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Application of an Enzyme-Linked Immunosorbent Assay for Screening of T-2 Toxin–Producing *Fusarium* spp.

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Culture filtrates of *Fusarium* species were subjected without clean-up procedures to an indirect competitive enzyme-linked immunosorbent assay with anti-T-2 toxin monoclonal antibody. *Fusarium sporotrichioides*, *F. poae*, *F. tricinctum*, and *F. culmorum* strains were positive for T-2 toxin, with a minimum detection limit of 5 pg per assay (100 pg/ml of culture filtrate), and the assay data correlated well with the gas-liquid chromatographic data.

For more than 10 years, several chemical and biological assay methods have been proposed for and applied in the detection and quantification of T-2 toxin (T-2) and related trichothecenes in foodstuffs and biological fluids (8, 10). Gas-liquid chromatography (GLC) and combined gas chromatography-mass spectrometry are widely used, but these methods require numerous clean-up steps before analysis. After the introduction of immunochemical methods for the detection of environmental toxicants, several enzyme-linked immunosorbent assays (ELISAs) for T-2 were proposed with polyclonal antibodies (1, 7) and monoclonal antibodies (MAbs) (2). We recently developed an ELISA with an anti-T-2 MAb having a high specificity and sensitivity for T-2, as reported in a preliminary form (6). In this study, we applied the ELISA to screening of T-2-producing *Fusarium* species.

T-2 was isolated from cultures of *Fusarium sporotrichioides* M-1-1. Anti-T-2-MAb 7D4 was prepared as described previously (6). Microtiter plates (Immuno Plate-II; Nunc, Roskilde, Denmark) and *p*-nitrophenyl phosphate were purchased from Inter Med, Roskilde, Denmark, and Wako Pure Chemicals, Osaka, Japan, respectively. Alkaline phosphatase (specific activity, 2,500 U/mg), sheep anti-mouse immunoglobulin G, and bovine serum albumin were purchased from Boehringer GmbH, Mannheim, Federal Republic of Germany, Organon Teknika, Malvern, Pa., and Nakarai Chemicals, Tokyo, Japan, respectively.

A total of 40 stock isolates from our laboratory, 57 isolates from Norwegian barley and wheat, and 6 isolates from Polish cereals were transferred to test tubes (1.5 by 15 cm) each containing 10 ml of peptone-supplemented Czapek medium (30 g of sucrose, 10 g of peptone, 3 g of $NaNO_3$, 1 g of K_2HPO_4, 0.5 g of $MgSO_4$, and 0.01 g of $FeSO_4$ in 1 liter of deionized water) (9). The test tubes were arranged on a stand at an angle of 30°; after 1 week at 25°C, 1-ml samples of the culture media were filtered through filter paper (no. 2; Toyo Roshi Co., Tokyo, Japan).

For the indirect ELISA, the method of Morgan et al. (4) was used with some modifications. To coat the solid phase with T-2, we added T-2–hemiglutarate–bovine serum albumin (1 μg/ml) dissolved in coating buffer (carbonate-bicarbonate buffer [pH 9.6], 0.02% NaN_3) to each well of a 96-well microtiter plate and incubated the mixture at 37°C for 2 h. After the wells were washed with phosphate-buffered saline–Tween (0.05 M sodium phosphate buffer [pH 7.4], 0.8% NaCl, 0.05% Tween 20) three times, 100 μl of 0.5% bovine serum albumin in phosphate-buffered saline was added to each well and incubated at room temperature for 1 h. A sample solution was prepared by the addition of ethanol to the culture filtrate (final concentration, 10%).

For the GLC analysis of T-2, 1-ml samples of the culture filtrates were twice extracted with 1 ml of ethyl acetate, and the contents of T-2 in the extracts were estimated by GLC as trimethylsilyl ether derivatives (5).

The standard curve for T-2 in our ELISA is shown in Fig. 1. The minimum detection limit was estimated to be 5 pg per assay (100 pg/ml of culture filtrate). Among 40 stock cultures in our laboratory, 6 isolates, including *F. tricinctum* (R2010 and R2016), *F. sporotrichiellas* var. *sporotrichioides* (R2131), and *F. sporotrichioides* (M-1-1), were positive in

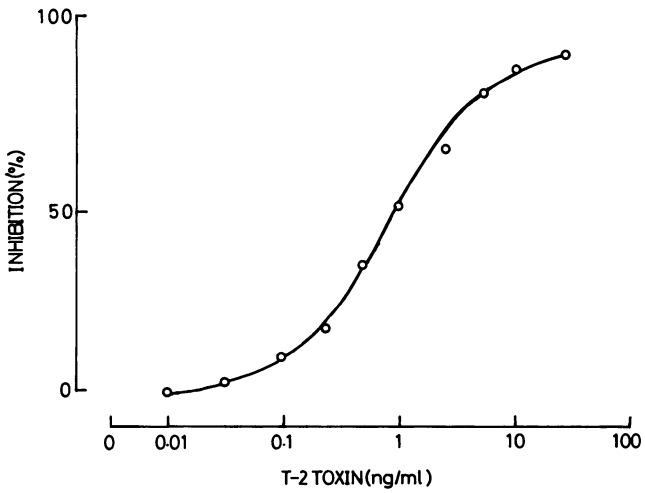

FIG. 1. Standard curve for T-2.

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TABLE 1. Estimation of T-2 in culture filtrates of *Fusarium* spp. by ELISA and GLC

Source of strains	Strains	T-2 toxin (μg/ml)[a] determined in:	
		ELISA	GLC[b]
Our laboratory	*F. tricinctum* R2010	0.41	0.49
	F. poae R2014	0.32	0.32
	F. tricinctum R2016	0.14	0.27
	F. sporotrichioides R2054	27.0	10.9
	F. sporotrichiellas var. *sporotrichioides* R2131	23.0	9.4
	F. sporotrichioides M-1-1[c]	41.0	37.7
Polish cereals	*F. sporotrichioides* KF196	81.4	85.0
	F. sambucinum KF701	0.55×10^{-3}	
	F. culmorum KF601	0.33×10^{-3}	
	F. sporotrichioides KF602	19.8	21.3
	F. culmorum KF603		
	F. culmorum KF604		
	F. sporotrichioides M-1-1[c]	132.0	140.0

[a] Detection limits: ELISA, 100 pg/ml; GLC, 100 ng/ml. Values are the means of duplicate determinations.
[b] Recovery, 80 ± 7% (n = 3).
[c] Positive control.

the ELISA. Quantitative analysis revealed a relatively good correlation between the ELISA and GLC results (Table 1).

Among six Polish isolates, two *F. sporotrichioides* isolates (KF196 and KF602) produced a large amount of T-2, a result which was confirmed by GLC analysis (Table 1). Notably, a minute amount of T-2 was detected in the culture filtrates of *F. sambucinum* KF701 and *F. culmorum* KF601 by the ELISA (Table 1). No T-2 was produced by the 57 *Fusarium* isolates from Norwegian cereals (data not shown).

These data indicate that the ELISA described in this study is an excellent tool for the mass screening of T-2-producing fungi. We recently developed a one-step ELISA for T-2 with an anti-T-2 MAb (3). An application of the ELISA in the determination of T-2 in agricultural products will be reported elsewhere.

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