DNA DAMAGE DETERMINATION IN GILLS OF THE MUSSEL *MYTILUS GALLOPROVINCIALIS* L. (MOLLUSCA: BIVALVIA) BY FAST MICROMETHOD®

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Abstract

Fast Micromethod® was applied in the determination of DNA damage as strand breaks alkali-labile sites and incomplete excision repair in cell suspensions or tissue homogenates in single microplates. The chemical xenobiotics (4-nitroquinoline-N-oxide and blepmycine) and different doses of γ-rays, generated by Cs^{127}, were used to induce DNA damage in native DNA and sponge cells. The method was used in a monitoring program in the Adriatic Sea to detect the effect of mixed marine pollutanta.

Keywords : Adriatic Sea, bio-indicators, bivalves, ecotoxicology, monitoring

Introduction

The estimation of genotoxic potential in the marine environment can be carried out by measuring genetic endpoints which exhibit primary DNA damage such as strand breaks. The Fast Micromethod® measures changes in the pattern of DNA denaturation by directly altering DNA integrety in cell or tissue lysates at alkaline pH, and following their time dependence (1). Two of the major advantages of the method are small amount of sample (cells suspension or solid tissues) needed and the short time of analyses. The aim of this study was to establish the applicability of the Fast Micromethod® for its use in marine ecotoxicological monitoring research.

Methods and materials

The Fast Micromethod® is performed according to Batel *et al.* (2). The time course and the extent of DNA denaturation are followed directly in the microplate by measuring the fluorescence of the DNA/PicoGreen complex for 30 mn. Results are calculated after 7-10 minutes of denaturations and expressed as strand scission factors (SSFs) calculated as:

$$SSF = \log_{10} (\%\ dsDNA_{\text{treated sample}} / \%dsDNA_{\text{control sample}})$$

Double-stranded DNA percentages were calculated in relation to fluorescence values at 0-time denaturations after correction for blank readings which represents relative 100% of ds-DNA before denaturation in samples. Thus SSF=0 assumes absence of DNA strand breaks and alkali labile sites, while SSF<0 indicates increasing frequencies of strand breaks and alkali labile sites in samples. For pratical reasons the SSF (strand scission factors) in graphical presentations were multiplied by (-1).

Results and discussions

The effect of 0-50μM Bleomycin-Fe(II) complex on the induction of DNA strand brakes in *Holothuria tubulosa* (3567A) cells has shown a dose dependent response with SSFx(-1) values from 0.047 to negative control to 0.537 respectively. The dose-response histogram for 0-500 rad γ-irradiated sponge *Suberites domuncula* cells has shown that average SSFx(-1) varied from 0.020 (control cells) to 0.082 for 500 rad (irradiated cells).

Mussels *Mytilus galloprovincialis*, injected with 0-1 μg NQO/g mussel, showed dose-dependent DNA damage with SSFx(-1) values from 0.000 for negative control to 0.168 for 1μg NQO/g. The effect of 0-83.3 μM Bleomycin-Fe(II) complex on the induction of DNA strand breaks in mussel gills has shown SSFx(-1) values 0.000 (control mussel) to 0.140 respectively.

A different amount of DNA damage (SSFx(-1) values from 0.738 to -0.610) from different locations along the Adriatic coast (Fig. 1) was detected during two years of observations (August 2000-June2000). In the cases where histograms (SSFx(-1- values) were negative, it was presumed, according to Vukmirovic (3), that the DNA-DNA and/or the DNA-protein crosslinks are presented.

This study represents a suitable Fast Micromethod® for DNA damage in native DNA isolated from cotton-spinner *Holothuria tubulosa,* marine sponge cells *Suberites domuncula* and mussel gill (*Mytilus galloprovincialis*) tissue.

The Fast Micromethod® is applicable for measurement of DNA integrity of small samples for genotoxicity assessment in environmental monitoring for genotoxic effects of lower taxa or sesile organisms (sponges, mussels). Sensitivity and preciseness of the Fast Micromethod® is comparable with the widely used Comet assay (4) which makes the former suitable for the assessment of pollution impact on aquatic organisms that can be used as bio-indicators.

References

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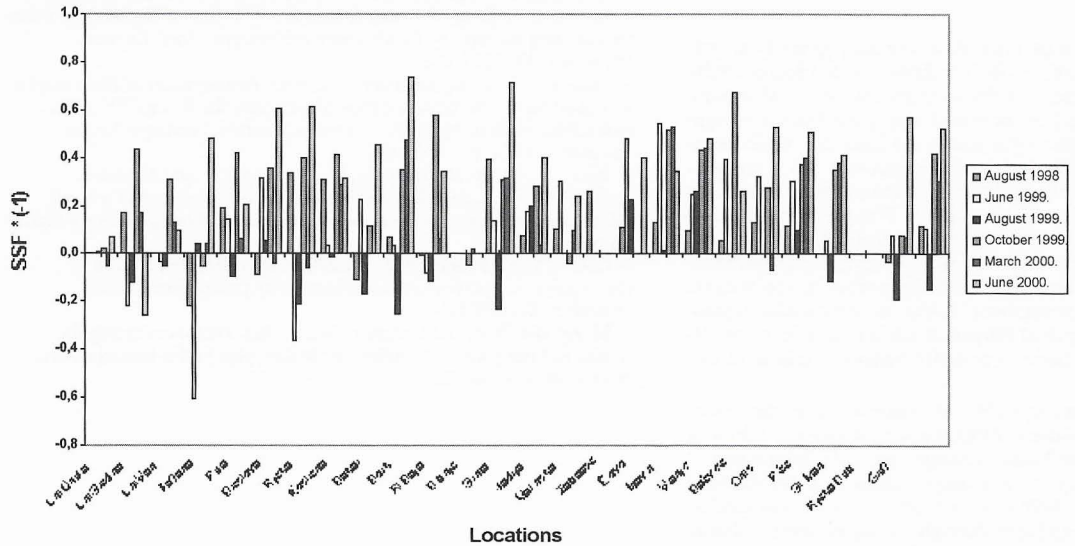

Fig. 1. - Results of SSF values for *Mytilus galloprovincialis* gill homogenates collected from different locations on the Adriatic coast during 2 years (August 1998 – June 2000).