Growth Inhibition of *Rhodopseudomonas capsulata* by Methylmercury Acetate

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Growth of *Rhodopseudomonas capsulata* was inhibited in a bacteriostatic manner by as little as $10^{-8}$ M methylmercury acetate (MeHgAc) in unsupplemented synthetic liquid medium or when cells were exposed to 8.0 nm of MeHgAc per mg of cell protein in a single exposure.

Several organo-mercurial fungicides have been reported to reduce photosynthesis by plankton at concentrations as low as 1.0 µg/liter (1), and mercuric chloride has been shown to reduce photosynthesis and growth of *Chlorella pyrenoidosa* at concentrations as low as 3.0 µg/liter (3). Mercuric ions introduced into marine sediments are converted to methylmercury compounds (2) and cell-free extracts of a methanogenic bacterium are capable of carrying out the transformation (7). We determined the minimal inhibitory concentrations of methylmercury acetate (MeHgAc) on the growth of an environmental isolate of *Rhodopseudomonas capsulata* under various nutritional conditions.

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Stock cultures were maintained in yeast-peptone (3.0 g/liter) agar stabs. The synthetic liquid medium (SLM) of Ormerod et al. (5), as modified by the addition of 50 µg of niacinamide and thiamine per liter, was used for growth and MeHgAc inhibition studies. Cells were grown in SLM with neutralized malic acid (4.02 g/liter) as a carbon source. Yeast extract (0.15 g/liter) and vitamin-free casein (Nutritional Biochemical Co., 0.44 g/liter) were sterilized along with the basal salts by autoclaving.

Vitamins (biotin, 15 µg/liter, niacinamide, 50 µg/liter, thiamine, 50 µg/liter) and cystine (10^{-4} M) were aseptically added to the SLM after passage through a filtration membrane. Cultures (100 ml of SLM per 140-ml square screw-cap bottle) were incubated at 25 C under a bank of fluorescent lamps at a distance of either 50 or 10 cm (saturating illumination).

Methylmercury acetate (MCB Scientific Products, Santa Ana, Calif.) was aseptically diluted from a 40% acetic acid stock solution and added directly to the media at the time of inoculation. Optical density at 580 nm was used as a measure of growth. Protein content of cell suspensions for inoculation was determined by the method of Lowry et al. (4).

The effect of the MeHgAc was primarily bacteriostatic at low concentrations. Cultures would begin to grow slowly at first and then at a rate nearly equal to that of the control. The differences in the initial growth rates are shown in Fig. 1. Cultures which began to grow within 50 h would usually reach a limiting cell density similar to that of the control (optical density, 3.0) within 300 h.

Preliminary experiments with cystine demon-

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**Fig. 1. Inhibition of growth rate by increasing concentrations of MeHgAc. Symbols: □, control (no MeHgAc); ○, 5 × 10^{-8} M MeHgAc; Δ, 10^{-7} M MeHgAc; △, 1.8 × 10^{-7} M MeHgAc.**
strated that it might offer some protection from MeHgAc inhibition. A final concentration of $10^{-8}$ M cystine permitted growth of *R. capsulata* (50% of control) at 3.5 times the concentration of MeHgAc observed in its absence. When inoculated with 0.5 μg of cell protein per ml, *R. capsulata* did not grow in the unsupplemented medium at concentrations greater than $10^{-8}$ M MeHgAc.

Growth inhibition in the presence of yeast extract was compared to growth inhibition in the presence of vitamin-free casein when each
were used at concentrations which provided 10^{-4} M cystine in the final medium, according to manufacturers analyses of their products. When inoculated with 5.0 \mu g of cell protein per ml, R. capsulata did not grow in the unsupplemented medium at MeHgAc concentrations above 1.7 \times 10^{-8} M. Medium supplemented with vitamin-free casein showed growth at 3.2 \times 10^{-8} M MeHgAc, and medium supplemented with yeast extract showed growth at 10^{-7} M MeHgAc. The higher minimal inhibitory concentration in unsupplemented SLM shown with this experiment as compared to the previous could be attributed to the fact that a 10-fold larger inoculum was used in this latter case.

To elucidate the time- and concentration-dependent relationship between cells and inhibitor, equal amounts of cell protein (450 \mu g) were exposed to various concentrations of MeHgAc in 40 ml of distilled water (4 C) for 10 to 80 min. After exposure, cells were washed once (40 ml of \text{H}_2\text{O}) and suspended in 50 ml of fresh SLM. The cultures were incubated under saturating illumination and observed for growth. No increase in inhibition was observed by lengthening the time of exposure at concentrations of MeHgAc less than 3 \times 10^{-8} M, and at higher concentrations the inhibition was virtually independent of contact time (Fig. 2). It was found that 8 nm of MeHgAc per mg of cell protein slowed growth and that 24 nm was bacteriostatic after a single 10-min exposure. The initial increase in growth rate for the 5 h immediately after MeHgAc exposure caused the cultures of cells exposed to 0.9 and 2.7 nm to exceed the optical densities of the unexposed controls.

The rapidity of the inhibition and the extremely small amounts required for inhibition in the absence of dissolved organic materials led us to suspect that either some component of yeast extract competed with the organisms for the binding of MeHgAc and thereby permitted their growth, or that the vitamins and cofactors stimulated growth under conditions in which cell syntheses were otherwise limiting.

Interaction of methylmercury or mercuric ions with cystine has not been previously reported. It is possible that, in the experiments reported here, the yeast extract supplement provided sufficient cyanocobalamin or methionine to overcome the deficiencies created by the presence of MeHgAc. Demethylation of the MeHgAc could permit the mercuric ion to react with free sulphydryl groups in the cell which were less essential to the metabolic functions. This could account for the reversibility of inhibition. The most puzzling observation was the apparent stimulation of growth during short-term exposure to low concentrations of the inhibitor. This effect could be related to the ability of the methylmercury to alleviate methionine auxotrophy in yeast (6).

In summary, the inhibition of MeHgAc was reversible and largely independent of the length of exposure. The ability of organic compounds to alleviate inhibition suggests either competition between cells and media constituents or co-metabolism and inactivation of the inhibitor.

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