Effect of Secondary Metabolites on the Organisms Producing Them: Effect of Nisin on *Streptococcus lactis* and Enterotoxin B on *Staphylococcus aureus*

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The effect of secondary metabolites added to cultures of the organisms producing them was investigated. Nisin was added to growing cultures of a nisin-producing strain of *Streptococcus lactis* (354/07) and enterotoxin B to strains of *Staphylococcus aureus* (S6 and 243) producing enterotoxin B. One quarter (12 $\mu\text{g/ml}$) of the amount of nisin formed by the culture of *S. lactis* inhibited lag-phase cells and lysed log-phase cells. The same amount of nisin added before inoculation or at a time when nisin synthesis had started (in late log phase), caused only transient delay in growth. Inhibition of growth of the two enterotoxin B-producing strains of *S. aureus* could not be demonstrated at any stage of their growth cycle with as much as 1 mg of enterotoxin B per ml of medium.

Nisin is known to be synthesized after the log phase of growth (5), and recently we obtained evidence that it may be synthesized in at least two stages (6). A nisin precursor is first made ribosomally, followed by enzymatic modifications necessary to obtain the active antibiotic from the precursor molecule. The nisin-generating enzyme(s) and the antibiotic were located on the surface of the producer cells (6, 12).

Surface synthesis of nisin might be necessary if the cells were sensitive to their own metabolic products. Also, if young cells were less able to exclude the toxic product from their internal environment than older cells, synthesis would have to be coordinated with the growth cycle. This could account for nisin synthesis occurring after the log phase of growth. We tested this hypothesis by adding nisin to cultures of the producer organism at different stages of the growth cycle and then followed growth turbidimetrically.

Nisin was prepared by the method of Bailey and Hurst (1), and we also used a highly purified preparation donated by R. H. Hall of Aplin and Barrett Ltd., Yeovil, England. Nisin was estimated by a turbidimetric bioassay (4).

We checked the purity of our preparations by a strongly dissociative disc-gel electrophoretic technique in 6 M urea (7). Our preparations were at least 95% pure; they gave a single major band with up to two additional faint bands detectable only at high concentration. The commercial and laboratory preparations had the same mobilities.

The effect of nisin on the producer organism (*Streptococcus lactis* strain 354/07) is shown in Fig. 1. In our fermentations, total nisin formed was usually 50 $\mu\text{g/ml}$ of medium. Figure 1 illustrates the effect of 12 $\mu\text{g/ml}$ added to 10 ml of medium inoculated with 0.5 ml of overnight culture. When nisin was added before the inoculum (tube 2), growth initiation was delayed, but the rate of growth and final optical density were the same as that of the control (tube 1). When nisin was added 30 to 105 min after inoculation, there was complete inhibition of growth (tube 3) or even lysis (tube 4). At 120 min, added nisin was no longer inhibitory (tube 5). Separate experiments showed that at this time nisin synthesis had begun. Its effect resembled that of nisin added before inoculation (tube 2). In similar experiments, nisin was added at 30-sec intervals after zero time. The sensitivity of the producer organism to nisin increased progressively between 0 and 4 min, the 4-min cells being inhibited to the same extent as the 30-min cells of Fig. 1, tube 2.

We thus established that young cells of *S. lactis* which do not synthesize nisin were sensitive to exogenous nisin (tubes 3 and 4). Separate experiments showed that such cells probably suffered membrane damage because they released 280-nm absorbing substances. Cells which had commenced to synthesize nisin did not respond to exogenously added nisin.

Secondary metabolites have "no obvious function in general metabolism" (2) and are produced

FIG. 1. Nisin-producing strain 354/07 of *Streptococcus lactis* grown overnight in LTB medium (5) was inoculated (5% v/v) into fresh LTB medium and grown without shaking at 30 C. Nisin was added to tubes 2 to 5 (12 µg/ml); tube 1 control, no nisin; tube 2, nisin added before inoculation; tube 3, after incubation for 30 min; tube 4, after incubation for 105 min; tube 5, after incubation for 120 min.

after the log phase of growth. We wondered whether our observations with nisin had a more general application, i.e., that other organisms not producing antibiotics were sensitive, at certain stages of growth, to their own metabolic products. We chose to work on the effect of enterotoxin B on *Staphylococcus aureus*, because there appear to be some similarities between enterotoxin B and nisin synthesis. Both are secondary metabolites synthesized from amino acids late in the growth cycle (8, 9). Enterotoxin B has been reported to be synthesized from a precursor (9); it may be associated with surface structures of the producer organism (3).

Enterotoxin B was prepared from the classical strain S6 by the method of Schantz et al. (10), and we also purchased commercial enterotoxin B from Makor Chemicals Ltd., Jerusalem, Israel. Enterotoxin B was estimated serologically by a single gel-diffusion assay (11), standardized with an antitoxin donated by M.S. Bergdoll, University of

Wisconsin, Madison. Disc-gel electrophoresis (7) showed that the laboratory and commercial preparations had the same mobilities and they were at least 95% pure enterotoxin B.

The medium used contained 3% (w/v) N.Z. Amine (Sheffield Chemical Co., Norwich, N.Y.), 1% (w/v) peptone (Evans, from British Drug Houses, Toronto, Ont.), and 0.001% (w/v) each of nicotinic acid and thiamine, pH 6.60. In this medium, enterotoxin B was produced up to 1 mg/ml. The effect of this terminal amount of enterotoxin B was tested on the growth of *S. aureus* (strain S6) by inoculating 10-ml amounts of medium with 0.1 and 0.01 ml of overnight cultures. Enterotoxin B was added before inoculation and at 30, 60, 120, 180, and 300 min after inoculation. Variables tested included the effect of glucose, effect of aeration, and growth from two initial pH values (pH 6 and 7). Inhibition was not detected in any of these tests. Similar results were obtained with strain 243.

Thus the antibiotic nisin inhibited the producer organism during stages of the growth cycle when nisin was not produced. However, enterotoxin B-synthesizing strains of *S. aureus* were insensitive to enterotoxin B at all stages of their growth cycle. Because the self-inhibitory effect was not demonstrated in the two cases examined, we do not have a general explanation for the surface synthesis of these compounds late in the growth cycle.

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