A NOVEL RAPD-PCR BASED DNA DAMAGE ASSESSMENT APPROACH FOR 'MUSSEL WATCH'

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Abstract

Detection of DNA damage can be a good genotoxicity biomonitoring tool that may find various levels of applications under *in vivo* conditions. In this work a novel RAPD-PCR approach has been proposed to assess the level of DNA damage in various exposed and less-exposed mussel (*Mytilus galloprovincialis)* samples from the Sea of Marmara for the more exposed and Bay of Saros, Aegean Sea for the less exposed.

Keywords : Bio-indicators, Bivalves, Biotechnologies, Monitoring, Pollution.

The increasing genotoxic risk in aquatic systems calls for novel strategies of biomonitoring. The detection of DNA damage could reflect the level of marine pollution by genotoxicants. In monitoring genotoxicity, it is important to have sensitive, but non-specific assays to indicate a wide range of DNA damage mechanisms. RAPD-PCR based assays have been shown to detect genotoxic effect by comparative changes occuring in RAPD profiles as variation in band intensities as well as gain or loss of bands following genotoxin exposures [1].To our knowledge, there is not a routine application that can assess the direct adverse effects of marine pollution on DNA.

Mussel species have been extensively utilized as a biological indicator of pollution in 'Mussel Watch' programs [2]. With the presumption that DNA of this organism becomes a mosaic in different cell and tissue types due to different genotoxic exposures and attacks that bioaccumulates throughout an organism's lifetime, a novel 'DNA mosaicism detection assay', RAMD-PCR (Random Amplified Mosaic DNA-PCR) based on RAPD-PCR analyses with as little as 2-5% of the cells having the same effected genotype to reveal unpredictable DNAdamage has been proposed [3],[4],[5].

DNA isolation was carried out using the Macherey & Nagel Nucleospin tissue DNA isolation kit according to the supplier's instructions. DNA concentrations and sizes were estimated by comparing them with a standard sample (GeneRulerTM 100bp DNA Ladder, ready-to-use, MBI Fermentas) in an agarose gel (fragment, (bp) and DNA quantity in band, (ng) were given, respectively, for 10 bands in descending order of the fragment sizes 1: 1031-169, 2: 900 - 147, 3: 800 - 131, 4: 700 - 115, 5: 600 - 98, 6: 500 - 164, 7: 400 - 65, 8: 300 - 49, 9: 200 - 33, and 10: 100-16). RAPD amplification was done with a 25 μl PCR mix, containing 1x PCR buffer $(NH_4)_2SO_4$, 0.2 mM from each dNTP (2 mM dNTP mix), 1μM of primer OPB-18 5'-CCACAGCAGT-3' from QIAGEN Operon RAPD 10mer Kits, 200-400 ng of genomic DNA, and 0.5 units of Taq DNA polymerase, and filled up with sterile deionized water to the final volume. nx RAPD-PCR that we have introduced the term RAMD-PCR (Random Amplified Mosaic DNA) was performed according to previous reference [6] with the preparation of a master mix with every component of the standard RAPD-PCR [7], including the DNA and subdividing it into several aliquots for amplification at the same PCR conditions. This novel approach enabled the visualization of the amplification products from the very same organism on the same gel, clearly showing the variation of the organism's genome (innate, induced or spontaneous). The RAPD profiles of the improved assay were evaluated on the same 2% agarose gel run for 30 min. at 150 volts.

In our approach RAPD-PCR is exploited as RAMD-PCR to see the extend of DNA mosaicism due to intrinsic genomic nature and life-long pollution exposed and less-exposed mussel (*Mytilus galloprovincialis)* samples from the Sea of Marmara and Bay of Saros, Aegean Sea respectively. The same organism's whole tissue DNA that is assumed to be a mosaic due to both intrinsic and extrinsic factors and RAPD-PCR profile variations within the same individual was used to assess the level of DNA change qualitatively. The originality of the approach is in the preparation of a nxPCR mastermix using the same DNA of the same organism and aliquoting it into n PCR tubes for standart RAPD-PCR, to evaluate the same organism's DNA change without a further need for checking reproducibility (Fig.1). The method has proved to be cheap, easy, useful and could be routinely applicable under *in vivo* conditions for genotoxicity biomonitoring of any aquatic systems when suitable model organism of watch is chosen.

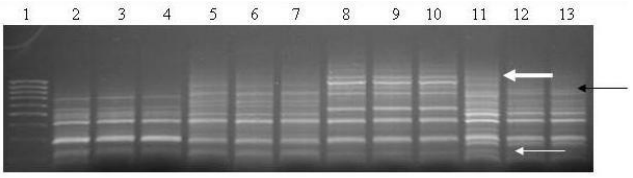

Fig. 1. Comparison of 3xRAMD-PCR profiles of small sized mussels (approximately 1.0 cm) collected from Saros Bay and Unkapani Shore, Haliç. Lanes 2-4, 5-7 and 8-10 are for 3 mussel samples collected from less polluted Saros Bay and Lanes 11-13 are for a mussel from highly polluted Unkapani shore, Haliç. Lane 1 is the DNA size marker. Arrows indicate the different patterns obtained for the same mussel DNA whereas for the other three mussels no RAPD-PCR profile changes are observed.

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