NOTES

METHIONINE SULFOXIDE AND SPECIFIC INHIBITION OF SPORULATION IN BACILLUS SUBTILIS

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Methionine sulfoxide has been shown to be a specific antagonist of glutamic acid in the conversion of glutamic acid to glutamine by Waelsch, Owades, Miller, and Borek (J. Biol. Chem., 166, 273, 1946) in growth inhibition studies with Lactobacillus arabinosus. Elliot and Gale (Nature, 161, 129, 1948) using cell-free extracts of Staphylococcus aureus have demonstrated that methionine sulfoxide inhibited the synthesis of glutamine through a competitive inhibition of glutamic acid.

Studies on the sporulation and growth of Bacillus subtilis, strain ATCC 6633, in a simple glucose-inorganic salts-glutamate medium have indicated that sporulation is related to the concentration of L(+) glutamic acid over and above that required for maximum growth (unpublished operations). This note confirms the specific effect of glutamic acid by inhibiting sporulation with methionine sulfoxide.

The basal medium of these experiments consists of: K_{2}HPO_{4}, 0.1 per cent; MgSO_{4}·7H_{2}O, 0.02 per cent; NaCl, 0.001 per cent; FeSO_{4}·7H_{2}O, 0.001 per cent; CuSO_{4}·5H_{2}O, 0.001 per cent; MnSO_{4}·7H_{2}O, 0.001 per cent; CaCl_{2}, 0.0046 per cent; (NH_{4})_{2}SO_{4}, 0.5 per cent; glucose, 1.0 per cent; L(+) glutamic acid, 0.44 per cent (2.99 × 10^{-2} M) in triple distilled water. Methionine sulfoxide was added to the basal medium in concentrations ranging from 1.80 × 10^{-2} M to 2.65 × 10^{-2} M in all but the control, giving molar ratios of methionine sulfoxide to glutamic acid in table 1.

The media were titrated to pH 7.2 and were sterilized through sintered glass filters except for glucose which was autoclaved and added separately. Two-tenths ml of a 20 hour culture incubated at 37 C and giving an optical density of 1.10 at 700 mμ on a Coleman spectrophotometer was used as a standard inoculum per 50 ml of medium. The flasks were incubated on a shaker under aeration at 37 C for 120 hours. Optical density readings at 700 mμ were taken hourly for 24 hours for a comparison of the effect of methionine sulfoxide on the lag periods and the growth rates of the cultures. Except in the culture at a ratio of methionine sulfoxide to glutamic acid of 6.03, growth in all cultures began in 8 hours, showed no significant difference in growth rates, and reached maximum growth within 24 hours. One hundred twenty hours were considered sufficient incubation since maximum sporulation in the control occurs within 48 to 72 hours and methionine sulfoxide presumably could be reduced (Waelsch, as above). The results summarizing the effect of methionine sulfoxide on sporulation and growth are presented in table 1.

Table 1 and the results of the hourly optical density readings indicate that sporulation has been inhibited without affecting growth within certain molar ratios. Apparently methionine sulfoxide separates the utilization of glutamic acid for growth and for sporulation, and provides a means to study a specific site in the chemical sequence necessary to sporulation. Experiments are now in progress to determine whether the specific antagonism to glutamic acid represents an inhibition of glutamine synthesis as is suggested by the methionine sulfoxide inhibition.

![Table 1: Inhibition of sporulation in Bacillus subtilis by methionine sulfoxide.](http://jb.asm.org/)

* 8% stimulation of growth.
† Reading at 48 hours.

Table 1 includes the following data:

- **Molar Ratio**
- **Methionine Sulfoxide**
- **Glutamic Acid**
- **Growth Optical Density, 24 HR**
- **Growth Per Cent of Inhibition**
- **Per Cent Spores, 120 HR**

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<th>Molar Ratio</th>
<th>Methionine Sulfoxide</th>
<th>Glutamic Acid</th>
<th>Growth Optical Density, 24 HR</th>
<th>Growth Per Cent of Inhibition</th>
<th>Per Cent Spores, 120 HR</th>
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