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Transformations of Inorganic Mercury by *Candida albicans* and *Saccharomyces cerevisiae*

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***Saccharomyces cerevisiae* and *Candida albicans* were incubated with 0.25, 0.5, or 0.75 μg of Hg (as $HgCl_2$) per ml of Nelson's medium in the presence of trace amounts of oxygen at 28°C for 12 days. Two control media were used, one without added Hg and one without yeast inoculum. Yeast cell growth was estimated after 1, 2, 3, and 8 days of incubation. The contents of organomercury in the system and of elemental mercury released from the media and collected in traps were determined at the end of the experiments. The results were as follows. (i) *C. albicans* was the more mercury-resistant species, but both yeast species failed to grow in the media containing 0.75 μg of Hg per ml. (ii) The amounts of organomercury produced by the two species were proportional to the amount of $HgCl_2$ added to the medium. In all cases *C. albicans* produced considerably larger amounts of methylmercury than *S. cerevisiae*. (iii) The amounts of elemental Hg produced were inversely proportional to the $HgCl_2$ level added in the case of *S. cerevisiae* but were all similar in the case of *C. albicans*. (iv) Neither organomercury nor elemental Hg was produced in any of the control media.**

Various microorganisms are capable of converting inorganic forms of certain heavy metals into organic derivatives (4, 10). Surprisingly, though, little information is available about such processes in yeast cells. *Saccharomyces cerevisiae* incubated with $HgCl_2$ for 3 days produced a small amount of CH_3Hg^+ (11). A *Cryptococcus* sp. was found to produce elemental Hg by reducing $HgCl_2$ (3). The mercury mentioned above is that found in the yeast cells themselves; it is not the Hg derivative found in the media or the liberated volatile forms of this metal. Also, the studies mentioned above were carried out under aerobic conditions. We failed to find any information on such processes taking place in the presence of only trace amounts of oxygen which occur in deep water bodies, especially in the presence of enrichment, such as from pollution or algal blooms, that greatly enhances yeast cell growth (5).

Both alkylation and reduction of mercurials to elemental Hg increase the volatility of the metal. Although organomercury derivatives are by far more toxic than Hg^{2+} (12), it is quite conceivable that elimination of some of the metal by volatilization due to microbial metabolism may decrease, rather than increase, the toxicity of this metal (6).

This study was undertaken to investigate the possible formation of organomercury and elemental Hg by yeast species in a medium containing trace amounts of oxygen. Of the yeast species tested earlier in our laboratory, *Candida albicans* was the most resistant to mercury, whereas *S. cerevisiae* was quite sensitive to this metal. Therefore, we included these two species in the present investigation.

MATERIALS AND METHODS

Media. Nelson medium (in grams per liter of distilled water) was as follows: glucose, 2.0; Casamino Acids (Difco Laboratories, Detroit, Mich.), 5.0; yeast extract (Difco), 1.0; NaCl, 10.0; $MgCl_2 \cdot 6H_2O$, 2.3; and KCl, 3.0. The pH was adjusted to 7.3, and the medium was autoclaved at 121°C for 15 min. Yeast extract agar (in grams per liter of distilled water) was as follows: glucose, 30.0; peptone, 5.0; yeast extract (Difco), 5.0; and agar (Difco), 25.0.

Growth conditions. The yeast cells were grown at 28°C in 2-liter Erlenmeyer flasks containing 1.5 liters of Nelson medium, into which $HgCl_2$ was added. The flasks were stripped with sterile nitrogen gas to remove all dissolved oxygen and then closed with rubber stoppers to eliminate most of the oxygen from the system. Microbial growth was estimated by viable counting of aliquots taken after incubation for 48 h on yeast extract medium. The concentrations of Hg (as $HgCl_2$) added were 0.25, 0.5, and 0.75 μg/ml of medium.

Two kinds of control flasks were employed, each in quadruplicate. The first one did not contain added mercury, and the second, containing the highest Hg^{2+} level used (0.75 mg/ml of medium), was incubated without yeast inoculum.

Each mercury level and controls were tested in four replicate flasks. The experiments were run twice, and the points in the figures represent the means.

Yeast species. *S. cerevisiae* ATCC 7752 and *C. albicans* CBS 682 were obtained from our Unit of Microbiology culture stock.

Analytical methods. Polyvinyl chloride tubing pierced through the stoppers of the growth flasks connected them to a series of three traps, as previously described (7), except that in our study the first two traps contained the same solvent (aqueous solutions of 5% Na_2CO_3 and 2.5% Na_2HPO_4 in equal volumes) to capture organic Hg, and the third trap contained 5% $KMnO_4$ in 2 N H_2SO_4, to remove elemental Hg, which is not captured in the first two traps, by oxidizing it to Hg^{2+}. The volume of the solution in all traps was 100 ml. Sterile nitrogen was passed through the system twice a day to drive the volatile forms of Hg through the traps and to remove as much of the oxygen in the system as possible. Samples from the growth media and traps were taken 12 days after the start of the experiment for determinations of inorganic and organic Hg. These assays were carried out in triplicate, as previously described by Levitan et al. (8) and Longbottom et al. (9).

Statistical analyses. The results were subjected to one-way

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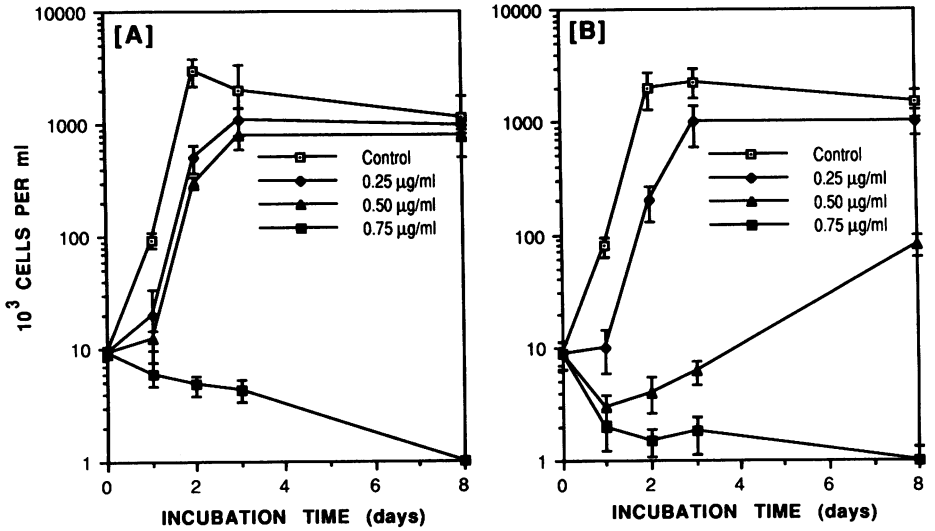

FIG. 1. Growth of *C. albicans* (A) and *S. cerevisiae* (B) in the presence of different levels of mercury. Vertical bars denote standard deviations of the mean.

analysis of variance, and in cases in which significant effects of the dose level were found, Fisher's test was performed to check for significant differences among the data for the various dose levels.

RESULTS AND DISCUSSION

Effects of mercury on growth rate. The growth performances of *C. albicans* and *S. cerevisiae* in media containing different concentrations of Hg^{2+} are shown in Fig. 1. All media containing Hg caused considerable growth retardation in both species during most of the incubation period. The media containing the highest Hg concentration (0.75 μg/ml) did not allow growth at all. *C. albicans* was the more resistant species; at 0.5 μg of Hg^{2+} per ml there was only a slight growth inhibition, and even in the presence of 0.75 μg of Hg^{2+} per ml the initial cell count did not decrease appreciably during the first 3 days of incubation. In the case of *S. cerevisiae*, however, 0.5 μg of Hg^{2+} per ml brought about marked growth inhibition, and in the medium containing 0.75 μg of Hg^{2+} per ml the cell population decreased drastically even within the first day.

Production of organomercury. The amounts of organic mercury (probably methylmercury) found in the growth media containing different concentrations of $HgCl_2$ and in the traps connected to them for the two yeast species after 12 days of incubation are shown in Fig. 2. The data established that the amounts of organomercury excreted into the media (Fig. 2A) and also the smaller amounts released from them and captured in the respective traps (Fig. 2B) are proportional to the amounts of $HgCl_2$ added to the medium at the start of the experiment. The differences were in all cases significant ($P < 0.01$). In both series of media and traps, *C. albicans*, which was the more mercury-resistant yeast species, produced significantly larger amounts of methylmercury than did *S. cerevisiae*. These findings are in agree-

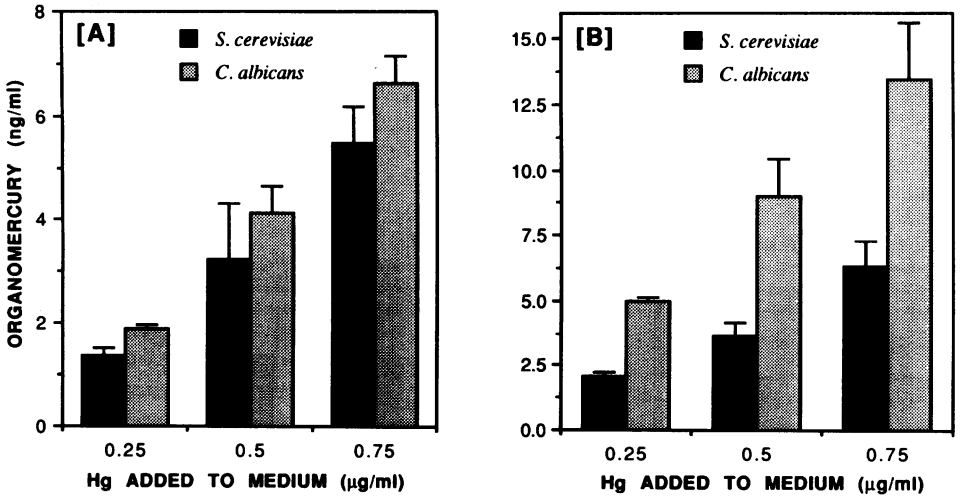

FIG. 2. Amounts of organomercury found in the growth medium (A) and organomercury in the traps (B) of *S. cerevisiae* and *C. albicans* after a 12-day incubation as a function of the concentration of $HgCl_2$ added. Vertical bars denote standard deviations of the mean.

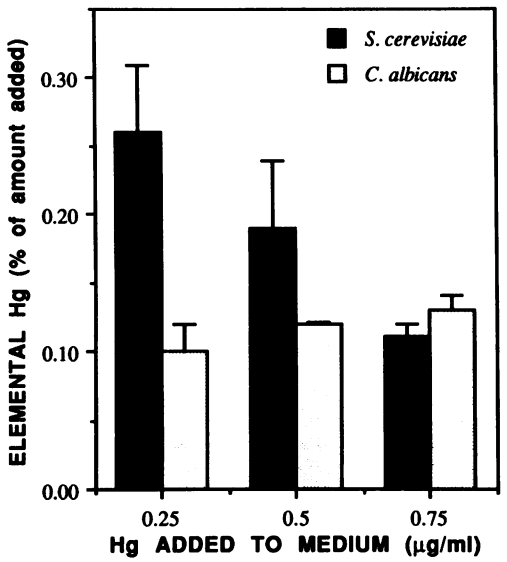

FIG. 3. Amounts of elemental Hg found in the traps connected to the different cultures after a 12-day incubation as a function of the concentration of $HgCl_2$ added. Vertical bars denote standard deviations of the mean.

ment with earlier observations for bacterial species; namely, the organisms exhibiting a higher resistance to toxic metals show a relatively high capacity to convert them to methyl derivatives (1, 2). The two control systems employed, one without added mercury and the other without yeast inoculum, did not contain detectable amounts of organomercury. It should be noted, though, that under the conditions prevailing in our experiments the amounts of organomercury found in the traps (Fig. 2B) were much smaller than those found in the medium (Fig. 2A). Yet, as mentioned above, the resistance of *C. albicans* was higher, despite the fact that it produced more of the highly toxic organomercury and that most of it was retained in the medium.

Production of elemental mercury. Both yeast species were also capable of reducing Hg^{2+} to elemental Hg (Fig. 3). However, while *C. albicans* produced a similar amount of elemental Hg in all media—except in the control (without yeast inoculum) which was devoid of any elemental Hg—*S. cerevisiae* exhibited a diminishing capacity for this reduction with increasing concentrations of Hg^{2+} ($P < 0.05$).

In many bodies of water the most prevalent microorganisms are bacteria. Yet, under certain environmental conditions yeast species may become the dominating species. This has been reported for enriched and polluted estuarine waters (5). Also, ecosystems with low pHs often favor yeast cell growth. Acid waters may be encountered in areas contaminated with acid rain, where biomethylation of mercury and other electrophiles is very likely (14). Therefore, we believe that the findings of this report indicate that biomethylation of this metal can be anticipated in such areas as well because of yeast species.

REFERENCES

1. **Berdicevsky, I., H. Shoyerman, and S. Yannai.** 1979. Formation of methylmercury in the marine sediment under in vitro conditions. Environ. Res. **20**:325–334.
2. **Brinckman, F. E., and G. J. Olson.** 1988. Global biomethylation of the elements: its role in the biosphere translated to new organometallic chemistry and biotechnology, p. 168–196. *In* P. J. Craig and G. Glockling (ed.), The biological alkylation of heavy elements. Royal Society of Chemistry, London.
3. **Bruncker, R. L., and T. L. Bott.** 1974. Reduction of mercury to the elemental state by a yeast. Appl. Microbiol. **27**:870–873.
4. **Glockling, F.** 1988. The bioalkylation of heavy elements: an historical introduction, p. 1–4. *In* P. J. Craig and G. Glockling (ed.), The biological alkylation of heavy elements. Royal Society of Chemistry, London.
5. **Hagler, A. N., and L. C. Mendonca-Hagler.** 1981. Yeasts from marine and estuarine waters with different levels of pollution in the state of Rio de Janeiro, Brazil. Appl. Environ. Microbiol. **41**:173–178.
6. **Hamdy, M. K., and O. R. Noyes.** 1975. Formation of methylmercury by bacteria. Appl. Microbiol. **30**:424–432.
7. **Kimura, Y., and V. L. Miller.** 1960. Vapor phase separation of methyl- or ethylmercury compounds and metallic mercury. Anal. Chem. **32**:420–424.
8. **Levitan, S., L. Rosner, and S. Yannai.** 1974. Mercury levels in some carnivorous and herbivorous Israeli fishes, and their habitats. Isr. J. Zool. **23**:135–142.
9. **Longbottom, J. E., R. Dressman, and J. Lichtenberg.** 1973. Gas chromatographic determination of methylmercury in fish, sediment and water. J. Assoc. Off. Anal. Chem. **56**:1297–1303.
10. **Robinson, J. B., and O. H. Touvinen.** 1984. Mechanisms of microbial resistance and detoxification of mercury and organomercury compounds: physiological, biochemical and genetic analyses. Microbiol. Rev. **48**:95–124.
11. **Vonk, J. W., and A. K. Sijpesteijn.** 1973. Studies on the methylation of mercury chloride by pure cultures of bacteria and fungi. Antonie van Leeuwenhoek **39**:505–513.
12. **Wood, J. M.** 1972. A progress report on mercury. Environment **14**:33–39.
13. **Wood, J. M.** 1974. Biological cycles for toxic elements in the environment. Science **183**:1049–1052.
14. **Wood, J. M.** 1988. Mechanisms for B_{12}-dependent methyl transfer to heavy elements, p. 62–76. *In* P. J. Craig and G. Glocking (ed.), The biological alkylation of heavy elements. Royal Society of Chemistry, London.