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NATICK, MASS.ACTIVATION OF *BACILLUS MEGATERIUM* SPORE GERMINATION
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It is well documented that spores, heated for extended periods of time at high temperatures, lose their viability, but when heated at sublethal temperatures, are activated for subsequent germination on various substrates. Here we report what we believe to be the first demonstration of an analogous activation of spores by exposure to water vapor. Bacterial spores, exposed to water vapor for long periods, germinated spontaneously (with no added substrate) and lost viability; when exposed for shorter periods, spores did not germinate spontaneously but were activated for germination on added substrate. Bullock and Tallentire (1952); Lewis (1961); and Marshall, Murrell and Scott (1963) found that, under certain conditions, spores stored at high water activity* levels for extended periods of time, germinated spontaneously, lost heat resistance and viability, but the possibility that shorter exposures might activate the spores for subsequent physiological germination was not examined. We now find that lyophilized *Bacillus megaterium* spores, exposed in an atmosphere over pure water (a_w 1.0) for 4 hr, germinated spontaneously and lost their viability upon addition of liquid water. Shorter exposure times (30 or 60 min) did not initiate spontaneous germination, but the spores were activated for glucose-induced germination.

B. megaterium QM B1551 spores, grown on the complex medium (omitting agar) of Arret and Kirshbaum (1959), were harvested, lyophilized, and stored under

* Water activity (a_w), a property of aqueous solutions, is the ratio of the vapor pressure of a solution to that of pure water. Equilibrium relative humidity (expressed as a decimal) is numerically equal to a_w .

vacuum at 4 C in a desiccator over anhydrous CaSO_4 . To ensure uniform exposure to water vapor, spores were coated onto the surface of No. 13 Ballotini beads (ca 0.1 mm diam.) in evacuated Thunberg tubes - 25 mg of spores mixed ("vortex" mixer) with 500 mg of beads. Spore-coated beads were evenly distributed in open 5 cm Petri dishes, and exposed at 25 C without evacuation, in a 160 mm desiccator (vol, ca 2750 ml) containing 100 ml of water (a_w 1.0) which had been added 24 hr previously. After exposure in this humid atmosphere, spores were suspended in 10 ml of liquid water and examined for spontaneous germination; for activation of germination induced by various substrates buffered with 50 mM potassium phosphate (pH 7.0); for heat resistance (10 min at 70 C); and for viability. Germination was measured by decrease in optical density of spore suspensions (0.4 mg of spores per ml) at 560 m μ (Klett-Summerson colorimeter) and by increase in stainability with 0.5% methylene blue. Heat resistance and viability were determined by microscopic observation of cell division after 120 min in Brain Heart Infusion broth (Levinson and Hyatt, 1966). Control spores, similarly coated on glass beads, but exposed in a desiccator over P_2O_5 , or mixed with liquid water without prior exposure in a desiccator, did not germinate spontaneously and were not activated for germination on any substrate tested.

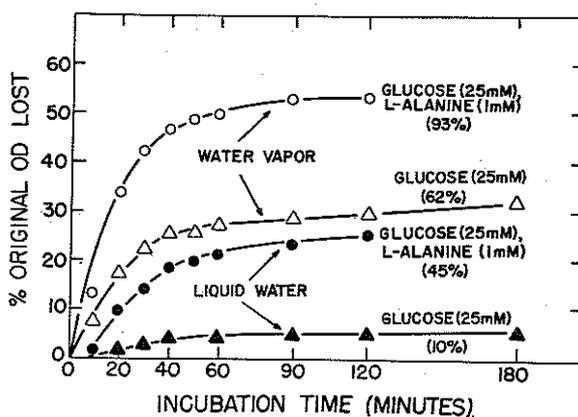


Figure 1. Activation of *Bacillus megaterium* spore germination by water in the vapor phase. Spores were exposed either to water vapor (a_w 1.0) or in liquid water, for 30 min at 25 C, then incubated at 30 C in 50 mM PO_4 (pH 7.0) with indicated substrates. Percent germination (stainability) at end of experiment indicated by numbers in parentheses.

Fifty percent of the spores exposed to water vapor (a_w 1.0) for 120 min germinated spontaneously after addition of liquid water; 50% were no longer heat resistant, but 80% remained viable. After 240 min exposure at a_w 1.0, 100% of the spores had undergone spontaneous germination and had lost heat resistance, but 50% were still viable.

Spores, exposed to water vapor (a_w 1.0) for 30 or 60 min, did not germinate spontaneously, did not lose heat resistance or viability, and were not activated either for ionic germination (5mM KI) or for germination in 100 mM L-alanine. Such spores, however, were activated for subsequent germination on 25 mM glucose, or on a combination of 25 mM glucose and 1mM L-alanine (Fig. 1). This activation affected both the total number of spores germinating, and the germination rate. The time for 50% completion of turbidity loss (Levinson and Hyatt, 1966) of spores germinating on glucose was reduced from ca 34 min to ca 18 min; for germination on the combined glucose-L-alanine substrate, the 50% completion time was reduced from 27.5 to 16.5 min.

Activation of spores by exposure to water vapor (a_w 1.0) for 30 min was not reversed by then exposing them for 2 or 24 hr over P_2O_5 , or to vacuum for 30 min.

Exposure times as short as 5 min resulted in almost maximal activation (Fig. 2). Activation by water vapor (30 min, 25 C) was maximal at a_w levels

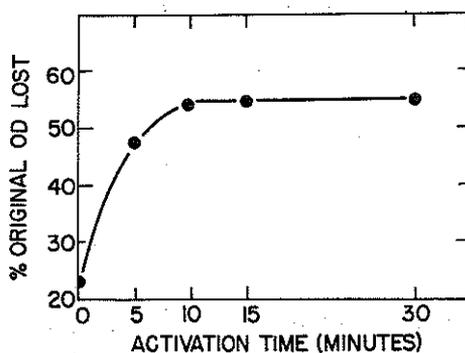


Figure 2. Effect of duration of exposure to water vapor on activation of *Bacillus megaterium* spore germination. Spores were exposed to water vapor (a_w 1.0) at 25 C, then incubated for 120 min at 30 C in 50 mM PO_4 (pH 7.0) with a combination of glucose (25 mM) and L-alanine (1 mM).

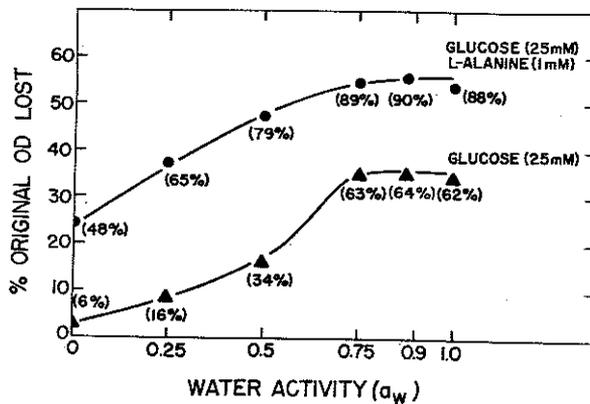


Figure 3. Activation of *Bacillus megaterium* spore germination at different water activity levels. Spores were exposed for 30 min at 25 C over H_2SO_4 mixtures giving various water activity levels (a_w 0.0 attained over P_2O_5), then incubated at 30 C in 50 mM PO_4 (pH 7.0) with indicated substrates. Incubation time in glucose was 180 min; in glucose-L-alanine, 120 min. Percent germination (stainability) at end of experiment indicated by numbers in parentheses.

from 0.75 to 1.0 (Fig. 3), but there was some activation as low as a_w 0.25. We are unable to determine from present data, whether the activation depends upon relative water activity, vapor pressure, or absolute mass of water present in the vapor phase.

The above problems, as well as the basis for activation of spore germination by exposure of spores to water vapor, and the failure of spores to be activated in liquid water (at 25 C) are the subject of current, more detailed investigations. Activation of spores by exposure to water vapor may be related to spore activation by such other treatments as sublethal heat and aging. Indeed, it is possible that we may be able to account for the often disparate germination responses of spore batches lyophilized or stored under differing conditions.

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