APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Oct. 1994, p. 3894–3896
0099-2240/94/$04.00+0

Vol. 60, No. 10

# Production of Beauvericin by a Strain of *Fusarium proliferatum* Isolated from Corn Fodder for Swine

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Received 11 March 1994/Accepted 26 July 1994

**Beauvericin, a cyclodepsipeptide, was produced by cultures of three strains of *Fusarium proliferatum*, M-5991, M-6992, and M-6993, grown on cracked corn. M-5991 produced approximately 1,000-mg/kg levels of fumonisins, moniliformin, and beauvericin.**

Toxic cultures of *Fusarium moniliforme* Sheldon and closely related species in the section *Liseola* have been known about for some time (11). One of the related species, *Fusarium proliferatum* (Matsushima) Nirenberg, is frequently found on toxic samples of corn and other grains (13). Although members of the section *Liseola* have never been reported to produce trichothecenes, which are produced by many species of *Fusarium*, this species is known to produce other toxic secondary metabolites, including moniliformin, fumonisins, and fusarins (11). Strain M-5991 was isolated by Ross et al. from corn associated with a case of pulmonary edema in swine in 1990 (12). Cultures of this strain and culture extracts from them have been examined for toxicity to horses, swine, and poultry by a number of workers (7, 11, 13), and the strain has been used for purification of large amounts of fumonisins for toxicity testing. In addition to high levels of fumonisin $B_1$ (>1,000 mg/kg) and fumonisin $B_2$ and $B_3$ (>100 mg/kg each), this isolate also is known to produce high levels of moniliformin (7).

Beauvericin, a depsipeptide (Fig. 1), was first isolated from *Beauveria bassiana* (6) on the basis of assays of toxicity to brine shrimp. It also has insecticidal properties (4). Beauvericin is a cyclic lactone trimer of the amide of *N*-methyl L-phenylalanine and D-α-hydroxyisovaleric acid, and it is structurally similar to the enniatins, which are produced by a number of *Fusarium* species (2). In the enniatins, the aromatic amino acid has been replaced with combinations of *N*-methyl derivatives of the aliphatic amino acids isoleucine, valine, and leucine. In 1991 Gupta and coworkers reported the production of beauvericin by two species of *Fusarium*, *Fusarium semitectum* Berk. et Rav. and *Fusarium moniliforme* var. *subglutinans* = *Fusarium subglutinans* (Wollenw. et Reinking) Nelson, Toussoun, et Marasas (5). Recently Logrieco et al. reported the production of beauvericin by isolates of *Fusarium subglutinans* from Peruvian maize at levels of up to 250 mg/kg (9) and the natural occurrence of beauvericin in preharvest *Fusarium subglutinans*-infected maize from Poland (10).

Two isolates of *Fusarium moniliforme* (M-3125 and M-5500), three isolates of *Fusarium proliferatum* (M-5991, M-6992, and M-6993), and an isolate from rice, identified as *Fusarium moniliforme* (M-1151) by the person who submitted it to the Fusarium Research Center, were grown on cracked corn as reported previously (8). Cultures were extracted with ethyl acetate (5 ml/g of culture material) and acetonitrile-water (1:1; 5 ml/g of culture material).

Extracts were analyzed for the presence of fumonisins by mass spectrometry (MS) and high-pressure liquid chromatography (HPLC) as reported previously (8). Moniliformin was measured by thin-layer chromatography (1) and by HPLC on a C18 column (acetonitrile-water [10:90]) with detection by UV at 230 nm and confirmation by the full UV spectrum with a diode array detector. The limit of detection by this method was 50 mg/kg. Beauvericin levels were determined by HPLC on a C18 column (methanol-water [67:33]) with detection at 204 nm. The detection limit for beauvericin by this method was 50 mg/kg. Positive samples were confirmed by MS and tandem MS. For structure confirmation, beauvericin was isolated from both the acetonitrile-water and ethyl acetate extracts of cultures of M-5991 and M-6992. An equal volume of ethyl acetate was added to 20 ml of the acetonitrile-water extract, and the upper layer was evaporated to dryness. The residue from 20 ml of ethyl acetate or the ethyl acetate partition of the acetonitrile-water extract was redissolved in 25 ml of hexane-ethyl acetate (5:1) and applied to a 10-g silica Sep-pack cartridge. This cartridge was then eluted with 100 ml of hexane-ethyl acetate (5:1) followed by 100 ml of hexane-ethyl acetate (1:1) and 100 ml of ethyl acetate. Fifty-milliliter fractions were collected, and aliquots were analyzed by thin-layer chromatography (hexane-ethyl acetate [5:1] or chloroform-methanol [95:5]) and MS. Beauvericin was visualized on developed plates by exposure to iodine vapors (2). Combined fractions containing beauvericin as determined by thin-layer chromatography were rechromatographed on silica, and the resulting beauvericin was analyzed by nuclear magnetic resonance spectrometry and MS. Significant amounts of beauvericin were found in the ethyl acetate extracts but more was extracted with acetonitrile-water from the residues of ethyl acetate extracts. For quantitative analysis the cultures were not extracted with ethyl acetate prior to the acetonitrile-water extraction, since fumonisins and moniliformin are also efficiently extracted in acetonitrile-water.

Kaurene, a diterpene that is a precursor to the gibberellins, was measured by gas chromatography (GC)-MS of the ethyl acetate extract of the cultures. Kaurene was identified by its mass spectrum. It was detected at significant levels (>100 mg/kg) only in the extract of M-1151. The ethyl acetate extract (20-ml, 4-g equivalents of culture) of M-1151 was evaporated to dryness and redissolved in hexane. This solution was chromatographed on a 500-mg silica Sep-pak cartridge, and fractions were monitored by GC-MS. Kaurene eluted in the first 2 column volumes. Approximately 500 μg of kaurene was col-

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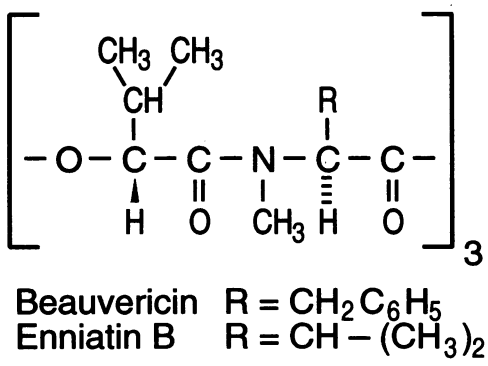


FIG. 1. Chemical structures of beauvericin and enniatin B.

lected and analyzed by nuclear magnetic resonance. The spectrum obtained was in close agreement with previously reported values for this terpenoid, with the most prominent signals being signals for three methyl groups at 1.00, 0.84, and 0.79 ppm, and the vinyl protons were observed as signals at 4.78 and 4.70 ppm. No kaurene was detected by GC-MS of the ethyl acetate extracts of the other five cultures. Additional substituted kaurenes were also observed in the mass spectra from the M-1151 extract, but these were not identified further. Kaurenes are gibberellin precursors. *Fusarium moniliforme* isolates are not generally gibberellin producers. The presence of kaurene indicates that this species probably has the ability to make gibberellins. Gibberellin-producing isolates are usually identified as *Fusarium subglutinans* or *Fusarium fujikouri*.

Because distinguishing the species within the section *Liseola* on the basis of macrocondidial morphology is difficult and differences of opinion as to species identifications occur even among experts, the use of the sexual stage to distinguish species (8) has been suggested. Strains that are fertile when crossed with testers from populations A and F are generally identified as *Fusarium moniliforme* on the basis of traditional morphology. Strains that are fertile when crossed with testers from populations B and E are generally identified as *Fusarium subglutinans*, while those that are fertile when crossed with testers from population D are identified as *Fusarium proliferatum*. Further work with strain M-1151, to identify which of the seven known mating populations it belongs to (8), is under way in this laboratory.

Fumonisin $B_1$ was detected at high levels (>1,000 mg/kg) in four cultures (Table 1). Moniliformin was detected in M-1151 and in M-5991 at high levels (Table 1). Beauvericin was detected in cultures of the three *Fusarium proliferatum* isolates and in that of M-1151 but not in the cultures of the other *Fusarium moniliforme* isolates. Moniliformin is not generally observed in cultures of *Fusarium moniliforme* mating population A, but it is frequently found in those of mating populations D, E, F, and G (11a). The two strains from mating population A (M-3125 and M-550) did not produce any detectable beauvericin or moniliformin. Previous work in this laboratory with members of this population has failed to reveal any isolates that produced detectable levels of moniliformin (data not shown). Extracts from four additional strains that were members of mating population A (M-3120, M-5538, KSU-1127, and KSU-1324) also had no detectable levels of beauvericin, nor did extracts from 12 additional isolates that were found to be members of mating population F (data not shown). Of 13 strains of *Fusarium proliferatum* that had been isolated from corn, 6 had no detectable level of beauvericin, while the other 7 had levels ranging from 54 to 110 mg/kg. Seven of 14 strains of *Fusarium proliferatum* isolated from rice produced between 100 and 600 mg/kg, while the remaining 7 did not produce detectable levels of beauvericin.

Beauvericin has been shown to act as an ionophore (5), but its avian and mammalian toxicities have not been reported. Its presence at significant levels in cultures of *Fusarium subglutinans* and *Fusarium proliferatum* suggests the potential for its presence in naturally contaminated grain samples associated with animal toxicoses. Porcine pulmonary edema has been experimentally reproduced by injection of swine with purified fumonisin $B_1$ (3) and by feeding of swine with culture materials, but it has been difficult to reproduce. The role of beauvericin, if any, in this animal toxicosis remains unknown. The presence of high levels of beauvericin in the cultures of M-5991, which has been widely used in toxicity studies, suggests that the toxicity of beauvericin to animals should be investigated further.

TABLE 1. Levels of fumonisin, moniliformin, and beauvericin in various culture extracts

| Strain | Amt of the following substance detected in culture extract (mg/kg): | | | Mating population |
|---|---|---|---|---|
| | Fumonisin $B_1$ | Moniliformin | Beauvericin | |
| M-3125 (*F. moniliforme*) | 4,250 | ND[a] | ND[b] | A |
| M-5500 (*F. moniliforme*) | ND[c] | ND[a] | ND[b] | A |
| M-1151 (*F. moniliforme*) | ND[c] | 8,000 | 300 | C |
| M-5991 (*F. proliferatum*) | 1,300 | ~1,000 | 1,100 | D |
| M-6992 (*F. proliferatum*) | 1,506 | ND[a] | 400 | D |
| M-6993 (*F. proliferatum*) | 1,990 | ND[a] | 300 | D |

[a] ND, not determined. Amount present was <50 mg/kg.
[b] Amount present was <10 mg/kg.
[c] Amount present was <5 mg/kg.

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