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Effect of Temperature on the Respiration and Cytochromes of an Extreme Thermophile

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The rate of oxygen uptake in an extreme thermophile at 70 C was three times greater than at 50 C. Cytochromes *a*, *b*, and *c* were present in cells grown at 50, 60, and 70 C. The content of these electron transport system elements remained relatively constant as the growth temperature was raised.

The extreme thermophile investigated is a gram-negative, nonsporeforming, rod-shaped bacterium resembling *Thermus aquaticus* (1) in many of its characteristics. The bacterium was isolated from a hot spring in Yellowstone National Park and grew optimally at 72 C but repeatedly failed to grow in an anaerobic environment. This finding prompted questions relating to the nature of the respiratory apparatus of this obligately aerobic bacterium since the oxygen solubility is low in the natural environment of this organism.

Respiration was measured at different temperatures by using a Gilson differential respirometer. The culture was grown to mid-exponential phase in complete medium (0.2% tryptone plus 0.2% yeast extract) at the temperatures that were used for the oxygen uptake experiments (50, 60, and 70 C). The cells were washed in potassium phosphate buffer containing basal salts, a mixture of 0.3 μg of each of the following growth factors per ml: biotin, B_{12}, lipoic acid, and paraaminobenzoic acid, and 150 μg of each of the following amino acids per ml: cystine, isoleucine, glutamate, aspartate, proline, and serine. This suspension was added to respirometer flasks in 3.0-ml volumes. Figure 1 shows that the rate of oxygen uptake of this thermophilic bacterium is four times greater at 70 C than at 50 C. This finding is noteworthy because the concentration of dissolved oxygen decreases from 2.2 mg/100 ml at 50 C to 1.4 mg/100 ml at 70 C. The addition of 0.2% glutamate and glucose effected a slight increase in the rate of oxygen uptake at 50, 60, and 70 C when compared with the control.

The increased respiratory activity observed at 70 C indicates that the electron transport system in this thermophile is unlike that of the mesophile with respect to temperature. In this connection, Smith (6) has reviewed examples of bacteria in which the cytochrome content is altered in cells grown under low oxygen tension. To test whether that phenomenon was occurring in this thermophile as a result of the depleted oxygen concentration in the environment that it finds optimal, cells were grown to late-exponential phase at 50, 60, and 70 C in the complete medium, and their cytochrome content was determined. The difference spectra at ambient temperature (2) were determined by using a Hitachi Perkin-Elmer model 356 spectrophotometer, and the cytochrome concentrations were estimated.

The difference spectrum at ambient temperature seen in Fig. 2 shows the bands that are characteristic for cytochromes $a + a_3$, *b*, and *c* in cells grown at 60 C. The alpha peaks at 603 nm and 555 nm represent cytochromes $a + a_3$ and probably a combination of *b*- and *c*-type cytochromes. The Soret peak at 430 nm with a shoulder at 445 denotes the cytochromes *b* and $a-a_3$. Low temperature spectra were measured at liquid nitrogen temperatures by using a Johnson Research Foundation split-beam spectrophotometer. These low temperature spectra revealed separate peaks for cytochromes *b* and *c* at 556 and 548 nm. The apparent failure to resolve separate bands for cytochromes *b* and *c* at ambient temperature may be explained by peak shift. Analysis of cells that were grown at 50, 60, and 70 C confirmed the presence of cytochromes $a-a_3$, and *b* and *c* throughout the 20-degree growth temperature range of this bacterium. These data reflect similarities between the cytochromes present in this thermophile and *Bacillus stearothermophilus* in that the predominant respiratory pigments of both bacteria are the same (4). The concentration of

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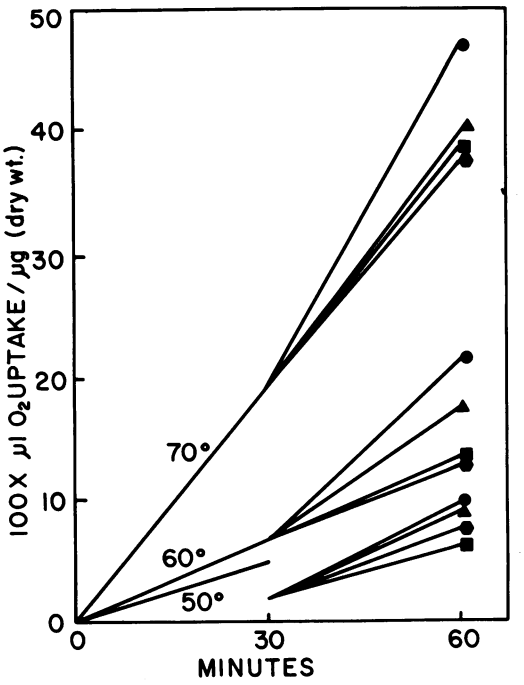

FIG. 1. *Effect of temperature on oxygen uptake in a defined medium. At 30 min, 0.2% (final concentration) glutamate (●), glucose (▲), and citrate (⬣) were added to separate flasks, and the oxygen uptake in the presence of these substrates was compared with a control culture in the defined medium (■). The 50 C curve was offset at 30 min. The values indicated on the ordinate scale have been multiplied by 100.*

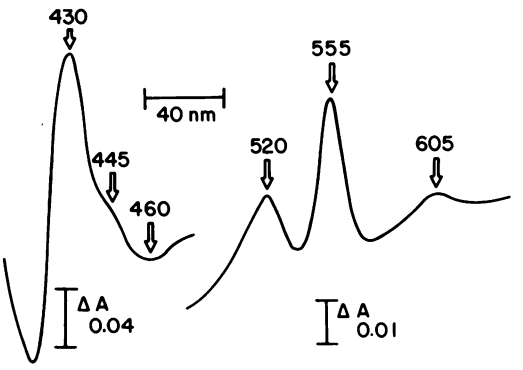

FIG. 2. *Oxidized versus dithionite-reduced difference spectrum done at room temperature. Cells grown at 60 C were suspended [1.25 mg (dry weight)/ml] in. 0.01 M phosphate buffer, pH 7.6.*

these cytochromes was estimated from the difference spectra recorded at ambient temperature with the appropriate extinction coefficients: (*a* $\mathcal{E}^{604-630}_{redox}$ = 24, a_3 $\mathcal{E}^{445-460}_{redox}$ = 164, *b* $\mathcal{E}^{563-575}_{redox}$ = 22, *c* $\mathcal{E}^{550-540}_{redox}$ = 19) (7, 8). The concentration of the cytochromes in this thermophile is not unlike that of many microbial systems (5, 6). Cells that were grown at 60 C contained the following concentrations of cytochromes expressed as nanomoles of cytochrome per milligram (dry weight): *a* = 0.02, a_3 = 0.06, *b* 0.29, and *c* 0.26. The values for *b*- and *c*-type cytochromes are only approximate, since separate absorption peaks were not resolved at ambient temperature.

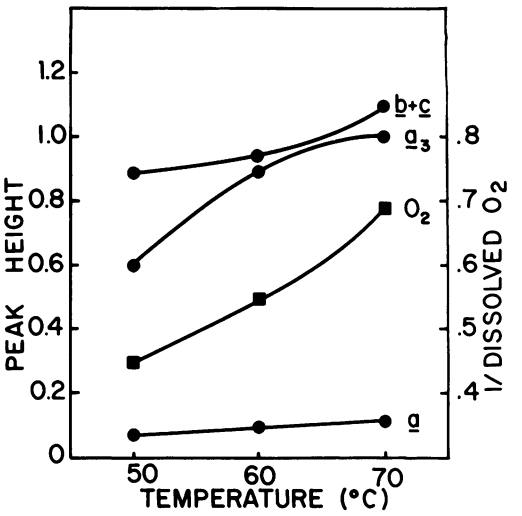

FIG. 3. *Effect of growth temperature on cytochrome concentration and oxygen solubility. Cytochrome concentrations are expressed as peak height [absorbancy/g (dry weight)] of the appropriate difference spectrum bands. The wavelength intervals that were used for the various cytochromes were: a = 604–630, a_3 = 445–460, and b + c = 455–475. The cells were grown at the temperatures indicated prior to analysis at ambient temperature. The dissolved oxygen was calculated by correcting the oxygen solubility in water at each temperature to the appropriate atmospheric pressure and is expressed as milligrams of O_2 per liter.*

The effect of growth at 50, 60, and 70 C on the cytochrome concentrations was only slight, as seen in Fig. 3. After comparison with the decrease in available oxygen as the temperature is increased, it was concluded that neither the composition nor the concentration of the respiratory enzymes of this bacterium becomes significantly altered in response to the lowered oxygen concentrations. In this connection, it is of interest to note that, when *B. stearothermophilus* was grown anaerobically, cytochrome a_3 was not detected (3). The increased respiratory activity described here for this obligately aerobic thermophile at 70 C may be due to one or more of the following factors at that temperature: the increased availability of reduced

cofactors as a reflection of overall metabolic activity; increased dehydrogenase activity or the resistance to heat inactivation of the respiratory enzymes found in this thermophilic bacterium, or both.

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