PI-9

RAPD Analysis of DNA Polymorphism and Genetic Species-Specificity Using PCR Technique in the Marine Microalgae

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ABSTRACT

Genomic DNA was isolated from the marine microalgae representing genetic characteristics and genomic polymorphisms by polymerase chain reaction amplification of DNA as arbitrary primers. The electrophoretc analysis of PCR-RAPD products showed hig levels of variation between different genus and little variation between different species. Outer of these primers, 6 generated 248 highly reproducible RAPD markers, producing almost seven polymorphic bands per primers. The degree of similarity frequency between *Chaetoceros gracilis* and *Chaetoceros calcitrans* species showed 90% as calculated by sharing analysis. The RAPD polymorphism generated by this primer may be used as a genetic marker for genus or species identification in important marine microalgae.

INTRODUCTION

The polymorphic genetic markers proved useful for individual identification, pedigree analysis, genetic relationship, gene cloning, pathological diagnostics, genetic structure of animal populations and marker assisted selection programs. Random primers based on the polymerase chain reaction(PCR) also produced a higher percentage of multiple-band profiles than RFLP probes. In this study, DNA isolated from marine microalgae was analyzed by 20 randomly amplified polymorphic DNA(RAPD) primers in order to identify genetic characteristics and genomic polymorphisms within the genus and to develop the species-specific genetic markers at the genetic and molecular level.

MATERIALS AND METHODS

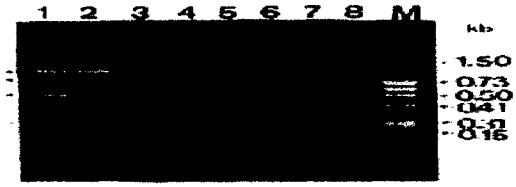
Microalgae collection, genomic DNA extraction, primer and amplification conditions

Microalgal DNA samples were obtained from a aquaculture facility at the Taean Hatchery. Preliminary RAPD analysis was performed on genetic DNA samples from microalgae as a reference species using 20 different random rimers. All glassware micropipette tips, centrifuge tubes, glass pipettes and solutions were autoclaved to avoid the amplification of contaminating DNA. DNA was isolated using a procedure as modified by Vierling et al(1994). In order to achieve reproducible results, DNA extractions should be undertaken with highest quality reagents. Of the 20 arbitrarily selected primers, six random primers were used on the basis of the number and frequency of the

polymorphisms producted. All primers have a 60% or 70% G+C content. Amplification was performed using a procedure as modified by Vierling et al(1994).

RESULTS AND DISCUSSION

When PCR amplification of genomic DNA is performed with short oligonucleotide primers of arbitrary sequence at low annealing temperatures, the amplification products can be generated as DNA fingerprint bands by gel electrophoresis, the patterns being characteristic of both the primers and the template DNA. It is used DNA extracted from *Chaetoceros* and *Pavlova* which have the genome size of from 10^3 to 10^4 bp. Six prime produced amplified fragments which were consistently polymorphic between the different genus. Fig. 1 shows genomic DNA fingerprints generated using 10 different primers to amplify DNA isolated from the *Pavlova* and *Chaetoceros* genus. These primers produc the sizes of polymorphic DNA bands ranged from approximately 310 to greater than 1,500 bp. On average, each random RAPD primer amplified 3.4 bands. The degree of similarity frequency between *Chaetoceros gracilis* and *Chaetoceros calcitrans* species showed 90 calculated by band sharing analysis. Six primers produced amplified fragments which were consistently polymorphic between the different stocks. The potential of RAPDs to identify diagnostic markers for strain identification in mice, in parasites, in livestock, in plants and in fish(Liu *et al*, 1998) has also been demonstrated. There were species-specif RAPD fragments in marine microalgae and there were differences in frequencies of two primer fragments, as have been reported in catfish and livestock.



<Figure>. RAPD fingerprints generated in *Chaetoceros* and *Pavlova* by arbitrary primer P-3. Each lane shows different individual DNA samples. Lane 1, 2: *Pavlova lutheri*, 3, 4: *Chaetoceros gracilis*, 5, 6: *Chaetoceros calcitrans*, 7, 8: *Pavlova gyrans*. M: ΦX174 DNA digested with *Hinf* I as molecular size markers.

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