Genetic Differences and DNA Polymorphism in Oyster (*Crassostrea* spp.) Analysed by RAPD-PCR

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INTRODUCTION

Seven primers were used generating a total of 481 scorable fragments in oyster (*Crassostrea gigas*) population from Buan and 264 in population from Geojedo, respectively, ranging in size of DNA fragments from larger than approximately 50 to less than 1,600 bp. The complexity of the banding patterns varied dramatically between primers and/or geographically separated locales. The only oligonucleotide random primer OPA-09 generated identical DNA fragments whose sizes of approximately 280 bp between oyster population from Buan and population from Geojedo. Primer OPB-07detected 44 identical major and/or minor bands in molecular weight, 50, 320, 400 and 600 bp, which were present in every individuals. Especially, this primer produced the highest number of fragments (a total of 92) in comparison with other primers used, with an average of 8.4. Also, bandsharing values between two oyster populations ranged from 0.116 to 0.494, with an average of 0.282 ± 0.008. As compared separately, the bandsharing values of individuals within oyster population from Buan were comparatively higher than those of individuals within population from Geojedo. The dendrogram resulted from reliable four primers, indicating three genetic clusters composed of group 1 (Case No. 01, 03 and 02), group 2 (Case No. 11, 06, 10, 08, 09, 07, 05 and 04) and group 3 (Case No. 14, 15, 18, 17, 12, 21, 13, 22, 16, 20 and 19). The genetic distances between two geographic populations ranged from 0.039 to 0.284.

MATERIALS AND METHODS

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

RAPD-PCR analysis was performed on genetic DNA samples from a total of 22 oyster 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).

RESULTS AND DISCUSSION

In the present study, seven decamer primers generated a total of 481 fragments in oyster population from Buan and 264 in oyster population from Geojedo, of which 143 polymorphic fragments (29.7%) in population from Buan and 60 (22.7%) in oyster population from Geojedo, respectively. The oligonucleotide random primer OPA-09 generated identical DNA fragments whose sizes of approximately 280 bp between oyster population from Buan and population from Geojedo. The similarity matrix based on the average bandsharing values of all of the samples within oyster population from Buan ranged from 0.493 to 0.879, whereas 0.244 ~ 0.889 within population from Geojedo. The average bandsharing value within oyster population from Buan showed 0.639 ± 0.013, whereas 0.537 ± 0.017 within population from Geojedo. Also, bandsharing values between two oyster populations ranged from 0.116 to 0.494, with an average of 0.282 ± 0.008. As compared separately, the bandsharing values of individuals within oyster population from Buan were comparatively higher than those of individuals within population from Geojedo. The average level of genetic difference was also approximately 0.710 ± 0.009 between two oyster populations. Accordingly, PCR analysis generated on the RAPD data showed that the oyster population from Buan in the West Sea was more or less separated from oyster population from Geojedo in the South Sea.The RAPD-PCR method using arbitrary primers was applicable to identify three endemic Spanish barbel species (Callejas and Ochando, 1998). Also, there were population-related RAPD fragments in catfish and there were differences in frequencies of six primer fragments, as have been reported in catfish (Liu et al., 1998). The percentages of polymorphic bands of the five geographic populations investigated in black tiger shrimp (*Penaeus monodon*) varied from 51.5 to 57.7% (Tassanakajon et al., 1998). Two primers yielded the highest level of polymorphism, which was 88.9% in black tiger shrimp.

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