J. Pharm. Pharmac., 1968, **20**. 626–629 Received February 9, 1968

The effect of storage conditions on the heat resistance and heat activation response of *Bacillus stearothermophilus* spores

A. M. COOK* AND R. J. GILBERT†

The heat resistance of spores was unaffected by storage for 52 weeks at 4° or 65 weeks at —16°. However, the magnitude of the heat activation response for heated spores fell with increase in storage time of the unheated spores.

SPORES of *Bacillus stearothermophilus* are widely recommended as bacteriological controls for autoclaving processes because of their extreme heat resistance. Paper strips impregnated with spores are commercially available and Cook & Brown (1965a, b) have shown that spore papers of reproducible resistance can be made and stored over long periods.

The phenomenon of heat activation of *Bacillus* spores is well documented especially with reference to *B. stearothermophilus* (Curran & Evans, 1945; Cook & Brown, 1964, 1965c). It is the purpose of this paper to record the effect of storage time on the heat activation response of *B. stearothermophilus* spores and to relate this effect with the overall heat resistance.

Experimental

Spores of *B. stearothermophilus* NCIB 8919 were obtained using the method of Cook & Brown (1964) except that the sporulation conditions were 7 days at 60° (suspension A) and 7 days at 55–60° (suspension B). Vegetative cells were removed by washing ten times and separating by differential centrifugation using a refrigerated centrifuge. The heating and counting techniques used were those described by Cook & Brown (1964) but the recovery medium, antibiotic assay medium with 0·1% starch, was of pH 7·3 and plates were incubated at 50° for 3 days.

Both aqueous spore suspensions were stored at 4° and heat resistance experiments at 115° made at various time intervals within the limits of 2–36 weeks for suspension A and <1–52 weeks for suspension B. Control counts on both unheated spore suspensions stored at 4° for 80 weeks indicated very little change in count.

The use of decimal reduction (D) values is a convenient method of expressing heat resistance when survivor curves are linear. However, D values do not take into consideration any initial heat activation effects on survivor curves, and care must therefore be exercised in their correct interpretation. In the present studies this has been overcome by quoting

From the School of Pharmacy, University of London, Brunswick Square, London W.C.1, England.

* Present address: Welsh School of Pharmacy, University of Wales Institute of Science and Technology, Cathays Park, Cardiff, Wales.

† Present address: Food Hygiene Laboratory, Central Public Health Laboratory, Colindale Avenue, London, N.W.9, England.

two inactivation times—a calculated D value for the exponential section of the curve, and the calculated time to give a 10^4 reduction of the initial (unheated) count (i.e. 99·99% kill).

Results and discussion

The results of 18 and 20 experiments made on suspensions A and B respectively are summarized in Table 1 and three time survivor curves for suspension B are shown in Fig. 1. Tests for parallelism indicated that there were no significant differences between the slopes of the exponential sections of the 18 survivor curves for suspension A, and of 20 curves for suspension B.

TABLE 1. HEAT RESISTANCE DATA OF *B. stearothermophilus* SPORES AT 115°

Suspension	Number of survivor curves compared	Range of calculated D values (min)	Range of calculated Times to inactivate 10^4 spores (min)
A	18	19·4–24·4	99·3–122·0
B	20	15·8–19·0	85·8–102·6

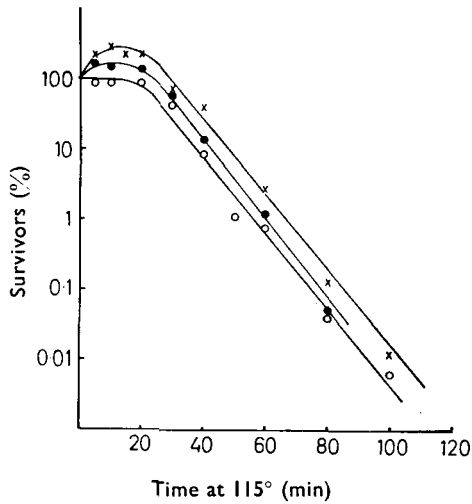

FIG. 1. Effect of storage at 4° on heat resistance at 115° of *B. stearothermophilus* spores (suspension B). ×, < 1 week; ●, 13 weeks; ○, 52 weeks.

Suspension A

calculated variance ratio $F_{17}^{65} = 1{\cdot}60$
tabulated (Documenta Geigy, 1962) $F_{17}^{65} = 2{\cdot}03 - 2{\cdot}06\ (P = 0{\cdot}05)$

Suspension B

calculated variance ratio $F_{19}^{65} = 1{\cdot}75$
tabulated (Documenta Geigy, 1962) $F_{19}^{65} = 1{\cdot}96 - 1{\cdot}98\ (P = 0{\cdot}05)$

Heat resistance experiments were also made within the temperature range 110–121° using suspension B after storage for 3 and 52 weeks at 4° and 65 weeks at −16°. A common regression line representing the three thermal death rate curves (Gilbert, 1966) is shown in Fig. 2. The slope of this line (z) is defined as the number of degrees of temperature to bring about a ten-fold change in decimal reduction time (D).

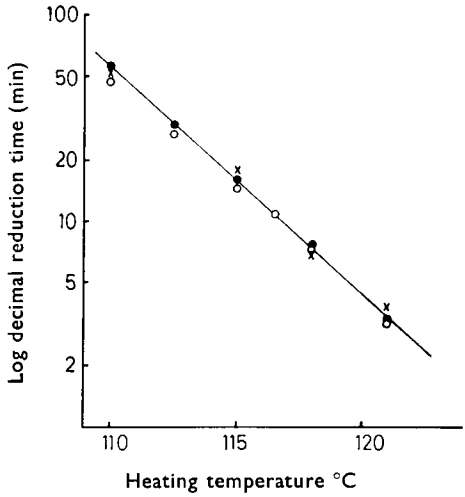

FIG. 2. Thermal death rate curves of stored spores of *B. stearothermophilus* (suspension B). ×, 3 weeks at 4°; ●, 52 weeks at 4°; ○, 65 weeks at −16°.

From the results of a large number of experiments it was apparent that the magnitude of the heat activation response fell with increase in storage time of the stock (unheated) spore suspensions (Fig. 3). Thus after <1–3 weeks storage at 4° both suspensions A and B showed four to six-fold increases in viable count on heating at 115°, compared with only one to three-fold increases after 13 weeks storage or one to two-fold increases after 28 weeks storage. Falls in the heat activation response of two other strains of *B. stearothermophilus* have been reported by Fields & Finley (1962). However, these workers only gave results before and after storage of spores for 16 months at 4°, there being no indication of the rate of loss of the heat activation response. Present results show that such losses were most marked in the first few weeks of storage. Similar results have been obtained recently with the spores of two heat resistant strains of *Clostridium welchii* associated with two food poisoning outbreaks (Sutton, Gilbert & Hobbs, unpublished).

The mechanism of the loss of the heat activation response is not known, but it must be concerned with a certain instability of the fraction of spores in a population which require activation before germination and outgrowth can occur. This loss in heat activation response may be connected with an alteration in the dipicolinic acid (DPA) content, a characteristic component of bacterial spores (Powell, 1953). Activation of *B. stearo-*

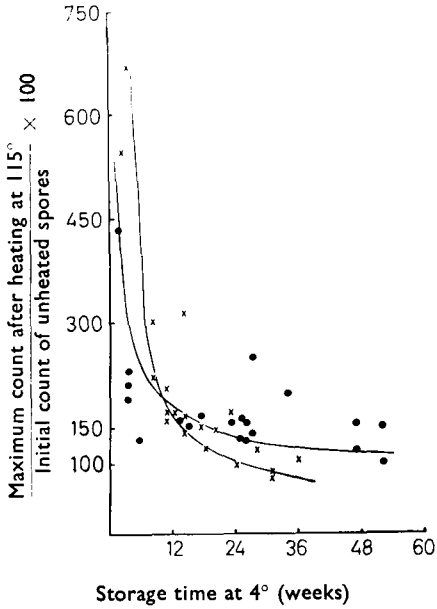

FIG. 3. Effect of storage at 4° on the heat activation response of *B. stearothermophilus* spores. ×, suspension A; ●, suspension B.

thermophilus spores and release of DPA after acid treatment has been reported recently (Brown, Brown & Porter, 1968).

Figs 1 and 2 clearly show that prolonged storage for 52 weeks at 4° or 65 weeks at −16° had little effect on the heat resistance of the spore suspensions. These results are at variance with those of Fields & Finley (1962) who reported a very significant fall in heat resistance after storage of spores for 16 months at 4°.

The calculated value of z of 9·4° from analysis of thermal death rate data (Fig. 3) was in reasonable agreement with values of 6·7° (Fields & Finley, 1962) and 7·0° (Briggs, 1966) for *B. stearothermophilus* spores heated in water.

Acknowledgement. One of us (R.J.G.) was a recipient of a Nuffield Foundation Food Science Scholarship.

References

Briggs, A. (1966). *J. appl. Bact.*, **29**, 490–504.
Brown, M. R. W., Brown, M. W. & Porter, G. S. (1968). *J. Pharm. Pharmac.*, **20**, 80.
Cook, A. M. & Brown, M. R. W. (1964). *Ibid.*, **16**, 725–732.
Cook, A. M. & Brown, M. R. W. (1965a). *Ibid.*, **17**, 1S–6S.
Cook, A. M. & Brown, M. R. W. (1965b). *Ibid.*, **17**, 7S–11S.
Cook, A. M. & Brown, M. R. W. (1965c). *J. appl. Bact.*, **28**, 361–364.
Curran, H. R. & Evans, F. R. (1945). *J. Bact.*, **49**, 335–346.
Documenta Geigy Scientific Tables (1962). 6th edn, p. 40. Manchester: Geigy.
Fields, M. L. & Finley, N. (1962). *Bull. Univ. Mo. Agr. Exp. Stn*, No. 807, p. 1–12.
Gilbert, R. J. (1966). Ph.D. thesis, University of London.
Powell, J. F. (1953). *Biochem. J.*, **54**, 210–211.