Thermal Inactivation of Aerosolized Bacillus subtilis var. niger Spores

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A hot-air sterilizer capable of exposing airborne microorganisms to elevated temperatures with an almost instantaneous heating time was developed and evaluated. With this apparatus, aerosolized Bacillus subtilis var. niger spores were killed in about 0.02 sec when exposed to temperatures above 260°C. This is about 500 times faster than killing times reported by others. Extrapolation and comparison of data on the time and temperature required to kill B. subtilis var. niger spores on surfaces show that approximately the same killing time is required as is necessary for spores in air, if corrections are made for the heating time of the surface.

The rate at which bacterial spores in air are inactivated by high temperature (dry heat) has been the subject of other publications (1, 2). However, the techniques employed in previous studies involved a system in which the aerosolized organisms were passed through a heated coil or tube and thus depended on heat transfer from the tube wall to the air and microorganisms passing through. Unless the air is turbulent, a temperature gradient will result across the airstream passing through such a tube. Even with turbulent airflow, the heating time involved (time to reach test temperature) is relatively long, which further complicates the precise measurement of the time-temperature kill rate.

In an effort to obtain more accurate data on the kill rate by high-temperature dry heat, an apparatus was constructed for the almost instantaneous mixing of air containing “wet” or dry aerosolized organisms with hot air and for sampling within a fraction of a second. Throughout this report, the term “wet aerosol” is used for aerosols produced from spores suspended in sterile distilled water as compared with aerosols generated with dry spores.

MATERIALS AND METHODS

Aerosols of dry Bacillus subtilis var. niger spores (1.1% moisture) were produced by using a pulse-jet generator which injected pulses of dry sterile air into a reservoir of micronized dry spores. The resulting aerosol passed through a 20-liter glass chamber (Fig. 1). The frequency of pulses and volume of air injected with each pulse were adjustable.

Spores from the same batch were prepared for the wet aerosol by adding a sufficient quantity to sterile water to give a slurry containing about $6 \times 10^9$ organisms per ml. The wet aerosol was generated in a 20-liter drying chamber (Fig. 1) by atomizing approximately 1 ml of an aqueous suspension per min with a Vaponefrin nebulizer by using a constant flow of 2.4 liters of primary air per min and 10.7 liters of secondary air per min. (Rotometers were used to monitor all airflows but are not shown.) The wet or dry aerosol passed through a 40-liter settling chamber, in which the pressure was held at 30 cm of water. Mean relative humidity of the wet aerosol airstream was 23%, whereas the dry aerosol airstream was about 10% relative humidity. The required volume of the concentrated aerosol for the test was metered into the exposure unit, and the excess aerosol was vented through a high-efficiency filter. The test aerosol entered the exposure unit through tube C (Fig. 2) and passed through holes (approximately 2.4 mm in diameter) in the tube wall into the mixing tube B. A metered volume of hot sterilizing air, obtained by passing an excess volume through an electric heater, entered the outer cavity A (Fig. 2) passing through the perforated tube wall into the mixing tube B. Both air-streams were forced into the mixing tube (volume, 20 cm$^3$) under slight pressure to cause more turbulence and to assure complete and very rapid mixing of the aerosol and heated air. The initial concentration of the aerosol, after being diluted in the mixing tube, was approximately 1 to 10 million spores per liter in the dry aerosol and 300,000 to 3 million spores per liter in the wet aerosol. Immediately after leaving the mixing and sterilizing tube, the heated aerosol passed over a thermocouple for measuring the test temperature and over a small port through which the treated sample was withdrawn. The temperature of the treated aerosol dropped very rapidly and was ambient when it passed through the liquid in the sampler.

Temperatures were taken at four positions with
iron-constantan thermocouples and a potentiometer. The positions are indicated in Fig. 1 and 2 as T₁, the test aerosol temperature; T₂, the hot-air temperature; T₃, the actual sterilizing temperature; and T₄ the secondary downstream temperature. Temperatures reported in Fig. 3 were recorded by thermocouple T₂ in the mixing and sterilizing tube B of Fig. 2.

In each test, the challenge aerosol was sampled just before it entered the sterilization chamber. Cotton collectors were used at this point, because it was desirable to use only a minimum amount of the incoming aerosol. All-glass impingers containing sterile water were used to sample the hot aerosol leaving the sterilizing chamber. The evaporation of water from this sample cooled the collecting fluid and the trapped microorganisms and thus prevented further kill. The number of viable spores collected was determined by standard dilution and plating techniques in Plate Count Agar (Difco Manual).

Although there was an extension tube on the apparatus to permit longer exposure times, only the small section of the mixing tube (exposure unit) was used, resulting in a very short exposure. This procedure was used because a considerable drop in temperature occurred along the extension tube regardless of whether it was insulated, and, furthermore, preliminary tests showed that the greatest kill took place in the mixing chamber. The stainless-steel exposure unit (Fig. 2) was approximately 12.7 cm in length and 2.5 cm in diameter and was insulated with asbestos packing.

The residence time was calculated by using the volume of the exposure unit, the flow volume of air entering, and the temperature of the incoming and exiting air volumes.

RESULTS AND DISCUSSION

Spores of B. subtilis var. niger in both wet and dry aerosols were killed rapidly when exposed to 204 to 260 C in the hot-air sterilizer for approximately 0.02 sec (Fig. 3). The difference in sterilizing temperatures between wet and dry spores was small, although it was slightly lower for wet spores (230 C) than for dry spores (260 C). The difference in recovery from wet versus dry aerosols is statistically significant at the 0.06 level at temperatures near 204 C, but at temperatures near the limits of testing, where there is less directly comparable data, the difference is less clear. With both wet and dry aerosols, sterility was generally obtained near 260 C with an exposure time of 0.02 sec. This is about 0.2% of the time reported by Decker et al. (1). The variation is undoubtedly due to the equipment employed. For example, our apparatus was smaller, used less airflow, and had a more efficient method of blending the hot air with the microorganisms to give essentially instantaneous heating. This type of mixing most likely causes the particles to be in turbulent motion with respect to the air and thus facilitates the rate of heat transfer from the surrounding air to the surface of the bacterial particle.

The steepness of the curves is of great interest. It indicates that, at temperatures above 204 C, a small increase in temperature gives a great increase in the kill rate. For temperatures below 204 C, however, the kill rate is much slower.

It was speculated that the rate at which unprotected microorganisms are killed in the air should correspond to the rate at which they are killed on a surface, after the heating time of the surface.
is negated. The latter rate was reported by Hoffman et al. (3). In their study, two strains of *B. subtilis* var. *niger* spores were tested after preequilibrating to 11, 33, or 85% relative humidity. The fastest and slowest times required to kill each strain of *B. subtilis* var. *niger* spores were taken for each temperature tested and treated by the least-squares method to obtain a combined equation for the best straight line when plotting log death-time against temperature.

The equation for this line is: $Y = 10.08 - 0.0512X$, where $Y$ = log time and $X$ = temperature. The plot for this equation is shown in Fig. 4.

The dotted portion of the line is a rather extreme extrapolation, made to determine where the line intersects the 0.02-sec exposure time. This occurs at approximately 230 °C, approximately the same temperature at which sterility is reached with wet *B. subtilis* var. *niger* spores in air. The closeness of the temperatures lends support to the theory that unprotected spores will be killed at the same rate whether in air or on a surface when subjected to the same temperature. Unfortunately, tests could not be run with contaminated surfaces at high temperatures, because the heating time of the surface approached the kill time somewhere between 164 to 192 °C. On the other hand, the design of the apparatus used in the tests reported here for aerosolized organisms did not permit the relatively long exposure time required to kill spores at 164 °C. For these reasons, it is possible to compare the kill times of the two techniques only by extrapolation.

In conclusion, it appears that utilization of the principle of rapid mixing of a bacterial aerosol with hot air permits accurate measurement of the very rapid sterilization of bacterial aerosols which occurs at elevated temperatures.

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