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Cell Culture Systems Are More Sensitive than *Saccharomyces cervisiae* Tests for Assessing the Toxicity of Aquatic Pollutants

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Cultured fish and human cells (Kocan *et al.* 1985, Marion and Denizeau 1983a, 1983b, Rachlin and Perlmutter 1968) have been used as bioassay systems for the evaluation of the toxicity of aquatic pollutants. Numerous assays using bacteria (Bitton 1983, McFeters *et al.* 1983, Liu and Dutka 1984) and yeast (Bitton 1984) have also been used for such purposes.

We now report the toxicity of aquatic pollutants (Cd, Hg and Ni), using cell culture systems and the yeast *Saccharomyces cervisiae* test. Cd, Hg and Ni were chosen as model compounds of pollutants because the related toxicity is now fairly well established.

MATERIALS AND METHODS

The yeast used in this test system (*Saccharomyces cervisiae* IFO 2260, obtained from the Institute for Fermentation, Osaka (IFO) Japan) was cultivated in yeast extract-malt extract (YM) medium (3 g yeast extract, 3 g malt extract, 5 g peptone and 10 g glucose in 1-L distilled water). Heavy metals inhibition of growth of the yeast was estimated in YM liquid cultures media. After 48-h incubation, the cells were suspended 1 X 10^5 cells per 1-mL of YM medium containing serial dilutions of heavy metals, and 5-mL of this YM medium were added to the test tubes (18 X 150 mm). Cultures were incubated at 25°C on a shaking incubator for up to 72-h. Cell density was determined by culture absorbance at 610 nm.

Cell lines used throughout this work were the human KB and HEL-R66 cells (Mochida and Gomyoda 1986). Toxicity test methods used were as described (Mochida 1986). KB and HEL-R66 cells were grown in a humidified, 5% CO_2 atmosphere at 37°C in Eagle's minimum essential medium (MEM), supplemented with 10% newborn calf serum, L-glutamine (292 μg/mL), penicillin G (100 unit/mL) and streptomycin sulfate (100 μg/mL). Cell suspension containing 1 X 10^5 cells/mL was seeded 5-mL volumes in 60 mm new Falcon plastic Petri dishes. After 24-h of incubation, the medium was replaced with 5-mL of MEM containing serial dilutions of the test metals. After a further 72-h of incubation, the cell number was

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determined, using a Bürker-Türk cell counter.

The following chemicals were used for the two test systems : cadmium chloride ($CdCl_2$), mercuric chloride ($HgCl_2$) and nickel chloride ($NiCl_2$)(Wako Pure Chemicals, Ind., Osaka, Japan). YM and MEM medium were used for the preparation of chemical solutions to *Saccharomyces cervisiae* and human cells tests, respectively. These solutions were filter sterilized. Chemical solutions spanned a 3 log concentration gradient.

Six replicate tests were carried out, for both systems. Results were expressed as percentage inhibitions compared with controls. ID50 values (concentrations exhibiting a 50% inhibition in growth) were calculated, using regression analysis (log concentration versus % inhibition). The ID50 values had 95% confidence limits of $\pm$ 5%.

RESULTS AND DISCUSSION

The growth inhibition assay was used to compare the relative sensitivity of *Saccharomyces cervisiae* and human KB and HEL-R66 cells toward Cd, Hg and Ni.

Table 1. A comparaison of the sensitivity to aquatic pollutants (Cd, Hg and Ni) using two toxicity systems (*Saccharomyces cervisiae* tests and cell culture systems).

metals	72-h ID50 (μM)[1]		
	Saccharomyces cervisiae	KB cells	HEL-R66 cells
Cd	76.0 $\pm$ 1.41	5.5 $\pm$ 0.33	3.1 $\pm$ 0.37
Hg	135.9 $\pm$ 0.66	32.9 $\pm$ 2.17	30.4 $\pm$ 3.61
Ni	1004.0 $\pm$ 5.63	120.0 $\pm$ 3.63	115.0 $\pm$ 3.31

1 Concentrations of heavy metals (Cd, Hg and Ni) producing a 50% growth reduction of *Saccharomyces cervisiae* and human cells (KB and HEL-R66) after 72-h incubation. The ID50 values had 95% confidence limits of $\pm$ 5%.

The 72-h ID50 values for the *Saccharomyces cervisiae* and human (KB and HEL-R66) cells tests are shown in Table 1. Cd, Hg and Ni were highly toxic to both the human cell lines, the 72-h ID50 values ranging from 3.1 to 120 μM. Cd was the most toxic and Ni the least toxic. The same ranking of toxicity was also obtained in the *Saccharomyces cervisiae* assay. This is in a agreement with results of Fischer (1976) and Skreb and Fischer (1984) who used human FH and HeLa cells and with the results of Grafl and Schwantes (1983) who used *Saccharomyces cervisiae*, *Saccharomycopsis lipolytica*, *Candida tropicalis and Candida utilis* to test effects of Cd, Hg and Ni.

Thus, the comparative data indicate that cell culture systems are more sensitive than the *Saccharomyces cervisiae* test for evaluating the toxicity of the aquatic pollutants (Cd, Hg and Ni). These ID50 values can serve as basis data for evaluating the hazards of chemicals on aquatic environments.

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