PROTEINASE PRODUCTION BY BACILLUS SUBTILIS[1]

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In a study on the proteinases of food spoilage organisms it was observed that *Bacillus subtilis* failed to produce substantial amounts of proteinase in aerated nutrient broths prepared from Difco peptone and beef extract and from Difco dehydrated nutrient broth but that the organism showed slightly increased growth and good proteinase production when grown in nutrient broth containing Armour or Witte products. The deficiency was traced to the ash constituents in the latter nutriments and was found to be due, at least in part, to a

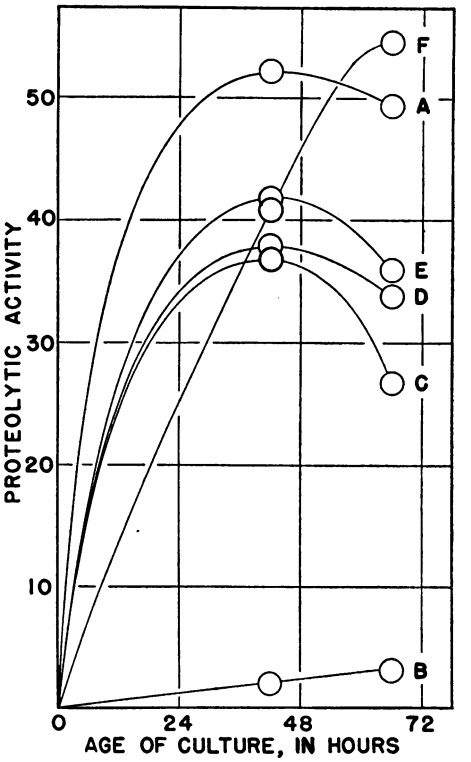

FIG. 1. EFFECT OF MN AND OTHER TRACE ELEMENTS ON PROTEINASE PRODUCTION

Digestion of casein in 5 ml of buffered 1% solution by 2 ml cell-free culture liquid acting 100 minutes at 30 C. Proteolytic activity measured as differences in percentage of transmittance of sulfosalicylic-acid-treated samples in Coleman Universal Spectrophotometer. *A*, Armour broth; *B*, Difco broth; *C*, Difco broth + Mn; *D*, Difco broth + Mn, Zn; *E*, Difco broth + Mn, Cu, Zn, B; *F*, Difco broth + Mn, Zn, Cu, B, Ti, Ni, Co, Mo, Br, I.

[1] The subject matter of this paper has been studied in co-operation with the Quartermaster Corps Committee on Food Research.

lack of manganous ion (figure 1). Substantial proteinase formation may be obtained in Difco broth merely by adding 0.05 to 50 mg of $MnSO_4 \cdot H_2O$ per liter. The optimal concentration of added manganous salt appears to be approximately 5 mg per L. When manganous sulfate was added in comparable quantities to poorly proteolytic culture liquid at the end of the growth period, no increase in proteinase activity resulted. Therefore, the added manganese appears to function during the elaboration of the enzyme and not merely as an activator of the proteinase system.

Elements which do not replace the maganese effect are zinc, copper, iron, boron, magnesium, and calcium. Proteinase production seemed slightly greater, however, when a mixture of $MnSO_4 \cdot H_2O$ and $ZnSO_4 \cdot 7H_2O$ were employed instead of $MnSO_4 \cdot H_2O$ alone. It was still further improved upon addition of various combinations of the elements Mn, Zn, Cu, B, Ti, Ni, Co, Mo, Br, and I, now being used by Professor C. R. Johnson in studies on plant nutrition (figure 1). The desirability of Mn (and also Fe) in culture media intended for the production of bacterial proteinases by *Bacillus subtilis* has previously been indicated (Wallerstein, 1939; Boidin and Effront, 1930). However, Haines (1931) described protease formation in synthetic media in the absence of added Mn and Fe.

When cultures are constantly agitated, the proteinase content of the centrifuged broth is maximal within 2 or 3 days. Stationary cultures require more than 6 days for maximum proteinase accumulation, and less total proteolytic activity is obtained. The rapid growth and increased crop of highly aerobic organisms obtained in aerated cultures may call attention to nutritional deficiencies in culture media that appear adequate for stationary growth.

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