E-11

Amplified Fragment Length Polymorphism Fingerprinting Analysis in the Three Populations of Olive Flounder (*Paralichthys olivaceus*)

방인철 · 이윤아 · 남윤권* · 김동수*
순천향대학교 생명과학부, *부경대학교 양식학과

Instroduction

Several types of markers have been used to assess genetic diversity and phylogenetic relationships of populations. DNA-based molecular marker, RFLP, RAPD, AFLP and microsatellite have been valuable tools for detecting patterns of DNA polymorphism among several species (Mackill, 1995). Especially, AFLP technique, one of the molecular marker technologies is a very powerful technique for marker-assisted breeding and genome mapping (Vos et al., 1995). Also this is very effective technique in detecting genetic variation since it can generate many marker bands in short time. This study was conducted to investigate genetic variability and to develop the DNA marker of thethree populations in olive flounder and to estimate phylogenetic relationships among the three population using a new AFLP technique.

Materials and methods

Genomic DNA was digested with a particular combination of two restriction enzymes with 4 base (Mse I) and 6 base (EcoR I) recognition sites, ligated to restriction specific adapters. Fifteen primer combinations were used for selective amplification. The PCR products of selective DNA fragment were runed at 5% denaturing polyacrylamide sequencing gel. The gel was stained with silver nitrate according to the manufacturer's instructions (Promega, USA). AFLP fingerprints were scored visually. Two different tyes of data files were made. In one type the presence or absence of polymorphic DNA fragments was given in binary characters (1 or 0). these were used as input files for genetic cluster analysis by UPGMA using the software program NTSYS-pc.

Results and discussion

In the result, the total number of scorable eastern coast, western coast and Japanese population AFLP bands were detected 627, 625 and 611 markers respectively. Also 191, 200, 158 bands were polymorphic bands and polymorphism is 31%, 32%, 25% respectively. In the eastern coast population, E/ACA-M/CAC primer combinations showed a high level of polymorphism with 0.43. E/ACG-M/CAC primer combinations were effective for AFLP analysis of eastern coast population and Japanese population.

Espacially, two bands (0.4~0.5 kb) identified in E/ACT-M/CAT primer combination were found to be unique in eastern coast population. Therefore, these AFLP DNA bands could be used as putative breed-specific DNA markers for eastern coast population identification.

In comparison of genetic distances, Eastern coast population was the most closely related to the western coast population. And western coast population was the most far related to the Japanese population.

References

Mackill, D. J., Z. Zhang, E. D. Renona and P. M. Colowit, 1996. Level of polymorphism and genetic mapping of AFLP markers in rice. *Genome* 39: 969-977.

Osten, M., M. Den Bieman, M. T. R. Kuiper, M. Pravenec, V. Kren, T. W. Kurtz, H. J. Jacob, E. Lankhorst and B. F. M. Van Zutphen, 1996. Use of AFLP markers for gene mapping and QTL detection in the rat. *Genomics* 37: 289-292.

Roa, A. C., M. M. maya, M. C. Duque, J. Tohme, A. C. Allem, M. W. Bonierbale, 1997. AFLP analysis of relationships among *Cassava* and other *Manihot* species. *Theor. Appl. Genet.* 95: 741-746.

Vos, P., R. Hodgers, M. Bleeker, M. Reijans, T. van de Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman, M. Kuiper, M. Zabeau, 1995. AFLP, a new technique for DNA fingerprinting. *Nucleic Acids Res.* 23: 4407-4414.