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FRESHWATER MUSSELS AS BIOINDICATORS IN THE ECOCENOOTOXICOLOGICAL STUDIES

Branka Vuković-Gačić¹, Stoimir Kolarević¹, Karolina Sunjog¹³, Jelena Tomović², Margareta Kračun², Jelena Knežević-Vukčević¹, Momir Paunović², Zoran Gačić³

¹University of Belgrade, Faculty of Biology, Chair of Microbiology, Centar for Genotoxicology and Ecogenotoxicology, Studentski trg 16, 11000 Belgrade, Serbia, e-mail: brankavg@bio.bg.ac.rs
²University of Belgrade, Institute for Biological Research Siniša Stanković, Bulevar Despot Stefa-
na 142, 11060 Belgrade, Serbia, e-mail: mpaunovi@ibiss.ac.rs
³University of Belgrade, Institute for Multidisciplinary Research, Kneza Višeslava 1, 11000 Bel-
grade, Serbia, e-mail: zorga@imsi.rs

Introduction

The simple detection of pollutants fails to provide the information on the relationship between contaminant exposure and biological effects in aquatic organisms and therefore usage of biomarkers becomes essential for assessing the condition of aquatic ecosystems (Nigro et al., 2006; Jha, 2008). Presence of pollutants in aquatic ecosystems can be detected by a range of physiological, histological and molecular responses, including abnormal morphology, alterations of antioxidative status and DNA integrity (Pavlica et al., 2001; Bolognesi et al., 2004, Kolarević et al., 2012).

The integrity of cellular DNA is continuously attacked by various agents in the environment resulting in DNA lesions such as strand breaks, modified bases, DNA–DNA cross-links and DNA–protein cross-links. Unrepaired DNA lesions may block replication and transcription, potentially leading to cell death, or may give miscoding information, generating mutations (Simić et al., 1998; Vuković-Gačić et al., 2006). As a result, a number of biological consequences can be initiated at the cellular, organ, and whole animal and finally community and population levels. Studying the origin of genotoxic pollution, as well as the effects of pollution on individuals and populations are the main objectives of ecogenotoxicology.

Freshwater mussels as bioindicators

Mussels are commonly employed in the ecogenotoxicological studies. They have several characteristics, such as wide distribution, filter feeding, a sessile life form and an ability to accumulate pollutants, which makes them favourable organisms for estimating the environmental pollution level and the bioavailability of various types of pollutants (Romé et al., 2003; Andral et al., 2004; Amiard et al., 2006). In response to environmental stress they show a range of physiological, histological and molecular responses, including abnormal morphology, alterations of antioxidative status, induction of DNA strand breaks, etc. (Pavlica et al., 2001; Binelli et al., 2007; Bolognesi et al., 2004; Coffinet et al., 2008).

In ecotoxicological studies, different approaches are used for assessing the conditions of ecosystems. Passive biomonitoring employs collection of the specimens from selected locations, while the active biomonitoring entails the use of bioindicator organisms obtained from unstressed populations and their subsequent exposure at polluted sites (De Kock and Kramer, 1994; Smolders et al., 2003). Active biomonitoring is increasingly used for quantifying the impact of pollutants on aquatic ecosystems because of the numerous advantages over the passive, such as avoiding the biological variability in the responses related to different age and the reproductive status of the organisms in situ. In addition, it can overcome the hydrological, hydrochemical and other abiotic and biotic factors that can influence species distribution, contaminant bioaccumulation and biomarker responses (Cossu et al., 2000; Arbuckle and Downing, 2002; Andral et al., 2004; Viarengo et al., 2007).
One of the major issues in ecogenotoxicological studies is providing data from the animals at unpolluted sites which can be used as control values of DNA damage for in situ assessment of genotoxicity. Active biomonitoring also requires the specimens from unpolluted sites to be used for translocation. However, finding an unpolluted site is not always possible. The acclimation of mussels in controlled laboratory conditions could provide an adequate solution for obtaining the control values i.e. the baseline DNA damage, as described in different mussel species (Fedato et al., 2010).

Studying the effect of exposure to certain compound or mixture of compounds in controlled laboratory conditions is employed in ex situ assessment of genotoxicity. The compounds that are mostly used are pollutants which are suspected to be threat to environment.

Residues of human pharmaceuticals are raising concerns about their potential effects in non-target species (Fick et al., 2010). Great attention is dedicated to anticancer drugs due to their cytotoxicity, genotoxicity, mutagenicity and teratogenicity (Kosjek and Heath, 2011).

Detection of genotoxic effect with Comet assay (SCGE)

The comet assay, also known as single cell gel electrophoresis (SCGE), is a sensitive and rapid technique for detection of DNA damage in individual cells based on the migration of denatured DNA during electrophoresis, in which damaged nuclei form comet-like shapes. Comet assay has been accepted as one of the major tools for assessing pollution related genotoxicity in aquatic organisms (Dixon et al., 2002). It has been used in many ecogenotoxicological studies on freshwater mussels (Pavlica et al., 2001; Klobučar et al., 2003; Guidi et al., 2010, Kolarević et al., 2013, Vuković-Gačić et al., 2013) and show correlation with other genotoxicity tests such as chromosomal aberration, sister chromatid exchanges and micronucleus assay (Dhawan and Bajpayee, 2009). The modified alkaline version of the comet assay, described by Sing et al. (1988), enables detection of both single and double DNA strand breaks, as well as alkali labile sites. In our studies the comets are scored and analyzed using Comet IV Computer Software (Perceptive Instruments, UK). Tail intensity - TI (the percent of DNA fluorescence in the comet tail) and Olive tail moment - OTM (calculated as a product of the TI and the distance between the means of the head and tail distributions, are most often used as a measure of DNA damage.

In mussels, detection of DNA damage is usually performed on haemocytes and gill cells. Gills have a high efficiency in genotoxicity monitoring due to their large surface and constant exposure to environment. Haemocytes have a role in processes such as the transport and digestion of nutrients, and elimination of toxic substances and small particles, which makes them constantly exposed to water-borne pollutants (Soares-da-Silva et al., 2002; Dhawan and Bajpayee, 2009). Haemolymph can be easily collected from the adductor muscle and, most importantly, collecting does not require sacrificing animals.

Assessment of genotoxicity - in situ assessment and active biomonitoring

The result of the in situ assessment performed on freshwater mussel Sinanodonta woodiana indicated presence of genotoxic pollution in the Velika Morava River (Kolarević et al., 2013). Employment of active biomonitoring with freshwater mussels Unio pictorum and Unio tumidus indicated significant genotoxic potential of untreated wastewaters in the urban area of the Belgrade city (Vuković-Gačić et al., 2013). Moreover, the results of these studies indicated correlation in the level of DNA damage in mussels with concentrations of dissolved heavy metals in water. Also, we have shown the ability of DNA damage recovery in mussel species from the Unionidae family.

Assessment of genotoxicity - ex situ assessment

Impact of acute exposure to the most frequently used cytostatics (5-FU, cisplatine, etoposide and vincristine) was studied on haemocytes of freshwater mussels Unio pictorum and Unio tumidus. The results of exposure show that majority of tested cytostatics can induce DNA damage in haemocytes of freshwater mussels U. pictorum and U. tumidus, even when present in concentrations which are far below administered in clinical treatment.
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References


