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The random amplified polymorphic DNA (RAPD) assay in assessment of genotoxic potential: the Sava River case study

K. Sunjog[1,2], S. Kolarević[1], M. Kračun-Kolarević[3], J. Kostić[1,2], J. Jovanović[1], P. Simonović[4], V. Simić[5], Z. Gačić[2], M. Lenhardt[2,3], M. Paunović[3], B. Vuković-Gačić[1]

[1] University of Belgrade, Faculty of Biology, Chair of Microbiology, Center for Genotoxicology and Ecogenotoxicology, Belgrade, Serbia
[2] University of Belgrade, Institute for Biological Research ¨Siniša Stanković¨, Belgrade, Serbia
[3] University of Belgrade, Institute for Multidisciplinary Research, Belgrade, Serbia
[4] University of Belgrade, Faculty of Biology, Institute of Zoology, Belgrade, Serbia
[5] University of Kragujevac, Faculty of Science, Kragujevac, Serbia

The Sava River is the major drainage basin of Southeastern Europe and the largest tributary to the Danube River [1]. With a population of about 8.2 million and poor legislation regarding the discharge of wastewaters in the countries of the region, the anthropogenic pressure in the basin is more than obvious. Genotoxic stressors are a group which has drawn increasing attention lately due to the possible adverse effect which they might have on the quality of the ecosystem [2]. The effects of the DNA alterations can be dramatic in means of drop of survival and fertility but can also lead to changes in the genetic code and become one of the primary drivers of genetic diversity among the populations [3].

The comet assay (single cell gel electrophoresis) is widely used for the evaluation of DNA-damaging effects in genotoxicity testing and population monitoring. It can be modified to enable the detection of specific classes of DNA damage, like oxidative damage (Fpg-modified comet assay). In the last decade, random amplified polymorphic DNA (RAPD) assay, a simple, fast, sensitive, and straightforward PCR-based method, has been used to detect genotoxic-induced DNA damage and mutations in different organisms, including fish. In the field of ecotoxicology, most RAPD studies describe the RAPD changes as differences in band intensity as well as gain/loss of stable bands.

In this study we have carried out a genotoxicological survey along the upper course of the Sava River. The specimens of chub (*Squalius cephalus*) were collected in August and September 2015 at seven sites (Litija-I, Vrhovo-II, Čatež-III, Zagreb-IV, Jasenovac-V, S. Brod-VI, Županja-VII) along the river. Blood was collected directly from the heart with 3 mL syringes. One drop of blood of each specimen was diluted 20x in 4°C cooled medium and immediately frozen in liquid nitrogen prior to the application of the comet assay and Fpg-modified comet assay analysis. Approximately 500 mg of muscle tissue was excised from each specimen and immediately frozen in liquid nitrogen upon RAPD analyses. The genomic DNAs from four individuals from each site were blended to suppress the intra-populational genetic polymorphism potentially revealed by RAPD. In our analysis 6 different primers were used.

Based on the results of the alkaline comet assay we have observed separation of the sites in Slovenia from the sites in Croatia. Increase of DNA damage was observed at sites situated downstream of the site Čatež and DNA damage reached the highest values in specimens collected at the site Jasenovac. The results of Fpg-modified assay showed a lack of correlation between the Net-contribution of 8-oxo-G sites and values obtained in the alkaline comet assay suggesting that the oxidative stress is not a major inductor of DNA damage in this case. Thus, it should be emphasized that the highest level of oxidative damage was also observed at the site Jasenovac. RAPD profiles evidenced substantial differences between examined sites. It was interesting that like the comet assay, RAPD band analysis

also singled out sites Zagreb and/or Jasenovac (5 out of 6 primers). Site Jasenovac (V) was excluded as the site with the highest net contribution of oxidative damage and the highest level of DNA damage detected by the comet assay but also as the site which was most frequently separated by others in clustering within the RAPD analysis. Based on the previous studies [3-5] occurrence of DNA damage such as single and double breaks as well as oxidized bases can lead to loss or appearance of bands in RAPD analysis, so it is reasonable to speculate the influence of DNA damage in overall change in bands. Besides the possible impact of the DNA damage in pattern of RAPD fingerprint is also the influence of other population of chub from the Bosna River (right tributary of this site), which could have changed genetic structure.

Nevertheless, one of the downsides of these kinds of studies is that RAPD detects both genetic variability and DNA damage, and it is very difficult to differentiate the real contribution of DNA damage. However, according to previous research [6] using both approaches would be advantageous. At the population level, concurrent responses between changes in population genetic structure and elevated levels of DNA damage may provide evidence that the population genetic changes are influenced by the exposure to genotoxic chemicals [3, 6].

References:

[1] Kolarević et al., 2016. *PloS one* 11(9), e0162450.
[2] Frenzilli et al., 2009. *Mutat. Res./Rev. Mutat*. 681(1), 80-92.
[3] Atienzar et al., 2006. *Mutat. Res./Rev. Mutat.* 613(2), 76-102.
[4] De Wolf et al., 2004. *Mutat. Res./Rev. Mutat.* 566(3), 249-262.
[5] Jin et al., 2009. *Toxicol. In Vitro* 23(7), 1209-1214.
[6] Theodorakis, 2001. *Ecotoxicology* 10, 245–256.