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. Grezcz, 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-tryphtose-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\(_{10, c}\) 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
NOTES

A, formis Bacillus after A, concentration Bacillus licheniformis. 4.4 min.
ultrasonic 1.2 1.2 108/ml. After x vw C 0 #n L- 0. m_.

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 x 10^9/ml); ▲, B. licheniformis (concentration of spores, 3.4 x 10^9/ml).

FIG. 2. Heat destruction of Bacillus cereus and Bacillus licheniformis. Heat treatment (110 C) of B. cereus without sonic treatment (—O—O—), and after ultrasonic treatment (—O—O—). Treatment: 20 kc, 1.2 A, 12 min, volume 4 ml; concentration of spores, 1.03 x 10^9/ml. Heat treatment (99 C) of B. licheniformis without sonic treatment (—O—O—) and after ultrasonic treatment (—O—O—). Treatment: 20 kc, 1.2 A, volume 4 ml, 12 min; concentration of spores, 4.4 x 10^9/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 x 10^9/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.

LITERATURE CITED

1. Adler, H. I., and M. S. Engel. 1961. Factors influencing the survival of bacteria after exposure to ionizing radiation. J. Cell. Comp. Physiol. Suppl. 1:85-105.

2. Davies, R. 1989. Observations on the use of ultrasound waves for the disruption of microorganisms. Biochim. Biophys. Acta 33:481-492.

3. Franklin, J. G., D. J. Williams, and L. B. L. Clegg. 1958. Methods of assessing the sporidical efficiency of an ultra-high-temperature milk sterilizing plant. III. Laboratory determinations of the heat resistance of spores of Bacillus subtilis in water and in milk. J. Appl. Bacteriol. 21:51-57.

4. Goodenough, E. R., and M. Solberg. 1972. A technique for producing large yields of vegetative cell-free refractile Clostridium perfringens spores of unaltered heat resistance. Appl. Microbiol. 23:429-430.

5. Heilgman, F., N. W. Desrosier, and H. Broumand. 1956. Spore germination. I. Activators. Food Res. 21:63-69.

6. Knaysi, G., and H. R. Curran. 1961. Effects of some mechanical factors on the endospores of Bacillus subtilis. J. Bacteriol. 82:691-694.

7. Levinson, H. S., M. T. Hyatt, and F. E. Moore. 1961. Dependence of the heat resistance of bacterial spores on the calcium-dipicolinic acid ratio. Biochem. Biophys. Res. Commun. 5:417-421.

8. Pisano, M. A., R. M. G. Boucher, and I. E. Alcamo. 1966. Sterilizing effects of high-intensity airborne sonic and ultrasonic waves. Appl. Microbiol. 14:732-736.

9. Williams, D. J., J. G. Franklin, H. R. Chapman, and L. F. L. Clegg. 1957. Methods of assessing the sporidical efficiency of an ultra-high-temperature milk sterilizing plant. I. Experiments with suspensions of spores in water. J. Appl. Bacteriol. 20:43-47.

10. Vinter, V., and B. Vechet. 1964. Spores of microorganisms. XV. The alteration of heat sensitivity and its relation to radiation resistance of bacterial spores. Folia Microbiol. 9:238-248.

11. Vinter, V., and B. Vechet. 1964. Spores of microorganisms. XVI. Contribution to the study of bacterial spores. Folia Microbiol. 9:352-357.