

Effect of Ultrasonic Waves on the Heat Resistance of *Bacillus cereus* and *Bacillus licheniformis* Spores

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Heat resistance of *Bacillus cereus* and *Bacillus licheniformis* spores in quarter-strength Ringer solution decreases markedly after ultrasonic treatments which are unable to kill a significant proportion of the spore population. This effect does not seem to be caused by a loss of Ca^{2+} or dipicolinic acid. The use of ultrasonics to eliminate vegetative cells or to break aggregates in *Bacillus* spore suspensions to be used subsequently in heat resistance experiments appears to be unadvisable.

Bacterial spores are so resistant to sonic and ultrasonic waves that such treatments have been used in the past to eliminate vegetative cells from spore suspensions (4, 5). Another useful application of ultrasonic treatment might be to break up aggregates. Ultrasonic treatment, however, induces changes in certain characteristics of the spore: swelling occurs, the surface is eroded (Sehgal, L. R., and N. Grezc, *Bacteriol. Proc.*, 1967, p. 5), and growth is stimulated (6). Some spores may be killed if the treatment is severe enough (1, 2, 8).

The work reported herein was undertaken to determine the effect of ultrasonic treatment on the subsequent heat resistance of the spores of *Bacillus cereus* and *Bacillus licheniformis*.

The spores were harvested after 4 days of incubation at 32 C on the manganese-containing medium of Williams et al. (9). The preparations were washed five times in sterile water and finally suspended in quarter-strength Ringer solution. The suspensions were stored at 2 C until used. Just before use, the vegetative cells were destroyed by heating at 80 C for 30 min. The killing effect of ultrasonics was determined by treating 4- to 5-ml volumes of the spore suspensions containing approximately 10^7 spores/ml for periods ranging from 1.5 to 12 min. During treatment, the temperature was maintained at 10 to 12 C. Survivors were estimated by colony counts on bromocresol-tryptose-dextrose-agar. Figure 1 shows the effect of ultrasonic treatment on the viability of spores of *B. cereus* and *B. licheniformis*. In both instances there was only a

slight decrease in count after treatment for 12 min.

The technique used for heat resistance determinations was that described by Franklin et al. (3). Initial and survivor spores were estimated by colony counts on starch-milk agar after incubation for 2 days at 32 C. Although under our test conditions ultrasonic treatments of 23 and 60 min were required to cause a log cycle reduction in the counts of spore suspensions of *B. cereus* and *B. licheniformis*, respectively, shorter treatments reduced markedly the number of spores surviving subsequent heat treatments. The effect of 12 min of ultrasonic treatment on the subsequent heat survival of *B. cereus* at 110 C and *B. licheniformis* at 99 C are shown in Fig. 2. Figure 3 illustrates the effect of 1.5 and 12 min of ultrasonic treatment on the heat resistance of *B. cereus* at 105 C. A marked decrease in heat resistance is observed in all instances. The effect, however, is greater with *B. cereus* than with *B. licheniformis*, as shown by the examination of the *D* values (death rate constants). It is appreciated that the *D* value for *B. cereus* is based on a part of the curve formed by two points only and is probably not very accurate. With *B. cereus* the $D_{110, C}$ decreased from 11.5 to approximately 1.5 min as a result of ultrasonic treatment; with *B. licheniformis* spores the $D_{99, C}$ decreased from 5.5 to 3 min.

From these results it can be deduced that ultrasonic treatment can not be used to eliminate vegetative cells or to break aggregates in *Bacillus* spore suspensions to be used in heat

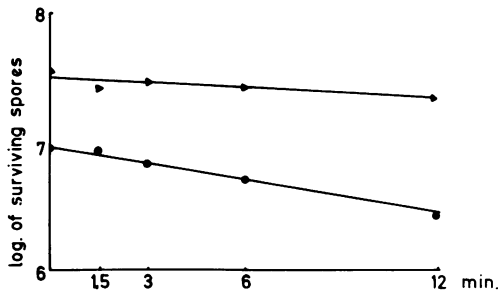


FIG. 1. Effect of ultrasonic treatment (20 kc, 1.2 A, volume 5 ml) upon spores of *Bacillus cereus* and *Bacillus licheniformis*. Symbols: ●, *B. cereus* (concentration of spores, 9.3×10^6 /ml); ▲, *B. licheniformis* (concentration of spores, 3.4×10^7 /ml).

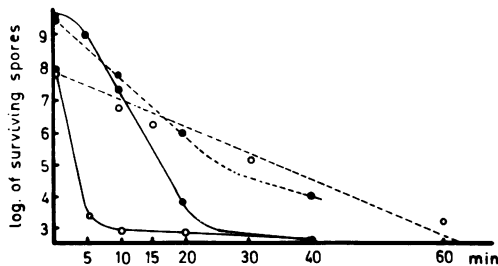


FIG. 2. Heat destruction of *Bacillus cereus* and *Bacillus licheniformis*. Heat treatment (110 C) of *B. cereus* without sonic treatment (—○—○—), and after ultrasonic treatment (—○—○—). Treatment: 20 kc, 1.2 A, 12 min, volume 4 ml; concentration of spores, 1.03×10^8 /ml. Heat treatment (99 C) of *B. licheniformis* without sonic treatment (—●—●—) and after ultrasonic treatment (—●—●—). Treatment: 20 kc, 1.2 A, volume 4 ml, 12 min; concentration of spores, 4.4×10^8 /ml.

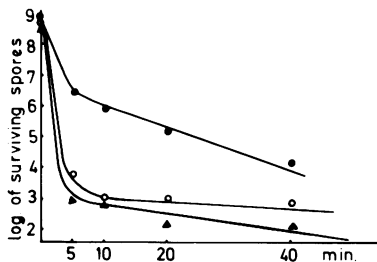


FIG. 3. Heat destruction of spores of *Bacillus cereus* at 105 C without sonic treatment (●), and after ultrasonic treatment (○). Treatment: 20 kc, 1.2 A, 1.5 min, volume 4 ml, concentration of spores, 6×10^8 /ml. After ultrasonic treatment (▲); time, 12 min.

resistance experiments, contrary to what has been reported for some *Clostridium* species (4).

Since heat resistance is known to be related to Ca^{2+} and dipicolinic acid (DPA) content (7, 10, 11), it was thought that the decrease in heat resistance could be caused by a loss of Ca^{2+} or DPA, or both. An investigation of Ca^{2+} and DPA in the spores prior to and after ultrasonic treatment and in the supernatant fluid of centrifuged spore suspensions after ultrasonic treatment, failed to reveal any detectable loss of Ca^{2+} or DPA as a result of these treatments.

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