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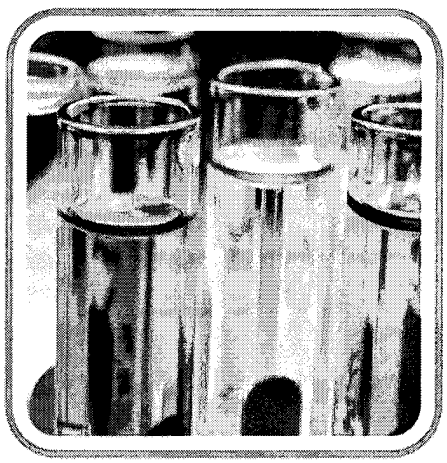
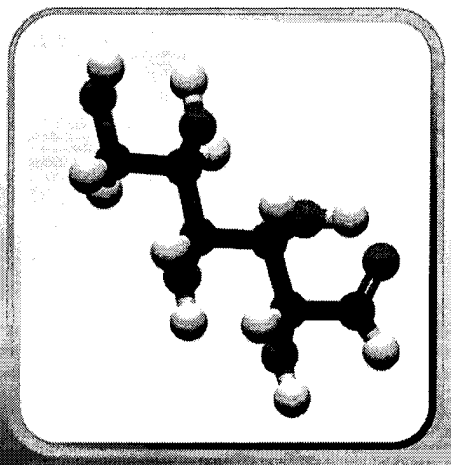


57 (suppl) 2011



СОДРЖИНА

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NEW TRENDS IN BIOMONITORING: APPLICATION OF RAPD-PCR AND PLANT MODEL SYSTEMS TO GENETIC ECOTOXICOLOGY

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Pollution of the environment has become a major concern of society. Perhaps one of the more serious concerns is the potential for exposure to substances that are genotoxic, potentially carcinogens and mutagens with the capacity to affect both the structural integrity of DNA and the fidelity of its biological expression [Wogan and Gorelick, 1985]. Genetic toxicology is an area of science in which the interaction of DNA-damaging agents with the cell's genetic material is studied in relation to subsequent effect(s) on the health of the organism. Structural changes to the integrity of DNA caused by DNA-damaging agents are useful endpoints for assessing exposure to hazardous environmental pollutants on human health [CBMNRC, 1987; Atienzar and Jha, 2006] and biota [Enan MR, 2006]. Genetic ecotoxicology is an approach that applies the principles and techniques of genetic toxicology to assess the potential effects of environmental pollution, in the form of genotoxic agents, on the health of the ecosystem. Because the techniques and methods unique to these disciplines are extremely sensitive and specific it is anticipated that their implementation into studies concerned with the mechanism of action of genotoxicants will provide a stronger scientific basis for the assessment of risk of exposure. Bioindicators of contamination make it possible to detect subtle forms of pollution that are hard to measure in the field.

In this paper we evaluated the application of RAPDs as molecular marker to detect DNA damage in plant model systems caused by environmental pollutants. Results showed that RAPD-PCR technique is a powerful tool for screening DNA damage induced by non-lethal levels of organic and inorganic contaminants.

Plants are good bioindicators because (i) they play a significant role in food chain transfer and in defining habitat, (ii) they are easy to grow and adaptable to environmental stress, (iii) they can be used for assaying a range of environmental conditions in different habitats. Furthermore, plant-based assays applied to toxicity screening in the environmental field would reduce animal sacrifice and testing costs. The use of plants as bioindicators of genetic toxicity of environmental pollutants has been reported in several studies [Grant WF, 1994; Knasmuller et al., 1998]. Mutagenic activity of chemicals has been analysed with different plant systems such as *Allium cepa, Vicia faba, Trifolium repens, Phaseolus vulgaris* and many others [Fiskesjo G, 1997; Enan MR, 2006; Cenkci et al., 2009] and chromosome aberration assays, mutation assays and cytogenetic tests were performed with these systems. The advantage of measuring the effect of genotoxic chemical directly on DNA is mainly related to the sensitivity and the short response time. Recently, enormous advances and developments in molecular biology have provided new ways of detecting DNA damage [Conte et al., 1998; Savva D, 2000]. Following the original description of the PCR [Mullis and Faloona, 1987], modifications were described enabling the generation of DNA fingerprints and their use in detecting genotoxicity. Random amplified polymorphic DNA (RAPD) of these techniques can be used to detect genotoxicity and differences in RAPD profiles and can clearly be shown when comparing DNA fingerprints from untreated and treated individuals to genotoxic agents [Atienzar and Jha, 2006; Enan 2006; Cenkci et al. 2009].

In the field of ecotoxicology, most RAPD studies describe the RAPD changes such as differences in band intensity as well as gain/loss of RAPD bands. RAPD profiles generated by samples exposed to genotoxic agents in all cases were different from those obtained using control DNA. One class of genetic effects includes alterations to the structure and function of DNA including DNA adducts, DNA breakage, and mutations as a result of chemical exposure (genotoxic effects). Biomonitoring should act as an early warning system providing information about the seriousness of the pollution by sensitive assays, especially for existing potential genotoxic problem assessment, since most polluting substances have shown genotoxic effect. A further consideration is that changes in the DNA fingerprints obtained may be used to identify target genes for particular genotoxic agents. Characterisation of these DNA molecules using DNA sequencing techniques may enable the identification of specific sequences that are hotspots for mutation by particular agents; this will open up possibilities of designing specific assays for detection of specific agents and may help explain the presence in the genome of preferential mutation points.

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