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Production and Antibacterial Activity of Malformin C, a Toxic Metabolite of *Aspergillus niger*

B. KOBBE, M. CUSHMAN, G. N. WOGAN, AND A. L. DEMAIN*

Department of Nutrition and Food Science and Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139*

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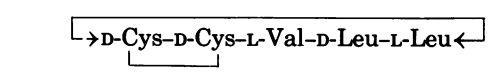
The production of the new mycotoxin malformin C by a solid substrate fermentation is described. Malformin C is highly toxic (mean lethal dose = 0.9 mg/kg) and exerts antibacterial activity against a variety of gram-positive and gram-negative organisms.

Aspergillus niger produces a group of cyclic pentapeptides called malformins, which cause malformations in bean plants and curvatures in corn roots (3, 8). Four malformins have been identified: malformins A_1 and A_2 (the mixture of which was formerly known as malformin A) were found in culture filtrates of *A. niger*, *A. ficuum*, *A. amawori*, and *A. phoenicis* (5); malformins B_1 and B_2 were produced only by *A. niger* (9). A new member of this group, malformin C, has recently been isolated from a strain of *A. niger* Van Tieghem, which originated from mold-damaged rice in Thailand (1). Its structure is as follows:



To produce sufficient amounts of crude malformin C for chemical purification, we screened a variety of grains by using the agitated solid substrate fermentation technique. Moist grains were inoculated with spores and incubated for 10 days on a rotary shaker at 30°C (except for a glutinous rice control, which was not shaken). The moldy grains were extracted with dichloromethane; the filtrate was evaporated, and the oily residue precipitated in petroleum ether to yield the crude toxin. Details of the fermentation and extraction procedure have been given elsewhere (4). All grains (300 g/flask) were supplemented with 150 ml of water and incubated for 12 days. They were compared for yield of crude toxin and degree of toxicity, the latter determined by administration to newborn rats (Table 1) (1). To ascertain the best fermentation process, we calculated the "potency index," which represents yield of crude toxin divided by the mean lethal dose (LD_{50}) value. The agitated grain technique represented a substantial improvement over the more conventional static rice method. White wheat gave the best results and was chosen as the medium for crude toxin production. The results of a series of such fermentations are summarized in Table 2. About 15% of the weight of the crude toxin represented malformin C. From 2.5 g of crude toxin, 369 mg of malformin C was isolated. The LD_{50} of this compound was 0.9 mg of malformin C per kg of body weight (1). Thus, by testing a variety of agitated grains for fermentation, we were able to improve our crude toxin yield considerably. The control (static glutinous rice) fermentation resulted in a potency index of 30, whereas the agitated wheat fermentation yielded a value of 360. The yield of purified malformin C (369 mg/kg) may be compared with figures reported for other malformins produced in liquid media. For example, 15 mg of malformin per liter was isolated from cultures grown in cornsteep liquor-glucose (8). In another report, a defined medium yielded 30 to 40 mg of malform C per liter, whereas 200 mg/liter was obtained from the cornsteep liquor-glucose medium (6).

The antimicrobial activity of malformin C against a number of gram-positive and gram-negative bacteria was determined as follows. Samples of 1 and 5 mg of malformin C were dissolved in 0.2 ml of glacial acetic acid and diluted with 0.8 ml of distilled water. Solutions of malformin A were prepared in the same way for comparative purposes. Paper disks (6.35 mm) were dipped into the solutions, and the acetic acid was allowed to evaporate. A disk with acetic acid alone served as a control. When dry, the disks were placed on agar plates seeded with the respective organisms. The plates were kept at 4°C for about 12 h to facilitate diffusion of the relatively insoluble malformins. After incubation for 20 to 24 h at 37°C, inhibitory zones were measured. The antibacterial activity of malformin C seemed comparable to that of malformin A. This observation agrees with earlier comparisons based on root curvature

TABLE 1. *Screening of grains for malformin C production by Aspergillus niger*

Grain	Crude toxin (mg/kg of grain)	LD_{50} (mg/kg)	Potency index[a]
Glutinous rice[b] (control)	150	5	30
Barley	300	Not tested	
Whole oats	360	5	72
Red wheat	950	5	190
White corn	1,070	5	214
White wheat	1,800	5	360

[a] Potency index = Crude toxin/LD_{50}.

[b] Unshaken; all other grains were incubated on the rotary shaker.

TABLE 2. *Malformin C production by Aspergillus niger on white wheat*

Fermentation no. designation	Crude toxin (mg/kg of grain)	LD_{50} (mg/kg)	Potency index
A	1,800	5	360
B	1,700	15	113
C	2,400	7	340
D	2,300	7	330
E	1,440	3	480
F	3,000	5	600

and bean sprout malformin assays (1). Disks dipped in solutions containing 1 mg of malformin A or C per ml inhibited only *Bacillus subtilis* and *B. megaterium*. At a fivefold higher concentration, the malformins also inhibited *Staphylococcus aureus*, *Streptococcus faecalis*, *Proteus mirabilis*, and *Sarcina lutea*. *B. megaterium*, which is frequently used as an assay organism for mycotoxins (2), was the most sensitive culture tested. Our results agree with the reported antibiotic activities of malformin A, as determined by growth inhibition of test bacteria in liquid culture (7).

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