SHORT COMMUNICATION

NON-CRYOGENIC PRESERVATION ON GROUPER MUSCLE TISSUE FOR DNA ANALYSIS

Andi Parenrengi

ABSTRACT

Sample preservation is one of the problems frequently faced in collecting materials such as muscle tissue and blood samples in the field and during transportation to the laboratory prior to DNA extraction. This study was carried out to evaluate the application of TNES-Urea (Tris-NaCl-EDTA-SDS-Urea) buffer as a non-cryogenic tissue preservative for grouper, *Epinephelus suillus* for Random Amplified Polymorphic DNA (RAPD) analysis. The preserved muscle tissue was kept at room temperature for 3, 6, 12, and 18 months before analysis. Genomic DNA was successfully extracted by the Phenol-Chloroform method from up to 18 months preserved muscle tissue. Clear and consistent RAPD banding patterns were amplified from genomic DNA of the preserved grouper tissue. The study suggests that the TNES-Urea buffer is convenient method for preserving muscle tissue of grouper prior to DNA extraction.

KEYWORDS: tissue preservation, grouper, *Epinephelus suillus*, RAPD

INTRODUCTION

Fresh tissue or blood sample provides the best source of DNA isolation for biological analysis, but when the samples are collected from remote areas, fresh samples are not feasible (Zhang and Hewitt, 1998). Thus, samples have to be preserved before DNA analysis is carried out. Tissue preservation for DNA isolation is usually done by freezing the sample in dry/wet ice or liquid nitrogen (cryogenic preservation). However, these preservation techniques are not suitable for transportation over long periods because the sample sublimates rapidly (Cann *et al.*, 1993) and liquid nitrogen is also classified as very dangerous under International Transport Association and Good Regulations (Seutin *et al.*, 1991). Thus, non-cryogenic preservatives such as DMSO-salt solution and high concentration of urea have been introduced. The high concentration of salts and urea has been used for a tissue or blood preservation at room temperature storage. Seutin *et al.* (1991) reported that high concentration of salts (saturated sodium chloride) is an efficient method in preserving bird tissues and blood samples. High concentration of urea has also been reported to be a convenient technique for tissue preservation of Japanese flounder, *Paralichthys olivaceus* and Atlantic herring, *Clupea harengus* (Asahida *et al.*, 1996). This study represents the first application of a non-cryogenic tissue preservation on grouper for Random Amplified Polymorphic DNA (RAPD) analysis.

Since RAPD analysis has been introduced, the technique has been an important genetic marker for several different applications (Williams *et al.*, 1990). The RAPD technique has been used for phylogenetic studies for different fish species in identification, sex chromosome differentiation, genetic population, genetic inheritance, positional cloning, molecular ecology, and conservation.

This study was carried out to evaluate the application of TNES-Urea buffer as a non-cryogenic tissue preservative on grouper, *Epinephelus suillus* for RAPD analysis.

MATERIALS AND METHODS

Muscle Tissue Preservation

A small amount of fresh muscle tissue (approximately 50 mg) from two samples of grouper (*E. suillus*) was placed in a 1.5 mL microcentrifuge tube containing TNES-Urea buffer preservation. This preservative consists of 6 M urea, 10 mM Tris-HCl, 125 mM NaCl, 10 mM EDTA, and 1% sodium dodecyl sulfate at pH 7.5 (Asahida *et al.*, 1996). The preserved muscle tissues were kept at room temperature in the laboratory for preservation periods of 3, 6, 12, and 18 months prior to DNA extraction.

DNA Extraction

Genomic DNA from the muscle tissue of grouper was extracted using the Phenol-Chloroform method (Parenrengi *et al.*, 2000). In order to compare the genomic DNA obtained from different preservation periods, the genomic DNA was electrophored at a 0.8% (w/v) horizontal agarose gel at 55 volts for 1-2 hours in 1 x TBE (Tris-Borate-EDTA). The gel was stained

*) Researcher at Research Institute for Coastal Fisheries, Maros, Indonesia

with 0.5 mg/mL ethidium bromide for 20-30 minutes and then washed with distilled water for 5 minutes prior to documentation.

DNA Amplification

DNA amplification was performed in a 25-mL volume reaction containing 1x PCR buffer; 4.5 mM $MgCl_2$; 0.4 mM dNTPs mixture; 0.4 mM primer; 2.0 units taq DNA polymerase; 50 mg genomic DNA and distilled water. The amplification was performed using a programmable temperature cycler of GeneAmp PCR system 2400 from Perkin Elmer. RAPD analysis was employed using arbitrary primers OPA-02 (5'-TGCCGAGCTG-3'), OPA-18 (5'-AGGTGACCGT-3'), and OPA-10 (5'-GTGATCGCAG-3'). The amplification was programmed at 45 cycles for 30 seconds of denaturation at 94°C, 30 sec of annealing temperature at 36°C, 1 min of primer extension at 72°C, and 2 min of final extension at 72°C.

A mixture of 10 mL PCR product and 2.5 mL loading dye was run on a 2.0% (w/v) agarose gel electrophoresis at 55 volts for 2–3 hours and then stained with 0.5 mg/mL ethidium bromide for 20-30 minutes. The gel was washed with distilled water for 5 minutes prior to photographing with Image Master VDS.

RESULTS AND DISCUSSION

Muscle Tissue Preservation

Genomic DNA of *Epinephelus suillus* was successfully extracted from preserved muscle tissues from three to 18 months using the Phenol-Chloroform technique. The genomic DNA detectable with ethidium bromide staining is presented in Figure 1. The tissue preservation up to six months showed a clear single band of genomic DNA. The 12 and 18 months preservation also showed a good band of genome but slight degradation occurred, which was indicated by a smearing band. Linacero *et al.* (1998) illustrated that the presence of degraded DNA was indicated by a smear from high to low molecular weight positions, while the presence of RNA was indicated by a broad band of very low molecular weight that appears beyond the dye front.

The clear band formed on agarose gel indicated that the buffer employed in this study preserved the DNA well. While preserving the DNA samples, this buffer perhaps also lyses the muscle tissue of grouper, since the buffer contains chemicals such as Tris-base, NaCl, EDTA, and SDS which are commonly used as lyses buffer for DNA extraction. Urea in this buffer also played an important role in preserving DNA for extraction purposes as well as in improving the DNA amplification. The addition of 7 M urea on the 8% (w/v) resolving gels in SDS-PAGE improved the amplified fragment separation considerably for tiger barb, guppy, tilapia, and salmon (Dinesh *et al.*, 1993a; 1993b). The TNES-Urea is also suitable for DNA extraction of fish muscle tissue that is rich in cellular end nucleases, since urea is an inhibitor of these enzymes and also an activator of proteinase K (Asahida *et al.*, 1996).

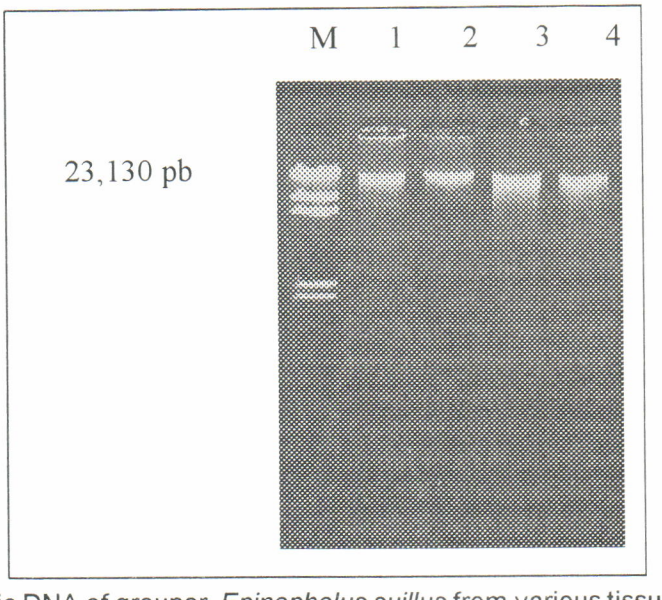

Fig. 1. Genomic DNA of grouper, *Epinephelus suillus* from various tissue preservation periods extracted by Phenol-Chloroform method and run at a 0.8% agorase gel. DNA marker of Hind III (lane M), a representative sample preserved in TNES-Urea buffer for 3 months (lane 1), 6 months (lane 2), 12 months (lane 3), and 18 months (lane 4)

Asahida *et al.* (1996) reported that the TNES-Urea was the most reliable buffer for tissue preservation and DNA extraction of Japanese flounder, *Paralichthys olivaceus* and Atlantic herring, *Clupea harengus*. They found that this preservative offered advantages for preserving fish muscle tissue with DNA yields of 0.5-2.6 mg of total DNA/mg tissue after the tissue was preserved for 3 years.

The TNES-Urea buffer is beneficial for preserving muscle tissues because this preservation modifies the cell lyses buffer for isolation of total cellular DNA from animal tissues. Nelson *et al.* (1997) also introduced the digestion buffer for muscle tissue preservation, by modifying a common lyses buffer for DNA extraction. This buffer, containing 50 mM Tris-HCl, pH 8.0, 100 mM EDTA, 100 mM NaCl, 100 mM DTT (dithiothreitol), 1% SDS and 0.5 mg/mL proteinase K, has been reported to be successful for preservation of muscle tissue of anemone fish, *Amphiprion ocellaris* up to 7 days.

RAPD Analysis

Amplification of genomic DNA generated RAPD banding patterns in the size range of 380-1100 bp for various preservation periods (Figure 2). Primer OPA-02, OPA-18, and OPA-10 generated good RAPD banding patterns and showed consistent RAPD banding patterns among periods of preservation. This result indicates that tissue preservation using TNES-Urea buffer for 18 months is still evident prior to DNA extraction of *Epinephelus suillus*. This preservative, when used to preserve samples in conjunction with Phenol-Chloroform extraction method for obtaining of genomic DNA, is found to be compatible for RAPD analysis.

CONCLUSIONS

The TNES-Urea buffer is a potential tissue preservative of grouper for DNA analysis. Genomic DNA was successfully extracted using the Phenol-Chloroform method from grouper muscle tissue preserved in TNES-Urea buffer for up to 18 months. Consistent RAPD banding patterns were obtained in DNA amplification from various tissue preservation periods.

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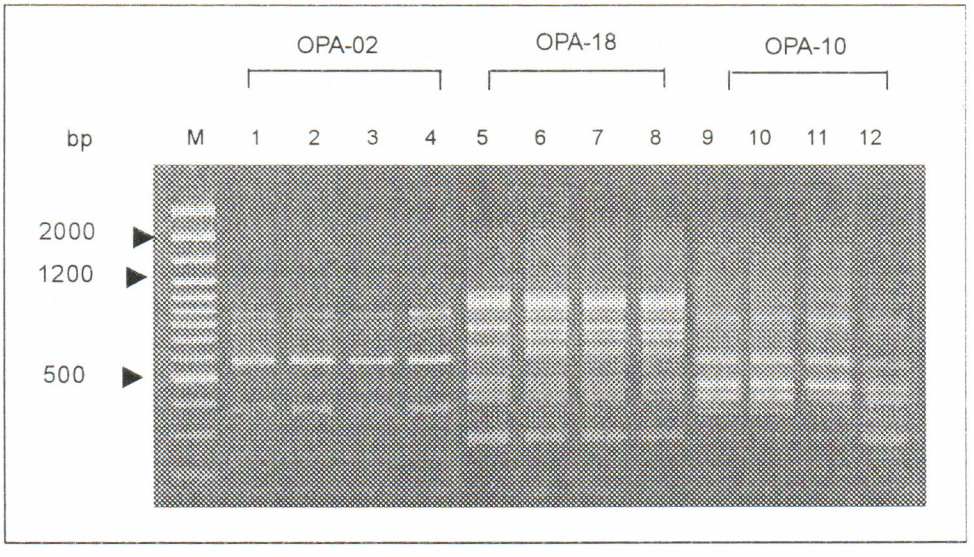

Fig. 2. RAPD banding patterns of genomic DNA from different periods of tissue preservation generated by primer OPA-02, OPA-18 and OPA-10. Gene Ruler 100bp DNA Ladder Plus from Ferment as (lane M), a representative sample preserved in TNES-Urea buffer for 3 months (lanes 1, 5, and 9), 6 months (lanes 2, 6, and 10), 12 months (lanes 3, 7, and 11), and 18 months (lanes 4, 8, and 12)

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