PD - 13

Genetic Differences of Two Wild Shortnecked Clam (*Ruditapes philippinarum*) Populations from the Yellow Sea Analysed by Random Amplified Polymorphic DNAs-Polymerase Chain Reaction

Jong-Man Yoon* and Yong-Ho Kim†

*Department of Marine Biomedical Science, College of Ocean Science and Technology, Kunsan National University, Kunsan 573-701, Korea

† Department of Marine Aquaculture and Biotechnology, College of Ocean Science and Technology, Kunsan National University, Kunsan 573-701, Korea

INTRODUCTION

Shortnecked clam is a commercially important mollusks species, which is distributed all over the Yellow Sea. Consequent of the rapid increase in seed production, there is a need to understand the genetic composition of wild shortnecked clam populations in order to evaluate exactly the latent genetic effects induced by seed production operations. Many genetic and molecular researches were made because RAPD-PCR is a simple and rapid method for determining genetic diversity and similarity in various organisms with the advantage that no prior knowledge of the genome under research is needed (Fischer et al., 2000). In this study, the research was made by RAPD-PCR using two decades of random primers, by bandsharing analysis and also by single linkage cluster analysis in order to identify genetic distances in two populations in wild shortnecked clam (*R. philippinarum*) from the Yellow Sea.

MATERIALS AND METHODS

Muscle collection, sources of genomic DNA, primer, marker, amplification conditions and analytical method

RAPD-PCR analysis was performed on genetic DNA samples from a total of 22 shortnecked clam using two decades of different random decamers. The cleared lysates were extracted with 2 volume of ice-cold 70% ethanol, then centrifuged at 6,289 g for 5 min, then precipitated. The primers, designed for other purpose and chosen arbitrarily for these experiments, were obtained from Operon Technologies, USA. All of these decamer random primers had a G+C content in the range 60-70%. The genomic DNAs were amplified using PCR with two decades of 10-base primers (5′ to 3′) in a DNA Thermal Cycler (Perkin Elmer Cetus, USA). Amplification products were separated by electrophoresis with ΦX174 DNA/*Hae*Ⅲ marker (Promega Co., USA) in 1.4% agarose gels with TBE. An average of whin-population similarity is calculated across all pairwise

comparisons between individuals within a population. Single linkage cluster analysis was performed on the similarity matrices in order to generate a dendrogram using pc-package program Systat version 10 (SPSS Inc., USA). Genetic distances within and between populations were calculated with dendrograms produced with Systat version 10.

RESULTS AND DISCUSSION

In this study, as investigated the genetic differences of two populations of shortnecked clam by RAPD-PCR method and bandsharing analysis, there were identified a number of polymorphic/specific bands generated using six random primers (OPA-08, -09, -11, -16, -18 and -20) to amplify DNA isolated from the muscle of 22 individuals from two sites. It were used DNAs extracted from shortnecked clam muscle which had the genome sizes of from larger than 50 to less than 1,500 bp, as shown in Fig. 1. The band patterns of Anmyeondo population (lanes 1 ~ 11) were considerably varied in comparison to those from Seocheon (lanes 12 ~ 22). In the concrete, a total of 1,111 amplification products were produced of which 533 were polymorphic (48.0%). Random primer OPA-11 produced a very high level of total polymorphic bands and average number of polymorphic bands (66 and 6.0, respectively) in population from Anmyeondo. The bandsharing values altered from 0.155 to 0.684 in population from Anmyeondo and also from 0.143 to 0.782 in population from Seocheon. the single linkage dendrogram resulted from three primers such as OPA-08, -09 and -20), indicating six genetic groupings composed of group 1 (No. 4, 8 and 10), group 2 (No. 18), group 3 (No. 2, 5 and 7), group 4 (No. 1, 3, 6, 9, 11, 12, 13, 14, 15 and 17), group 5 (16, 19 and 20) and group 6 (No. 21 and 22). The genetic distances between two populations ranged from 0.094 to 0.275. Especially, the genetic distance between individuals No. 22 and the remnants among individuals in two geographical populations was highest (0.275). These results illustrated that individual No. 22 be distinct from other individuals within two shortnecked populations. The geographical distance (120 km) between two sampling sites displayed significant molecular differences based on RAPD method.

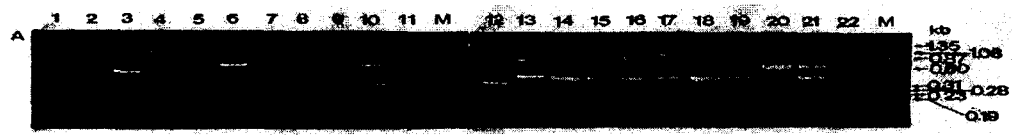

Fig. 1. PCR-RAPD fragments of shortnecked clam amplified by arbitrary primer OPA-08 (A). Each lane (1~22) shows different individual DNA samples (lanes 1~11 from Anmyeondo and lanes 12~22 from Seocheon).

REFERENCES

Fischer M, Husi R, Prati D, Peintinger M, Kleunen M V and Schmid B 2000 RAPD variation among and within small and large populations of the rare clonal plant *Ranunculus reptans* (Ranunculaceae). American J. Botany, **87 (8)**: 1128-1137.