SOMATIC AND GONADAL MOSAICISM DETECTION IN CULTURED FISH BY RAPD-PCR

Irem Uzonur [1]*, Kubra Sari [1], Tugba Senel [1] and Arzu Erol [1]
[1] Fatih University, Biology Department - iuzonur@fatih.edu.tr

Abstract

DNA samples of organs and tissues from cultured fish: *Oncorhynchus mykiss*, *Sparus auratus* and *Dicentrarchus labrax* have been analyzed by PCR amplification with random primers. Among the 20 random 10-mer nucleotide primers tested, four that yield stable, well reproducible profiles of amplification products were chosen for further genome-wide mosaicism analysis. With these primers, the differences in the RAPD profiles of some tissues were detected in several individuals. These differences were associated with the modification of mobility or with the gain/loss of the fragment in the RAPD profile and could be caused by either genomic rearrangements or mutations involving the regions of DNA-primer pairing. Different epigenetic factors may also contribute to this process.

Keywords: Aquaculture, Fisheries, Fishes, Genetics, Monitoring

Introduction

The random amplified polymorphic DNA (RAPD) method has initially been used to detect polymorphisms in genetic mapping, taxonomy and phylogenetic studies and later genotoxicity and carcinogenesis studies. Despite its extensive use, this technique has also attracted some criticisms, mainly for lack of reproducibility [1]. RAPD assays have a great potential for the detection of DNA effects, alterations and damages including DNA adduct formations, breaks, point mutations, large rearrangements, and other changes, such as structural distortions induced by chemical or physical agents following the direct and/or indirect interactions with the genomic DNA. It has proven to be an efficient molecular tool for the identification of differences among natural populations, when provided with reliable reference samples [2-4]. Molecular evaluation of DNA effects by RAPD profiling can be a method capable of identifying environmental threats earlier than the used standard methods. Traditional approaches to the assessment of the impact of pollution on aquatic communities have generally been based on ecological criteria, such as changes in biomass, diversity and in species composition, whereas genetic changes may occur in natural populations as well as aquacultured organisms exposed to pollution. Many of the pollutants found in aquatic environments are known to be genotoxic and carcinogenic, and may interact, directly with DNA or after metabolic activation [5, 6]. Genotoxicity is an organism-specific and quantitative measure of the potential of a particular environment that causes damage to a cell's DNA. Target organ genotoxicity in this context can be defined as how target organs' DNA responds to genotoxicants [3, 4].Somatic mosaicism and gonadal mosaicism as an extension of the phenomenon imply the presence of genetically different cell lines in a single organism. Our work relies on RAPD analysis to show the extend of the somatic and gonadal mosaicism in freshwater cultured *Oncorhynchus mykiss* and mari-cultured *Sparus auratus* and *Dicentrarchus labrax* to be a basement for further epigenetic and genetic work that is to be done to improve fish-culture work quality.

Materials and Methods

A comparative analysis was carried using RAPD assay to assess the genotypic differences among the same fish's various organs/tissues. Furthermore, inter and intra-tissue variations were assessed together by an improved RAPD approach proposed in this work to detect target organ genotoxic effects on various organs and tissues of aquacultured fish: *Oncorhynchus mykiss*, *Sparus auratus* and *Dicentrarchus labrax*. Genomic DNA was isolated from dissected organs of the three mentioned cultured fish. Their genetic diversity has been assessed by RAPD-PCR and intra-specific genetic variation detection due to intrinsic and extrinsic factors realized as somatic and gonadal mosaicism were shown with xn replicates of RAPD-PCR using primers OPA-8 and OPB-18. The combination of these methodologies enabled the detection of various RAPD profile changes in the DNA as a mosaicism of tissues/organs extracted separately, when the RAPD-PCR mastermix with the same organ's/tissue's DNA was aliquoted into multiple tubes and thoroughly assayed together under identical conditions.

Results and Discussion

Preliminary data obtained in this work show that the RAPD profile changes of various tissues (Figure 1, liver, muscle, gonad respectively for two different primer OPA-8 and OPB-18) of the same aquacultured fish can easily and reliably be monitored for various DNA effects described as somatic and gonadal mosaicism which might be an early indication for the heritable in the case of gonadal mosaicism and genotoxic, mutagenic and carcinogenic potentials of the cultured fish populations with variable target organ genotoxicities detected as somatic mosaicism.

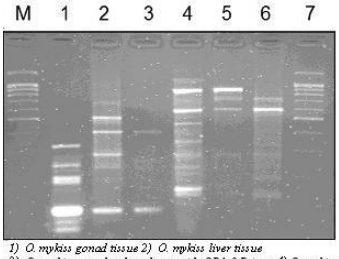

Fig. 1. Comparison of various tissue DNA mosaicism with two different 10-mer primers; OPA-8 and OPB-18.

References

1 - Atienzar F.A., Venier P., Jha A.N. and Depledge M.H., 2002. Evaluation of the Random Amplified Polymorphic DNA (RAPD) Assay for the detection of DNA damage and mutations. *Mutat. Res*., 521, 151-163.

2 - Uzonur I., Abasiyanik M.F., Bostanci B., Eyidemir M., Ocba N., Yanik C. and Petek M., Re-exploring planaria as a model organism for genotoxicity monitoring by an 'Improved Random Amplified Polymorphic DNA' approach, *Fres. Environ. Bull*., 13, 12a, 1420-1426, 2004a.

3 - Uzonur I., Abasiyanik M.F., Cam S., Cobanli K., Elmas A., Erdogan H., Hizal S., Karabulut D.S., Ozdemir M., Yesil F.A. and Petek M., A Preliminary report on target organ genotoxicity biomonitoring by an 'Improved Random Amplified Polymorphic DNA Assay'. *Fres. Environ. Bull*., 13, 12a, 1453-1456, 2004b.

4 - Uzonur I., 2005. Genotoxicity monitoring with Improved RAPD Assay on *Mytilus galloprovincialis* as a model. PhD Dissertation, Istanbul University, Institute of Marine Sciences and Management, Physical Oceanography and Marine Biology Department, Istanbul.

5 - Nadig, S. G., Lee, K. L. and Adams, S. M., 1998. Evaluating Alterations of Genetic Diversity in Sunfish Populations Exposed to Contaminants Using RAPD Assay, *Aquat Toxicol*, 43.

6 - Butovskaia, P. R., G. V. Pavlova, et al., 2009. "Somatic mosaicism in mice detected using the RAPD-PCR method." Mol Gen Mikrobiol Virusol(1): 3-7.