Bull. Environ. Contam. Toxicol. (1987) 39:86–91

Bulletin of Environmental Contamination and Toxicology

Survey of Sensitivity of 22 Strains of Yeasts to T-2 Toxin in Relation to Growth on Glucose and Glycerol Medium

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Yeasts have served as valuable test organisms for investigating the biological activity of trichothecene mycotoxins (McLaughlin et al. 1977; Hernandez and Cannon 1982; Schappert and Khachatourians 1983). Trichothecenes such as T-2 toxin are occasionally found as contaminants in grain infected with Fusarium fungi and these toxins are associated with a variety of disorders in animals and man (Ueno 1983a). In general, trichothecenes are not toxic to bacteria but exert potent cytotoxic activity against eukaryotic organisms (Ueno 1983b). Consequently, sensitive yeast species could be highly adaptable as test organisms in biological assays for trichothecenes while offering the convenience and economy of microbial test organisms.

Burmeister and Hesseltine (1970) conducted an early survey of yeast susceptibility to T-2 toxin but were unable to isolate strains with adequate sensitivity to serve in practical biological assays. A recent survey, released while our present study was in progress, revealed that one particular yeast, Kluyveromyces fragilis, was remarkably sensitive to T-2 toxin in relation to 75 other isolates, all tested on a medium with glucose as the major carbon source (Sukroongreung et al. 1984). For Saccharomyces cerevisiae, however, sensitivity to T-2 toxin was significantly higher when tested on a glycerol medium as compared to a glucose medium possibly as a result of anti-mitochondrial activity of the toxin (Schappert and Khachatourians 1983). In this regard, we have evaluated the sensitivity of 22 strains of yeasts to 25 ug of T-2 toxin using agar diffusion tests with both glucose and glycerol media to extend the search for a suitable organism for biological detection of toxic trichothecenes.

MATERIALS AND METHODS

Twenty two strains of yeasts (Table 1) were cultured at 28 °C on agar slants prepared with 10.0 g glucose, 5.0 g malt extract (Difco Laboratories, Detroit MI) , 5.0 g yeast extract (Difco Laboratories, Detroit MI), 5.0 g peptone (Difco Laboratories, Detroit MI) and 15 g agar per 1000 mL water. The cultures were stored at 7 °C.

Yeast sensitivity to 25 ug of T-2 toxin was evaluated with agar diffusion tests on glucose or glycerol medium using the soft agar overlay method described by Saubolli (1976) and Schappert and Khachatourians (1983). The media consisted of 20.0 g of either glucose or glycerol, 2.0 g of yeast extract and 2.0 g of peptone per 1000 mL of distilled water. For soft agar, 6 g of agar was added, for hard agar, 15 g was added.

Inoculum was prepared in glucose and glycerol broth and incubated at 28 °C for 48 h. Before each test, the broth culture was diluted with fresh broth to obtain an optical density of 0.3 as determined on a Beckman DB spectrophotometer with wavelength at 610 nm.

Exactly 10 mL of molten hard agar was dispensed into 100 mm x 15 mm petri dishes and allowed to solidify. A tube containing 10 ml of soft agar kept molten at 45 °C was inoculated with 0.1 ml of standardized inoculum, mixed for 2 s with a vortex mixer, and poured onto the corresponding hard agar. T-2 toxin (Sigma Chemical Co., St. Louis MI) prepared in a stock solution of 10 mg toxin per mL dimethylsulfoxide, was applied to sterilized filter paper disks (6.5 mm dia) in 2.5 ul aliquots (25 ug toxin per disk). The disks were allowed to air dry and were secured onto the surface of the seeded agar within 15 min of inoculation.

Inoculated plates were incubated overnight at 35 °C since yeast susceptibility to T-2 toxin has been shown to increase at elevated temperatures (Schappert and Khachatourians 1984). Zones of growth inhibition were measured accurately to the nearest millimeter using a precision scale ruler as soon as the boundaries had become distinct. For yeasts that did not grow at 35° C, tests were repeated with overnight incubation at 28 °C.

Further tests were conducted on the most sensitive species of yeast to determine the effect of incubation temperature on degree of response and to evaluate the minimum detectable level for T-2 toxin.

Table 1. Sensitivity of 22 species of yeasts to 25 ug of T-2 toxin using agar diffusion tests with glucose and glycerol media.

Yeast Species	ID No.	Diameter of Zone (mm)[a]				
		glucose		glycerol		
		x	s.d.	x	s.d	
Candida albicans	OMH	13.7	1.5	20.7	1.2	b
Candida boidinii	UWO8215	13.7	0.6	17.0	1.0	
Candida boleticola	UWO791OO	n.z.		n.z.		
Candida diddensii	UWO79223	22.3	2.1	19.7	0.6	
Candida guilliermondii	UWO837561	n.z.		n.z.		
Candida ingens	UWO79S781OO	31.3	0.6	29.7	0.6	
Candida lipolytica	UWO799	12.7	0.6	13.3	0.6	c
Candida norvijica	UWO8214	19.0	1.0	18.6	1.5	
Candida parasilosis	UWO792O9	9.6	0.6	8.7	0.6	b
Candida sake	UWO8243	14.3	0.6	14.0	1.0	
Candida tropicalis	UWO793O	n.z.		n.z.		
Hanensula anomala	UWO79148	14.3	1.5	17.7	0.6	
Kluyveromyces fragilis	UWO79O7158	36.5	1.0	37.6	0.6	
Rhodotorula glutinis	UWO811OO	13.3	0.6	14.7	0.6	c
Rhodotorula rubra	UWO8O8	12.7	0.6	13.0	1.0	c
Rhodotorula rubra	M 3O1	11.0	1.0	11.0	0.8	
Saccharomycoides ludwigii	M 299	12.7	0.6	14.3	0.6	
Saccharomyces cerevisiae	UWO838832	16.3	0.6	21.0	1.0	
Saccharomyces cerevisiae	M 135	14.3	0.6	18.6	0.6	
Saccharomyces cerevisiae	M 288A	12.6	1.2	18.0	1.0	
Saccharomyces carlsbergensis	ATCC 8O9O	19.7	1.5	23.3	1.2	
Trichosporon cutaneum	UWO8233	n.z.		n.z.		c

OMH, Ontario Ministry of Health; UWO, University of Western Ontario; M, Malloch collection, University of Toronto; ATCC, American Type Culture Collection.
x = mean of three replicates; s.d. = standard deviation.
n.z. = no zone of growth inhibition evident.
a, Total diameter of zone including 6.5 mm diameter disk.
b, Diffuse zone, retarded growth, not completely inhibited.
c, Results determined with incubation at 28 ^{o}C instead of 35 ^{o}C.

Table 2. Inhibition of growth of Kluyveromyces fragilis in agar diffusion tests on glucose and glycerol medium in relation to level of T-2 toxin.

T-2 Toxin (ug/disk)	Diameter of Zone (mm)[a] glucose x	s.d.	glycerol x	s.d.
25.ØØ	35.2	Ø.8	34.7	1.5
1Ø.ØØ	24.5	Ø.5	23.3	1.5
5.ØØ	23.3	1.5	22.3	1.2
1.ØØ	14.3	1.5	12.3	Ø.6
Ø.5Ø	1Ø.7	Ø.6	12.Ø	1.Ø
Ø.2Ø	7.5	Ø.5	7.5	Ø.5
Ø.1Ø	7.3	Ø.3	7.3	Ø.3
Ø.Ø5	n.z.		n.z	

x, s.d. and a, as in Table 1.

Table 3. Sensitivity of Kluyveromyces fragilis to 25 ug of T-2 toxin in agar diffusion tests on glucose and glycerol medium in relation to incubation temperature.

Temperature (°C)	Diameter of Zone (mm)[a] glucose x	s.d.	glycerol x	s.d.
2Ø	33.Ø	2.Ø	33.Ø	1.3
24	36.5	Ø.9	35.5	1.Ø
28	34.6	Ø.3	34.8	Ø.3
35	35.2	Ø.7	35.2	Ø.8

x, s.d. and a, as in Table 1.

RESULTS AND DISCUSSION

As shown in Table 1, most strains of yeasts displayed moderate sensitivity to 25 ug of T-2 toxin (diameter of zone of growth inhibition approximately 10 - 20 mm). Three strains were resistant but one strain in particular, *K. fragilis*, showed outstanding sensitivity to the trichothecene. In a previous study (Sukroongreung et al. 1984), another strain of *K. fragilis* was found to be the most sensitive of 75 strains of yeasts exposed to T-2 toxin which suggests that sensitivity to the toxin is a stable characteristic of this species.

For most species, there was no difference in sensitivity to T-2 toxin whether tested on glucose or glycerol medium (Table 1). Four species, *C. albicans*, *C. boidinii*, *S. carlsbergensis*, and *S. cerevisiae*, showed higher sensitivity on glycerol as compared to glucose medium. Results for the latter species confirm those of Schappert and Khachatourians (1983) and further substantiate the suggestion that T-2 toxin exerts an anti-mitochondrial effect. This component of T-2 toxicity has been demonstrated in other biological systems (Schiller and Yagen 1981; Pace and Murphy 1981; Pace 1983), but for most species of yeasts in the present study, evidence of an anti-mitochondrial activity was not apparent.

For agar diffusion tests with *K. fragilis*, the minimum detectable level for T-2 toxin was 100 ng per disk whether tested on glucose or glycerol medium (Table 2). Response was similar for both media throughout the range from 25 ug to 100 ng of T-2 toxin per disk (Table 2). Incubation temperature exerted remarkably little effect on the magnitude of response of *K. fragilis* to T-2 toxin (Table 3) suggesting that, for this species, dynamics of membrane fluidity do not impose significant restrictions on toxin entry into the cell as suggested for other species of yeast (Schappert and Khachatourians 1984). Thus, *K. fragilis* appears to be an ideal candidate for further research directed to development of simple biological assays for these economically important fungal toxins.

Acknowledgments. This research was supported in part by an Industrial Research Assistantship grant (coded FUSMYCO 1483) from the Natural Sciences and Engineering Research Council of Canada. NSERC Industrial Research Fellowships to J.A.B. and D.W.S. are also gratefully acknowledged.

REFERENCES

Burmeister HR, Hesseltine CW (1970) Biological assays for two mycotoxins produced by Fusarium tricinctum. Appl Microbiol 20: 437-440

Hernandez F, Cannon M (1982) Inhibition of protein synthesis in Saccharomyces cerevisiae by the 12,13-epoxytrichothecenes trichodermol, diacetoxyscirpenol and verrucarin A: reversibility of the effects. J Antibiotics 35: 875-881

McLaughlin CS, Vaughan MH, Campbell IM, Wei CM, Stafford ME, Hansen BS (1977) Inhibition of protein synthesis by trichothecenes. In: Rodricks JV, Hesseltine CW, Mehlman MA (eds) Mycotoxins in human and animal health. Pathotox Publishers, Park Forest South, IL p 263

Pace JG (1983) Effect of T-2 mycotoxin on rat liver mitochondria electron transport system. Toxicon 21: 675-680.

Pace JG, Murphy PE (1981) Effect of T-2 mycotoxin on the respiratory function of rat liver mitochondria. J Cell Biol 91: 285a.

Saubolli MA (1976) Antimicrobic susceptibility of yeasts. In: Barry AL (ed) The antimicrobiotic susceptibility test: principles and practices. Lea and Febiger, Philadelphia, PA p 142

Schappert KT, Khachatourians GG (1983) Effects of fusariotoxin T-2 on Saccharomyces cerevisiae and Saccharomyces carlsgerbensis. Appl Environ Microbiol 45: 862-867.

Schappert KT, Khachatourians GG (1984) Influence of the membrane on T-2 toxin in Saccharomyces spp. Appl Environ Microbiol 47: 681-684

Schiller CM, Yagen B (1981) Inhibition of mitochondrial respiration by trichothecene toxins from Fusarium sporotrichioides. Fed Proc Fedn Amer Soc Exp Biol 40: 1579.

Sukroongreung S, Schappert KT, Khachatourians GG (1984) Survey of sensitivity of twelve yeast genera toward T-2 toxin. Appl Environ Microbiol 48: 416-419

Ueno Y (1983a) Historical background of trichothecene problems. In: Ueno Y (ed) Trichothecenes - chemical, biological and toxicological aspects. Elsevier, New York, NY p 1

Ueno Y (1983b) Biological detection of trichothecenes. In: Ueno Y (ed) Trichothecenes - chemical, biological and toxicological aspects. Elsevier, New York, NY p 125

Received January 26, 1987; accepted March 17,1987.