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Genetic Characterization based on Partial 28S rRNA Gene Sequence of Korean Two Scallops

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= 국문요약 =

한국산 가리비 2종의 28S rRNA 유전자 염기서열에 의한 유전적 특성

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한국산 가리비, 큰가리비(*Patinopecten yessoensis*)와 주문진가리비(*Chlamys swifti*), 2종에 대한 28S ribosomal RNA 유전자의 PCR-산물을 이용 RFLP 및 염기서열을 밝히고, 이미 보고된 2과 3종의 염기서열과 상동성을 비교 분석하였다. 그 결과, 28S rRNA 유전자를 이용하여 7가지 제한효소를 처리한 PCR-RFLP의 종간 차이에서 *Taq* I 제한효소에서만 차이를 볼 수 있었다. 한편 두 종간에 28S rRNA 유전자의 D1 부위의 염기서열에서 231개 부위 중 14군데에서 변이를 보였다.

Key words: Scallop, 28S rRNA, RFLPs, Sequence, *Patinopecten yessoensis*, *Chlamys swifti*, Korea

Introduction

Scallops are among the best known shellfishes and are widely distributed through out the world. They are of worldwide economic importance and support both commercial fisheries and mariculture efforts. They have been the subject of numerous research efforts and their high economic stature encourages aquaculture efforts by both academicians and industrial researchers (Adamkewicz & Castagna, 1988; Beaumont & Gruffydd, 1974; Fevolden, 1989).

Characters of soft anatomy in mollusks are used to separate the families, subfamilies and, in a few cases, genera and species. However, there remains considerable confusion over species recognition and relationships among of Pectinidae because soft anatomy differences are few. This study was designed to address this question at the molecular genetic level.

The chromosome numbers of 13 pectinid species have been reported and although most species have 2n=38, there is considerable variation in chromosome number within the group and particularly in the genus *Chlamys*, *C. varia*, *C. farreri farreri*, *C. distorta* and *C. islandica* have 2n=38 while *C. nobilis* has 2n=32 and *C. glabra* has n=14 (Bermont & Gruffydd, 1974; Ieyama, 1975; Wada, 1978; Komaru & Wada, 1985).

Much effort has been devoted to the study of the population genetics of scallops using protein variation

(Marthers, 1975; Beaumont & Beveridge, 1984; Foltz & Zouros, 1984; Beaumont *et al.*, 1985). Electrophoretic studies have been used to investigate genetic diversity among genera of bivalve molluscs and to support species identity among sympatric populations (Hedgecock & Okazaki, 1984). However, there is still some debate and conflict in resolving species identify on the grounds of genetic similarity, particularly when these data are combined with information on hybridization between species (Gosling, 1992a, b). Gosling (1992a) suggested that the solution lies in deciding how much hybridization is permissible before two taxa can no longer be regarded as separate species.

Techniques in molecular biology are also useful in clarifying inter- and intraspecific relationships (Barker, 1989). So far, few molecular sequence data have been used to resolve scallop phylogenetic. Recently, Roche *et al.*, (1990) discriminated in *Placopecten magellanicus* using mitochondrial sequence data. They reported that the scallop *P. magellanicus* exhibited large inter- and intraindividual length variation owing to the varying copy number of a repeated element.

Genomic DNA analysis has an advantage over biochemical analysis of gene products in identifying species according to genotypic variations. Recently, Restriction fragment length polymorphism using the DNA polymerase chain reaction (RFLP-PCR) technique has been developed for molecular genetic identification and examination of the genetic diversity in population (Bowditch *et al.*, 1993; Liu & Mitton, 1993). Hillis and Dixon (1991) noted that, if chosen carefully, many divergent domains in the gene coding for large subunit ribosomal RNA (28S rRNA) are useful for reconstructing relatively recent events.

For this reason, In this study, genomic DNA analysis was carried out using an 28S rRNA probe to compare RFLPs between *Patinopecten yessoensis* and *Chlamys swifti*, which have different geographical origins. I also present preliminary results of phylogenetic study of scallops using partial nucleic acid sequences from the 28S rRNA gene. Aligned sequences were compared with sequence data of other molluscs and animal species to determine homology.

Materials and Methods

1. Source of scallop material

Patinopecten yessoensis (Jay), 1857 and *Chlamys swifti* (Bernardi, 1858) from east coast of Kangwon Do in 1996 were used as reference samples in the study. DNA was extracted from frozen, and freshly obtained isolates by a standard method using SDS and proteinase K treatment, phenol/chloroform extraction, and ethanol precipitation (Sambrook *et al.*, 1989).

2. Sequencing of 28S rRNA

Oligonucleotide primers JB9 and JB10 were designed based on evolutionarily conserved regions detected in aligned eukaryote sequences for the 5' end of the 28S rRNA gene. Forward primer JB10 (5' GATTACCCGCTGAACTTAAGCATAT 3') corresponds to positions 21-45 of 28S rRNA gene of mouse and reverse primer JB9 (5' GCTGCATTCACAAACACCCCGACTC 3') corresponds to positions 278-302 (Qu *et al.*, 1988). The PCR amplication of approximately 300 bp 28S 5' region was performed with DNA from a simple representative of each available scallop species, including *P. yessoensis* and *Ch. swifti* from East coast of Korea. Single-stranded DNA template was prepared by alkaline denaturation and sequenced using the Sequenase kit. Sequences were visually aligned and sequence difference values were calculated for each possible pairwise comparison.

3. PCR-product analysis

2% Metaphor agarose (FMC Bioproducts, Rockland, ME) gel electrophoresis was performed to analyze polymerase chain reaction (PCR) - restriction fragment length polymorphism of the 5' 28S rRNA gene sequences using the restriction endonuclease. Genomic DNA was digested with seven different restriction endonucleases, *Alu* I, *Msp* I, *Hae* III, *Mbo* I, *Cfo* I,

Rsa I and *Taq* I. PCR conditions were as follows: 95 ℃ for 1 min. initially, 95℃ for 20 sec (denaturation), 55℃ for 30 sec (annealing) and 72℃ for 30 sec (extension), then for 72℃ 6 min at the end of all 40 cycle. Generally, 10 μl of unpurified PCR product was used, the total volume was increased to 50 μl by the addition of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) and no adjustments were made to digestion buffers to allow for the presence of PCR buffer. Fragments were separated by agarose gel electrophoresis on a 2% (w/v), TBE agarose gel stained with ethidium bromide.

4. Sequencing data analysis

Sequencing data were aligned using Clustal V program (Higgins *et al.*, 1992), and then highly divergent regions that could not be reliably aligned were excluded from the analyses. Phylogenetic analyses were performed using both parsimony and distance methods. In parsimony method, the branch and bound search option in PAUP 3.1.1 (Swofford, 1993) with 100 bootstraps was used.

Results

The analysis of PCR-RFLP was performed to distinguish two scallops from east coast of Korea. Blots using both species with seven restriction endonucleases-digested displayed similar but distinct patterns in the migration of rRNA sequences between *P. yessoensis* and *Ch. swifti* DNAs (Fig. 1 lanes 1-7, respectively). Six of seven restriction endonucleases, *Alu* I, *Msp* I, *Hae* III, *Mbo* I, *Cfo* I, and *Rsa* I, yielded the same patterns in both species (Fig. 1). One enzyme, *Taq* I, detected one different band. *Taq* I-digested in *P. yessoensis* DNA resulted in an additional 50 bp rRNA fragment that was not present *Ch. swifti* DNA(Fig. 1A, 7).

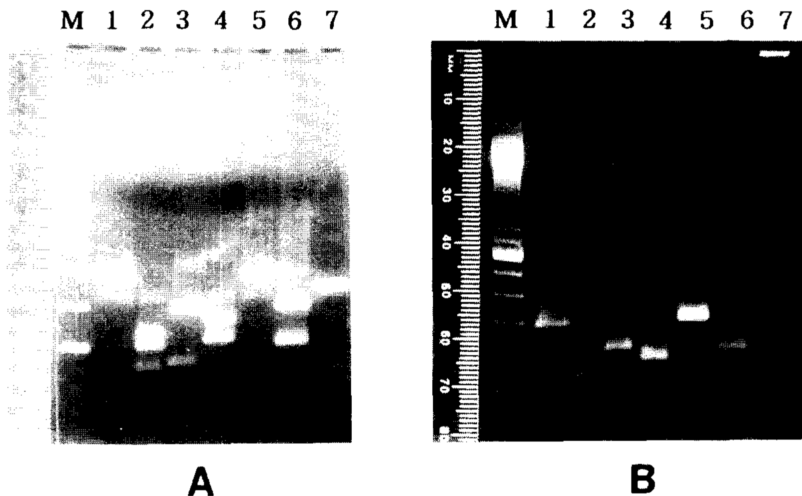

Fig. 1. DNA gel electrophoretic patterns of 2% Mataphor-agarose PCR-RFLP for 5' 28S rDNA gene using the restriction endonucleases. Running at 40 min in 100V (Mulpid-2 Kit). Lane M, molecular weight markers(0.1 μg/μl, 100 bp DNA ladder, 1GIBCO BRL); lane 1: Alu I ; lane 2: Msp I; lane 3: Hae III; lane 4: Mbo I; lane 5: Cfo I; lane 6: Rsa I; lane 7: Taq I. bp: basepair. (10 μl of unpurified PCR product, 2-3 hrs incubation) A: *Patinopecten yessoensis;* B: *Chlamys swifti*

Fig. 2. Sequence comparison of the aligned partial of 5' 28S rRNA gene of five species in the D1 region. The nucleotide positions were numbered from the 5'-terminus. A dashes represent gaps in the alignment. The underlining aligned sequences were compared with sequence data of other molluscs, Ostreidae(*O. edulis* and *C. gigas*) and Mytilidae(*M. edulis*), for homology. Number of total nucleotide was 231 bp. The first nucleotide position corresponds to position 43 of the mouse 28S rRNA gene (Hassouna *et al.*, 1984).

Fig. 2 shows aligned sequence of the D1 region for each of the scallop species examined and with previously reported bivalves. Sequence for the related scallop is included for comparison. Nucleotide sequence differences for the various pairs of scallop are presented in Table 1. For this gene segment, interspecies differences for recognized species within the scallops was 14 bp nucleotide. Parsimony analysis of the aligned sequences using the branch and bound option yielded a single most parsimonious tree shown in Table 1. The distances between the various scallop interspecies are not significantly greater than some interspecies distances within the genera *Patinopecten* and *Chlamys*. Bootstrap analysis showed strong support for three clades in both distance and parsimonious trees: a clade grouping two species of Pectinidae (100%) and a clade grouping two species of Ostreidae (100%) (Fig. 3).

In the D1 region (sequence position number 1-231), the two species of scallop are distinguished by the insertions and/or deletions of 14 base pairs. The results indicates interspecific differences between the two species. Pairwise distance between taxa for the 5' 28S rRNA region was 0.036 (Table 1).

Discussions

Scallops have been cultured successfully in a number of geographic locations. The eight species in three different genera of marine clams belonging to the family Pectinidae have been reported from Korea (Kwon *et al.*, 1993).

In recent years, molecular techniques have been used to the systematics for more objective

phylogenetic analysis. In particular, DNA sequence data provide powerful tools to deduce the phylogenetic relationships among various organisms (Hassouna *et al.*, 1984; Littlewood, 1994; Smith *et al.*, 1992). Although this technique does not necessarily detect all genetic variation of DNA, it is much simpler than complete DNA sequencing of the whole genome and thus has a great utility for population genetics and evolutionary studies.

Application of mitochondrial DNA has already generated important information on the rate of nucleotide substitution in evolution and genetic structure of populations (Shah & Langley, 1979). Nei & Tajima (1981) have been reported that the nucleotide sequence of DNA often changes due to deletion and insertion, particularly in noncoding regions of DNA. In higher animals, most changes of mitochondrial DNA seem to result from nucleotide substitution, but, in nuclear DNA, deletion and insertion play an important role.

Sequence variation in the 28S rRNA and polymerase chain reaction - restriction fragment length polymorphism (RFLP) pattern differences were used as markers of genetic identity. In this study, PCR-RFLP analysis was used for studying the relationship between *P. yessoensis* and *Ch. swifti*. No intra-specific variation was detected and one of seven restriction enzymes revealed RFLPs of rRNA between the two species when rRNA was used as a probe. Data from restriction maps of rDNA genes have been used to construct phylogenetic trees (Hillis & Davis, 1986). The scallop species studied here have too few variable sites for a meaningful phylogenetic of the seven enzymes used.

A region encompassing the D1, D2, and part of the D3 domains (Hassouna *et al.*, 1984) of the gene coding for large subunit ribosomal RNA (28S rRNA) was chosen for sequencing after preliminary results suggested that a resolved phylogeny could be achieved with this gene. Nuclear 28S rRNA sequence data for homology with two species including two species of Ostreidae and one of Mytilidae with previous studies were analysed together to clarify the relationships of them. Of the 231 sites that varied, in this study, 14 were different sites. Molecular data from domains D1 region of the 28S rRNA gene supply sufficient phylogenetic information to determine systematic relationships among the extant scallop taxa. These conclusions are strongly supported among family by high bootstrap values for both distance and parsimony analyses.

A measure called 'nucleotide diversity' has been

Table 1. Pairwise distances between taxa for the 5' 28S rRNA gene D1 region

Species	1	2	3	4	5
1. *Ch. swifti*	-	0.065	0.233	0.205	0.233
2. *P. yessoensis*	14	-	0.208	0.191	0.207
3. *O. edulis*	52	46	-	0.191	0.048
4. *M. edulis*	45	42	43	-	0.213
5. *C. gigas*	52	48	11	48	-

Below diagonal represent absolute distances and above diagonal represent mean distances

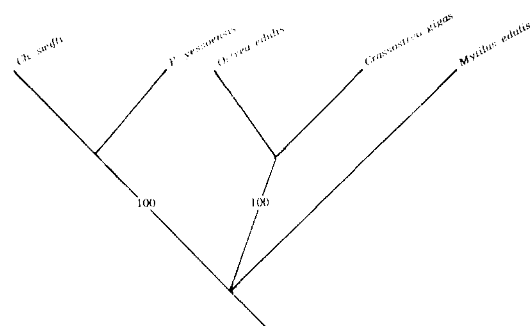

Fig. 3. The one most-parsimonious tree obtained using 28S rRNA gene sequences.
Branch lengths and ranges of boot strap values obtained using statistical package number on branches indicate the percentage of 100 bootstraps supporting the branching pattern shown (Power Macintosh 6100/66, PAUP Ver. 3.1.1.).

proposed to express the degree of polymorphism in a population at the nucleotide level (Nei & Tajima, 1981). From these results, the relationship between two species are considered to have close similarity at the species level. It is necessary to accumulate further data in nuclear DNA to compare nucleotide diversity among various molluscs.

Summary

In this study, the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) of 28S ribosomal RNA were compared between *P. yessoensis* and *Ch. swifti* using seven different kinds of restriction endonucleases. No intra-specific variation in six restriction enzyme fragment profiles was shown within both species. One enzyme, *Taq* I, detected one different band.

Partial sequences of 28S rRNA were amplified using PCR and sequenced for two species. Of the 231 sites that varied, 14 were different sites. Molecular data from domains D1 region of the 28S rRNA gene supply sufficient phylogenetic information to determine systematic relationships among the extant scallop taxa.

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