Heat-Induced Increase in the Number of Viable Cells in Lyophilized Preparations of Bacillus popilliae

A. J. LINGG, K. J. McMAHON, AND R. A. CONSIGLI
Division of Biology, Kansas State University, Manhattan, Kansas 66506

Received for publication 21 August 1974

Viable cells detectable in lyophilized preparations of Bacillus popilliae were increased by 72% when dry cells were heated at 60 to 65 °C for 2.5 h.

This is a report of a study that shows that application of mild heat to lyophilized cultures of Bacillus popilliae causes an increase in the number of viable cells that can be detected.

The strain of B. popilliae used and the medium for growth have been described (7). Cells were grown for 9 to 12 h, removed from the growth medium by centrifugation, and suspended in 5% sodium glutamate plus 0.5% gum tragacanth. They were then placed in 10-ml glass ampoules (American Instrument Co., Inc., Silver Spring, Md.) in 0.5-ml amounts, frozen in liquid nitrogen, and lyophilized according to the method of Lingg et al. (8).

Sealed vials containing lyophilized cells were immersed in a water bath at 60 to 65 C for 2.5 h. Unheated vials that contained the same number of viable cells initially and were lyophilized at the same time as those used for each heating experiment served as controls. Heated and unheated cells were reconstituted with growth medium, serially diluted with 0.1% tryptone, and plated. Plates were incubated at 25 C and counted after 5 days. Counts were recorded as log averages of two or three replicate counts. Results obtained from 17 experiments were analyzed by Student's t test to determine validity of the difference between means.

In other experiments, heating time and temperature were varied. Portions of reconstituted cultures were used to inoculate growth medium, and optical density of the cultures was read with a Spectronic 20 colorimeter at 525 nm to determine rate of growth.

The viable count of lyophilized cells was increased by heating at 60 to 65 °C for 2.5 h before reconstitution. In 17 experiments, the average viable count of heated cells was 72% higher than that of unheated control cells (7.68 × 10⁹/ml and 4.46 × 10⁹/ml, respectively). The t test demonstrated that the difference between means was significant at the 0.005 level. Medium inoculated with heated lyophilized cells showed detectable growth approximately 2 h earlier than medium inoculated with unheated cells.

After heating lyophilized cells at 60, 70, and 80 °C for 2 h, the optimum temperature was found to be 60 °C (Table 1). The number of viable cells detected at 70 °C was lower than at 60 °C, but both were higher than unheated controls. After heating at 80 °C, no cells were recovered at the lowest dilution plated (10⁻⁶).

Results of heating cells at 50 °C for 2 h were not significantly different from unheated controls.

Optimum heating time was determined by heating lyophilized cells for 1 to 4 h at 60 °C and determining viability by plate count and optical density. Cells heated for 2 or 3 h gave the earliest visible sign of growth. Those heated for either 1 or 4 h gave a slower response and appeared to be about 2 h slower in reaching peak growth rates. Unheated cells exhibited a longer lag period and were 6 to 8 h behind cells heated 2 or 3 h.

The response of lyophilized B. popilliae to mild heat appears to be similar to that of heat activation of spores. However, the culture was examined many times under the phase-contrast microscope, and spores were not seen. In addition, it is unlikely that spores were present because even low levels of sporulation on artificial media require use of selected strains of B. popilliae and highly specific conditions (1, 2, 9, 10, 12, 13). The increased count obtained after heat treatment did not appear to be due to dispersion of clumps of cells. Clumping was not detected when unheated cells were examined microscopically.

Other workers have suggested that dry bacterial cells were stimulated or activated by heat. Hiscox (3) reported a higher viable count in

---

1 Contribution no. 1065, Division of Biology, Kansas Agricultural Experiment Station.

2 Present address: Department of Bacteriology and Biochemistry, University of Idaho, Moscow, Idaho 83843.

3 Present address: Department of Bacteriology, North Dakota State University, Fargo, N.D. 58102.
spray-dried milk on rehydration at 50°C than when rehydrated at 20°C, and Speck and Myers (11) found that a spray-dried skim milk culture of Lactobacillus bulgaricus gave a substantially higher count when reconstituted at 50°C than when reconstituted at 21 to 25°C. A freeze-dried culture, however, when reconstituted at 50°C gave a lower count than when reconstituted at 21 to 25°C, and heating the dry culture to 50°C did not cause activation but a destruction of some of the cells.

Although results obtained with liquid cultures may not be applicable to dried cultures, a stimulatory effect of heat on growth rate has been reported. Voss and Frazier (14), using a skim milk culture of Streptococcus thermophilus, found that the generation time of the culture was reduced from 40 to 42 min to 24 and 29 min, respectively, after heating at 60°C for 30 min. However, Lindolo and Ordal (4), Jackson and Woodbine (5), and Kaufmann et al. (6) exposed Staphylococcus aureus to sublethal heating and observed a longer lag phase for heated cells, or a delayed lag phase as Jackson and Woodbine (5) indicated would more clearly describe their findings. It was also reported by Lindolo and Ordal (4) and Jackson and Woodbine (5) that heating produced a decline in the number of viable organisms.

The data are not sufficient for us to offer a hypothesis for increased viability of lyophilized cells after heat treatment; however, Hiscox (3) has suggested that, as with spores, the dormancy of dried cells may be broken by the application of heat.

R.A.C. is a recipient of Public Health Service career development award I-K3 CA 12056 from the National Cancer Institute.

LITERATURE CITED

1. Haynes, W. C., and L. J. Rhodes. 1966. Spore formation of Bacillus popilliae in liquid medium containing activated carbon. J. Bacteriol. 91:2270-2274.

2. Haynes, W. C., and L. J. Weih. 1972. Sporulation of Bacillus popilliae in liquid cultures. J. Invertebr. Pathol. 19:125-130.

3. Hiscox, E. R. 1945. The effect of the method of reconstitution and of the temperature of incubation on the plate count of spray-dried milk powder. J. Dairy Res. 14:175-183.

4. Lindolo, J. J., and Z. J. Ordal. 1966. Repair of thermal injury of Staphylococcus aureus. J. Bacteriol. 91:134-142.

5. Jackson, H., and M. Woodbine. 1963. The effect of sub-lethal heat treatment on the growth of Staphylococcus aureus. J. Appl. Bacteriol. 26:152-158.

6. Kaufmann, O. W., L. G. Harmon, O. C. Paithorp, and I. J. Pflug. 1969. Effect of heat treatment on the growth of surviving cells. J. Bacteriol. 78:834-838.

7. Lingg, A. J., and K. J. McMahon. 1968. Survival of lyophilized Bacillus popilliae in soil. Appl. Microbiol. 17:718-720.

8. Lingg, A. J., K. J. McMahon, and C. Herzmann. 1967. Viability of Bacillus popilliae after lyophilization of liquid nitrogen frozen cells. Appl. Microbiol. 15:163-165.

9. Rhodes, R. A., M. S. Roth, and G. R. Hrubant. 1965. Sporulation of Bacillus popilliae on solid media. Can. J. Microbiol. 11:779-783.

10. Sharpe, E. S., G. St. Julian, and C. Crowell. 1970. Characteristics of a new strain of Bacillus popilliae sporogenic in vitro. Appl. Microbiol. 19:681-688.

11. Speck, M. L., and E. P. Myers. 1946. The viability of dried skim-milk cultures of Lactobacillus bulgaricus as affected by the temperature of reconstitution. J. Bacteriol. 52:657-665.

12. Steinkraus, K. H., and M. L. Provvidenti. 1958. Studies on the milky disease organisms. III. Variability among strains of Bacillus popilliae sporulating on artificial media. J. Bacteriol. 75:38-42.

13. Steinkraus, K. H., and H. Tashiro. 1955. Production of milky-disease spores (Bacillus popilliae Dutky and Bacillus lentimorbus Dutky) on artificial media. Science 121:873-874.

14. Voss, J. G., and W. C. Frazier. 1945. Influence of incubation at low temperatures on heat resistance of Swiss cheese starter cultures. J. Dairy Sci. 28:545-553.