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Effects of Oxygen and Methyl Viologen on *Thermus aquaticus*

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Under increased oxygen tensions, *Thermus aquaticus* exhibited a lag period in growth of 80 min, during which the specific activities of catalase, peroxidase, and superoxide dismutase increased. Methyl viologen increased the lag period, decreased the maximum population density, increased cell lysis, and induced catalase and superoxide dismutase. Methyl viologen, in conjunction with chloramphenicol, decreased cell viability.

Oxygen toxicity results from the partial reduction of oxygen to hydrogen peroxide and the superoxide radical, which form the hydroxyl radical by the Haber-Weiss reaction (5–7). Aerobic organisms are protected from these substances by catalase (CAT), peroxidase (PER), and/or superoxide dismutase (SOD) (5–7, 13). Many aerobes respond to elevated oxygen tensions by increasing the specific activity of one or more of these enzymes (9, 10, 13, 16).

The superoxide radical may be responsible for the induction of these defensive enzymes. Its effects can be determined by increasing its concentration under a constant oxygen concentration. This can be achieved with compounds such as flavins, quinones, and pterins, which are capable of penetrating cell membranes and univalently reducing oxygen (14). With the use of streptonigrin, increased concentrations of the superoxide radical were shown to induce SOD in *Escherichia coli* K-12 (11).

Because gases are less soluble in water at elevated temperatures, thermophilic bacteria evolved under low oxygen tensions. However, the rates of reactions producing compounds such as hydrogen peroxide and the superoxide radical would be expected to be higher at elevated temperatures. Therefore, thermophiles could have defensive enzymes similar to those of their mesophilic counterparts. A previous investigation of the effects of oxygen on *Thermus aquaticus* and eight other thermophiles confirmed that they all possessed CAT, PER, and SOD (13). With the exception of *Thermomicrobium roseum*, all the aerated cultures displayed significantly higher levels of CAT, PER, and/or SOD than the static cultures. However, the aerated cultures demonstrated a lag period before the resumption of growth (13).

This investigation attempted to determine how increased oxygen tensions affect the specific activities of CAT, PER, and SOD in *T. aquaticus* during the lag phase. To determine if the organism responded in a manner similar to that of *E. coli* K-12, we used methyl viologen, a compound capable of univalently reducing oxygen (8), in conjunction with chloramphenicol to determine the effect of increased concentrations of the superoxide radical on the growth and viability of this organism under aerated and static culture conditions. In addition, methyl viologen was used to determine how increased concentrations of the superoxide radical affect viability and the specific activities of CAT, PER, and SOD.

T. aquaticus YT-1 (ATCC 25104) (2) was grown at 65°C in a medium of inorganic salts (L salts) (13) containing yeast extract (1% [wt/vol]) and tryptone (0.1% [wt/vol]). Cell extracts were prepared as described by Beauchamp and Fridovich (1). Protein concentrations were determined by the biuret procedure (4). CAT activity was determined by the potassium permanganate titration procedure (3). PER activity was determined by the ability of PER to oxidize *p*-phenylenediamine in the presence of hydrogen peroxide (12). SOD activity was determined by the ability of SOD to inhibit the radical-mediated autooxidation of epinephrine (15).

To determine the effects of increased oxygen tensions, I used statically grown cells for the inoculum. The cells were rapidly chilled to 5°C, centrifuged at 16,000 × *g* for 20 min, and suspended in 100 ml of medium at 25°C. The suspension was used to inoculate flasks containing fresh medium at 65°C, and the flasks were then agitated at 250 rpm. At selected intervals, flasks were removed and the cell densities were determined by measuring the A_{595}. Cell extracts were assayed for CAT, PER, and SOD activities.

To determine the effects of methyl viologen (paraquat or 1,1′-dimethyl-4,4′-bipyridium dichloride) on the growth of *T. aquaticus*, we grew the bacterium in the absence or presence of methyl viologen (5 and 20 μM) in both static and aerated cultures. Growth was determined by measuring the A_{595}. Methyl viologen was also used with 750 μM chloramphenicol to determine the effect of increased levels of superoxide radicals on cell viability in the absence of protein synthesis. *T. aquaticus* was grown in both static and aerated cultures without or with methyl viologen (5, 20, and 40 μM). Cell viability was determined by using a most-probable-number dilution assay.

The effect of methyl viologen on the specific activities of CAT, PER, and SOD was determined by using statically grown cells for the inoculum. The cells were exposed to methyl viologen at 25, 50, 100, 200, 500, and 1,000 μM. The flasks were agitated at 250 rpm and removed after 2.5 h. The cultures were assayed for cell viability and enzyme activity.

When statically grown *T. aquaticus* cells were aerated, they displayed an 80-min lag phase before resuming growth (Fig. 1). During this period, the specific activities of CAT, PER, and SOD increased by 300, 100, and 70%, respectively. After the cells began growing, PER and SOD activities leveled off and CAT activity continued to increase. There appears to be a correlation between growth and increased CAT, PER, and SOD activities when oxygen tensions are increased.

The growth of *T. aquaticus* was affected by methyl viologen (Fig. 2), the toxicity of which was directly related to concentration. At 20 μM methyl viologen, the lag times were up to twice those of the controls, and maximum population

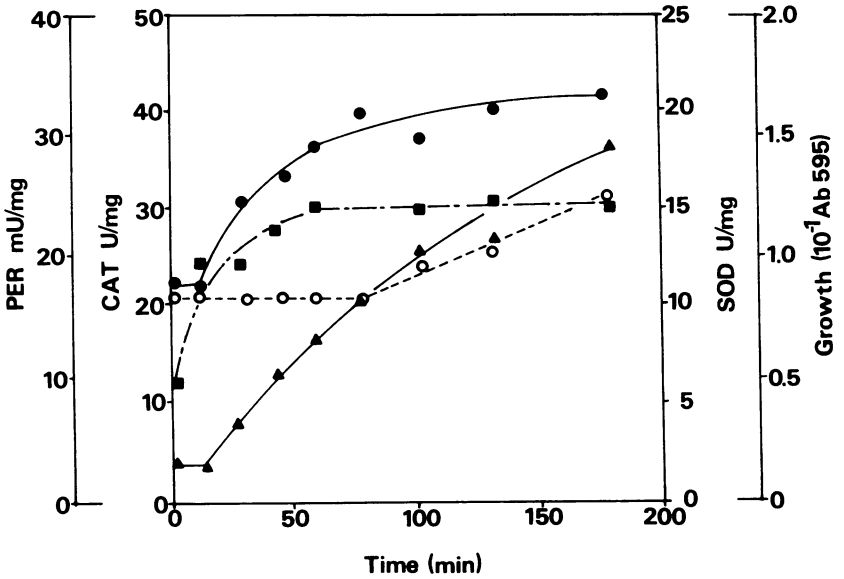

FIG. 1. Effect of aerating statically grown *T. aquaticus* on growth, as measured at 595 nm (○), and the specific activities of CAT (▲), PER (■), and SOD (●). Ab, Absorbance.

levels were reduced by over 60%. Furthermore, the cultures exposed to methyl viologen entered the death phase more rapidly than did the controls. Aeration increased the effects of methyl viologen on the organism. The decreased maximum population densities and the early onset of the death phase may have been due to the increased damage caused by the high levels of the superoxide radical. This damage would cause the cells to use cellular resources for repair instead of growth.

The specific activity of CAT increased over 300% at 100 μM methyl viologen, above which it rapidly fell (Fig. 3). SOD followed a similar pattern, increasing over 300% at 200 μM methyl viologen, above which its activity rapidly declined. The specific activity of PER decreased by 40%. Cell

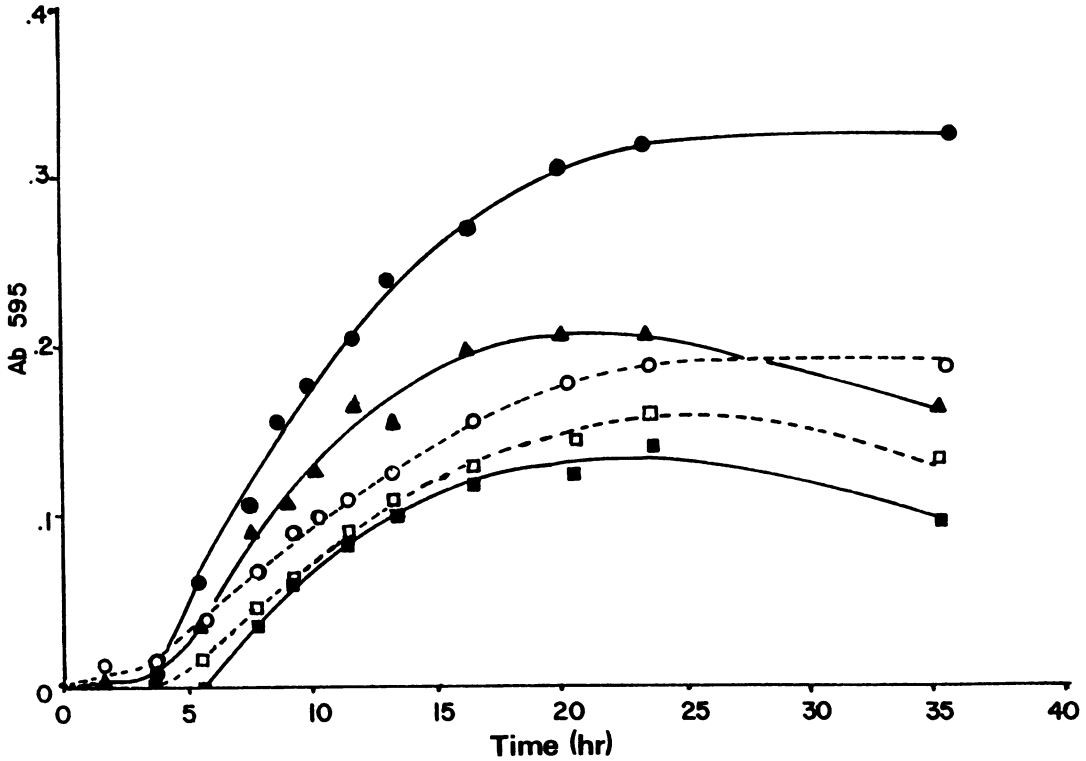

FIG. 2. Effect of methyl viologen at 5 μM (△) and 20 μM (□) on the growth of *T. aquaticus* under aerated (solid symbols) and static (open symbols) conditions. ○, Control. Ab, Absorbance.

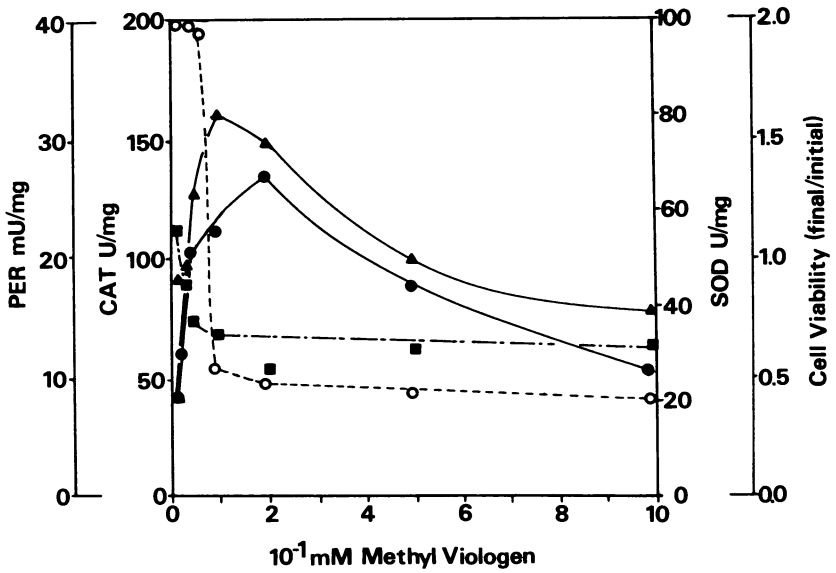

FIG. 3. Effect of methyl viologen on cell viability, as determined by the most-probable-number dilution assay (○), and the specific activities of CAT (▲), PER (■), and SOD (●) in *T. aquaticus*. Statically grown cells were exposed to methyl viologen for 150 min while being agitated at 250 rpm.

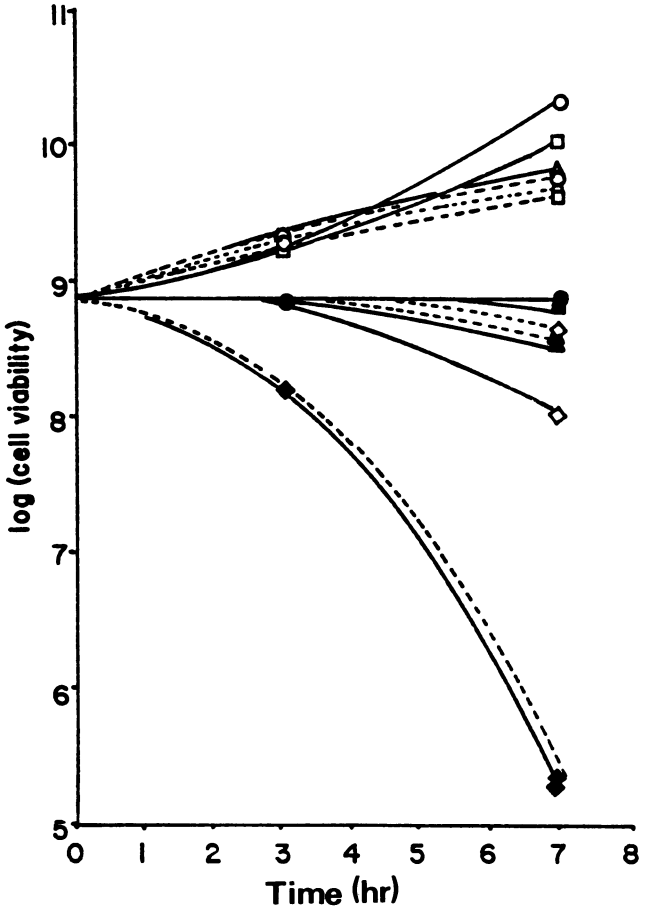

FIG. 4. Effect of methyl viologen at 5 μM (□), 20 μM (△), and 40 μM (◇) on the cell viability of *T. aquaticus*, as determined by the most-probable-number dilution assay, under aerated (solid lines) and static (broken lines) conditions. The cells were cultured in the absence (open symbols) or presence (solid symbols) of 750 μM chloramphenicol. ○, Control (no methyl viologen).

viability dropped off dramatically above 100 μM methyl viologen. Above 100 μM, the cells became overwhelmed by radical-mediated reactions.

The exposure of *T. aquaticus* to increased levels of the superoxide radical had effects similar to those observed in *E. coli* K-12 (11). The increased lag time was apparently due to the need for the induction of CAT and SOD, indicating that the superoxide radical or possibly the hydroxyl radical is responsible for the induction of these enzymes.

When protein synthesis was inhibited with chloramphenicol, the cultures exposed to methyl viologen exhibited decreased viability as the concentration of methyl viologen was increased (Fig. 4). Apparently, the inhibition of protein synthesis prevented the organism from adequately defending itself against increased concentrations of the superoxide radical. With unchecked radical-mediated reactions occurring, the cells were rapidly killed. This killing was further enhanced by aeration, indicating that the toxicity was due to the production of the superoxide radical and not to some other toxic property of methyl viologen.

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