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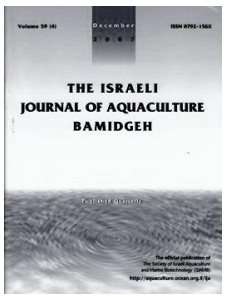

Genomic DNA Extraction from *in vivo* Sampled Tissue of Pacific Oyster, *Crassostrea gigas*

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Abstract

As opposed to other animals, live bivalve shellfish cannot be sampled because of the high mortality associated with opening closed shells. In this paper, we present a new anesthesia method using an $MgSO_4$ seawater solution for sampling live bivalve shellfish. In our experiment, genomic DNA was successfully extracted from mantle tissues of sampled Pacific oyster (*Crassostrea gigas*) and the DNA was used as a template for polymerase chain reaction (PCR), which indicated that 3 mm^3 of mantle tissue was enough for DNA extraction and subsequent molecular biology testing. The survival rate after the *in vivo* sampling was high (83.3%), indicating that the sampling method caused only slight harm to the oysters. Thus, $MgSO_4$ anesthesia is a practical method for *in vivo* sampling of oysters and probably could be applied to other bivalve shellfish species as well, significantly promoting the application of molecular marker-assisted selection in bivalve shellfish breeding and the study of tissue injury and repair mechanisms in bivalve shellfish.

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Introduction

Many molecular markers have been developed for bivalve shellfish (Scott et al., 2005; Wang et al., 2008; Bai et al., 2009; Wang et al., 2009; Zhang and Guo, 2010), but much work remains to realize the application of molecular marker-assisted selection in bivalve shellfish breeding. Unlike other animals, live bivalve shellfish still cannot be sampled because their closed shells prevent examining internal tissues and mortality is very high if traditional aggressive sampling methods are used. Here we put forth a new method of anesthesia using a $MgSO_4$ seawater solution for *in vivo* sampling of bivalve shellfish, based on clinical studies in which $MgSO_4$ was used as anesthetic (Koo et al., 2004; Arcioni et al., 2007).

Materials and Methods

Sixty Pacific oysters from a single population, with a shell height exceeding 6 cm, were chosen and dried for 30 min in the shade at room temperature (25°C). Thirty oysters in the experimental group were anesthetized in an 8% $MgSO_4$ seawater solution and another 30 oysters were held in sand-filtered sea water as a control. After 12 h, the oysters in the experimental group were opened while the ones in the control group remained undisturbed. The experimental oysters were removed from the $MgSO_4$ seawater solution and about 3 mm^3 of mantle tissue was scissored off each individual. Mantle samples were stored at -20°C for subsequent treatment. Oysters whose mantles were sampled were rinsed twice in sand-filtered sea water and returned to the $MgSo_4$ solution. Survival of each group was statistically calculated after one month by χ^2 test in SAS 8.2.

Genomic DNA of the sampled mantle tissue was isolated by the phenol-chloroform method (Sambrook et al., 2001). The quality and quantity of the extracted DNA was determined with a spectrophotometer at 260 nm and 280 nm. The DNA yield was calculated as follows: DNA yield per mm^3 of mantle tissue = A260 × (50 μg DNA/ml) × (dilution factor) × (solution volume)/3 mm^3. Using the extracted DNA as a template, PCR was carried out to further detect the DNA quality with a set of primers: ACCCTATCACATTCTCTCCC and AGAAAACCCAGCCTACCCG. The PCR was performed in a total volume of 25 μl containing 100-200 ng of genomic DNA, 5 × PCR buffer, 0.2 mM deoxynucleotide triphosphate (dNTP), 2.5 mM $MgCl_2$, 0.5 mM of each primer, and 0.5 U of Taq DNA polymerase (Tiangen Biotechnology Co., Beijing, China). The PCR conditions were as follows: 94°C for 5 min followed by 35 cycles of 94°C for 30 s, 58°C for 30 s, 72°C for 30 s, and a final extension at 72°C for 7 min. The PCR products were run on a 1.0% agarose gel in 1× TBE buffer.

Results

After one month, there were 25 oysters still alive in the experimental group and 29 in the control group. The survival rates between the two groups did not statistically differ at the 5% level ($\chi^2 = 0.16$, $p = 0.69$), indicating that the sampling method did little harm to the oysters. The nucleic acid purities monitored by A260/A280 ratios of DNA samples were 1.8-1.97, indicating that the extracted DNA samples were of high purity. The average DNA yield was 3.15±0.27 μg/mm^3, which is within the normal range. Specific bands appeared in all lanes, indicating that all PCR readings were accurate (Fig. 1).

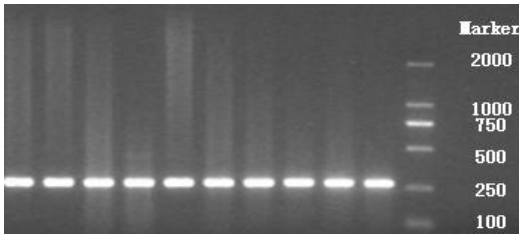

Fig. 1. Agarose gel electrophoresis of PCR products, with DNA templates extracted from tissues of live Pacific oysters.

Discussion

The high survival rate (83.3%) after *in vivo* samplings suggest that the sampling method caused only slight harm to the oysters and that the mantle is an appropriate organ for sampling. Genomic DNA was extracted from all sampled mantle tissues and all the DNA could be used as templates for PCR, indicating that 3 mm^3 of the mantle tissue was enough for DNA extraction and subsequent molecular biology testing. Our results suggest that $MgSO_4$ anesthesia is a practical method for *in vivo* sampling of oysters, and that it could probably be applied to other bivalve shellfish species. If so, this method could significantly promote the application of molecular marker-assisted selection in bivalve shellfish breeding and the study of tissue injury and repair mechanisms in bivalve shellfish.

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