Bull. Environ. Contam. Toxicol. (1992) 49:444–448

Environmental Contamination and Toxicology

Influence of Temperature and Nutrient Strength on the Susceptibility of *Saccharomyces cerevisiae* to Heavy Metals

T. Hsu, L. W. Lee, and T. H. Chang

Cell Biology and Immunology Division, Development Center for Biotechnology, Taipei, Taiwan 10671, Republic of China

Saccharomyces cerevisiae is not only a key microorganism in brewing or fermentation processes, it has also been employed for monitoring aquatic pollutants (Bitton et al. 1984 ; Mochida et al. 1988a ; Mochida et al. 1988b; Haubenstricker et al. 1990). The major advantage of using *Saccharomyces cerevisiae* as a bioassay system is that this yeast can be easily obtained as dry pellets from commercial sources at low cost. In addition to its economical aspect, *Saccharomyces cerevisiae* , like other microorganisms, is easy to handle, grows rapidly, and provides a large number of homogeneous individuals for utilization in toxicity tests.

Although cell growth (Mochida et al.1988a), cell viability (Mochida et al. 1988b), electron transport (Bitton et al. 1984) and mitochondrial respiration (Haubenstricker et al. 1990) of *Saccharomyces cerevisiaes* have all been selected as parameters for toxicity assessment, measuring cell growth by absorbance is by far the most convenient and rapid method when large amounts of water samples are to be tested. Mochida et al. (1988 a) ,however, reported that *Saccharomyces cerevisiae* was five to ten times less sensitive than cell culture systems to cadmium, mercury and nickel, when cell growth of both systems was monitored. This relative insensitivity to heavy metals might handicap the practical use of this yeast strain for bioassays.

Since previous studies indicated that the susceptibility of microorganisms to enviromental toxicants can be influenced by incubation temperature and nutrient strength (Slabbert 1986; Baxter et al. 1987; Prahalad and Seennayya 1988), we attempted to examine the effect of incubation temperature and nutrient strength on the susceptibility of *Saccharomyces cerevisiae* to heavy metals in order to obtain the optimum bioassay sensitivity. In this study, we used cadmium and mercury as model toxicants.

MATERIALS AND METHODS

Saccharomyces cerevisiae dry pellet, produced by Taiwan Sugar Co., was purchased from a local commercial supplier. Cadmium chloride and mercuric chloride were obtained from Sigma Co. (MO, U.S.A.). YM broth (3 gm yeast extract, 3 gm malt extract, 5 gm peptone and 10 gm glucose in 1 liter distilled water) was a product of Difco Co. (Detroit MI, U.S.A.).

Send reprint requests to T.H. Chang at the above address.

Inoculum was prepared in YM broth (pH 5.2) and incubated at 37℃ overnight. Before each test, the broth culture was diluted with phosphate buffered saline, and the cell number was counted on a hemocytometer. *Saccharomyces cerevisiae* was then added to sterile 12X75 mm tubes containing serial dilutions of heavy metals in 2ml YM broth. The initial cell density was always kept at $1X10^6$/ml. The cell cultures were incubated at 37 °C with shaking for 6hrs, and the optical density of each culture was determined on a Hitachi 150- 20 spectrophotometer with wavelength at 660 nm. Yeast cells were incubated at 37℃ because we desired to compare our data with mammalian cell bioassays conducted at this temperature. Another inoculm was grown and tested for its susceptibility to heavy metals at 25℃ according to the same procedure mentioned above.

To compare the effects of heavy metals on *Saccharomyces cerevisiae* under different nutrient strengths, yeast cells were added to test tubes containing heavy metals in full, half, and quarter strength of YM broth followed by shaking incubation at 37℃. Results were expressed as percentage inhibition compared with controls. IC_{50} values were calculated using regression analysis (log concentration versus % inhibition).

RESULTS AND DISCUSSION

After a 6-hour incubation period at 37℃ , $1x10^6$/ml *S.cerevisiae* usually gave an absorbance reading of 0.7 to 0.8, whereas the absorbance reading was around 0.2 at 25℃ , indicating that growth rate is regulated by temperature. Dilution of medium to half and a quarter strength caused a reduction of growth rate by 10 and 30%, respectively.

The susceptibility of *S. cerevisiae* to cadmium was dramatically enhanced by elevating incubation temperature, the IC_{50} values for this yeast was 6.43uM at 25℃ and 0.42uM at 37℃. Dilution of YM medium did not significantly alter the effect of cadmium on *S.cerevisiae* , except that the sensitivity of *S. cerevisiae* to cadmium was slightly increased as IC_{10} value for yeasts grown in quarter stength medium shifted from 0.13uM to 0.075uM. *S. cerevisiae* grown in half medium strength showed almost identical response(Fig.1).

Elevation of incubation temperature also enhanced the susceptibility of *S. cerevisiae* to mercury. The degree of enhancement, however, was less than that for cadmium. The IC_{50} value was 4.8uM for *S. cerevisiae* grown at 25℃ versus 3.2uM for those grown at 37℃. Dilution of medium did not affect the response of *S. cerevisiae* to mercury (Fig.2). Our results indicated that temperature played a major role in altering the susceptibility of *S. cerevisiae* to cadmium and mercury, yet complexation of nutrient components with metals imposed only very minor effects.

Schappert and Khachatourians (1984) reported that *S.carlbergensis* and *S. Cerevisiae* can be rendered more sensitive to T-2 toxin by elevating incubation temperature. They proposed that elevated temperature enhanced the fluid dynamics of yeast membrane, and, therefore, provided toxin molecules an easier accessibility to their target sites. It is very likely in our study that the increased fluid membrane dynamics facilitated the entry of heavy metals into yeast cells, then resulted in a more profound inhibitory effects. The differential effects of elevated temperature on the susceptibility of *S. cerevisiae* to cadmium and mercury were possibly due to different entry restrictions.

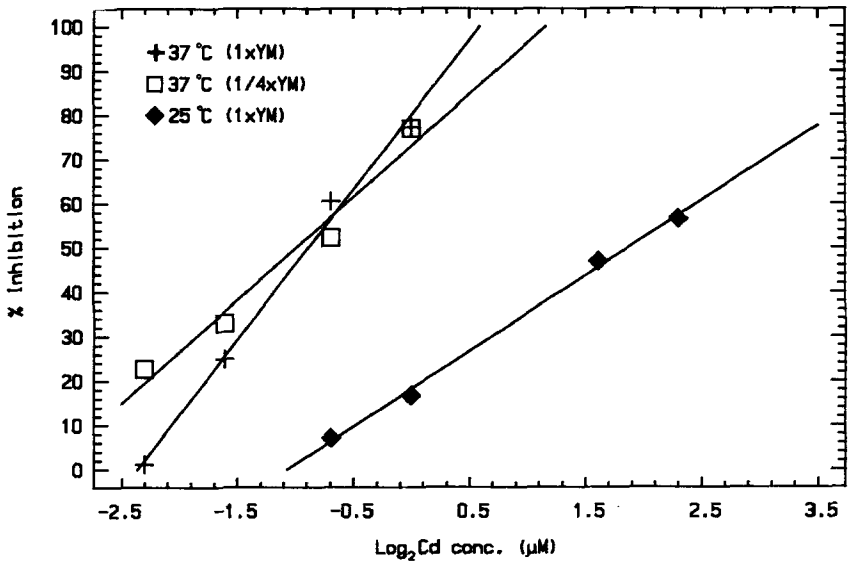

Figure 1. Effects of temperature and nutrient strength on the susceptibility of *S. cerevisiae* to cadmium. Each point represents the mean of 5 replicates with S.D. less than 10%.

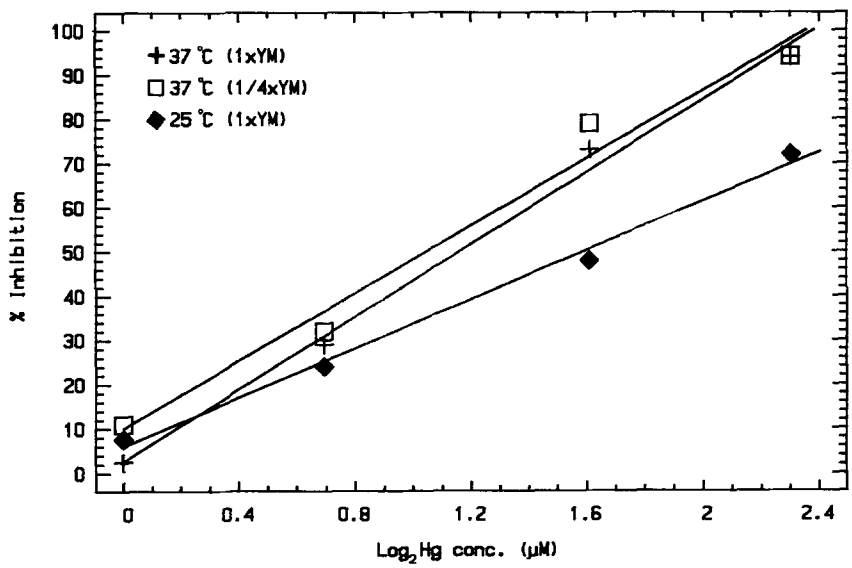

Figure 2. Effects of temperature and nutrient strength on the susceptibility of *S. cerevisiae* to mercury. Each point represents the mean of 5 replicates with S.D. less than 10%.

In contrast to the results of Mochida et al. (1988 a) who concluded that *S. cerevisiae* is less sensitive than mammalian cell culture systems, our 6-hour *S. cerevisiae* bioassay showed a better sensitivity, at least, than 72-hour cell culture assays. We also confirmed that the order of magnitude between the sensitivity for cadmium and the sensitivity for mercury is similar for both *S. cerevisiae* and mammalian cell cultures(Table 1). Thus, *S. cerevisiae* can be regarded as a valuable organism for predicting the hazardous effects of chemicals on mammals. We have tested two different batches of *S. cerevisiae* dry pellet produced by the same company and obtained very close results.

Table 1. Comparison of the IC_{50} values (mg/l) determined according to this 6-hr *S. cerevisiae* toxicity test with other *S. cerevisiae* and mammalian cell bioassays.

	6hrs	72hrs	72hrs	72hrs	72hrs
Chemical	S-C[1]	S-C[2]	KBcells[2]	HEL-R66 cells[2]	AGMK cells[3]
Cd^{2+}	0.05	8.53	0.62	0.35	0.36
Hg^{2+}	0.66	27.25	6.59	6.09	N.D.[4]

1. Assays were conducted at 37℃ in full strength medium.
2. Mochida et al. (1988 a)
3. Mochida (1986)
4. not determined

It is easy and convenient to perform *S. cerevisiae* toxicity tests by monitoring the influence of toxicants on its growth since this can be done in any laboratory equipped with a simple model spectrophotometer. It would be more feasible to apply this test for assessing large amounts of heavy metal-containing industrial effluent samples if reasonable sensitivity and short test periods could be obtained. Our results suggest that higher incubation temperatures such as 37℃ and lower incubation strength should enable the practical use of this 6-hour *S. cerevisiae* bioassay.

Acknowledgments. This work was supported by the Republic of China Enviromental Protection Agency grant No. EPA-79-003-13-052. We thank Ms. Pui-Yee Ng for typing this manuscript.

REFERENCES

Baxter JA, Chia LS, Hsieh DW, Datta SK (1987) Survey of sensitivity of 22 strains of yeasts to T-2 toxin in relation to growth on glucose and glycerol medium. Bull Environ Contam Toxicol 39: 86-91

Bitton G, Koopman B, Wang HD (1984) Baker's yeast assay procedure for testing heavy metal toxicity. Bull Environ Contam Toxicol 32: 80-84

Haubenstricker ME, Meier PG, Mancy RH, Brabec MJ (1990) Rapid toxicity testing based on yeast respiratory activity. Bull Environ Contam Toxicol 44: 669-674

Mochida K (1986) Aquatic toxicity evaluated using human and monkey cell culture assays. Bull Environ Contam Toxicol 36: 523-526

Mochida K, Gomyoda M, Fujita T, Yamagata K (1988 a) Cell culture systems are more sensitive than *Saccharomyces cervisiae* tests for assessing the toxicity of aquatic pollutants. Bull Environ Contam Toxicol

41: 1-3
Mochida K, Gomyoda M, Fujita T, Yamagata K (1988 b) Toxicity assessment of bis(tri-n-butyltin)oxide (TBTO) using yeast *Saccharomyces cerevisiae* and human KB cells. Bull Environ Contam Toxicol 41: 847-850
Prahalad AK, Seenayya G (1988) Bioavailability of zinc and cadmium and their effect on microbial growth and metal uptake. Bull Environ Contam Toxicol 41: 921-927
Schappert K, Khachatourians GG (1984) Influence of the membrane on T-2 toxin toxicity in *Saccharomyces* spp. Appl Environ Microbiol 47: 681-684
Slabbert JL (1986) Improved bacterial growth test for rapid water toxicity screening. Bull Environ Contam Toxicol 37: 565-569

Received December 30, 1991; accepted March 30, 1992.