Sambutoxin-Producing Isolates of *Fusarium* Species and Occurrence of Sambutoxin in Rotten Potato Tubers

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A total of 50 *Fusarium* isolates representing 13 species from various sources were surveyed to determine their potential to produce sambutoxin. Sambutoxin production was restricted to *Fusarium sambucinum* and *F. oxysporum*, with the exception of one isolate of *F. semitectum*. Sambutoxin was produced by high percentages of *F. sambucinum* (80.0%) and *F. oxysporum* (84.6%) isolates at levels of 1.1 to 101.0 µg/g. In addition, 9 (42.9%) of 21 rotten potato samples were contaminated with sambutoxin at levels of 15.8 to 78.1 ng/g.

Sambutoxin (Fig. 1) was recently isolated from a wheat culture of *Fusarium sambucinum* PZF-4 which was obtained from a rotten potato tuber (5). The toxin was elucidated as 3-{5-methyl-6[(*E*)-1,3,5-trimethylhept-1-enyl]tetrahydropyranyl}-5-(*p*-hydroxyphenyl)-1-methyl-4-hydroxy-2(1*H*)-pyridone (6). It had toxic effects on rats, including body weight loss, feed refusal, hemorrhage in the stomach and intestines, and finally death, when the rats were fed diets supplemented with 0.05 and 0.1% sambutoxin (5). The toxin was also toxic to cultured human tumor cell lines Molt-4, CCRF-CEM, and KATO III (unpublished data). Most of the toxic isolates of *F. oxysporum* and *F. sambucinum* obtained from rotten potatoes produced sambutoxin (5). This fact suggests that some *Fusarium* species frequently produce sambutoxin and that the toxin occurs naturally in agricultural products infected with *Fusarium* species, including rotten potatoes. Therefore, we surveyed the occurrence of sambutoxin-producing isolates of *Fusarium* species from various sources and sambutoxin in rotten potatoes in Korea.

A total of 50 *Fusarium* isolates representing 13 species isolated from various sources were used. The isolates of *Fusarium* species were classified in accordance with the method of Nelson et al. (8). Erlenmeyer flasks (500 ml), each containing 100 g of wheat grains and 60 ml of distilled water, were autoclaved for 1 h at 121°C twice with a 24-h interval. The wheat media were inoculated with mycelium plugs from 5-day-old potato dextrose agar plates of the fungi. The flasks were incubated for 4 weeks at 25°C. The fungus-infested wheat was spread on a screen-bottom tray, allowed to air dry in a fume hood, and ground to a powder. A 10-g portion of each culture was moistened with 3 ml of distilled water and extracted with 100 ml of ethyl acetate for 30 min in a wrist action shaker. After filtration through Whatman no. 2 filter paper, an aliquot of the filtrate (50 ml) was concentrated to dryness. The residue was redissolved in 2 ml of chloroform and applied to a Florisil column (2 cm [inside diameter] by 20 cm). The column was packed with 10 g of Florisil (60/100 mesh) in *n*-hexane and topped with 5 g of anhydrous sodium sulfate. After being washed with 100 ml of *n*-hexane, the column was eluted with 100 ml of ethyl acetate-*n*-hexane (2:1, vol/vol). The eluate was concentrated to

dryness and redissolved in 10 ml of methanol. The extract was analyzed by high-performance liquid chromatography (HPLC). For the HPLC analysis, the following equipment and conditions were used: instrument, Shimadzu SCL-10A; column, Zorbax C₁₈ (4.6 mm [inside diameter] by 150 mm; particle size, 5 µm; Dupont Co., Kyoto, Japan); mobile phase, CH₃OH-H₂O (82:18, vol/vol); flow rate, 1 ml/min; UV light detection wavelength, 254 nm. The retention time of sambutoxin was 15.6 min. The detection limit was 100 ng/g. When control wheat samples were spiked with 500 ng/g of each sample, mean recovery was 91.0% ± 1.4%. To confirm the presence of sambutoxin in wheat cultures, the extract of each of the three *Fusarium* species which were positive in the HPLC analysis was chosen. The total residue of the extract was chromatographed on an analytical thin-layer chromatography plate (Kieselgel 60, 10 by 10 cm, 0.25-mm thickness; E. Merck, Darmstadt, Germany) with a sambutoxin standard and developed in ethyl acetate-*n*-hexane (3:1, vol/vol). The region corresponding to sambutoxin was scraped off and eluted with 0.5 ml of methanol, and the eluate was evaporated to dryness. The residue was analyzed with a JEOL AX 505 mass spectrometer equipped with an MSMP-DAP-2 data system. The analytical conditions used were as follows: ion source temperature, 270°C; ionizing voltage, 70 eV; ionizing current, 300 µA; scanning rate, 3 s per scan.

To survey the natural occurrence of sambutoxin in rotten potato tubers, a total of 21 samples (at least three tubers each) were collected from farmers' stocks in Korea during January 1995. The potatoes had been harvested in July 1994 and stored for 7 months. Fifty grams of rotten potato tissue was obtained from each potato sample and extracted with 200 ml of methanol for 1 min in a laboratory blender. The extract was filtered, and 100 ml was concentrated to dryness. The residue was dissolved in 2 ml of methanol and applied to a Florisil column (2 cm [inside diameter] by 20 cm). After being washed with 100 ml of *n*-hexane, the column was eluted with 100 ml of ethyl acetate-*n*-hexane (2:1, vol/vol). The eluate was concentrated to dryness and redissolved in 5 ml of methanol. The extract was analyzed by HPLC as described above. The detection limit was 10 ng/g. When control potato samples were spiked with 200 ng per g of each sample, mean recovery was 92.5% ± 2.5%.

Of 50 isolates, 15 (30%) produced sambutoxin (Table 1). Sambutoxin levels ranged from 1.1 to 101.0 µg/g. None of the 50 isolates produced more than the amount (204 µg/g) pro-

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FIG. 1. Chemical structure of sambutoxin.

duced by *F. sambucinum* PZF-4, the strain from which sambutoxin was first isolated in our laboratory. Sambutoxin production was restricted to *F. sambucinum* and *F. oxysporum*, with the exception of one isolate of *F. semitectum*; sambutoxin was produced by 4 (80.0%) of 5 *F. sambucinum* isolates, 10 (83.3%) of 12 *F. oxysporum* isolates, and 1 (20%) of 5 *F. semitectum* isolates. None of eight *F. graminearum* isolates, one *F. reticulatum* isolate, one *F. equiseti* isolate, one *F. acuminatum* isolate, three *F. culmorum* isolates, two *F. chlamydosporum* isolates, two *F. sporotrichioides* isolates, four *F. poae* isolates, three *F. moniliforme* isolates, and three *F. solani* isolates tested produced sambutoxin. To unequivocally verify the presence of sambutoxin, the extract of each of the three *Fusarium* species which were positive for sambutoxin in the HPLC analysis was chosen and prepared as described above. The mass spectrum of sambutoxin from the culture extract gave the diagnostic ions at m/z s of 396, 354, 272, and 243 and the molecular ion at an m/z of 453.

TABLE 1. Sambutoxin production by *Fusarium* species

Organism	Isolate	Source	Content ($\mu\text{g/g}$)
<i>F. sambucinum</i>	RPF1	Potato	6.2
	RPF2	Potato	7.8
	RPF5	Potato	101.0
	MK02	Soil	82.9
<i>F. oxysporum</i>	PJP2	Ginseng	8.2
	ASP1	Asparagus	12.0
	MK01	Soil	19.4
	TSF20	Radish	1.1
	FF401	Strawberry	28.3
	FS410	Sesame seed	41.9
	TSF110	Watermelon	7.5
	TSF182	Tomato	38.8
	RPF11	Potato	10.0
	RPF12	Potato	47.6
<i>F. semitectum</i>	SCYM6	Corn	6.8

A survey of the natural occurrence of sambutoxin in rotten potato samples showed that 9 (42.9%) of 21 samples were contaminated with sambutoxin at levels of 15.8 to 78.1 ng/g and the mean of the positive samples was 49.2 ng/g. Because low concentrations of sambutoxin were contaminated in rotten potato samples, it was impossible to obtain the full-scan mass spectrum of the toxin. Therefore, the identity of sambutoxin for each positive potato sample was confirmed by the full UV light spectrum with a photodiode array detector. The UV light spectrum of sambutoxin from the sample extract showed maxima at 213, 233, and 254 nm.

The results indicate that sambutoxin is produced by high percentages of *F. sambucinum* and *F. oxysporum* isolates and that sambutoxin-producing isolates of the two species may be widespread. *F. sambucinum* and *F. oxysporum* are cosmopolitan soil organisms with a wide host range (3, 4). Many isolates of the two species from various sources have been reported to cause toxic effects in experimental animals. The toxic effects in some cases were due to a known mycotoxin(s) and sometimes were due to an uncharacterized or unknown mycotoxin(s) (1, 2, 5, 7, 9, 10). Sambutoxin should be considered as a possible source of toxic effects on experimental and farm animals, although its toxicity is relatively low.

This is the first report of the natural occurrence of sambutoxin in rotten potato tubers. Additional surveys of the natural occurrence of sambutoxin and further toxicological examinations of sambutoxin are expected to provide valuable information for sambutoxin risk assessment of agricultural products.

This work was supported in part by a grant from the Research Center for New Biomaterials in Agriculture, Seoul National University.

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