Inhibition of Growth and Patulin Synthesis in *Penicillium expansum* by Potassium Sorbate and Sodium Propionate in Culture

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Potassium sorbate and sodium propionate brought about a marked inhibition in the growth of *Penicillium expansum* and a proportionally greater inhibition in the synthesis of patulin by the mold. At inhibitor concentrations used commercially in bakery products, propionate inhibited growth less efficiently than sorbate did but was a more effective inhibitor of patulin synthesis.

Patulin is a toxic, carcinogenic, unsaturated lactone produced by a number of molds, including certain species of *Penicillium*, *Aspergillus*, and *Byssochlamys*. The toxicity of patulin for microorganisms has been recognized since its discovery in the 1940s and led initially to its being tested as a possible chemotherapeutic agent. The subsequent demonstration of its toxicity and carcinogenicity not only terminated these efforts but led to rising concern, as patulin-producing molds were shown to be widespread, particularly as pathogens of fruits and contaminants of grains (16, 17).

As some patulin-producing molds are natural pathogens of apples, considerable effort has been expanded in determining the extent of the risk to public health in consuming contaminated fruit or juices prepared from such fruit (1, 6, 8, 10). Collateral studies demonstrated that the contamination of flours and bakery products might also present a health hazard. Investigators were able to isolate toxigenic fungi from cornmeal (2), cereal and legume crops (14), and flours, dough, and bread products (4, 9). In two studies (4, 14), toxigenic strains of fungi known to be patulin producers were isolated, and in one study (4), the toxic agent was shown to be patulin.

In one investigation (18), a number of mold strains, mostly of the genera *Aspergillus* and *Penicillium*, were isolated from naturally contaminated samples of rye and white breads. Of 23 moldy bread samples tested, 21 were found to contain patulin. The concentrations varied from 27 to 160 μg/kg of bread, with the highest concentrations being found in the dark bread samples. Experimental inoculation of whole wheat bread with *Penicillium expansum* resulted in the production of patulin (12).

The potential for mycotoxin synthesis in certain foods, such as breads and other bakery products, has encouraged investigation of the effects of mold inhibitors on growth and patulin production in a number of known patulin-producing fungi. In *P. patulum*, Bullerman (2) demonstrated a general suppression of growth and a virtual elimination of patulin production with potassium sorbate at 0.10%. Other concentrations permitted some patulin production after prolonged incubation periods. The effect of sorbate on *P. roqueforti* was quite different. This organism, in comparison with *P. patulum*, was only slightly inhibited, and its production of patulin was actually enhanced.

In *Byssochlamys nivea*, Roland et al. (13) found that potassium sorbate at 50 μg/ml completely eliminated patulin production at 37°C. The effects of this concentration of sorbate on growth were less striking. At 21°C, higher concentrations (75 to 100 μg) of sorbate were required to bring about the inhibition of growth, and even these levels had relatively little effect on patulin production (13).

In this investigation, the effects of potassium sorbate and sodium propionate on the growth of and patulin biosynthesis in a known toxigenic strain of *P. expansum* are reported.

**MATERIALS AND METHODS**

The culture used was a toxigenic strain of *P. expansum* (NRRL 2304) obtained from the Northern Regional Research Laboratory, Peoria, Ill. Cultures were maintained at 25°C on CYD medium (Czapek solution agar with 0.2% yeast extract and 0.8% glucose added) or YAG medium (0.5% yeast extract, 1.5% agar, and 2% glucose per liter).

The liquid medium used for the production of patulin was CYD broth (Czapek Dox broth with 0.2% yeast extract and 0.8% glucose added; 100 ml per flask). The pH of the liquid medium was 6.9. This medium was used for controls and with potassium sorbate (Sigma Chemical Co., St. Louis, Mo.) or sodium propionate (Sigma) added. For 0.1 M inhibitor concentrations, the inhibitor was added directly to the broth which was then sterilized by being autoclaved. Media with other concentrations were prepared by adding dilutions of filter-sterilized inhibitor stock solutions (1 M) to presterilized CYD broth.

Conidia for the inoculum were harvested by agitation from 2- to 3-week-old cultures after the addition of 1 ml of sterile Tween 80, 9 ml of sterile distilled water, and several sterile glass beads. Conidial numbers were estimated with a cell-counting chamber, and 10⁴ conidia were added to each flask. The cultures were incubated at 16°C without agitation for 9 days.

Dry weights were obtained by filtering the cultures through preweighed Whatman no. 1 filter paper. The filters and mycelia were dried to a constant weight at 80°C. The constant weight was determined when successive weighings at 24-h intervals agreed within 10 mg.

Standards for high-pressure liquid chromatography were obtained from Sigma. High-pressure-liquid-chromatography-grade ethyl acetate and methanol used in the extractions were from Fisher Scientific Co., Pittsburgh, Pa.

The spent culture medium was further filtered through a 0.45-μm HA filter (Millipore Corp., Bedford, Mass.). The filtrate was extracted twice in a 125-ml separatory funnel with 25 ml of ethyl acetate, and the combined extracts were

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dried over anhydrous calcium sulfate (5 g). The extract was then decanted into 100-ml round-bottomed flasks and flash evaporated to dryness at 50°C under a reduced atmosphere. The residue was rehydrated with 10 ml of 50% aqueous methanol and filtered through a 0.5-μm fluorepore filter (FHLP; Millipore).

High-pressure liquid chromatography was conducted with a model 6000A solvent delivery system, a model 440 UV detector (254 nm), a reverse-phase μ-Bondapak/C18 column (3.9 mm [inside diameter] by 30 cm) (all three from Waters Associates, Inc., Bedford, Mass.), and a Rheodyne 7125 injector with a 20-μl loop (Rheodyne Inc., Cotati, Calif.). The solvent system was methanol-water (50:50) at 1.0 ml/min.

A standard curve was prepared by averaging the actual units full scale produced from three consecutive injections of serially diluted patulin standard. The patulin concentrations in extracted filtrates were determined by averaging the actual units full scale of at least three consecutive injections per sample and extrapolating to the standard. The retention time for patulin in this system was 3 min.

RESULTS AND DISCUSSION

The effects of sodium propionate and potassium sorbate on the growth of and patulin production in P. expansum are shown in Fig. 1. In a complex medium (CYD), 0.1 M sodium propionate (0.96%) caused nearly a 48% reduction in growth but a 98% reduction in the synthesis of patulin. More dilute propionate concentrations, such as 10⁻² (0.096%) and 10⁻³ (0.0096%), resulted in less inhibition of growth but still resulted in a marked inhibitory effect on the production of patulin (Fig. 1).

The results obtained with 0.1 M potassium sorbate (1.5%) were similar to those obtained with sodium propionate (Fig. 1). This concentration of sorbate reduced growth by 83% and patulin synthesis by 98%. At a 10⁻² (0.15%) concentration of sorbate, there was a 50% reduction in mold growth; however, the inhibition of patulin synthesis was only 32%. At sorbate concentrations of 10⁻³ (0.015%) through 10⁻⁵ (0.00015%), there was little effect on either fungal growth or patulin production.

An upper limit of 0.32% sodium propionate is accepted for use in flours and white breads and rolls, although up to 0.38% may be used in whole wheat breads (7). Concentrations of 0.03 to 0.3% potassium sorbate may be used in bakery products, depending upon the specific product in question (5, 15).

Extrapolation of these values to our data indicates that the concentrations of propionate used commercially in bakery products would result in only a 31% reduction in growth but a 91% reduction in patulin biosynthesis. A sorbate concentration of 0.03% would result in a reduction in growth of 15% and a reduction in patulin biosynthesis of 30%. Sorbate at a concentration of 0.3% would reduce growth by 57% and patulin synthesis by 67%. Furthermore, these data suggest that sodium propionate at the concentrations used commercially would bring about a greater decrease in the production of patulin per unit weight of mycelium (0.21 μg/mg) than would potassium sorbate (1.22 μg/mg) (Table 1).

Our results indicate that sodium propionate at the concentrations used commercially inhibits growth less effectively than potassium sorbate and yet is more effective in preventing patulin synthesis. It should be noted, however, that effective levels of sodium propionate and potassium sorbate may differ significantly in foods as opposed to culture media.

The inhibition of mold growth and patulin synthesis in bread by these inhibitors and the possible concomitant synergistic effects are now under investigation.

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LITERATURE CITED

evaluation of potential mycotoxin-producing molds in corn
ing potential of molds isolated from flour and bread. Cereal Sci.
Today 18:346–347.
5. **Chichester, D. F., and F. W. Tanner, Jr.** 1972. Antimicrobial
food additives, p. 115–184. In T. E. Furia (ed.), Handbook of
food additives, 2nd ed. CRC Press, Cleveland.
P. M. Davidson (ed.), Antimicrobials in foods. Marcel Dekker,
8. **Fritz, W., and R. Engst.** 1981. Survey of selected mycotoxins in
10. **Lovett, J., R. G. Thompson, Jr., and B. K. Boutin.** 1975. Patulin
production in apples stored in a controlled atmosphere. J.
11. **Lueck, E.** 1980. Antimicrobial food additives; characteristics,
uses, effects. Springer-Verlag, Berlin.
of temperature, acidity, and light on the formation of aflatoxins
13. **Roland, J. O., L. R. Beuchat, R. E. Worthington, and H. L.
Hitchcock.** 1984. Effects of sorbate, benzoate, sulfur dioxide and
temperature on growth and patulin production by *Byssochlamys
14. **Scott, D. B.** 1964. Toxigenic fungi isolated from cereal and
A. L. Branen and P. M. Davidson (ed.), Antimicrobials in
17. **Stott, W. T., and L. B. Bullerman.** 1975. Patulin: a mycotoxin of
potential concern in foods. J. Milk Food Technol. 38:695–705.
18. **Tyllinen, H., M. Raevuori, E. Karppanen, and A.-S. Garry-And-
derson.** 1977. A study on the toxicity of spontaneously molded