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Detection of Genotoxicants in a Polluted Watercourse by Means of a Yeast System

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Genotoxic activity has been mainly studied for samples of pure chemicals or agents with known chemical concentrations; less attention has been given to the alterations induced in man and environment by complex mixtures like air, industrial wastes, etc. Such mixtures contain a wide variety of compounds potentially capable of causing additive, antagonistic, or synergistic genotoxic response in living organisms (Raymond et al. 1988). Although biological assays have been employed to detect either mutagenicity or carcinogenicity of complex environmental samples, information regarding usefulness and sensitivity of different assays is still scarce. The aim of the present study was to show the usefulness of a biological test system in the monitoring of streams polluted by industrial effluents.

The determinations were performed during 1988, on samples of a heavily polluted stream (the Riachuelo) located near Buenos Aires city area. The river under study has been receiving effluents from industrial plants as well as domestic waste waters for more than 40 yr. Discharges from tanneries, iron foundries, oil refineries, and chemical plants are daily mixed within the stream. The lack of adequate protection measures together with a flexible industrial control policy interfered with the building of waste treatment plants and, consequently, the discharge of effluents without treatment at all. The Riachuelo flows into the Rio de la Plata approximately 10 km below the point of water intake for the Buenos Aires city supply. Variations in the Rio de la Plata flow induced by climatic conditions, may allow the arrival of certain contaminants to the intake area with a potential risk for human health.

A strain of Saccharomyces cerevisiae that allows the simultaneous detection of mutation and genetic recombination was chosen as test organism for this study. Although the genotoxicity yeast assays have been usually considered to be of low sensitivity and difficult performance, the publications of Nestman and Lee (1983,1985) demon-

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strated the advantages of this kind of assays for the detection of genotoxicants in waste waters.

MATERIALS AND METHODS

The Saccharomyces cerevisiae D7 strain was obtained from Dr. Giorgio Bronzetti, Istituto di Mutagenesi e Differenziamento CNR, Pisa Italy. This strain is able to detect simultaneously mitotic gene conversion at the trp 5 locus, point (reverse and suppresor) mutation of the allele ilv 92, and mitotic recombination between the centromere and the ade 2 locus (Zimmermann et al. 1975). In this work we consider mitotic gene conversion and reverse point mutation which are sufficient to give indications of genetic effects (Bronzetti et al. 1983; Del Carratore et al. 1984).

Surface river samplers were collected with a Grab Sampler (Cole-Parmer Instrument Company) from three sampling stations located within the industrial area (Figure 1). The sampling was performed at 5 m from the coast during Sundays, in order to avoid direct influence of effluents from operating plants. Samples were analyzed within 24 hr, after filtration through Millipore 0.22 um membrane.

Yeast cells were washed twice in phosphate buffer (0.1 M, pH 7.4), suspended in 4 mL of raw water samples or the corresponding dilution (10^8 cells/mL) and incubated during 24 or 48 hr on a rotatory saker at 28°C. After incubation the cells were washed twice in phosphate buffer and plated on isoleucine-free medium, about 10^6 cells per plate, on a medium without tryptophan, about 10^5 cells per plate and finally on a complete medium, 200 cells per plate. All plates were incubated at 28° C. Plates could be scored for the number of survivors and convertant colonies starting on the third day after treatment. Revertants for ilv 92 allele were scored after 6-8 days (Zimmermann et al. 1975).

RESULTS AND DISCUSSION

Table 1 shows the average values for biochemical oxigen demand, dissolved oxigen, pH and number of viable bacteria determined in samples from the Riachuelo by the National Institute of Hydrologic Science and Technology and by our laboratory during May and June 1988.

Table 1. Results obtained with samples from the Riachuelo

Sampling Station	BOD (ppm)	pH	DO (ppm)	Viable bacteria/mL
1	57	7.4	1.5	3.3×10^6
2	60	7.0	0.3	1.9×10^6
3	47	7.0	0.3	2.9×10^4

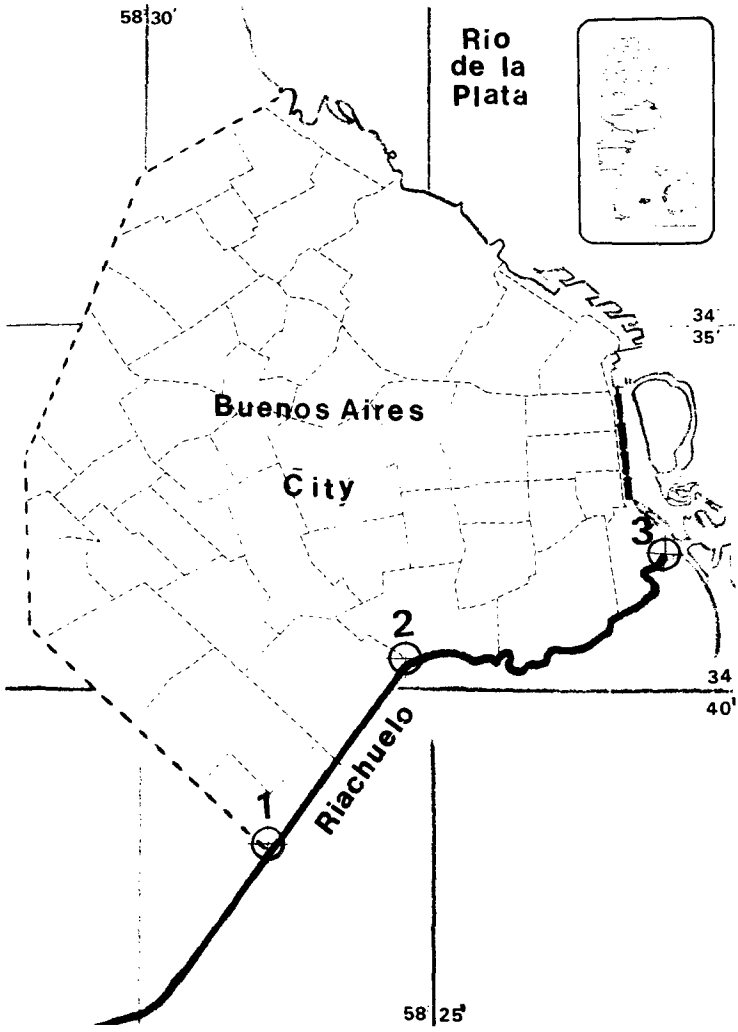

Figure 1. Location of sampling stations.

The data consigned in the table 1 clearly showed the degree of contamination of the stream selected for genotoxicity studies.

In this first part of our study we have analyzed the genetic effects induced by unconcentrated waste water samples, in order to determine the actual genotoxic characteristics of the mixture.

Since no alterations in the cell viability of the strain employed in the test were observed during a prolonged incubation period, the study was performed incubating Saccharomyces cerevisiae D7 during 24 and 48 hr with samples of raw water. These exposure periods gave a more precise picture of the potential effects on the procaryotic

genoma of cells present in the stream. The determination performed with dilutions of the samples provided information on the variations in the original mixture mainly originated by the city run-off discharged into the river.

Table 2 shows the results obtained from six representative samples. In all the cases the genotoxic effect was considered positive when the experimental frequencies exceeded those from the controls by at least two-fold (De Serres 1981).

Table 2. Mitotic gene conversion and reverse mutation induced in Saccharomyces cerevisiae D7 by raw water samples from the Riachuelo.

Sampling Station	Incubation (hr)	Dose (a)	Frequencies (b)		
			Survival%	GC	R
1 June 1988	24	0	100.0	0.10	0.25
		1	90.3	0.29	0.22
		1/2	95.1	0.11	0.30
		1/10	100.5	0.07	0.20
	48	0	100.0	0.68	0.33
		1	90.0	1.47	0.30
		1/2	96.9	0.73	0.27
		1/10	99.0	0.72	0.20
1 August 1988	24	0	100.0	0.24	0.22
		1	82.6	9.68	0.25
		1/2	53.3	0.98	0.31
		1/10	94.6	0.14	0.33
	48	0	100.0	0.16	0.30
		1	77.5	4.24	0.10
		1/2	87.9	1.68	0.16
		1/10	100.3	0.05	0.19
2 May 1988	24	0	100.0	0.22	0.25
		1	112.0	0.24	0.35
		1/2	98.2	0.24	0.22
		1/10	100.7	0.19	0.30
	48	0	100.0	0.24	0.26
		1	130.0	0.28	0.10
		1/2	102.5	0.15	0.31
		1/10	105.3	0.19	0.30

Sampling Station	Incubation (hr)	Dose (a)	Frequencies (b)		
			Survival%	GC	R
3 June 1988	24	0	100.0	0.10	0.22
		1	134.1	0.13	0.30
		1/2	111.4	0.16	0.32
		1/10	99.0	0.11	0.37
	48	0	100.0	0.50	0.22
		1	147.0	2.52	0.23
		1/2	121.8	0.26	0.25
		1/10	99.3	0.11	0.30
3 July 1988	24	0	100.0	0.18	0.32
		1	102.3	0.19	0.23
		1/2	71.8	0.21	0.25
		1/10	100.1	0.11	0.20
	48	0	100.0	0.52	0.12
		1	83.5	1.34	0.20
		1/2	66.2	1.39	0.19
		1/10	99.8	0.45	0.22
3 August 1988	24	0	100.0	0.10	0.31
		1	103.2	1.10	0.25
		1/2	87.6	0.09	0.29
		1/10	63.2	0.11	0.22
	48	0	100.0	0.11	0.35
		1	95.6	0.62	0.30
		1/2	71.8	0.15	0.27
		1/10	65.7	0.18	0.29

(a) Yeast cells suspended in: 0, 4mL of buffer; 1, 4mL of raw water; 1/2, 2mL of raw water in 2mL buffer; 1/10, 0.4mL raw water in 3.6mL buffer.

(b) Percen of survival is relative to control in each experience Convertant (GC) and Reversion (R) frequencies are expressed per 10^4 and 10^5 survivors respectively.

Results obtained with raw water samples Station 1 June 1988 and Station 3 July 1988 are considered as weak positive because the frequency values obtained for gene conversion just duplicated those of the controls. Raw water samples from Station 1 August 1988 and Station 3 August 1988 showed a clear positive response after 24 hr incubation; in both cases the frequencies for induced gene conversion fell after 48 hr incubation of cells and raw water samples. Sample from Station 2 showed no induction of gene conversion neither after 24 hr nor 48 hr incubation.

From the three different samples obtained at Sampling Station 3 area, only one (August 1988) showed a positive response in gene conversion after 24 hr incubation period; the other two samples required 48 hr incubation with the cells.

None of the samples tested showed induction of mitotic revertion.

It is apparent from the data obtained that no correlation between the gene conversion induced by the samples and the place of sampling can be made. Marked variations in the genotoxic response of different samples were observed even in those obtained in the same sampling station. Whenever a clear positive response was observed, a two-fold dilution of raw water produced a ten-fold decrease in the gene conversion frequencies induced. These results demonstrated that the mixture found in the stream varies in concentration and composition, probably due to alterations in the flow and dilutions provoked mainly by the city run-off. In all the cases a ten-fold dilution turned the genotoxic effect undetectable by this system. These results are relevant from the viewpoint of potential genetic alterations induced in wildlife downstream from the Rio de la Plata.

Since the methodology employed for these assays allows only the testing of water soluble genotoxic agents, some research is in progress in order to determine the genetic activity of concentrates obtained using XAD2 resines.

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