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Tyrocidin inhibition: effect of Tween 80 and conidial density

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Abstract

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Charlang, G.W. and N.H. Horowitz. Tyrocidine inhibition: effects of Tween 80 and conidial density.

Mach and Slayman (1966 Biochim. Biophys. Acta 124:351) have shown that the decapeptide antibiotic tyrocidine irreversibly damages the cell membrane of N. crassa conidia and mycelia, resulting in loss of K^+, 260nm absorbing and Lowry positive material. Under their experimental conditions, relatively small concentrations of tyrocidine (1 µg/ml) inhibit conidial germination. We have found that when these conditions are changed somewhat, quite different results are obtained.

N. crassa wild type 74A was grown on slants of Vogel's minimal medium N supplemented with 0.5% yeast extract and 0.25% casein hydrolysate. Four-day-old cultures were used as the source of conidia for all experiments. Conidia were harvested by suspension in distilled water, using a Vortex mixer, and filtered through 4 layers of cheesecloth to remove mycelial debris. They were then washed once and resuspended in 0.067M Sodium phosphate buffer (pH6.0), before being counted in a hemocytometer. Two types of experiments were done: (1) Growth experiments using the conditions of Mach and Slayman (20 ml minimal N in 125 ml Erlenmayer flasks, harvested after 3 days of stationary incubation at 25°C) with an inoculum of 10^5 conidia per ml of medium, and (2) experiments using shaken suspensions of up to 10^8 conidia per ml in 0.067 M Sodium phosphate buffer (pH 6.0) at 30°C.

When 28 mg of Tween 80 (2 drops of syrup added before autoclaving) are added to each flask under condition (1), good growth is observed, even at concentrations of 100 µg tyrocidine per ml of medium (see Table 1).

Table 1. Response (mg dry wt) of N. crassa 74A to different concentrations of tyrocidine in presence or absence of Tween 80.

Tyrocidine (µg/ml)	None	1	4	10	25	50	75	100
Minimal medium	73.5	n.g*	n.g.	n.g.				
Minimal medium with Tween 80	70.6	69.1	69.9	65.7	66.9	64.7	53.2	39.3

* no growth

When 10^8 conidia per ml are incubated without tyrocidine under condition (2), there is some leakage of 260nm absorbing and ninhydrin-positive material, but no GF (germination factor; see Charlang and Horowitz 1971 Proc. Nat. Acad. Sci. U.S.A. 68:260) is released into the medium, and germination does not occur even after 48 hours of incubation. There is no loss of viability. When tyrocidine is added at 20 µg per ml., there is an immediate and rapid loss of 260nm absorbing and ninhydrin-positive material, as well as GF into the medium. But after 1 hour of incubation this reaction is reversed, and the lost material begins to disappear from the medium. After 3 hours, germination begins. (The carbon source is either the tyrocidine or some contamination in the commercial product (Nutritional Biochemicals Corp.). We did not attempt to clean up the preparation prior to use.) At 6 hours, only a trace of GF, about 50% of the 260nm absorbing and 20% of the ninhydrin-positive material remain in the medium; at the same time germination is

essentially complete. After 24 fours of incubation, no further growth has occurred and there is no change in GF level, but the amounts of 260nm absorbing and ninhydrin-positive material have been further reduced.

When 10^7 conidia per ml are incubated under condition (2) with 20 µg tyrocidine per ml, there is no recovery. However, if the concentration of tyrocidine is reduced to 2 µg per ml, the reversal occurs, and germination is observed. When Tween 80 (1.4 mg per ml) is added to 10^8 conidia per ml incubating in buffer with 20 µg tyrocidine per ml, no detectable GF is released into the medium. The conidia germinate, but much more slowly than when Tween 80 is not present.

Tyrocidine is believed to bind to the cell membrane, although the exact nature of the binding is not known. The results of our experiments suggest that a certain minimal number of binding sites must be occupied for inhibition to occur. Mach and Slayman found that a concentration of 0.5 µg of tyrocidine per mg of cells was sufficient for complete inhibition of log-phase cultures. In our experiments a level of 2 µg tyrocidine per mg of conidia was not inhibitory. Conidia thus seem to be less sensitive to tyrocidine than are hyphal cells. The antagonistic effect of Tween 80 on tyrocidine action may be related to the fact that both substances are surfactants. Possibly they compete for the same binding sites on the membrane.

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