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Polluted Water Concentrates: Induction of Genetic Alterations in *Saccharomyces cerevisiae* D7 Strain

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In a previous paper we showed that samples of raw water obtained from the Riachuelo (a heavily polluted watercourse) induced genetic effects in Saccharomyces cerevisiae D7 strain (Moretton et al. 1990) In those tests the raw water samples were assayed within 24 hr and only the mutagenic activity of the non-volatile, water soluble constituents could be detected.

The detection and quantitation of genetic toxicity in the organic water-insoluble fraction becomes much more difficult. This organic material consists of thousands of unidentified compounds in dilute mixtures which are not amenable to current analytical technology. The individual concentrations of these compounds are such that a great number of the mutagenic/carcinogenic components are below the limits of detection for short-term bioassays. Consequently, concentration of organic compounds is required before performance of biological tests.

The development of analytical methods involving XAD resins for the isolation and identification of pesticides and other compounds led to the use of XAD resins in the concentration of organics in water (Dressler 1979). This methodology combined with the use of short-term tests allows the identification of biohazardous fractions to be submitted to further analysis.

In the experiments reported here the genotoxic potential of XAD2 concentrates, obtained from samples of a heavily polluted stream, were evaluated by the induction of gene conversion and point mutation in Saccharomyces cerevisiae D7 strain.

MATERIALS AND METHODS

The Saccharomyces cerevisiae D7 strain was obtained from Dr. Giorgio Bronzetti, Istituto di Mutagenesi e Differenziamento CNR, Pisa, Italy. This strain can be used 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). Mitotic gene conversion and reverse point mutation are the two effects con-

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sidered as indicators of genotoxicity (Bronzetti et al. 1983; Del Carratore et al. 1984).

Surface river samples were collected with a Grab Sampler (Cole Parmer Instruments USA) along June 1989 from three sampling stations located within the industrial area near Buenos Aires city (34º 40' S lat.; 58º 25' W long.) as described previously (Moretton et al. 1990). This heavily polluted stream (Riachuelo) has been receiving effluents from tanneries, iron foundries, oil refineries and chemical plants as well as domestic waste waters for more than 40 years. Those effluents are daily mixed within the stream without treatment at all.

The macroreticular resin XAD2 was obtained from Rohm and Haas (Philadelphia, USA). The resin beds were purified by sequential solvent extraction with methanol, acetonitrile and diethyl ether, as described by Dressler (1979). The concentration column was prepared as follows: In a 25-mL glass burette a clean glass wool plug was inserted near the stopcock, XAD2 purified resin was added as a methanol slurry until a resin bed of approximately 9 cm high was obtained; then, a second glass wool plug was inserted above the resin. The methanol was drained through the stopcock and the resin was washed with three 20 mL portions of distilled water.

Samples of 500 mL of waste water were passed through the XAD2 resin column by gravity flow at a rate of 2 mL/min, then the column was washed with 20 mL of distilled water and elluted with 20 mL of diethyl ether.

Ether extracts were evaporated to dryness under low pressure and dissolved in 3 mL of dimethyl sulfoxide, and tested for genotoxicity withouth further sterilization.

Late log-phase cultures of yeast growing in liquid medium were washed with two 3-mL portions of phosphate buffer (0.1M, pH 7.4) and suspended in the same buffer at a concentration of 10^8 cells/mL. Suitable dillutions of XAD2 concentrates were then added to samples of yeast suspension. Each sample was incubated for 4 and 24 hr in a rotatory shaker at 28º C. After incubation each culture was washed with phosphate buffer, the final suspension was diluted appropriately and 0.1 mL samples were plated upon complete and slective medium (Zimmermann et al. 1975). The reproducibility of the technical procedure was checked by repeating each treatment in a second experiment conducted on a separate day (Loper 1980).

RESULTS AND DISCUSSION

The recovery of organic acids and phenols from Amberlite XAD2 resines is related to dissociation and is dependent on the pH (Dressler 1979; Loper 1980). The recovery increases in acidic media and the aqueous solutions of these compounds have to be acidified prior to the extraction. In order to determine the influence of the pH on the recovery of mutagenic activity, each water sample was divided into

two 500-mL portions. One was passed through the XAD2 column at its original pH (7-7.4); the other portion was acidified with 1N HCl to pH 3 before the extraction.

The cells were incubated with the concentrates during 4 and 24 hr because extended periods of incubation led to toxic effects. The biological tests were performed with pure and diluted samples in order to obtain information on the maintenance of the genotoxic response when the original mixture varies.

Table 1 presents the results obtained with concentrates of samples from three sampling stations tested for genotoxicity with Saccharomyces cerevisiae D7 strain. The genotoxic effects were considered positive when the experimental frequencies exceeded those of the controls by at least two fold (De Serres 1981).

All the concentrates tested showed a positive response in gene conversion induction. These results give a clear idea of the contamination degree in the samples examined. Concentrates from the sample 3 showed a clear possitive response after 24 hr incubation. This response was associated with a fall in the survival of the yeast induced for the XAD2 pH 7-7.4 concentrate but not for the acidic one. The other two samples showed a genotoxic response after 4 hr incubation. A slight toxic response, not associated with genotoxicity, was detected when pure concentrate of sample 2 were assayed. The genotoxicity was detected when the toxic effect disappeared with dilution.

None of the samples showed induction of mitotic reversion.

Resin concentrates from waste water are complex mixtures and genotoxic responses are often complicated due to the interaction of various toxic and mutagenic components. The future identification of even a few of the compounds involved in the genotoxic response will permit a systematic analysis for answers to such questions as the contribution of point sources to the origin of the genotoxic,seasonal effects and procedures for monitoring purposes.

The meaning of results such as those presented here in relation to mutagen exposure of human populations is still a mather of discussion. The advantages of the use of point mutation and gene conversion induction in yeast, as a genotoxicity bioassay, have been largely discussed by Zimmermann et al. (1975).

A relevant factor that emerges from this paper and the previous one (Moretton et al. 1990) is that we can positively say that a number of compounds, either water-soluble or water-insoluble, found in the stream are capable of inducing genetic changes in laboratory yeast cultures. Such compounds may be capable of inducing genetic changes in microbes that cause diseases in men and animals. In particular, characteristics such as the virulence of fungal pathogens and anti-

Table 1. Mitotic gene conversion and reverse mutation induced in Saccharomyces cerevisiae D7 strain by XAD2 concentrates.

Sampling Station	Incubation (hr)	Dose (a)	pH (b)	Frequencies (c) Survival%	GC	R
		0		100.0	0.42	0.28
		P	7-7.4	54.2	1.50	0.32
		1/10	7-7.4	108.5	0.46	0.10
	4	P	3	42.3	1.22	0.32
		1/10	3	115.4	0.35	0.29
1		0		100.0	0.39	0.30
		P	7-7.4	3.2	--	--
		1/10	7-7.4	76.7	0.48	0.30
	24	P	3	2.5	--	--
		1/10	3	118.9	0.53	0.28
		0		100.0	0.19	0.25
		P	7-7.4	56.8	0.22	0.27
		1/10	7-7.4	120.2	0.56	0.32
	4	P	3	81.5	0.30	0.35
		1/10	3	126.3	0.58	0.30
2		0		100.0	0.18	0.30
		P	7-7.4	3.8	--	--
		1/10	7-7.4	98.3	1.20	0.28
	24	P	3	33.1	0.25	0.35
		1/10	3	75.5	1.65	0.32
		0		100.0	0.24	0.30
		P	7-7.4	104.0	0.30	0.27
		1/10	7-7.4	86.4	0.10	0.20
	4	P	3	105.7	0.13	0.32
		1/10	3	111.3	0.29	0.30
3		0		100.0	0.10	0.28
		P	7-7.4	9.5	--	--
		1/10	7-7.4	63.8	0.43	0.30
	24	P	3	54.6	0.83	0.39
		1/10	3	115.5	0.37	0.27

(a) Yeast cells suspended in: 0, 1mL of dimethylsulfoxide in 3mL of buffer; P, 1 mL of concentrate in 3mL of buffer; 1/10, 0.1 mL of concentrate in 3.9 mL of buffer.
(b) Final pH of water samples.
(c) Percent of survival is relative to control in each experiment. Convertant (GC) and reversion (R) frequencies are expressed per 10^4 and 10^5 survivors, respectively.

biotic resistance may be influenced by induced mutation and recombination produced by these pollutants. If such genetic changes occur within an urban area, they may be of considerable importance for public health.

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