (Gunderson *et al.*, 1987; Mylvaganam and Dennis, 1992; Nübel *et al.*, 1996). In the present study of Korean *Alexandrium* isolates, multiple classes of the LSU rDNA molecule were also found in all four *A. tamarense* (SJC95a and SJW9704-15) and *A.* sp. cf. *catenella* (DPC95a and YSC98a) isolates analyzed, when at least four PCR-amplified clones were sequenced. However, the positions at which we detected the sequence polymorphisms did not always coincide with those previously reported (e.g., Scholin *et al.*, 1994; Medlin *et al.*, 1998; Higman *et al.*, 2001). Instead, the polymorphisms were irregularly dispersed throughout the LSU rDNA molecule.

Over-representation of rare classes of rRNA genes by clonal biasing (Scholin and Anderson, 1996) may result in exaggeration of divergence among closely related taxa, producing incorrect phylogeny. Randomly distributed sequence substitutions and/or indels indicate that some of these sequences may not code for functional rRNA transcripts (Gunderson *et al.*, 1987; Scholin *et al.*, 1993). After excluding non-functional genes, the homology value would be much higher. To obviate the possibility of cloning bias and to clarify the authentic nature of heterogeneity, further characterization by sequence analyses of cellular rRNA molecules will be necessary. This will also be important for designing rRNA-trageted DNA probes for HAB monitoring (Scholin *et al.*, 1994).

Relationship between morphology and genetic data

Korean *A. tamarense* and *A.* sp. cf. *catenella* isolates used in this study, in which species discrimination was based on the shape of the sulcal plate (sp) and the sulcal width (Kim *et al.*, 2002), corresponded to the North American and templated Asian ribotypes, respectively. *A.* sp. cf. *catenella* with and/or without a ventral pore (vp) in the first apical plate (1') was genetically identical to *A. catenella* Dino-6 (Lee *et al.*, 1998), of which the morphology was typical owing to the consistent lack of the vp (see Kim *et al.*, 2002, for more details). Thus, the LSU rDNA sequence data supported the validity of the sp and sulcal width as being more informative taxonomic criteria upon which to differentiate *A. catenella* and *A. tamarense* than the presence or absence of the vp is (Kim *et al.*, 2002). Moreover, *A. tamarense* SJW9704-5 (this study), BAH ME 182 (Medlin *et al.*, 1998), and UW4 and UW61 (Higman *et al.*, 2001) had high sequence homogeneity to *A. fundyense* AFNFA3 with the nearly identical sequence polymorphisms. Thus, we suggest that *A. fundyense* and *A. tamarense*, of which discrimination relies on solely the presence or absence of the vp (Balech, 1995), are actually conspecific. Hansen *et al.* (2003) also showed *A. angustitabulatum* Taylor and *A. minutum* Halim, which are

distinguishable depending on the presence or absence of the vp (Balech, 1995), had high genetic homogeneity and suggested that they are conspecific. Considerable variation of the 1' of *A. taylorii* Balech with and without the vp was also reported from the type locality (Delgado *et al.*, 1997).

A. catenella, A. fundyense and *A. tamarense* were previously reported to comprise a strongly supported terminal taxon ramifying into distinct ribotypes in the LSU rDNA phylogeny (Scholin *et al.*, 1994). To prove validity of the ribotypes as morphologically and genetically monophyletic, however, further taxonomic and phylogenetic studies on *A. acatenella* (Whedon and Kofoid) Balech, *A. excavatum* (Braarud) Balech and Tangen, *A. tropicale* Balech and toxic *A. tamarense* CU13, which are morphologically difficult to distinguish from *A. tamarense* (Balech, 1995), are required; it is necessary to confirm whether these species are distinct morphospecies with significant genetic divergence or morphological variants inhabiting distinct ecological niches.

Geographical distribution

Korean *A. tamarense* and *A. catenella* (= *A.* sp. cf. *catenella*) isolates were distributed at all sites of the Korean coasts that we sampled. *A. tamarense* isolates belonging to the North American ribotype were observed in all three major coastal waters of Korea; and *A. catenella* isolates belonging to the temperate Asian ribotype were present along the southern coasts and off the coasts of the South Sea (Fig. 4). However, because of high sequence homogeneity, it was uncertain whether the North American populations were introduced into Korea by natural dispersal during the last geological period or by recent human activities (Scholin *et al.*, 1994; 1995). Likewise, it was also difficult to assert that the temperate Asian populations are endemic (Scholin *et al.*, 1994). The LSU rDNA sequences of Chinese, Japanese, Korean *A. catenella* and/or *A. tamarense* populations belonging to the temperate Asian ribotype (Scholin *et al.*, 1994; Scholin and Anderson, 1996; Yeung *et al.*, 1996; Lee *et al.*, 1998) showed high homogeneity and also produced the shorter PCR product. Recent reports of Chilean *A. catenella* isolates characterized by this additional PCR product (Uribe *et al.*, 1999) and of French *Alexandrium* sp. X3 containing the same site-specific deletion of 87 bp extended enormously the scope of distribution of the temperate Asian populations. The true origin of the temperate Asian ribotype has not yet been confirmed, but the ribotype certainly is not limited to temperate Asia.

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