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Effects of Fusariotoxin T-2 on *Saccharomyces cerevisiae* and *Saccharomyces carlsbergensis*

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A *Fusarium* metabolite, T-2 toxin, inhibits the growth of *Saccharomyces carlsbergensis* and *Saccharomyces cerevisiae*. The growth inhibitory concentrations of T-2 toxin were 40 and 100 μg/ml, respectively, for exponentially growing cultures of the two yeasts. *S. carlsbergensis* was more sensitive to the toxin and exhibited a biphasic dose-response curve. Addition of the toxin at 10 μg/ml of *S. carlsbergensis* culture resulted in a retardation of growth as measured turbidimetrically, after only 30 to 40 min. This action was reversible upon washing the cells free of the toxin. The sensitivity of the yeasts to the toxin was dependent upon the types and concentrations of carbohydrates used in the growth media. The sensitivity of the cells to the toxin decreased in glucose-repressed cultures. These results suggest that T-2 toxin interferes with mitochondrial functions of these yeasts.

T-2 toxin [4β,15-diacetoxy-8α-(3-methylbutyryloxy)-12,13-epoxytrichothec-9-en-3α-ol] is a member of the trichothecene family of mycotoxins. The trichothecenes are produced by *Fusarium*, *Trichoderma*, and *Cephalosporium* spp. These fungi are known to contaminate agricultural products worldwide (9).

T-2 toxin is a potent inhibitor of protein and DNA synthesis and acts as a c-mitotic agent in eucaryotic cells (6, 9). Although the effect on DNA synthesis has not been detailed, it is suspected that the toxin breaks or damages the chromosome (4).

The effect of the toxin on procaryotes has only been investigated to a limited extent. Burmeister and Hesseltine (2), in their survey, demonstrated that the toxin had no effect on the growth of 54 strains of bacteria. However, a recent report shows a pH-dependent inhibition of growth of *Bacillus thuringiensis* (1).

The effect of T-2 toxin on the growth and metabolism of yeasts has only been investigated to a very limited extent (2). The survey (2) reported the fungistatic properties of the toxin. The effects of this toxin on yeast physiology and its mechanism of action have not been detailed. In the present paper we describe the results of our studies on the effect of T-2 toxin on the growth and metabolism of *Saccharomyces cerevisiae* and *Saccharomyces carlsbergensis*.

(The material presented in this paper is part of an M.Sc. thesis to be submitted by K.T.S. to the University of Saskatchewan.)

MATERIALS AND METHODS

Yeast strains. *S. cerevisiae* (ATCC 24858) and *S. carlsbergensis* (strain L5) came from the culture collection of W. M. Ingledew, of this department.

Media. All experiments were conducted in yeast extract-peptone-glucose medium, except where indicated. This medium consisted of (per 1,000 ml) 2 g of yeast extract, 2 g of peptone (Difco Laboratories, Detroit, Mich.), and 20 g of glucose. If desired, 15 g of agar was added per liter of yeast extract-peptone-glucose medium.

Optical density measurements. The optical density of the cultures was measured at 610 nm with a Horizon spectrophotometer (model 5965-50; Horizon Ecology Co., Chicago, Ill.).

Filter disk tests. Disks (6 mm) were cut from Whatman 3MM filter paper (W. R. Balston Ltd.). The disks were sterilized and impregnated with 100 μg of T-2 toxin by the addition of 10 μl of a stock solution (10 mg/ml) of T-2 toxin prepared in dimethyl sulfoxide. The disks were allowed to air dry.

Growth inhibitory concentration tests. The macrodilution method of Washington and Sutter (10) was used. T-2 toxin was prepared in either dimethyl sulfoxide or ethyl alcohol (95%, vol/vol). The initial toxin concentration was 100 μg/ml, and serial dilutions thereof were made as indicated below. The tubes were inoculated with exponentially growing cultures of either *S. cerevisiae* or *S. carlsbergensis*. The tubes were incubated at 30°C for 20 h, and growth was measured at 610 nm. The resultant optical density ratios of T-2-treated cultures to the untreated controls, referred to as relative growth, were plotted against the concentration of toxin.

Yeast growth and growth rate measurements. Yeast cultures were grown to the log phase (absorbance at

610 nm of 0.3) at 30°C at 280 rpm in a New Brunswick gyratory shaker incubator (New Brunswick Scientific Co., Edison, N.J.). When the cells reached an absorbance at 610 nm of 0.3, the toxin was added to yield the final concentrations as indicated. Incubation was continued, and the optical density of the culture was monitored frequently.

Carbohydrate response. The response of yeast cell sensitivity to T-2 toxin in the presence of various carbohydrates was tested by a soft agar overlay method. Solid agar plates of yeast extract-peptone supplemented with different carbohydrates were prepared. A soft agar overlay (0.6%, wt/vol) of the same composition was also prepared and kept molten at 45°C. A 0.1-ml sample of mid-log-phase yeast cells was mixed with the soft agar and poured on top of the appropriate yeast extract-peptone plate. After the soft agar had hardened at room temperature, a filter disk containing a sample of T-2 toxin was secured on the soft agar. The plates were incubated aerobically or anaerobically (GasPak jar; BBL Microbiology Systems, Cockeysville, Md.) at 30°C, and the results were recorded after 48 to 72 h.

RESULTS

Growth inhibitory concentration tests. To quantitate the effects of T-2 toxin, minimal inhibitory concentrations were determined. The results indicate that *S. carlsbergensis* and *S. cerevisiae* have different sensitivity profiles toward T-2 toxin (Fig. 1). *S. cerevisiae* was noticeably more resistant to the effect of the toxin at any given concentration of toxin. Compared with *S. carlsbergensis*, which had a biphasic response curve, the effect of the toxin on the inhibition of the growth of *S. cerevisiae* was linear.

Effect of T-2 toxin on exponentially growing cells of *S. carlsbergensis*. To understand the results described above, studies of the effect of T-2 toxin on exponentially growing *S. carlsbergensis* were undertaken. The growth inhibitory effect of the toxin on exponentially growing cultures occurred after 30 to 40 min, depending on the concentration of toxin. When the inhibition occurred, it was complete (Fig. 2). An analysis of these results appears in Table 1. The toxin had a concentration-dependent effect on the growth rate of the yeast. Between toxin concentrations of 1 and 25 μg/ml, there was a dose-dependent decrease in the growth rate, from 0.002 to 0.0005 optical density units per min. At higher toxin concentrations, between 25 and 90 μg/ml, the effect on the growth rate was only twofold when the toxin concentration increased by almost fourfold. A graphic analysis of the data shown in Table 1 indicated a very similar sensitivity of the yeast cells to T-2 toxin whether they were from a 3-h or a 20-h culture. With exponential cultures there was an initial sensitive response to T-2 concentrations of up to 15 to 18 μg/ml and then a second, less responsive, phase. Thus, it is possible to measure the effects of the toxin on the growth of yeast cells at the end of 20 h of incubation or when the toxin is added to exponentially growing cultures with subsequent growth rate calculations.

Effect of T-2 toxin on yeasts in the presence of various concentrations of glucose. When yeasts are cultured in media containing glucose at concentrations higher than 5% (wt/vol), the synthesis of the respiratory enzymes is repressed. Thus, the yeasts grow anaerobically. The effect

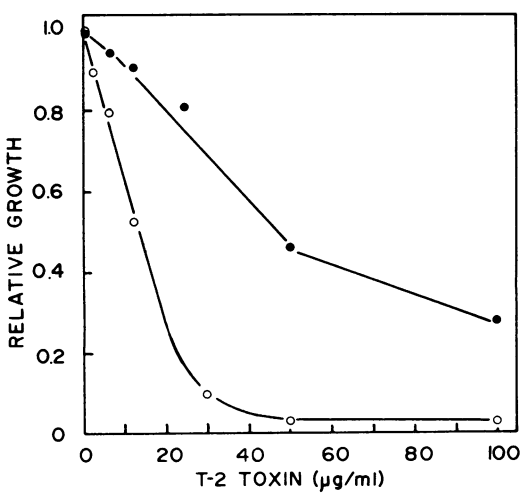

FIG. 1. Effect of T-2 toxin on the growth of *S. carlsbergensis* (○) and *S. cerevisiae* (●). Relative growth values, determined by a growth inhibitory concentration assay, are plotted as a function of T-2 toxin concentration.

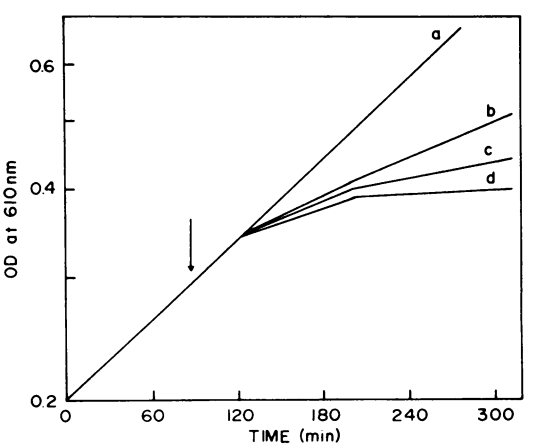

FIG. 2. Effect of T-2 toxin on exponentially growing cells of *S. carlsbergensis*. T-2 toxin was added in the early log phase (arrow), and incubation was continued. The following T-2 toxin concentrations were used: 0 (a), 10 μg/ml (b), 25 μg/ml (c), and 50 μg/ml (d). OD, Optical density.

TABLE 1. Effect of T-2 toxin on growth rate and generation time in *S. carlsbergensis*[a]

Treatment	Concn (μg/ml)	Growth rate (ΔOD/min [×10⁻⁴])[b]	Generation time[c] (h)
None		19.0	3.5
Ethanol (solvent control)[d]		17.0	3.9
T-2 toxin	1	20.0	3.4
	10	9.1	7.3
	25	5.1	13.1
	50	4.5	14.8
	75	2.2	30.3
	90	1.6	41.7

[a] Data taken from Fig. 2 and similar experiments.
[b] ΔOD, Change in optical density units at 610 nm.
[c] Generation times in T-2-treated cultures were measured 2 h after addition of toxin.
[d] 0.15-ml amount of 95% (vol/vol) ethanol was added per 10 ml of culture.

of T-2 toxin (10 μg/ml) on yeast cells growing in cultures containing various concentrations of glucose was tested (Fig. 3). As the concentration of glucose was increased, the toxin had less of an effect. At 1% glucose the relative growth was 0.52, whereas when the concentration of glucose was 10%, the relative growth was 0.78.

Effect of T-2 toxin on aerobic or anaerobic growth of yeasts. The effect of T-2 toxin on the growth of *S. cerevisiae* and *S. carlsbergensis* was studied by using the filter paper disk assay method. The surfaces of agar plates were spread with yeast cultures to yield a lawn of growth. Disks impregnated with toxin were then added and incubated either aerobically or anaerobically (Table 2). The two yeasts differed markedly in their sensitivity to T-2 toxin under aerobic conditions. *S. cerevisiae* had a zone of growth inhibition of 17 mm, whereas *S. carlsbergensis* had a zone size of 30.5 mm. Under anaerobic conditions, the zones were 14.5 and 22.5 mm, respectively.

Although the zones of growth inhibition were well defined, some graded growth occurred within the zones. Minimum growth occurred near the disk, and maximum growth occurred at the edge of the zone. The edge of the zone was well defined and sharp. The sharpness of the zone reflects a critical concentration of toxin necessary for growth inhibition. The graded growth within each zone indicates that both yeasts are capable of growth under the influence of the toxin.

Effect of T-2 toxin on the growth of yeasts utilizing different carbohydrate sources. The results described above may indicate mitochondrial involvement in T-2 sensitivity. The mitochondrion is an essential organelle for respiration and induction of certain chromosomal enzymes.

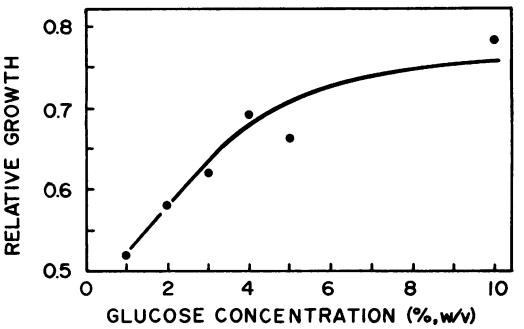

FIG. 3. Effect of T-2 toxin (10 μg/ml) on the growth of *S. carlsbergensis* in various concentrations of glucose. Relative growth was measured after the cells were incubated statically at 30°C for 48 h.

Puglisi and Algeri (8) have shown that petite mutants cannot utilize galactose, raffinose, maltose, or glycerol as a sole carbon source. That is, for the yeast to utilize maltose, galactose, and raffinose, the mitochondrion must be competent and functioning.

If the toxin affects the mitochondrion, then the zones of growth inhibition should be larger on agar plates containing maltose, galactose, or raffinose than on those containing glucose. The results of such an experiment appear in Table 3. The zones of growth inhibition were considerably larger for maltose, galactose, and raffinose than for glucose for both yeasts, as would be predicted on the basis of the hypothesis. Thus, T-2 toxin interferes with mitochondrial functions such that, among other possibilities, the induction of certain metabolic enzymes is prevented. To further support this reasoning, glycerol was used as a sole carbon source. The zones of growth inhibition on these plates were 2.25 times the zone size on glucose plates.

Figure 4 is a photograph of the plates used to determine the results listed in Table 3 for *S. cerevisiae*, showing the variation in zone sizes as described above. When sucrose was the sole carbon source, there was a graded growth pattern in the zone of growth inhibition. Just out-

TABLE 2. Comparative effect of T-2 toxin under aerobic and anaerobic growth conditions

Yeast	Incubation condition	Zone of growth inhibition[a] (mm ± SD)
S. cerevisiae	Aerobic	17.0 ± 0.0
	Anaerobic	14.5 ± 0.7
S. carlsbergensis	Aerobic	30.5 ± 0.7
	Anaerobic	22.5 ± 2.1

[a] The results are averages of two determinations. Filter disks alone or with dimethyl sulfoxide showed no zones of growth inhibition.

TABLE 3. Relationship between zones of growth inhibition and different carbohydrate sources[a]

Carbohydrate	Zone of growth inhibition (mm)	
	S. cerevisiae	*S. carlsbergensis*
Glucose	18.7	20.2
Glycerol	43.0	NG[b]
Maltose	28.6	39.6
Sucrose	23.5	28.5
Galactose	25.3	34.4
Raffinose	32.0	49.3

[a] Details of the inhibition test are given in the text.
[b] NG, No detectable growth.

side the zone of growth inhibition, there was an apparent zone of growth stimulation. Although not clearly evident in this photograph, all of the carbohydrate sources possessed a ring of growth stimulation adjacent to the growth inhibition zone. With galactose there were two distinct zones of growth inhibition, an inner zone of 15.3 mm and an outer zone of 27 mm. *S. carlsbergensis* grown under conditions identical to those used for Fig. 4 showed similar results (Fig. 5).

When the plates were incubated for several days at 4°C, we noticed that the zones of growth stimulation increased significantly. This probably indicates the reduced action of the toxin at low temperatures. We are presently examining this observation and testing the effect of T-2 toxin on psychrophilic organisms.

Recovery of cells from T-2 toxin treatment. Exponentially growing cells of *S. carlsbergensis* were subjected to 10 μg of T-2 toxin per ml for 30 or 120 min and then washed free of the toxin and incubated in fresh medium (Fig. 6). The addition of the toxin had observable effects only 30 min later. The growth rate of the treated culture was significantly less than that of the untreated control culture (Fig. 6A). When the treated cultures were washed free of the toxin after 30 min (Fig. 6A), there was an apparent lag period of 5 to 10 min before the cells regained a growth rate equivalent to that of the untreated controls. The cells that were treated for 120 min (Fig. 6B) showed a similar reduction in growth for about 50 min after the wash. The growth rate then returned to normal values, indicating recovery from the effects of the toxin.

It was also observed in both instances that after washing of the treated cultures and resuspension in fresh medium, the optical density of these cultures dropped slightly. The untreated control cultures, when washed in a similar fashion and returned to fresh medium, did not show such changes in optical density. Therefore, the observed effect could not be due to the washing procedures, and as yet we have no explanation for this effect.

DISCUSSION

In this paper we have described the effects of T-2 toxin on the growth of the eucaryotic microorganisms *S. carlsbergensis* and *S. cerevisiae*.

The growth inhibitory concentration tests showed that both yeasts were capable of some growth in the presence of up to 100 μg of T-2 toxin per ml. *S. cerevisiae* showed a linear inhibitory response to increasing concentrations of the toxin. *S. carlsbergensis* showed a biphasic response to the effects of the toxin. *S. carlsbergensis* is clearly more sensitive to the effects of the toxin than is *S. cerevisiae*.

The effects of the toxin after addition to the

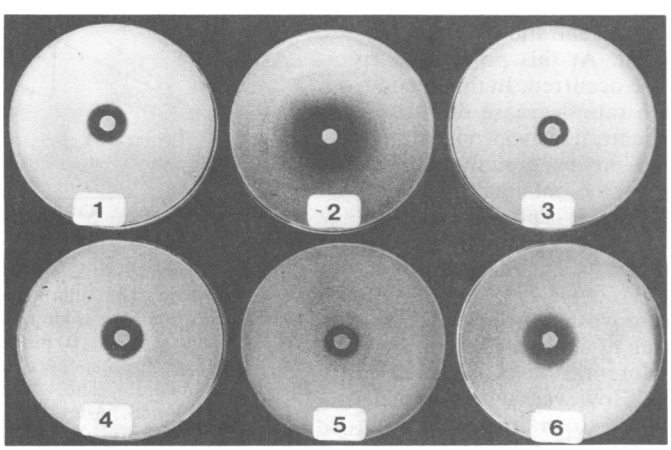

FIG. 4. Effect of T-2 toxin on the growth of *S. cerevisiae* in the presence of various carbon sources. Experimental details are given in the text. Plates: 1, maltose; 2, glycerol; 3, glucose; 4, sucrose; 5, galactose; 6, raffinose.

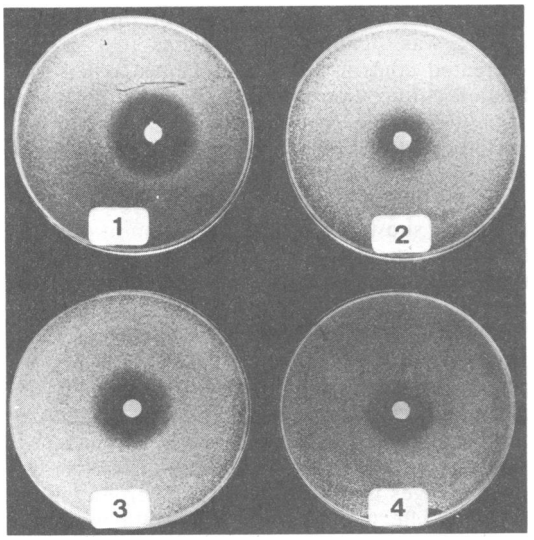

FIG. 5. Effect of T-2 toxin on the growth of *S. carlsbergensis* in the presence of various carbon sources. Experimental details are given in the text. Plates: 1, maltose; 2, glucose; 3, sucrose; 4, raffinose.

exponentially growing cells were not immediately measurable. The growth rate of the treated cells remained the same as that of the control for 30 to 40 min. At this point the growth rate of the treated cultures began to decrease. The point at which the effect was observable was immediate. A similar delay of 30 min was observed by LaFont and co-workers (5) in their study of the effects of T-2 toxin on yeast metabolism by Warburg respirometry.

The addition of T-2 toxin to exponentially growing *S. carlsbergensis* produced a curious effect on the growth rate. The *S. carlsbergensis* growth rate was very sensitive to the effects of the toxin up to toxin concentrations of approximately 15 to 18 μg/ml. At this point a sharp change in the response occurred. In this secondary phase the growth rate decrease due to the increasing toxin concentrations was not as rapid as before. The effect on the growth rate was remarkably similar to the minimal inhibitory concentration tests. Thus, it is possible to quantitate the effects of the toxin either by an endpoint assay or by measuring changes in the growth rates.

When the cells were washed free of the toxin (10 μg/ml) after 30 min of exposure, there was a short lag period before the cells returned to control growth rates. However, when the cells were washed free of the toxin after 120 min of exposure, the growth rate value was commensurate with that achieved after 60 min. Thus, the effects of the toxin clearly are fully reversible within 2 h after exposure. We also observed that cells recovering from T-2 toxin had a loss in optical density soon after the washing and resuspension in fresh medium. This occurred for both the 30-min and the 120-min exposures. This may reflect additional physiological changes occurring in cells recovering from treatment with T-2 toxin.

The zones of growth retardation observed during the carbohydrate tests were also quite unique. Galactose, maltose, and raffinose exhibited much larger zones of growth inhibition than glucose. For each carbohydrate tested, a ring of growth stimulation occurred just outside the zone of growth retardation. The consistency of

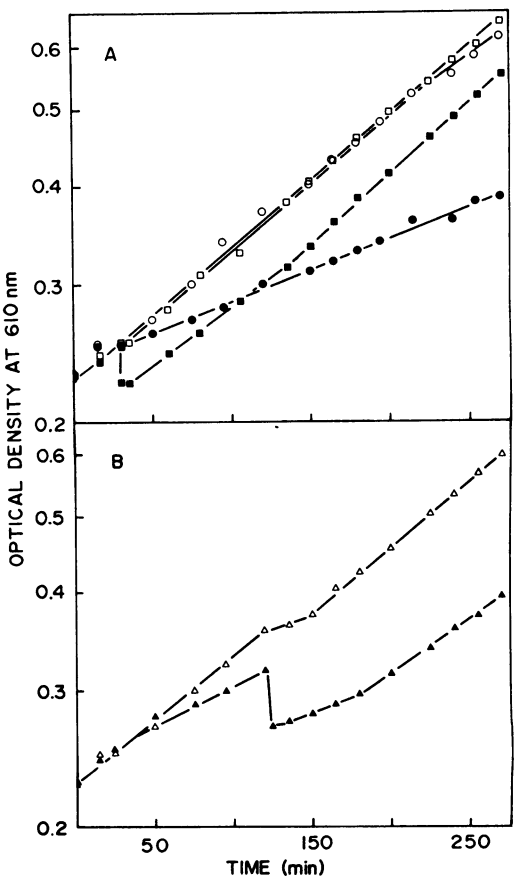

FIG. 6. Recovery of *S. carlsbergensis* after exposure to T-2 toxin. The cells were grown to early log phase. At this point the toxin (10 μg/ml) was added (t = 0). After 30 (A) or 120 (B) min of exposure, the cells were washed and resuspended in yeast extract-peptone-glucose medium. The wash procedure took approximately 15 min. After being washed, the cells were returned to the incubator, and the optical density was followed. Symbols: ○, no toxin control; ●, toxin control; □, 30-min wash control; ■, 30-min toxin wash; △, 120-min wash control; ▲, 120-min toxin wash.

this observation among the different carbohydrates suggests a common cellular target, such as sugar transport systems through the cell membrane, for the action of T-2 toxin.

The zones of growth inhibition observed are probably better described as zones of growth retardation, because the zones did contain some growth of the yeast cells. The outside of the zone was definite, and measurements of the zone size were easily determined.

The growth inhibition observed on the disk assays reflects the antimitochondrial action of the toxin. Under aerobic conditions the zones of growth inhibition were larger than under anaerobic conditions for both *S. carlsbergensis* and *S. cerevisiae*. This may be merely a function of growth rate or may reflect an intracellular target, such as the mitochondrion, which would not be functional under anaerobic conditions.

It has been observed by many authors that inhibition of mitochondria delays or affects the expression of nuclear genes. The effect of T-2 toxin on the expression of certain nuclear genes for the utilization of carbohydrates detects an antimitochondrial effect. The zones of growth retardation were much larger on those carbohydrates that need a functional mitochondrion than on glucose. Similarly, the zones of growth retardation were significantly larger on those carbon sources that need a functional mitochondrion for their metabolism. Such was the case when glycerol was the sole carbon source.

In terms of the environmental and animal toxicological aspects, reports have established that T-2 affects rodent, bovine, and fowl species (3, 7, 9). However, with regard to cellular or molecular targets, which would enable us to understand the mechanism of the action of T-2 toxin, the necessary model system had not been discovered. We believe that the yeast studies described here would enable us to establish the mode of action and molecular target(s) of T-2 toxin.

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