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Thermal Profile of a *Bacillus* Species (ATCC 27380) Extremely Resistant to Dry Heat

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Spores of *Bacillus* sp. ATCC 27380 were exposed at intervals to dry-heat temperatures ranging from 125 to 150 C. D-values from 139 to 2.5 h were obtained.

Prior to the expression of concern by the National Academy of Science in 1957 over the problem of interplanetary contamination and subsequent studies in the areas of spacecraft decontamination and sterilization, relatively little was known of the precise bacterial inactivation kinetics effected by dry heat. Early studies (4) showed that the dry-heat resistance of indigenous soil flora was much higher than that of pure cultures of known sporeformers and pure cultures of resistant spore isolates from soil, presumably due in most part to the physical-chemical environment of soil during heating. Bond et al. (2), employing thin layers of soil heated on stainless-steel surfaces, observed that no spore isolates obtained from the heated soils had resistance equal to or greater than the mixed flora in the soils from which they were isolated. Furthermore, addition of sterile parent soil to subcultured spore isolates did not affect their resistance to dry heat in the test system employed; i.e., it appeared that the subculturing of the isolates had a drastic effect on dry-heat resistance levels.

While studying the comparative dry-heat resistance levels of naturally occurring bacterial spores in soil, an isolate was recovered in broth after a sample from Cape Kennedy, Fla., was heated for 48 h at 125 C. Subsequent examinations of the unusual surface morphology of the spores, growth characteristics, biochemical reactions, and resistance to 125 C (dry heat, D = 139 h) and 80 C (moist heat, D = 61 min) have been reported (1). The extraordinary resistance to 125 C dry heat is the highest reported value for bacterial spores and in our comparative studies is the only instance in which the resistance level of an isolate was greater than that of the mixed flora in parent soil. A spore preparation was submitted to the American Type Culture Collection and was given an accession number of 27380.

It was therefore of interest to determine the thermal response of the isolate to dry-heat temperatures higher than 125 C. Methods of spore preparation, heating, and assay have been described previously (1). Figure 1 shows the mean values when five test units were heated at each interval and temperature. The concave downward (6) shapes of all survivor curves indicate that the spores require heat activation for maximum germination; note that all mean unheated control values are approximately 5×10^2/test unit. D-values were determined from best-fit regression lines of data points (ignoring the unheated control values) by using a least-

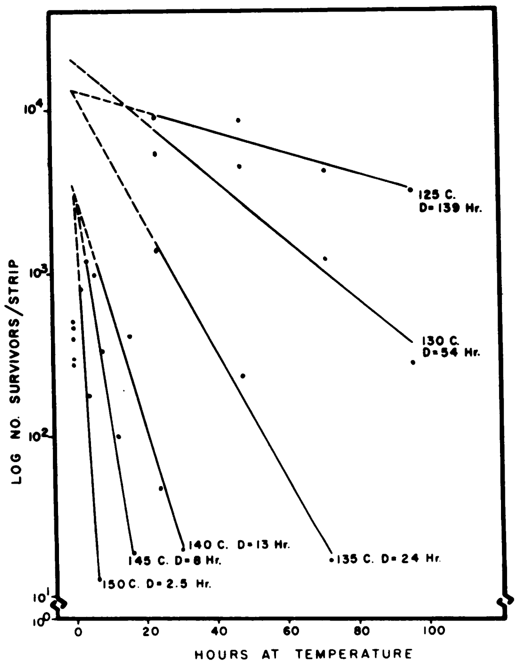

FIG. 1. *Survival of Bacillus sp. ATCC 27380 spores exposed to dry heat. Note that the mean values for the unheated controls are ca.* 5×10^2.

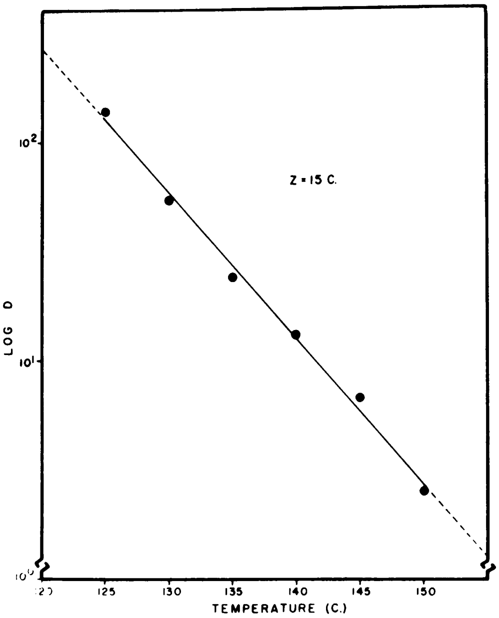

FIG. 2. *Thermal resistance curve of Bacillus sp. ATCC 27380 spores exposed to dry heat.*

squares method, and the results were as follows: D_{125C} = 139 h, D_{130C} = 54 h, D_{135C} = 24 h, D_{140C} = 13 h, D_{145C} = 8 h, D_{150C} = 2.5 h. When log D was plotted against temperature (Fig. 2), a Z-value of 15 C was obtained from the best-fit regression line of data points (correlation coefficient = −0.997). This value was somewhat lower than expected, since the dry-heat Z-values of several commonly studied and much less resistant spore cultures range from 18 to 27 C (6).

In a review of factors influencing the heat destruction of microorganisms, Pflug and Schmidt (6) stated that it may be possible for a physically smaller spore to be more resistant to dry heat since initial size is perhaps indicative of a tighter molecular structure with greater resistance to denaturation reactions. Birdsell (Abstr. Annu. Meet. Am. Soc. Microbiol. 1974, p. 55; unpublished data), in his ultrastructure study of the relatively large spore of *Bacillus* sp. ATCC 27380, found that sectioning revealed an irregular thick outer spore coat composed of globular subunits and a laminated inner spore coat containing up to nine distinct layers. It may be that these thick spore coats serve as barriers to moisture loss during heating and thereby maintain the proper moisture levels within the spore critical to maximum dry-heat resistance. The relationship of moisture loss to dry-heat inactivation of spores has been discussed by Pflug and Schmidt (6).

When first isolated in 1971, *Bacillus* sp. ATCC 27380 was rather physiologically inactive and fit no described pattern of reaction (1). From a battery of standard biochemical tests (1, 3, 5), the only positive reactions shown were growth in 2, 4, or 10% NaCl broth and production of catalase. Recently, R. E. Gordon (personal communication) observed that five colonies of the isolate selected in 1971 and subsequently transferred at intervals on soil extract agar have gained physiological capabilities not observed initially but still do not fit currently described reaction patterns. Likewise, during extensive examination of both heat-stressed and untreated environmental *Bacillus* spp. isolates, Puleo (personal communication; unpublished data) has observed that the heat-injured sporeformers tend to be much less physiologically reactive than the unheated isolates, and that many of these injured isolates also gained biochemical capabilities upon sequential subculture in the laboratory. There are no firm data available to correlate increases or decreases in physiological reactivity of an actively growing culture to heat resistance of spores.

The magnitude of the dry-heat resistance, the need for heat activation, and also the difference in resistance levels between dry and moist heat may make the spore of this apparently new species a valuable tool in the study of germination and heat inactivation mechanisms.

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