NOTES

Survival of Cocci After Exposure to Ultrahigh Vacuum at Different Temperatures

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Received for publication 26 September 1966

In a previous study, it was found that microbial spores were capable of withstanding ultrahigh vacuum at temperatures as high as 60 °C for 5 days but that above 60 °C inactivation became accelerated (N. S. Davis, G. J. Silverman, and W. H. Keller, Appl. Microbiol. 11:202, 1963).

This study on the relationship between temperature and vacuum is an extension of the previous study. An improved vacuum chamber having a more precise temperature control was utilized, and vegetative species were examined rather than microbial spores.

Three cocci were studied: Streptococcus faecalis 10C1, Straphylococcus aureus (department culture collection), and a radiation-resistant coccus, strain 248, isolated in the laboratory of one of the authors (N. S. Davis, J. G. Silverman, and E. B. Masurovsky, J. Bacteriol. 86:294, 1963). Strain 248 has been shown to be related to another red-pigmented cocc, Micrococcus roseus, by base ratio analysis (Y. Okazowa and G. J. Silverman, Intern. Congr. Radiation Res., 3rd, 1966). S. faecalis and S. aureus were grown as stationary cultures at 37 °C for 22 hr in tryptone-glucose extract broth (TGE). Strain 248 was propagated as a 36-hr shake culture (50 ml in a 500-ml baffled Erlenmeyer flask) at 30 °C in plate count broth supplemented with 0.5%, N-Z case (Sheffield Chemical, Norwich, N.Y.).

Aqueous samples of washed stationary-phase cells (0.1 ml) were placed directly upon glass-fiber filters (3.7 cm, no. 934-AH, Reeve Angel, Clifton, N.J.) and dried overnight over silica gel. This treatment produced a stabilized population of from 40 to 60% of the initial spore population. The number of initial viable cells was usually 10⁶ to 3 × 10⁷ per filter, although, for certain vacuum experiments with strain 248 conducted at temperatures of 60 °C or greater, 7 × 10⁶ to 8 × 10⁷ viable cells were used. This was necessitated by the greater susceptibility of this strain at these temperatures.

Filters were subjected to ultrahigh vacuum at a specific temperature for 5 days. When the vacuum was broken with dry nitrogen, the filters were removed and assayed for viable cells or were stored over silica gel if not assayed immediately. Assays were usually completed on the same day. Each filter was blended in 100 ml of chilled, sterile, distilled water (5 °C) for 2 min. Samples of serial dilutions were placed on prepoured plates of agar of the same composition as the propagation medium of the particular organism. The incubation temperatures for each organism were also the same as their propagation temperatures. The incubation period was usually 5 days for vacuum-treated organisms and 3 days for the control plates containing unexposed cells. The control filters were maintained over silica gel for 5 days (the duration of the experiment) before being plated.

The vacuum apparatus was an improved model of the chamber previously used for similar studies (N. S. Davis et al., Appl. Microbiol. 11:202, 1963) and is described in Fig. 1. The stainless-steel chamber contained series of tantalum wire coil heaters supported on a frame by ceramic insulators. A stainless-steel reflector surrounded the frame. The heater assembly was 10.5 inches (26.7 cm) in diameter and 11.5 inches (29.2 cm) in height. Two circular, stainless-steel sample trays, each 8.5 inches (21.6 cm) in diameter, were centrally located, and the assembly was insulated from contact with the bottom of the chamber by positioning the trays on insulating rods. The temperature was controlled (±1 °C) by a Pyr-O-Vane temperature-indicating controller. The temperature in the sample area was monitored by several chromel-alumel thermocouples, a number of the thermocouples being positioned in the immediate vicinity of the fiber-glass filters containing the dried organisms. The vacuum chamber was encased in refrigerated coils, and the entire unit was insulated. The vacuum system was rou-
Temperature equilibrium was attained in approximately 1 day, as compared with the test period of 5 days during which the organisms were exposed to vacuum.

The survival of the three vegetative species of organisms, S. faecalis, S. aureus, and strain 248, was determined in ultrahigh vacuum over a temperature range of 4 to 80 °C (Fig. 2). At temperatures below 40 °C, prolonged exposure in ultrahigh vacuum caused a decrease in viability to a level which varied with each of the organisms. S. aureus was stabilized at 4 to 8%; S. faecalis, around 10%; and strain 248, the most resistant at these low temperatures, at 10 to 30%. A. A. Imshhevetskey and S. V. Lysenko (In M. Florkin [ed.], Life Sciences and Space Research, North-Holland Publishing Co., Amsterdam, Netherlands, 1965) also had noted variability in the resistance among vegetative cells exposed for 72 hr at ambient temperature to an ultrahigh vacuum of 10⁻⁹ torr. Of the strains they examined, Sarcina lutea and Mycobacterium rubrum were the most resistant with approximately 30% of the cells surviving. Pseudomonas pyocyanea and Escherichia coli were less resistant, with 0.34 and 4.7%, respectively, surviving the vacuum treatment. In contrast, none of the inoculum of P. fluorescens, Serratia marcescens, and Vibrio metchnikovii survived this treatment. Lower inocula were used by Imshhevetskey and Lysenko, and the support material was filter paper rather than glass filters. The destructive effect of vacuum per se on the test organisms as indicated by the survival at 4 °C is higher than that noted previously for microbial spores (N. S. Davis et al., Appl. Microbiol. 11:202, 1963).

Spores were much more resistant to vacuum, which has little effect on spore survival at low temperatures, for periods of up to 7 days.

A sharp decrease in viability occurred at temperatures above 40 °C. That small numbers of S. aureus appeared to be quite resistant to elevated temperatures may have been due to the presence of a number of clumps, although this effect was not noticed for S. faecalis or strain 248.

The nature of the survival data for both spores and vegetative cells exposed to ultrahigh vacuum over a temperature range indicates that at some transition temperature lethality becomes significant. This occurred around 60 °C for spores (N. S. Davis et al., Appl. Microbiol. 11:202, 1963) and between 40 and 50 °C for vegetative cells. The factor responsible for the increased lethality at this transition temperature range is difficult to postulate, since conditions are established in the vacuum chamber which permit the molecular distillation of cell constituents. Whether it is a cell constituent such as a lipid or merely the removal of water closely associated with cell structure that irreparably destroys a cell's integrity certainly warrants further investigations. S. J. Webb (Can. J. Microbiol. 7:621, 1961) has characterized certain aspects of the damage to E. coli caused by

**FIG. 1. Ultrahigh-vacuum chamber.**

Tinily capable of attaining a pressure of 10⁻¹⁰ torr at ambient temperatures and was capable of achieving vacuums of 10⁻⁸ torr at 180 °C.

**FIG. 2. Survival of vegetative cells in ultrahigh vacuum (5 days) at various temperatures. Staphylococcus aureus, •; Streptococcus faecalis, ○; and strain 248, △. Survival fraction equals the ratio of surviving cells over the original cell population after 5 days of storage over silica gel (20 °C).**
desiccation. He has suggested that loss in viability results from damage incurred by ribosomes involved in protein synthesis and is not primarily due to the loss of the integrity of the cell membrane. In this regard, J. B. Bateman and F. E. White (J. Bacteriol. 85:915, 1963) have suggested that water must intervene in the cells’ organizations. As water is removed at some elevated temperature, an irreversible modification of essential cellular constituents may result, culminating in a loss of viability.

The organisms subjected to ultrahigh vacuum and temperature in this study were harvested from their stationary phase of growth. It is assumed that this is their most resistant physiological state, but other growth media or different techniques for predrying might have rendered the test organisms more stable to the vacuum-temperature treatments.

This investigation was supported by the National Aeronautics and Space Administration under contract NASW-773.