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Survey for Fusaria That Elaborate T-2 Toxin[1]

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Of the 136 strains of *Fusarium* examined, T-2 toxin was confirmed by thin-layer chromatography in 13 of the 21 extracts that inhibited either *Rhodotorula rubra* or *Pencillium digitatum*.

During the past decade of intensive mycotoxin research, numerous molds have been isolated from feedstuffs causing suspected toxicoses in farm animals. Strains of the genus *Fusarium* are frequently isolated from these feeds, and several identified mycotoxins are produced by certain strains of some species (5). One of the more potent fusariotoxins is T-2 toxin (4β,15-diacetoxy-8α-[3-methylbutyryloxy]-12, 13-epoxytrichothec-9-en-3α-ol), a metabolite first isolated from cultures of *Fusarium tricinctum*, strain T-2 (2) and later detected in other *Fusarium* isolates (8). The oral LD_{50} of the toxin is 4.0 mg/kg of body weight in rats (1) and 6.1 mg/kg in rainbow trout (6). Because fusaria are prevalent on grains and forages and because some of them produce potent mycotoxins, a survey of selected strains from the Agricultural Research Service (ARS) culture collection was made to determine whether T-2 toxin was a metabolite common to certain species within the genus and to detect strains that produce this toxin. Fusaria strains selected for study in this survey were collected over a period of 30 years and only strain NRRL 3299 (strain T-2 from E. B. Smalley, Univ. of Wisconsin) was known to produce T-2 toxin prior to deposit in the ARS culture collection. The strains were isolated from many products, but the principal sources were fescue hay (39 strains), corn (22 strains), and wheat and flour (12 strains).

Ten grams of white corn grits (WCG) was sterilized in petri dishes with 15 ml of 1.5% agar. The medium was inoculated with each of 136 strains of *Fusarium* and incubated at 15 C for 21 days. After incubation, the molds were killed by steaming for 30 min. A block of substrate about 1 cm^2 was placed on the surfaces of yeast extract-malt extract agar seeded with each of the T-2 toxin-sensitive fungi, *Rhodotorula rubra* NRRL Y-7222 or *Penicillium digitatum* NRRL 1202 (4). The 21 strains that inhibited at least one of the indicator fungi (Table 1) were grown on 50 g of moistened WCG incubated at 15 C for 21 days, conditions known to be favorable for toxin production (3). Molded WCG was extracted with 200 ml of chloroform-acetone (1:1), filtered through paper toweling, evaporated to remove the solvent, and taken up in 20 ml of acetone. The extracts were tested for T-2 toxin by the antibiotic disc method (4) and compared to a T-2 standard by thin-layer chromatographic (TLC) procedures (7). Standard T-2 toxin was prepared by the method of Burmeister (3), and its purity was confirmed by a melting point determination (150-152 C), and by nuclear magnetic resonance spectroscopy.

Twenty-one of the 136 strains inhibited at least one of the microbiological indicators, and the presence of T-2 toxin in extracts from 13 of these was confirmed by TLC. The toxin-producing strains belonged to four species, *F. tricinctum*, *F. lateritium*, *F. equiseti*, and *Gibberella zeae* (Table 1). Thirty-seven strains of the four named species, 51 strains representing 20 other species of *Fusarium* and *Gibberella* (these 51 strains retained the name given them before receipt by the ARS culture collection maintained at the Northern Laboratory), and 40 unidentified strains produced no T-2 toxin. In addition to the T-2 toxin-positive strains confirmed by TLC, five extracts were active against *P. digitatum* only, and three were weakly active against *R. rubra* but active against *P. digitatum*. Each strain that inhibited the growth of *R. rubra* inhibited also the growth of *P. digitatum*. The lack of TLC evidence for T-2 toxin in eight extracts suggests that the microbiological test is more sensitive than the TLC method for detecting toxin in crude extracts of some strains. Alternatively,

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TABLE 1. *Detection of T-2 toxin-producing Fusaria by microbiological and thin-layer chromatography (TLC) methods*

Fusarium strain	Source	Inhibition with indicator microorganism		TLC
		Rhodotorula rubra	*Penicillium digitatum*	
F. tricinctum NRRL 3299 (22)[a]	Corn	31[b]	46[b]	+++[c]
F. tricinctum NRRL 3510	Proso millet	31	41	+++
F. tricinctum no. 1	Fescue hay	32	35	++
F. tricinctum no. 2	Fescue hay	15	26	++
F. tricinctum no. 3	Fescue hay	15	22	++
F. tricinctum no. 4	Fescue hay	15	32	None
F. tricinctum no. 5	Fescue hay	20	29	None
F. tricinctum NRRL 3511	Proso millet	15	26	None
F. tricinctum NRRL 3287	Unknown	0	24	None
F. equiseti no. 1 (12)[a]	Fescue hay	25	43	++
F. equiseti no. 2	Fescue hay	32	45	++
F. equiseti no. 3	Fescue hay	30	43	++
F. equiseti no. 4	Fescue hay	20	35	++
F. equiseti no. 5	Fescue hay	22	33	+
F. equiseti no. 6	Fescue hay	0	20	None
F. lateritium no. 1 (10)[a]	Fescue hay	31	46	++
F. lateritium no. 2	Fescue hay	27	37	++
F. lateritium no. 3	Fescue hay	0	33	None
Gibberella zeae (6)[a]	Corn	28	40	++
Fusarium sp. no. 1 (40)[a]	Fescue hay	0	30	None
Fusarium sp. no. 2	Wheat flour	0	24	None
	T-2 toxin, 1 mg[d]	45	50	

[a] Total number of strains of this species in the survey.
[b] Diameter of inhibition zone in millimeters.
[c] Relative intensity of fluorescence after spraying with anisaldehyde (7).
[d] Crystalline product.

fusaria active against *P. digitatum* may be producing a second antibiotic not active against *R. rubra*.

Our data suggest that *Fusarium* strains producing T-2 toxin more likely belong to *F. tricinctum*, *F. equiseti*, *F. lateritium*, and *Gibberella zeae* than to other species. Fifteen of the 21 strains producing products active against the indicator fungi were isolated from fescue hay (*Festuca arundinaceae* Schreb.) although only 39 strains were from this source. In addition to the strains from fescue, 36 isolates were from grain or grain products, and the remainder came from a variety of products.

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