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Methyl Anthranilate, an Inhibitor for the Germination of Spores of Aerobic Bacilli

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Methylanthranilate inhibited the germination of spores of aerobic bacilli without affecting growth and sporulation. The inhibition of germination could not be reversed by removal of methylanthranilate.

The conversion of the cryptobiotic state of the spore to a vegetative cell is associated with a number of biochemical changes. Three main phases of this process are defined as activation, initiation, and outgrowth (2). The term germination, as used in this paper, refers only to the initiation step.

The need for germination inhibitors in prevention of food spoilage by bacterial spores cannot be overemphasized (1). A variety of metabolic inhibitors have been reported in recent years which can inhibit one or more steps in the process of germination (4-6). During the course of our investigations into tryptophan catabolism, we noticed that methylanthranilate (MA) inhibited the germination of *Bacillus cereus* T spores. In this communication the inhibitory effect of methylanthranilate for germination of spores of four different baclli is reported.

B. subtilis NCTC 2116, *B. coagulans* 110, *B. megaterium* C8 (obtained from T. B. Holland, Leicester, England), and *B. cereus* T used in these experiments were allowed to sporulate on nutrient agar supplemented with 8 mg of $CaCl_2 \cdot 2H_2O$ per 100 ml, except for *B. cereus* T, which was grown on G agar (3). Spores were harvested and cleaned by the method of Whitehouse and Clegg (7). The rate of germination was followed by measuring the change in optical density.

The effect of various concentrations of MA on the inhibition of germination of *B. subtilis* spores is shown in Table 1. The percentage of inhibition of germination increases logarithmically with the increasing concentrations of the drug until a maximum level is reached. The inhibition of germination by MA is irreversible. The inhibited spores (incubated for 30 min in the presence of 0.5 mM MA) of *B. subtilis* could not be brought back to normal germination after washing with 10% ethyl alcohol and water. A similar washing of untreated spore suspension did not affect its germination characteristics. Figure 1 shows that MA (0.5 mM) inhibited the germination of spores of *B. subtilis* NCTC 2116, *B. coagulans* 111, and *B. cereus* T. However, there were marked differences among the different bacilli in response to various levels of MA. *B. megaterium* showed only about 50% inhibition of germination in 30 min at a concentration of 10 mM. According to the degree of resistance of the spores to MA, the four bacilli can be placed in the following sequence, *B. megaterium* being the most resistant and *B. subtilis* the least: *megaterium* → *cereus* → *coagulans* → *subtilis*.

We also compared the relative inhibitory potency of MA and its two analogues for germination of *B. subtilis* NCTC 2116 spores in G medium. When the potency was calculated on the basis of the concentration required for 50% inhi-

TABLE 1. *Effect of different concentrations of methylanthranilate on the germination of Bacillus subtilis NCTC 2116 spores*[a]

Concn of MA	Initial absorbancy decrease	Inhibition
mM	%	%
Control	47	
0.1	41	13
0.2	33	23
0.3	25	45
0.4	12	74
0.5	0	100

[a] Heat-shocked (65 C for 1 hr) spores were incubated in G medium with various levels of MA. The per cent decrease in initial absorbancy at 630 nm was determined after incubation for 30 min at 30 C.

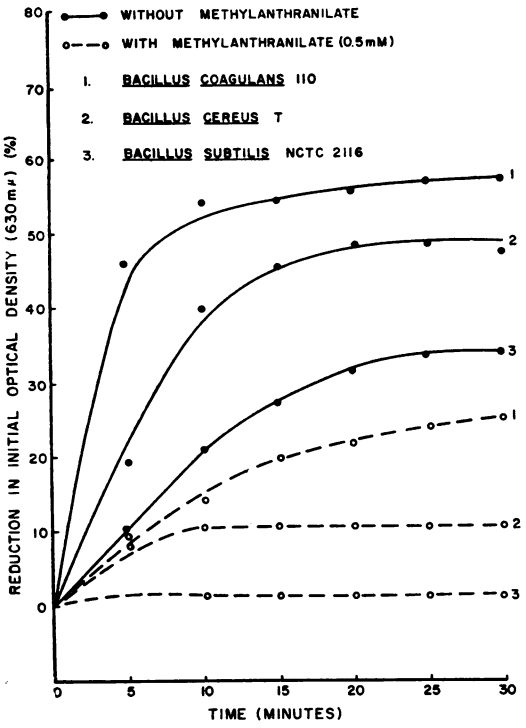

FIG. 1. *Inhibition of germination of Bacillus spores in G medium with methylanthranilate.*

bition of germination, methyl- and ethyl-anthranilates were five and seven times, respectively, more potent than *p*-aminoethylbenzoate.

Our experiments showed that MA inhibits only the germination of *Bacillus* spores without affecting their growth and sporulation; the concentration of MA needed is less than any other inhibitor reported previously in the literature.

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