Short Communication

HEAT AND GAMMA-RADIATION RESISTANCE OF *BACILLUS MEGATERIUM* SPORES

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Received May 1, 1963

The experiments reported are part of a series designed to investigate whether or not the heat resistance of bacterial spores can be correlated with resistance to other inimical physical agents such as ionising or ultra-violet radiation and dehydration.

It has been shown that heat resistance is affected by the divalent metallic ion content of growth and sporulation media (Slepecky and Foster, 1959; Black, Hashimoto and Gerhardt, 1960), and an investigation of this effect was the starting point for the present work.

A chemically defined liquid medium containing Mg and Fe as the sole divalent metallic ions was formulated for the production of spores of *Bacillus megaterium* (ATCC8245). It is referred to as "GS" medium and consists of K_2HPO_4 0·3 g., KH_2PO_4 0·1 g., NH_4Cl 0·05 g., NH_4NO_3 0·01 g., Na_2SO_4 0·01 g., $MgSO_4 \cdot 7H_2O$ 0·001 g., $FeSO_4 \cdot 7H_2O$ 0·0001 g., glucose 0·1 g., L-glutamic acid and L-asparagine·H_2O 10^{-2} M, water to 100 ml.; the pH is adjusted to 7·0–7·2. The general procedure for spore production is as follows: a drop of a standard spore suspension is added to 20 ml. of GS medium. This culture is grown aerobically at 37° until the appearance of filamentous cells and then subcultured into a second 20 ml. of GS medium. Aerobic incubation of the subculture is carried out for 36 hr.; sporulation is by then complete. The spores are washed three times in sterile water, resuspended in water and stored at 4°.

Samples of such a suspension were heated for different periods in a bath at 100° and survivors estimated from colony counts. Exponential time/survival curves were constructed and the slopes of these used as a measure of heat resistance, the greater the value of the slope the less resistant are the spores to heat. Similarly, slopes of exponential dose/survival curves were used as a measure of gamma-radiation resistance. Exposures to cobalt-60 gamma-rays were carried out at 22° with spores suspended in aerated water.

The GS medium was modified by substituting Mn and/or Ca for Fe, and also by adding Mn or Ca or both. This gave batches of spores with different resistances to heat.

We find: (1) Mg is essential for sporulation, together with Fe or Mn. No spores are obtained if Fe is substituted by Ca alone; (2) spores produced in GS medium (Mg and Fe) are the least resistant to heat, slope = 0·14 sec.$^{-1}$; (3) substitution in the GS medium of Fe by Mn plus Ca gives spores with about twice this resistance to heat, slope = 0·060 sec.$^{-1}$; (4) addition of Ca to GS medium has no effect on heat resistance, slope = 0·15 sec.$^{-1}$; (5) addition of Mn to GS medium gives spores with

an intermediate heat resistance, as does the addition of both Mn and Ca, slopes of both batches 0·092 sec.$^{-1}$; (6) the gamma-radiation resistance is unchanged by these changes in the divalent metallic ion content of the GS medium, slopes between 0·014 and 0·012 Krad^{-1}.

These preliminary results confirm earlier reports that the high resistance of spores to heat depends to some extent on the divalent metallic ion content of the sporulation medium. At this stage the specific roles of these ions in sporulation and in regulation of heat resistance cannot be recognised, but clearly they are not similarly involved in regulation of gamma-radiation resistance. However, we cannot as yet reject the concept that these resistances are correlated. It should be noted that of the spores produced by these techniques, those with the lowest heat resistance are many times more heat resistant than the corresponding *B. megaterium* vegetative cells. It may well be that an unidentified mechanism responsible for this disparity is a common regulatory mechanism for the usual high resistance of spores to both heat and radiation.

References

Black, S. H., Hashimoto, T. and Gerhardt, P. (1960). *Can. J. Microbiol.*, **6**, 213–224.
Slepecky, R. and Foster, J. W. (1959). *J. Bact.*, **78**, 117–123.

The paper was presented by DR. TALLENTIRE.