PH-6

DNA Polymorphism and Genetic Similarity in Cultured Catfish by Polymerase Chain Reaction-Random Amplified Polymorphic DNAs

Jong-Man Yoon · Kwan-Ha Park and Sung-Woo Park
Department of Marine Biomedical Science, Kunsan National University,

ABSTRACT

Out of 20 primers, 6 generated 1349 highly reproducible RAPD markers, producing approximately 5.2 polymorphic bands per primer in catfish(*Silurus asotus*) population from Kunsan. The electrophoretic analysis of polymerase chain reaction-random amplified polymorphic DNAs (PCR-RAPD) products showed the middle levels of similarity between different individuals in population from Kunsan. That is to say, the degree of similarity varied from 0.40 to 0.54, with the average of 0.46, as calculated by bandsharing analysis. The RAPD outlines obtained with DNA of different two catfish populations from Kunsan and Yesan were more or less different(0.35 and 0.48, respectively).

INTRODUCTION

DNA markers that are shown to be genetically linked to a trait of interest can be used for gene cloning, pathological diagnostics, and for trait estimate in fish breeding programs. Also, a genetic linkage map in fish is needed to improve the efficiency of breeding by marker-assisted selection and for the identification of economically important genes such as disease resistance genes. There were so far used various molecular biological methods including restriction fragment length polymorphism(RFLP)(Geldermann and Ellendorff, 1990), randomly amplified polymorphic DNAs (RAPD)(Simpson et al., 1993), and microsatellite (MS)(Taggart et al., 1995) based on the polymerase chain reaction. Also, the genetic similarity and polymorphisms were identified by not only RFLP but also RAPD markers (Roche et al., 1997). Especially, most fisheries applications of RAPD's have been at the fish and shellfish species level. More recently RAPD techniques provided a new means for species or strain identification of microorganisms in aquaculture science(Oidtmann et al., 1999). In this study, DNAs isolated from cultured catfish were analyzed by 20 random amplified polymorphic DNAs (RAPD) primers in order to identify the genetic similarity and diversity of populations.

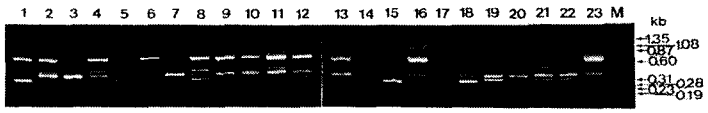
MATERIALS AND METHODS

Cultured catfish(*Silurus asotus*) DNA samples were obtained from a few of aquaculture facilities in the periphery of Kunsan and Yesan(apart from approximately 180km) in Korea. RAPD analysis was performed on genetic DNA samples from a total of 60 crucian carp using 20 different random primers. Amplification was performed in a DNA Thermal Cycler with highest quality reagents to achieve reproducible results. Amplification products were separated by electrophoresis in 1.4% agarose gels with TBE and detected by staining with

ethidium bromide. The gels were illuminated with UV light and taken photographs by photoman direct system. If the comparison between the three lanes, the formula would be: BS=3(Nabc)/(Na+Nb+Nc) and so forth.

RESULTS AND DISCUSSION

The random primer OPA-15 showed genomic DNA fingerprints generated using a primer to amplify DNA isolated from the blood of 23 individuals from Kunsan(lanes 1~12) and Yesan(lanes 13~22)(Fig. 1). The specific bands from 0.19 to 0.31kb were present, which were polymorphic. This primer also produced the sizes of polymorphic DNA bands ranged not only from greater than 0.31 to 0.60kb but also from greater than 0.87 to 1.35kb. The number of polymorphic RAPD fragments was shared between individuals from catfish population. A total of 1349 amplification products were produced of which 224.8 were polymorphic (52.9%). 5.2 of the 31.4 amplified bands were found to be polymorphic. A high level of genetic variation within the populations to deemed sufficient to separate individuals within population. In other words, bandsharing values were calculated as an expression of similarity of RAPD fingerprints of animals from either the same or different breeds. In general, the potential of RAPDs to identify diagnostic markers for strain or species identification in organisms has also been demonstrated. In conclusion, in addition to mapping and breeding applications, PCR-RAPD system could be very useful for the rapid certification and quality control of seed production and for all projects based on PCR amplification of specific fish DNA fragments. Further analysis is required to identify primers that amplify sufficient bands shared by the species to permit a quantitative analysis.



<Fig. 1>. Specific RAPD fingerprints generated in catfish by arbitrary primer OPA-15(TTCCGAACCC). Each lane(1~23) shows DNA samples of different individuals (lanes 1~12 from Kunsan and lanes 13~23 from Yesan). M: Two standard markers(Φ X174 DNA marker digested with HaeIII).

REFERENCES

Geldermann, H., Ellendorff, F., 1990. Genome analysis in doemstic animals. VCH Verlags gesellschaft mbH, Weinheim, Deutschland.

Roche, P., Alston, F. H., Maliepaard, C., Evans, K. M., Vrielink, R., Dunemann, F., Markussen, T., Tartarini, S., Brown, L. M., Ryder, C., King, G. J., 1997. RFLP and RAPD markers linked to the rosy leaf curling aphids resistance gene(*Sd1*) in apple. Theor. Appl. Genet. 94, 528-533.

Oidtmann, B., Cerenius, L., Schmid, I., Hoffmann, R., Söderhäll, K., 1999. Crayfish plague epizootics in Germany-classification of two German isolates of the crayfish plague fungus *Aphanomyces astaci* by random amplification of polymorphic DNA. Dis. Aquat. Org. 35, 235-238.

Simpson, A. J. G., Dias Neto, E., Steindel, M., Caballero, O. L. S. D., Passons, L. K. J., Pena, S. D. J., 1993. The use of RAPDs for the analysis of parasites. DNA fingerprinting, 331-337.

Taggart, J. B., Ferguson, A., 1990. Minisatellite DNA fingerprints of salmonid fishes. Anim. Genet. 21, 377-389.