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DNA Fingerprinting by Amplified Fragment Length Polymorphism Markers in Rainbow Trout(*Oncorhynchus mykiss*)

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ABSTRACT

The objective of the present study was to analyze genetic variation and characteristics in rainbow trout(*Oncorhynchus mykiss*) using amplified fragment length polymorphism(AFLP) method as molecular genetic technique, to evaluate the usefulness of AFLP as genetic markers, and to compared the efficiency of agarose and polyacrylamide sequencing gels. The amplified products were performed by agarose and sequencing gel electrophoresis to detect AFLP band patterns, respectively. Using 9 primer combinations, total of 141 AFLP bands were produced, 108 bands(82.4%) of which were polymorphic in agarose gels. In sequencing gels, total of 288 bands were generated, and 220 bands (76.4%) were polymorphic. The level of bandsharing(BS) ranged from 0.18 to 0.32 for the 9 primer combinations tested, with a mean of 0.24. Consequently, AFLP markers of these rainbow trout could be used as genetic information such as species identification, genetic relationship or analysis of genome structure, and selection aids for genetic improvement of economically importment traits in fish species.

INTRODUCTION

The AFLP technique provides a novel and very powerful DNA fingerprinting technique for DNAs of origin or complexity. Also, AFLP has numerous potential applications such as individual identification, the monitoring of animal and plant breeding, diagnostics of genetic diseases, pedigree analysis and the screening of DNA markers for marker-assisted selection(Blears et al., 1998). Despite the important roles of amplified fragment length polymorphism, applications of PCR-based AFLP to the teleost or shellfish species have so far been a little. Therefore, in this study, genomic DNAs isolated from rainbow trout were digested by restriction enzymes, ligated by adapters and amplified by selective primers in order to measure the AFLP variations, to determine genetic relationships and to detect the intra-species genetic markers.

MATERIALS AND METHODS

Sources of genomic DNA, digestion, adapter ligation, amplification and data analysis

A piece of samples of Rainbow trout(*Oncorhynchus mykiss*) were used as DNA sources for PCR amplification. The cleared lysates were extracted with 2 volume of ice-cold ethanol, then centrifuged for 5 min. at 3,000 rpm, then precipitated. The DNA pellet was air-dried for 30min, and then dissolved 200$\mu\ell$ of TE buffer. Purity and concentration of DNA were

estimated by calculating the ratio of A_{260}/A_{280} measured with a spectrophotometer. In order to achieve reproducible results, DNA extractions should be undertaken with highest quality reagents. The original AFLP protocol developed by Zabeau and Vos was followed with the minimum modifications. Polymorphisms are revealed by analysis of amplified fragments on a denaturing polyacrylamide gel, and comparison of the patterns generated for each sample. Eight selective primers were synthesized to be complementary to the adapter/restriction-site sequences and to carry selective 3' nucleotides. There were detected by staining with ethidium bromide and silver staining kit. Bandsharing(BS) of DNA sequences was quantified using the formula of Jeffreys and Morton(1987): BS=2(Bab)/(Ba+Bb) and so on.

RESULTS AND DISCUSSION

The DNA from rainbow trout was isolated, digested, ligated and preamplified at various times with PCR machines. The high degree of reproducibility of AFLP markers between experiments has been shown in this study and among different laboratories. The amplified products were separated by agarose gel electrophoresis with nine AFLP primer combinations and stained with ethidium bromide(Fig. 1). Substantial amounts of polymorphism were seen for all the primers used. Each sample had unique banding patterns ranged from 0.4 to 1.5kb showing individual identification in agarose gel electrophoresis. Individuals could be distinguished by the presence of unique bands. The characteristics and polymorphisms of AFLP fragments were analyzed by each primer combination by between agarose gel and sequencing gel electrophoresis. A total of 131 AFLP fragments amplified by each AFLP primer pair, ranging in size from 0.5 to 1.5kb. The number of polymorphic loci detected per AFLP primer pair showed an average of 15 polymorphic fragments. Among 131 polymorphic fragments, the 108 fragments account for 82.4% of the total amplified fragments in rainbow trout.

<Fig. 1>. Polymorphic AFLP profiles of rainbow trout amplified with primer combinations (M11+H11) in agarose gel.

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