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Baker's Yeast Assay Procedure for Testing Heavy Metal Toxicity

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As a result of the entry of thousands of chemicals into the aquatic environment, there is a need for rapid screening of chemical toxicity. A wide range of biochemical and microbial tests have been developed to respond to this need. This topic has been reviewed by Bitton (1983). One of the most successful bacterial tests is the Microtox assay which is based on the reduction of bacterial luminescence following exposure to aquatic toxicants (Bulich et al. 1980, Chang et al. 1981, Dutka and Kwan 1981). The practical aspects of this assay are based on the use of freeze-dried cultures of Photobacterium phosphoreum. Bakers' yeast (Saccharomyces cerevisiae) is another microorganism which is commercially available and sold as packaged dry pellets in any food store at low cost. Studies have been undertaken on the effects of organic xenobiotics (Karenlampi et al. 1982, Nelson and Williams 1971) as well as heavy metals (Brunker 1976, Gadd 1983) on yeast metabolism. This type of study has been generally useful in examining the mechanism(s) of chemical toxicity. However, a rapid and quantitative toxicity test using S. cerevisiae as the test organism has not been developed.

The purpose of this study was to develop a toxicity assay for heavy metals, using commercial dry yeast as the test microorganism. This rapid and simple procedure is based on the reduction of 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyltetrazolium chloride (INT) to INT-formazan by the yeast electron transport system (Trevors 1982, Zimmermann et al. 1978). The scoring of active cells following exposure to heavy metals was undertaken according to the MINT (malachite green-INT) method developed by Bitton and Koopman (1982).

MATERIALS AND METHODS

The heavy metals (Cu^{++}, Hg^{++}, Zn^{++}, Ni^{++} and Ag^{+}) were dissolved in deionized water. Stock solutions were stored at 6 C until they were used.

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A commercial brand of dried bakers' yeast (Red Star Dry Yeast, Universal Food Corp., Milwaukee, WI) was employed throughout the entire study. This brand was selected because, according to the manufacturer, no antioxidants (e.g., BHT, BHA) are added to the dried cultures.

A 1% (w/v) yeast suspension was prepared using sterilized saline (0.85% NaCl) as the suspending fluid. In toxicity tests with Ag^+, the yeast cells were suspended in deionized water in lieu of saline. The yeast suspension was stirred for 15 min using a magnetic mixer in order to break up the yeast flocs, yielding a more homogeneous suspension. 0.8 mL of yeast suspension and 0.2 mL of the appropriate toxicant concentration were combined in a series of 2-mL plastic tubes. Control tubes contained 0.8 mL of yeast suspension and 0.2 mL of deionized water. The tubes were capped and shaken at 30 C for a period of 30 min. After the exposure period, each tube was amended with 0.1 mL of 0.2% (w/v) INT (Eastman Kodak Co., Rochester, NY) and 0.1 mL of a 10% solution of yeast extract which served as a substrate. Incubation with INT and yeast extract was carried out in the dark at 30 C with shaking. The yeast suspension turned red upon reduction of INT to INT-formazan by the yeast dehydrogenase enzymes. INT reduction was stopped after 60 min by adding 0.1 mL of 37% formalin.

The proportion of respiring (i.e., active) cells was determined according to a modification of the method developed by Bitton and Koopman (1982): One or two loopfuls of the yeast suspension was spread on a glass slide and allowed to air dry prior to heat fixing. The smear was then counterstained with 0.025% (w/v) malachite green (MC/B, Norwood, OH). After 1 min, the malachite green was drained completely (do not wash) off the slide and the smear blotted prior to examination under oil immersion (1000X or 1600X), using bright field microscopy. The scoring consisted of counting separately the number of respiring cells (green with red formazan crystals present) and non-respiring cells (green only). Approximately 500 total cells were examined for each count. Figure 1 shows a micrograph of respiring and non-respiring yeast cells. The proportion of respiring cells was calculated following exposure to each toxicant concentration. The EC_{50} (effective concentration which inhibits 50% of the cells) was derived from the best fit line via regression analysis of the data. All data presented in this study represent means of triplicate runs.

RESULTS AND DISCUSSION

EC_{50} values for five heavy metals, determined according to the bakers' yeast assay procedure, are given in Table 1. Comparisons are made for copper, mercury, zinc and nickel to alternative toxicity assays employing microbes as indicator organisms. These include Microtox, Spirillum volutans, Pseudomonas fluorescens and Aeromonas hydrophila assays as reported by Dutka and Kwan (1981) and Qureshi et al. (1982). The proposed procedure was the most sensitive assay for copper and was intermediate in sensitivity for mercury, zinc and nickel. Only in the case of mercury was the

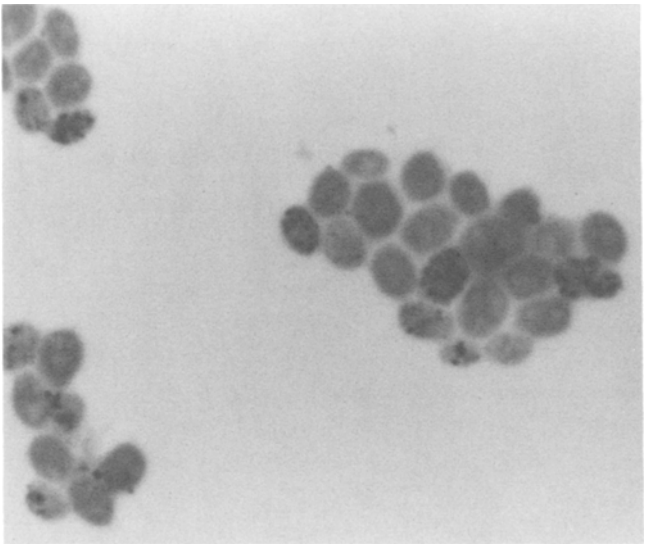

Figure 1. Yeast cells stained according to the MINT technique. Shown are a total of 36 cells, of which 11 (31%) contain INT-formazan crystals and are thus considered to be active.

Table 1. Comparison of EC_{50} values determined according to the bakers' yeast assay procedure with toxicities from alternative assays. All values are in mg/L.

Metal	Bakers' Yeast Assay	Alternative Assays[a]
Cu^{++}	5.6 ± 0.3	8.6(S)-9.2(M)-17(P)-21(A)
Hg^{++}	0.8 ± 0.1	0.031(P)-0.049(A)-0.060(M)-0.86(S)
Zn^{++}	19.5 ± 3.2	9.3(S)-13(M)-360(P)-500(A)
Ni^{++}	19.6 ± 3.8	8.7(P)-17(A)-20(S)-23(M)
Ag^{+}	6.3 ± 0.5	-

[a]Alternative assays were Microtox (M), S. volutans (S), P. fluorescens (P) and A. hydrophila (A) as reported by Dutka and Kwan (1981) and Qureshi et al. (1982). Multiple values for the same assay are represented by their geometric mean.

sensitivity of the bakers' yeast assay procedure less than 40% that of the most sensitive alternative toxicity assay. Cenci and Morozzi (1979) and Ryssov-Nielsen (1975) observed a similar lack

of sensitivity of dehydrogenase activity assays to mercury, measuring EC_{50} values of 2.6 and 1.5 mg/L, respectively, in activated sludge.

It was important to investigate if the extent of heavy metal toxicity varied from one batch of yeast to another. Table 2 shows that, with regard to copper and zinc toxicity, there were no significant differences between two separate batches of yeast.

Table 2. Comparison of EC_{50} values for copper and zinc obtained using two different batches of yeast.

Batch No.	Copper	Zinc
1	5.6 ± 0.3	19.5 ± 3.2
2	6.2 ± 1.0	20.6 ± 0.6

We have described a simple, rapid and inexpensive assay to test heavy metal toxicity. Bakers' yeast, readily available in dry pellets from commercial sources, is inexpensive and stable at room temperature for relatively long periods. No tedious and time-consuming culture techniques are necessary since the commercial preparations may be used simply by suspending them in saline or deionized water. The cells can be easily observed using a low-cost compound microscope. The assay can also be easily adapted to field conditions.

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