CATALASE ACTIVITY IN ESCHERICHIA COLI

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Witkin (1946) reported a mutant, designated *Escherichia coli*, strain B/r, which was more resistant to direct ultraviolet radiations than the parent strain B of *E. coli*. The same mutant also was found to be more resistant to X-rays, penicillin, sodium sulfathiazole (Witkin, 1947), and nitrogen mustard (Bryson, 1948). Morse and Carter (1949) found *E. coli*, strain B/r, to contain 3 to 4 times more desoxyribose nucleic acid than *E. coli*, strain B. Bryson (1949) also found the B/r strain to be more resistant to the lethal actions of substrates which had been irradiated with ultraviolet light or treated with hydrogen peroxide. However, he found no difference between the B/r and B strains in their resistance to bactericidal concentrations of hydrogen peroxide.

In view of the effect catalase has on such treated substrates (Wyss *et al.*, 1948) it was decided to compare the catalase activity of the two strains of *E. coli* to determine if any of the differences between the strains could be attributed to increased catalase activity.

EXPERIMENTAL METHODS AND RESULTS

The B and B/r strains of *E. coli* were carried routinely in stock on nutrient agar. Log phase subcultures in nutrient broth were used for experiments. Twenty-five ml aliquots of nutrient broth containing from 0 to 20 ppm hydrogen peroxide were inoculated with 0.1 ml of a log phase culture of *E. coli*, strain B/r, and a duplicate set inoculated with an equal amount of *E. coli*, strain B. The growth of the inoculum was determined by turbidity readings taken at 6 and 24 hours. The results of the 24 hour readings are shown in figure 1; the six hour readings gave similar results. This confirms the report of Bryson (1949) on the comparative resistance of the strains to peroxide treated substrates. When washed cell suspensions were placed into phosphate buffer containing 0.5 per cent hydrogen peroxide, and aliquots were removed at two minute intervals and placed in a sterile catalase solution to destroy any residual peroxide before plate counts were made, no difference was found between the two strains in their resistance.

Catalase activity of the cells was determined by measuring the rate of oxygen output from a dilute hydrogen peroxide solution in a Warburg constant volume respirometer at 5 C. Washed cell suspensions of approximately 100 million cells per ml (checked by plate count) were suspended in M/15 phosphate buffer at pH 6.9, and 5 ml were placed in the flask; 0.1 ml of a 0.1 per cent H_2O_2 solution was placed in the side arm. After equilibration the peroxide was dumped in the flask and the oxygen evolution measured at two minute intervals. The endogenous respiration of the cells and the spontaneous decomposition of the H_2O_2 were checked and found to be negligible. Figure 2 shows a comparison of the rate of

O_2 evolution by essentially equal masses of cells of the B and B/r strains. Since the rate of the decomposition of the hydrogen peroxide is proportional to catalase activity, it is obvious that *E. coli*, strain B/r, has a much higher catalase activity than *E. coli*, strain B.

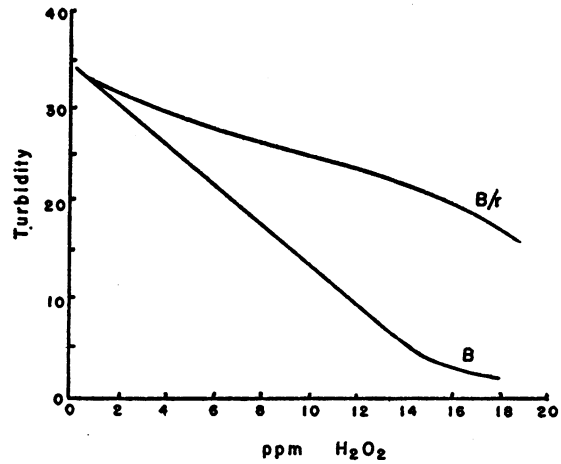

Figure 1. The growth of *Escherichia coli*, strains B and B/r, in nutrient broth treated with hydrogen peroxide. (Turbidity figures are arbitrary galvanometer readings with 0 = 100 per cent transmission.)

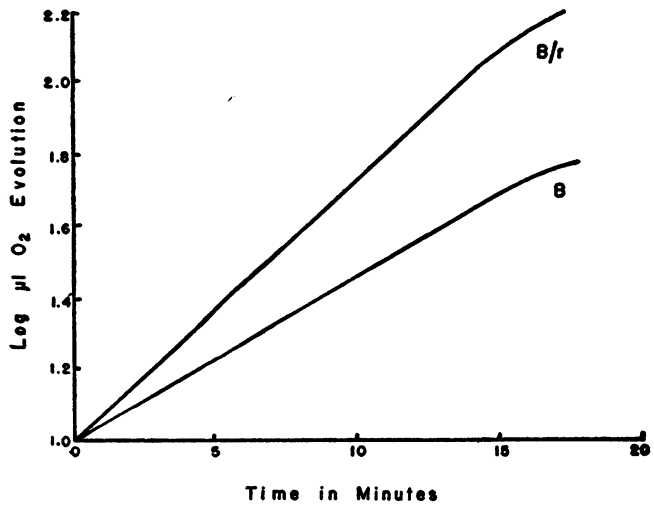

Figure 2. The catalase activity of cell suspensions of *Escherichia coli*, strains B and B/r.

The catalase activity was calculated per cell from a duplicate experiment by the method of Virtanen and Karström (1925). Using the formula,

$$\text{activity} = \frac{k}{\text{number of cells}},$$

E. coli, strain B, was found to have a catalase activity of 6.10×10^{-11} per cell compared to 1.34×10^{-10} per cell of *E. coli*, strain B/r. Such calculations may not be absolute since they are based on the assumption that the decomposition of H_2O_2 by catalase is monomolecular. Although the first part of the reaction appears to be monomolecular, Molland (1947) has pointed out that the reaction is more complicated and cannot be expressed properly by either monomolecular or bimolecular rate constants.

DISCUSSION

It is probable that the increased catalase activity in *E. coli*, strain B/r, could at least in part account for the increased resistance of this strain to substrates that are irradiated with ultraviolet light or treated with hydrogen peroxide. In both treatments of the substrate the toxic principle is due to the production of minute amounts of hydrogen peroxide which react with molecules of the substrate. This effect is not due to the presence of hydrogen peroxide, *per se* (Wyss *et al.*, 1947). However, catalase will remove this toxicity by decomposing the peroxide and thus decomposing the toxic molecules which are in equilibrium with the peroxide. The excess catalase activity in *E. coli*, strain B/r, probably is not sufficient to afford any detectable protection against concentrations of hydrogen peroxide that are definitely bactericidal.

The excess production of catalase could not account for the other properties displayed by this organism, however. There are many similarities between the effects of direct and indirect ultraviolet irradiation, and the basic mechanisms of these effects may be related closely. Catalase has been found to have no demonstrable effect in protecting bacterial cells from the lethal effects of direct ultraviolet irradiation (unpublished data). Therefore, it must be assumed that the various other increased resistances found in *E. coli*, strain B/r, are due to other cellular factors.

The excess catalase activity of *E. coli*, strain B/r, could be due either to an increased production of the enzyme molecules or to an increased availability of the enzyme. Since the catalase determination is based on activity and not on molar concentration of the enzyme protein, it is impossible to determine which is the case. It is conceivable that a mutation in a cell could result in increased enzyme production. This could be considered a quantitative mutation rather than the usual qualitative phenotypic expression.

SUMMARY

Escherichia coli, strain B/r, has higher catalase activity than *Escherichia coli*, strain B. This can account for its increased resistance to the toxic effects of substrates which have been irradiated with ultraviolet light or treated with hydrogen peroxide. The catalase activity cannot explain the other characteristics of this organism.

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