

THE DETOXIN COMPLEX,
SELECTIVE ANTAGONISTS OF
BLASTICIDIN S

Sir :

In the course of our investigations on the biological transformation of blasticidin S¹⁻²⁾, we found that a strain of streptomycetes produced a number of antagonists, which negated the inhibitory action of blasticidin S selectively against *Bacillus cereus*, but did not against *Piricularia oryzae*.

This interesting phenomena led us to study *in vivo* activity, involving the test for rice blast disease in the green house with a combined preparation of blasticidin S and the antagonists. It has been observed that the phytotoxicity to the rice plant was depressed markedly without a decrease in curative effect of the antibiotic. Moreover, the combined preparation showed less eye irritation, one of the troublesome defects of blasticidin S in practical use.

In view of such an interesting activity to bring about detoxification, both in animal and plant cells, the name detoxin complex is given to the mixture of antagonists.

It is noteworthy that detoxin complex is a selective antagonist with unique biological activities, discovered for the first time among natural products.

This communication deals with the production, extraction and some of the biological activities of detoxin complex.

The producing organism of detoxin complex was classified as a species of *Streptomyces caespitosus* and it was named *Streptomyces caespitosus* var. *detoxicus* 7072 GC₁. Furthermore, we found that the complex was produced by several strains of streptomycetes, notably, by *Streptomyces mobaraensis*⁵⁾.

Production of detoxin complex was carried

out in a jar fermentor at 27°C for 40 hours with aeration. The medium consisted of glucose (3%), peptone (1%), corn steep liquor (2%) and sodium chloride (0.5%) and was adjusted to pH 7.0 before sterilization. A 48-hour shake cultured seed of *Streptomyces caespitosus* var. *detoxicus* 7072 GC₁ was used as inoculum.

Detoxin complex was assayed by a paper disk method of measuring the growth zone of *Bacillus cereus* IAM 1729, which was inoculated into peptone agar containing 10 mcg/ml of blasticidin S in petri dish. After incubation of the agar plate for 16 hours at 37°C, a distinct growth zone of test organism developed around the paper disk. A satisfactory linear relation between the diameter of growth zone and logarithm of detoxin complex concentration was observed.

A sample of an arbitrary lot of relatively purified detoxin complex was assigned a potency of 1,000 units per mg, and was used as a standard for purification.

Preliminary purification of detoxin complex was carried out in reasonable yield by cation-exchange procedure followed by carbon chromatography. The filtered broth (100 liters) was absorbed on the column of Amberlite IRC-50 (H⁺ form, 5×50 cm), successively washed with water and eluted with 0.1N aqueous ammonia containing 10% methanol. The active fractions were combined and concentrated *in vacuo* to yield a colored solution (200 ml), which was absorbed on a column of carbon (3×30 cm, Shirasagi activated carbon). The column was washed thoroughly with water, and then eluted with 60% aqueous methanol containing 3% acetic acid (v/v).

Active fractions of the eluate were concentrated *in vacuo*, and the last traces of acetic acid was removed in alkaline desiccator

Table 1. Antimicrobial activity of blasticidin S and detoxin (Inhibition diameter, mm)

Test organism	Blasticidin S (1,000 mcg/ml)	Detoxin (250 u/ml)	Blasticidin S+Detoxin (1,000 mcg/ml+250 u/ml)
<i>Pseudomonas fluorescens</i>	20.5	0	20.5
<i>Mycobacterium phlei</i>	24.5	0	29.5
<i>Candida albicans</i>	20.0	0	0
<i>Bacillus cereus</i>	28.0	0	0
	(40 mcg/ml)	(10 u/ml)	(40 mcg/ml+10 u/ml)
<i>Piricularia oryzae</i>	27.0	0	30.0

Table 2. Curative and preventive effects of mixture of blasticidin S and detoxin complex

Concentration		Pot test in green house				Test on bed				
Blasticidin S (mcg/ml)	Detoxin (u/ml)	Curative test		Preventive test		Curative test		Phytotoxicity		
		spot/leaf	Effective- ness (%)	spot/leaf	Effective- ness (%)	spot/leaf	Effective- ness (%)	1 st	2 nd	3 rd
100	—	0.11	98.4	0.13	97.1	0	100	+++	+++	+++
50	—	0.17	97.5	0.47	89.7	0.17	97.7	+++	++	++
20	—	0.33	95.2	1.15	74.7	0.61	90.8	+	+	+
5	—	1.03	84.9	1.90	58.2	1.40	80.7	—	—	±
100	100	0.02	99.7	0.18	96.0	0	100	—	—	±
50	100	0.21	96.9	0.28	93.8	0.33	95.5	—	—	—
20	100	0.31	95.5	0.31	93.2	0.37	94.9	—	—	—
5	100	0.23	96.6	0.80	82.4	0.57	92.2	—	—	—
0	100	1.00	85.4	1.08	76.3	2.00	72.5	—	—	—
100	10	0.07	98.9	0.27	94.1	0.17	97.7	—	±	±
50	10	0.18	97.4	0.37	91.2	0.43	94.1	—	—	—
20	10	0.28	95.9	0.31	93.2	0.40	94.5	—	—	—
5	10	0.33	95.2	0.85	81.3	0.67	90.8	—	—	—
0	10	2.47	63.8	1.41	69.0	2.83	61.1	—	—	—
0	0	6.82	0	4.55	0	7.27	0	—	—	—

under reduced pressure until the substance formed a dry powder. The resulting pale yellow powder (20 g) showed about 200 units/mg, and the yield was 60 % overall. The crude powder (20 g) was triturated twice with methanol (100 ml) at 50°C and the insoluble mass was removed by filtration and discarded. The solution of methanol, containing over 90 % of the active substance, was concentrated *in vacuo* and dried in a desiccator to yield a light colored fine powder (12 g) with an activity of 500 units/mg.

The product at this stage is called detoxin complex.

It is of particular interest that the antagonistic actions of detoxin complex are quite selective toward the kind of microorganisms, and that detoxin complex itself does not exhibit antimicrobial activity nor toxicity so far as tested. As shown in Table 1, the inhibitory action of blasticidin S is completely negated with *Bacillus cereus* and *Candida albicans* but not with *Piricularia oryzae* and *Mycobacterium phlei*.

The curative and preventive effects of the combined preparation of blasticidin S and detoxin complex against rice blast disease were examined together with the phytotoxicity on rice plants. As shown in Table 2, the effectiveness of the antibiotic was not decreased by addition of detoxin, but the phytotoxicity was depressed markedly compared with that of the control.

Further purification and studies of the mode of action of detoxin are in progress.

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