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MANGANESE AND THE PROTEOLYTIC ACTIVITY OF SPORE EXTRACTS OF BACILLUS MEGATERIUM IN RELATION TO GERMINATION

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Levinson and Sevag (J. Gen. Physiol., 36, 617, 1953) have demonstrated the stimulatory effect of manganese on spore germination. In attempting to elucidate this effect, we have been working on the hypothesis that manganese activates a key enzyme or enzyme system. In the present note, it is shown that spores do contain proteolytic enzymes and that the activity of these enzymes is accelerated by manganese.

Spores of *Bacillus megaterium*, strain QM B-1551, were harvested according to the method outlined by Levinson and Sevag (J. Gen. Physiol., 36, 617, 1953) and dried from the frozen state. Homogenates were made by grinding a watery paste of spores with powdered pyrex glass in a Potter mill, and taking up the ground paste in distilled water so that 1.0 ml of homogenate was derived from 30 mg of spores. The extracts used were the clear supernates obtained after centrifugation of the homogenates in an angle centrifuge. Manganese, when used, was in a concentration of 10 ppm as manganous sulfate.

Hydrolysis of the protein substrates (gelatin, egg albumin, spore homogenates) and its acceleration by manganese were demonstrated by: (a) detection on paper partition chromatograms (figure 1) of amino acid spots resulting from gelatin hydrolysis (similar results with egg albumin); (b) by reduction in the viscosity of gelatin (the initial rate with manganese is 1.5 times the rate without manganese); and (c) by increase in the color intensity (using Klett filter no. 56) developed when 1.5 ml of the reaction mixture was heated in a boiling water bath for 5 minutes with 0.5 ml of a 0.1 per cent aqueous solution of ninhydrin (figure 2).

The above data show that, at the very least,

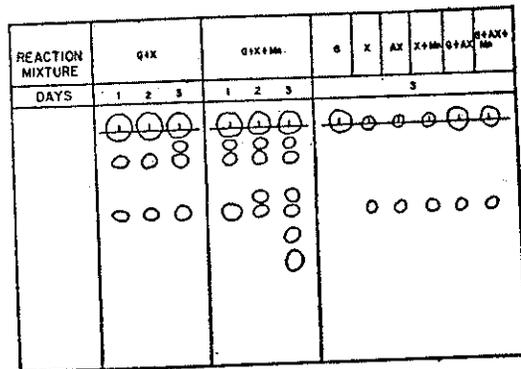


Figure 1. Paper partition chromatogram tracing showing hydrolysis of gelatin by spore extract. G = gelatin (1.0 per cent); X = spore extract (equivalent to 2.7 mg of ground spores per ml of the reaction system); AX = autoclaved spore extract; Mn = manganese (10 ppm) as manganous sulfate. Streptomycin present in a final concentration of 110 µg per ml to maintain sterility. Gelatin reaction mixtures chromatogrammed after 1, 2, and 3 days of incubation at 30 C.

proteolytic enzymes are present in spores. This may be at variance with the conclusion of Hardwick and Foster (J. Gen. Physiol., 35, 907, 1952; J. Bact., 65, 355, 1953) that "enzymes in the vegetative cell are destroyed or lost during sporogenesis."

On incubation of spore homogenates without added protein, the ninhydrin color intensity increases with time. Manganese accelerates and increases this effect (figure 3). The magnitude of the reaction, as compared with that in figure 2, may be attributed at least partly to the homologous nature of the substrate specific for the spore enzymes. The above discussed reaction in-

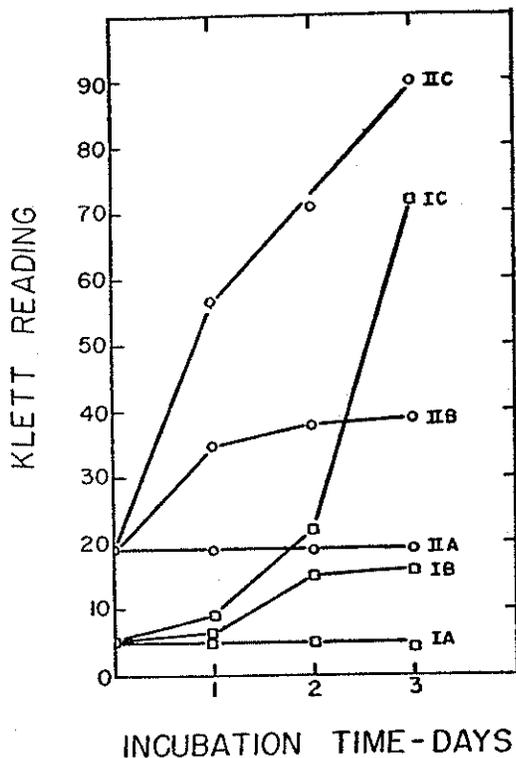


Figure 2. Colorimetric estimation of proteolysis by spore extracts. Curve IA, gelatin and auto-claved spore extract; curve IB, gelatin and spore extract; curve IC, gelatin and spore extract with manganese. Curve IIA, egg albumin and auto-claved spore extract; curve IIB, egg albumin and spore extract; curve IIC, egg albumin and spore extract with manganese. The spore extract concentration was equivalent to 2.7 mg of ground spores per ml of the reaction system. Protein concentration was 1.0 per cent. Streptomycin present in a final concentration of 110 μ g per ml to maintain sterility. Incubation was at 30 C.

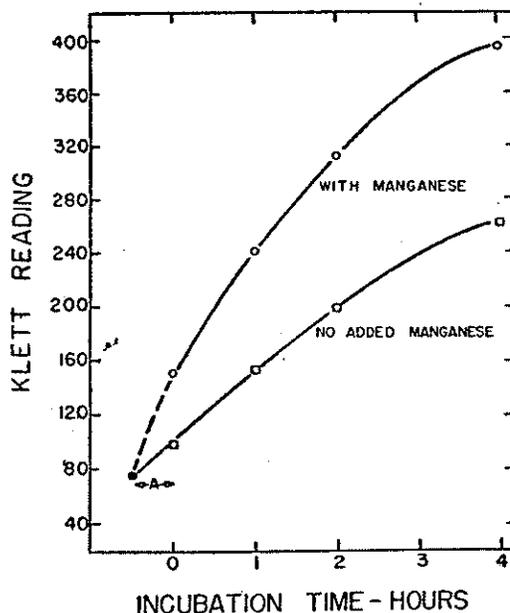


Figure 3. Proteolysis in spore homogenates. Each ml of the homogenate was derived from 30 mg of ground spores. Ninhydrin color of homogenates developed after centrifugation following various times of incubation at 37 C. Streptomycin was added to give a final concentration of 500 μ g per ml to maintain sterility. The closed circle indicates the estimated time of addition of manganese to one of the aliquots, and the distance, A, represents the time required for centrifuging. The dashed portions of the curve therefore may be somewhat approximate.

volving the hydrolysis of the native substrate is paralleled by concomitantly increasing germination of the spore. This indicates that the spore material itself can act as a substrate for the production of substances stimulatory to germination.