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Review

The Comet assay for the evaluation of genotoxic impact in aquatic environments

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ABSTRACT

This review considers the potential of the Comet assay (or Single Cell Gel Electrophoresis, SCGE) to evaluate the environmental impact of genotoxins in aquatic environments. It focuses on *in vivo* and *in situ* studies that have been carried out in various marine and freshwater sentinel species, published in the last 5 years. A large number of the studies reviewed report that the Comet assay is more sensitive when compared with other biomarkers commonly used in genetic ecotoxicology, such as sister chromatid exchanges or micronucleus test. Due to its high sensitivity, the Comet assay is widely influenced by laboratory procedures suggesting that standard protocols are required for both fish and mussel cells. However, there are still a wide variety of personalised Comet procedures evident in the literature reviewed, making comparison between published results often very difficult. Standardization and inter-laboratory calibration of the Comet assay as applied to aquatic species will be required if the Comet assay is to be used routinely by national bodies charged with monitoring water quality.

Contents

1. Introduction

The demand for a clean and safe supply of water for drinking, agriculture and recreation has rapidly increased over the last few decades. Receiving waters, such as lakes, rivers and marine coastal areas are the receptacles for huge amounts of wastes derived directly from industry, agriculture and urban settlements or indirectly from the atmospheric deposition of airborne emissions. Present amongst these waters are a complex environmental mixture of well-known toxicants along with an increasing number of emerging contaminants, which pose a threat to both aquatic ecosystems and the health and welfare of human populations [1]. It is known that a number of chemicals present are highly persistent and have mutagenic and/or clastogenic properties [2,3]. The relevance of detecting the mutagenic/genotoxic risks associated with water pollution was firstly perceived in the late 1970s, when methods based on

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Salmonella bioassay [4] or sentinel species, such as mussels [5] and fish [6,7] were set up for monitoring the presence of mutagens and genotoxicants in aquatic environments. Since that time several tests have been developed for evaluating DNA alterations in aquatic animals, these are based on potentially pre-mutagenic lesions such as, DNA adducts, base modifications, DNA–DNA and DNA–proteins cross-linking and DNA strand breaks [8].

The analysis of DNA alterations in aquatic organisms has been shown to be a highly suitable method for evaluating the genotoxic contamination of environments, being able to detect exposure to low concentrations of contaminants in a wide range of species. In general, these methods have the advantage of detecting and quantifying the genotoxic impact without requiring a detailed knowledge of the identity and the physical/chemical properties of the contaminants present. Tests directly assessing DNA strand breaks, or downstream alterations following DNA strand damage, are commonly used to assess genotoxic impact in aquatic animals. The early procedures for

Table 1
Assessment of DNA damage by Comet assays after in vivo exposure of aquatic animals to genotoxicants.

Organism	Cell type	Agent	Exposure time	Concentration range	Parameter	Response	Reference
Invertebrates							
D. polymorpha	Haemocytes	Lake water (Italy)	3 h; 20 days	+ or − disinfectants (NaCl, PAA, ClO_2)	LDR (migration length/head diameter)	PAA − NaCl and ClO_2 + (reduction)	[48]
D. polymorpha	Haemocytes	Lake water (Italy)	20 days	Different seasons (Autumn, Winter, Summer)	LDR, TL	+	[49]
L. fortunei	Haemocytes	Sediment samples (urban sites, Brazil)	7 days	100 ± 5 g of sediment sample	DI (damage index); DF (damage frequency)	+	[50]
D. polymorpha	Haemocytes	3 strains MC toxins (*Microcystis aeruginosa*)	7–14–21 days	104 cells/ml freshwater for each strain	%Tail DNA	+	[52]
U. tumidus	Haemocytes, gill cells, digestive gland cells	B(*a*)P	6 days	50–100 μg/l	%Tailed DNA cells	+	[44]
		Fe^{3+}		20–40 mg/l			
U. tumidus	Digestive gland cells	Polyphenols	24 h	60–500 μM	TM	+	[45]
			48 h				
L. fortunei	Haemocytes	PCP	2 h + repair	10–150 μg/l	IL (image length, μm); DI	D–R	[51]
		$CuSO_4$		3.75–20 μg/ml			
P. felina	Whole animals	Norflurazon (herbicide)	7 days	0.2–2 μM	TL	+	[54]
					TM		
					%Tail DNA		
G. schubarti	Tail	Diluvio's Basin (Brazil)	13 days	Urban waste water (products of automobile fumes, human urban activities)	Damage index (DI)	+	[55]
C. gigas	Embryos	B(*a*)P	16 h	0.2 nM to 2 μM	%Tail DNA	+	[20]
		EE2		0.02–1.70 nM	OTM	−	
		ES				+	
S. sachalinensis	Digestive gland cells	MNNG	2 days	0.01–1 ppm(mg/l)	TL	+	[63]
	Haemocytes	B(*a*)P		0.1–1 ppm	TM		
M. edulis	Haemocytes	Tritiated water, HTO	96 h	12–485 μGy/hr	TM	D–R	[21]
P. viridis	Haemocytes	Water-borne B(*a*)P	3–12 days	0.3–30 μg/l	TL, OTM, %Tail DNA	D–R	[17]
P. viridis	Male gonad cells	Extracts of cigar tobacco	2–16 days	2.5–15 μg/ml	%Tail DNA	+	[18]
M. edulis	Haemocytes	TBT	7 days	0.1–5 μg/l TBTO	%Tail DNA	+	[19]
M. edulis	Gill cells	Cd	10 days	10–200 μg/l	%Tail DNA	+	[29]
		Cr	7 days	10–200 μg/l		+	
		Cr VI	Injection	10.4 μg/animal		+	
M. edulis	Haemocytes	Styrene	7 days	2 mg/l	%Tail DNA	+	[36]
T. semidecussatus	Haemocytes	Estuarine sediments	7–21 days	1 kg sediment added to 2 l of seawater	%Tail DNA	+	[37]
	Gill cells Digestive cells						
S. droebachiensis	Coelomocytes	Dispersed crude oil	4–5 weeks	0.06–0.25 mg/l	%Tail DNA	+	[24]
M. edulis	Haemocytes			0.15–0.25 mg/l			

(+) Positive response, (−) negative response, (D–R) dose–response.