Effects of Tylosin and Nisin on Canned Food Spoilage Bacteria

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Many antibiotics have been screened for inhibition of sporeforming bacteria during the last 10 years in this laboratory. The effect of some of these antibiotics on canned food spoilage bacteria has previously been described (Denny and Bohrer, 1959). Recently, two antibiotics which eventually may be of use in food processing have been through a preliminary screening in this laboratory. These antibiotics are nisin and tylosin.

Nisin has been recommended for approval for use in certain foods in Great Britain (Her Majesty's Stationery Office, 1959). Our sample was obtained through the courtesy of Dr. H. B. Hawley of Alpin and Barrett, Ltd., Yoevil, Somerset, England.

Tylosin is undergoing extensive animal toxicity tests in the manufacturer's laboratory. These are nearly complete and appear promising with respect to its suitability for use in food. Dr. Bernard Malin of Eli Lilly and Company in Indianapolis, Indiana, graciously supplied the sample for our tests.

MATERIALS AND METHODS

The initial screening procedure used in this laboratory has made use of three stock solutions of the antibiotics at concentrations of 1,000, 500, and 250 ppm based on dry weight (not units). Nisin was reported to contain about 1,000,000 units per g and tylosin about 900,000 units per g. The stock solutions were diluted serially in media. For purposes of comparison in this procedure, approximately equal numbers of spores of each of the test suspensions were inoculated into both the tylosin and nisin. The initial number of spores used was determined from dilutions of suspensions which had previously been heated at 212 F for 5 min.

The spore-antibiotic broth tubes were steamed at 212 F for 5 min, cooled, stratified with sterile Vaseline, and incubated at optimal temperatures. Gas production, turbidity, or change in indicator color was used to denote growth.

To determine the adjunctive effect of heat and each of the antibiotics, only Bacillus stearothermophilus was used. Each sealed thermal death time tube contained 2 ml of the inoculated medium or inoculated antibiotic-medium. The inoculum contained 920 spores per ml based on a boiled, 5-min count obtained on Dextrose Tryptone agar4 incubated at 131 F for 48 hr. The heating substrate was Tryptone-yeast extract broth. The tubes were heated in an oil bath at 250 F. Tubes were withdrawn at 5-min intervals (allowing for initial lag time), cooled, and incubated at 131 F.

A mushroom experimental pack was made in 211 x 212 cans at a commercial cannery. Stems and pieces were used for reasons of economy. The product was commercially filled, a 40-grain salt tablet was added, and the antibiotic added so that each can contained either none, 1, or 2.5 ppm of tylosin. Boiling water then completed the fill and the cans were sealed on commercial equipment. The heat processing was conducted in the cannery by plant personnel. A stopwatch was used to time the heat process. The cans were cooled in the retort and incubation at 131 F was begun on the same day that the cans were packed.

RESULTS AND DISCUSSION

The results obtained in the preliminary screening are shown in table 1. Concentrations below the ones indicated for growth were all positive. Note that most of these results showed much greater inhibitory potency for tylosin as compared to nisin. Results with a butyric anaerobe and Bacillus coagulans are not included, however, these tests were also conducted. Tylosin inhibited 20,000 spores per ml of a butyric anaerobe (suspension no. 59-165) in a concentration of 1 ppm, whereas nisin failed to inhibit at 100 ppm. In three tests with B. coagulans spores in tomato juice, both antibiotics show comparable inhibitory powers. Dilution of negative tubes into media allowed the spores to grow and demonstrated only inhibitory action following mild heat.

The results of the test to determine the adjunctive effect of heat and each of the antibiotics are shown in table 2. Nisin was effective at the moment the heating substrate reached 250 F. However, its effectiveness was lost at 5 min at most concentrations which probably demonstrates its partial heat lability. The combined effect of heat and nisin is shown at all concentrations after heating 15 min at 250 F. This adjuvant effect of heat and nisin has been shown previously with spores of other food spoilage organisms (Campbell, Sniff, and O'Brien, 1959). If tables 1 and 2 are compared, it will be noted that 10 ppm nisin is effective with extreme heat but not with mild heat.

The results in table 1 indicate that tylosin is much
more active than nisin against *B. stearothermophilus*. Since the experiment shown did not involve attempts to make quantitative dilution recovery counts, the question of whether complete absence of growth in the presence of the antibiotics is due to acceleration of the rate of spore destruction or merely the inhibition of the spores cannot be answered at this time. However, spores recovered by dilution cultures following 5 min heating at 250 °F in the presence of tylosin demonstrated that its inhibitory effect on *B. stearothermophilus* was retained.

To determine the effectiveness of tylosin against natural spore contamination, a mushroom pack was prepared. Mushrooms generally contain many spores of harmless thermophilic bacteria. At the present time, excessive heat processes must be used to eliminate spoilage. By decreasing the process for the smaller cans to 10 min at 250 °F, a better appearing product might be obtained than the product now generally processed 18 min at 250 °F. The results of an experimental mushroom pack are shown in table 3. It is evident that 1.0 ppm tylosin prevented the thermophilic spoilage in mushrooms which were so contaminated as to give 100 per cent spoilage with the reduced heat process without the antibiotic. Flat sour, sulfide spoilage, and thermophilic anaerobe spoilage were all demonstrated in the spoiled control cans. Also, spores of these organisms were demonstrated in cans subcultured after filling and before processing. The reduced heat process used in this experiment was sufficient to destroy the spores of *Clostridium botulinum*, so no health hazard problem was involved. Storage assays for residual potency are under way.

At the time of the writing of this paper, neither of the antibiotics has been approved by the Food and Drug Administration for use in canned foods.

**Summary**

The antibiotics nisin and tylosin were screened for inhibitory powers against several types of food spoilage...
bacterial spores. The results indicate that tylosin is strongly inhibitory. Additional tests confirm the adjuvant effect of nisin and heat on spores of *Bacillus stearothermophilus*. These tests also show that tylosin has marked stability to heat as compared to the lesser heat stability of nisin.

The ability of tylosin to prevent the outgrowth of naturally occurring thermophilic spores in a commercial product following a reduced heat process was demonstrated.

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**Growth of *Aspergillus oryzae* in Irradiated Sucrose-Nitrate Medium**

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In determining the death of fungi by radiation, the growth medium used to determine survivors is an important factor to be considered. Beraha et al. (1959) used sucrose-nitrate medium to suspend mold spores during radiation. When the spores were suspended in this medium, the lethal dose was lower than when the spores were suspended in Tochinai's medium. They also found that when *Penicillium italicum* and *Penicillium digitatum* were grown on sucrose-nitrate medium prior to radiation the dosage needed to kill the fungi was less than when these organisms were grown on Tochinai's medium.

The purpose of this study was to determine the effect of irradiated media on growth and spore germination of *Aspergillus oryzae*.

**Materials and Methods**

**Radiation facility.** The sucrose-nitrate medium was radiated in a glass chamber with aluminum windows 1 mil in thickness. The cell was 2 cm thick and 3.8 cm in diameter. The irradiation chamber was held in a fixed position 6 in. from the end of the accelerator by a clamp device which was bolted to the end of the accelerator. The linear electron accelerator, which was built to operate between 2.0 and 6.0 Mev, was controlled at 5.6 Mev. Ten per cent of the electrons in the unscanned cathode beam were lower in energy than 5.6 Mev (MacKay, 1953). Dosimetry was performed using the ceric sulfate method (Weiss, 1952).

**Preparation of medium.** Sucrose-nitrate medium (Czapek-Dox, Difco4), was radiated in liquid and dry form. A Seitz-filtered sucrose-nitrate broth (35 g per L) was prepared on the day of radiation, placed in a sterile flask, and refrigerated until the time of radiation. The medium in the form of a dry powder was radiated, rehydrated (35 g per L), and sterilized by filtering through a Seitz filter. Both media were dispensed aseptically into sterile 250-ml Erlenmeyer flasks.

**Preparation of spore suspension.** *A. oryzae* (Ahlburg) Cohn ATCC strain 11601 was obtained from the American Type Culture Collection, Washington, D. C. This organism was grown in Roux flasks at room temperature and the spores were harvested in about 1 week after spore production was observed. The spores were removed from the surface of the agar by moving a sterile glass rod over the surface of the agar which had been flooded with sterile, distilled water. A glass column, which was filled with glass wool and attached to a suction flask, was used to filter the spore suspension gently. After filtration, the spore suspension was transferred to a sterile Erlenmeyer flask containing glass beads, and the suspension was shaken thoroughly to break up spore clumps.

**Growth determinations.** Flasks containing sucrose-nitrate medium were inoculated with 1 ml of the spore suspension within 2 hr after radiation and were incubated at 30 ± 1 C for 1 week. At the end of the incubation period, the mycelial mat was separated from the broth by filtering through previously weighed Whatman no. 2 (9 cm) filter paper. The filter paper and mycelial mat were placed in one half of a Petri

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