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Glucosylation and Other Biotransformations of T-2 Toxin by Yeasts of the *Trichomonascus* Clade

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Trichothecenes are sesquiterpenoid toxins produced by *Fusarium* species. Since these mycotoxins are very stable, there is interest in microbial transformations that can remove toxins from contaminated grain or cereal products. Twenty-three yeast species assigned to the *Trichomonascus* clade (Saccharomycotina, Ascomycota), including four *Trichomonascus* species and 19 anamorphic species presently classified in *Blastobotrys*, were tested for their ability to convert the trichothecene T-2 toxin to less-toxic products. These species gave three types of biotransformations: acetylation to 3-acetyl T-2 toxin, glycosylation to T-2 toxin 3-glucoside, and removal of the isovaleryl group to form neosolaniol. Some species gave more than one type of biotransformation. Three *Blastobotrys* species converted T-2 toxin into T-2 toxin 3-glucoside, a compound that has been identified as a masked mycotoxin in *Fusarium*-infected grain. This is the first report of a microbial whole-cell method for producing trichothecene glycosides, and the potential large-scale availability of T-2 toxin 3-glucoside will facilitate toxicity testing and development of methods for detection of this compound in agricultural and other products.

The fungal T-2 toxin (see Fig. 1) is a sesquiterpenoid trichothecene produced by *Fusarium sporotrichioides* and related species. Trichothecenes inhibit protein synthesis in eukaryotes and can cause both acute and chronic health problems for humans and animals that ingest contaminated food or feed. T-2 toxin in overwintered wheat was the cause of outbreaks of alimentary toxic aleukia in the 1930s in the former Soviet Union and has been associated with other gastrointestinal problems (42).

Trichothecenes are very stable mycotoxins. Consequently, there is interest in identifying chemical treatments or microbial transformations that effectively remove the compounds from contaminated grain or cereal products. For example, treatment of contaminated grain with the preservative sodium bisulfite converts the trichothecene deoxynivalenol (DON) into nontoxic DON sulfonates (13). Several types of microbial bioconversions of trichothecenes have also been reported (49), including oxygenation (3), acetylation (1, 24), deacetylation (5, 47), oxidation (41), deepoxidation (16, 20, 25, 43, 44, 48), and epimerization (19, 22). Some of these transformations lead to a complete loss of toxicity of the trichothecenes (e.g., deepoxidation) or reduce their toxicity (e.g., acetylation, oxidation) and thereby protect organisms from the deleterious effects of the toxins. Changes to the C-3 position and the epoxide have the greatest impact on toxicity (49). Indeed, microbes or enzymes that degrade or detoxify mycotoxins have possible practical applications as additives in animal feeds. These microbes can also be a source of genes that can be used to engineer disease-resistant crop plants. For example, the trichothecene acetyltransferase gene has been used to confer resistance to DON (33), which is a major virulence factor in *Fusarium graminearum* wheat head blight disease (37).

The most widespread microbial biotransformations of T-2 toxin are deacetylation and deepoxidation, the latter by anaerobic ruminal microorganisms (43, 44). Aerobic microorganisms that can biotransform trichothecenes have been found in environmental samples of water, soil, or plant surfaces using enrichment culture techniques (5, 20, 41). Species of the *Trichomonascus* yeast clade were selected as potential candidates for trichothecene modification because they were earlier found to metabolize compounds such as adenine, xanthine, glycine, uric acid, putrescine, and branched-chain aliphatic compounds (32).

In this study, 23 species of the *Trichomonascus* clade (Saccharomycotina, Ascomycota) were tested for their ability to biotransform, bind, or otherwise degrade T-2 toxin. We report here three types of bioconversions of the trichothecene T-2 toxin by members of this group.

MATERIALS AND METHODS

Yeast strains and media. The 23 yeast species in the *Trichomonascus* clade used in this study and their strain numbers are listed in Table 1. Five of the species (*Trichomonascus farinosus*, *Trichomonascus ciferrii*, *Blastobotrys nivea*, *Blastobotrys arbuscula*, and *Blastobotrys parvus*) were grown at 17°C; the remaining species were grown at 25°C. Fifty-milliliter Erlenmeyer flasks containing 20 ml of filter-sterilized glucose yeast nitrogen base (Difco) medium (G-YNB) (stage 1) were inoculated with a loopful of cells from a 2-day-old YM agar slant (26) and grown in an incubator shaker at 200 rpm in the dark. After 5 days, the cultures were centrifuged (10 min at 3,000 rpm), and the pellets were resuspended in either 20 ml (stage 2) G-YNB or 20 ml YNB (glucose-free medium). Stage 2 cultures were grown for up to 6 days at 25 or 17°C at 200 rpm in the dark. Supernatants from each stage 1 culture were transferred to sterile 50-ml conical tubes and incubated at 25 or 17°C.

Mycotoxin biotransformation studies. T-2 toxin in methanol was added to stage 2 cultures and to stage 1 supernatants to achieve a final concentration of 300 μM. The amount of methanol was less than 1% of the final culture volume. The T-2 toxin used was isolated from yeast extract-peptone-dextrose (YEPD) liquid cultures of *Fusarium sporotri-*

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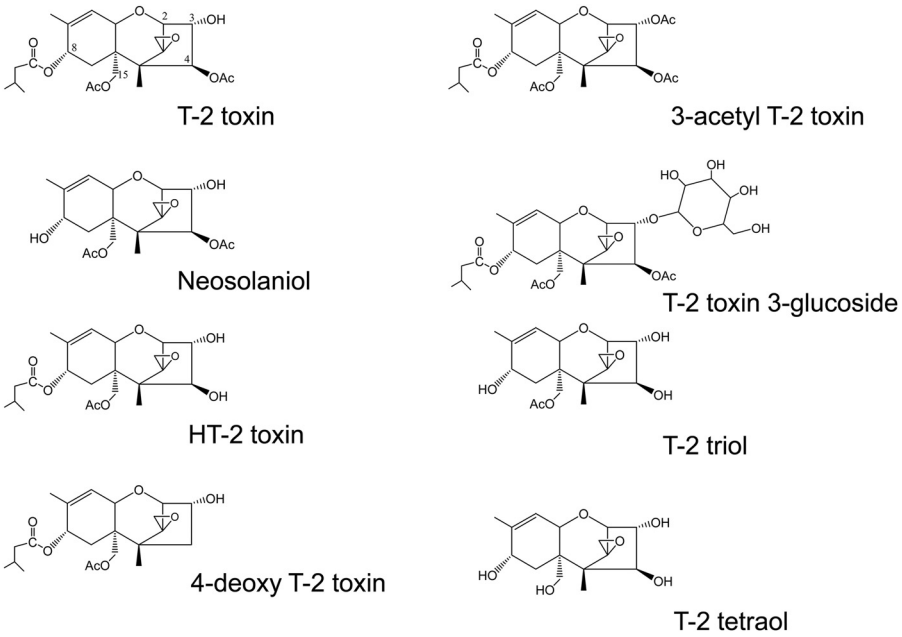

FIG 1 Chemical structures of T-2 toxin, 3-acetyl T-2 toxin, neosolaniol, T-2 toxin 3-glucoside, HT-2 toxin, T-2 triol, 4-deoxy T-2 toxin, and T-2 tetraol.

chioides strain 5493cos9-1#11 (18) and contained about 5% 8-butyryl neosolaniol, which differs from T-2 toxin only in the substituent at C-8; T-2 toxin is 8-isovaleryl neosolaniol (see Fig. 2A). Samples (3 ml) were taken from the culture or supernatant immediately after the addition of T-2 toxin and at later time points for up to 6 days and were extracted with ethyl acetate (1 ml).

Blastobotrys muscicola cultures were also grown on G-YNB, and fed T-2 toxin immediately after loop inoculation from a YM agar slant. In addition, stage 2 *B. muscicola* cultures were fed either HT-2 toxin (Fig. 1), isolated from *F. sporotrichioides TRI7* mutant strain 7-4-7 (9) or 4-deoxy T-2 toxin (Fig. 1), isolated from *F. sporotrichioides TRI13* mutant strain D10 (10), at an initial concentration of 300 μM.

Chemical analysis. Extracts were analyzed for the presence of T-2 toxin, HT-2 toxin, 4-deoxy T-2 toxin, or their metabolites by gas chromatography/mass spectrometry (GC/MS) with an Agilent 6890 gas chromatograph fitted with an Agilent J&W HP-5ms column (30 m; 0.25-mm film thickness) and a 5973 mass detector. The carrier gas was helium with a 20:1 split ratio and a 20-ml/min split flow. The oven temperature was ramped from 120°C to 260°C at 25°C/min and then held at 260°C for 10.4 min (total run time of 16 min). Under these conditions, HT-2 toxin elutes at 15.8 min, T-2 toxin elutes at 15.7 min, 3-acetyl T-2 toxin elutes at 15.4 min, 8-butyryl neosolaniol elutes at 13.6 min, 4-deoxy T-2 toxin elutes at 12.4 min, and neosolaniol elutes at 11.5 min. Compounds were identified based on comparison of their mass spectra and retention times with those of authentic standards. T-2 toxin concentrations were determined using a standard curve.

Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS). MALDI-TOF mass spectra were recorded on a Bruker-Daltonics Microflex LRF instrument (Bruker-Daltonics, Billerica, MA) operating in reflectron mode. The system utilizes a pulsed nitrogen laser, emitting at 337 nm. Typically, 1,000 to 2,000 shots were acquired at a frequency of 60 Hz and 78% laser power, with the laser attenuator offset at 16% for 30% range. The matrix was saturated 2,5-dihydrobenzoic acid in acetonitrile and was premixed with the samples prior to spotting onto a standard 96-position stainless steel target. Ion source 1 (IS 1) was set at 19.0 kV, and source 2 was set at 15.9 kV (83.7% of IS 1), with lens and reflector voltages of 9.79 and 19.99 kV, respectively. During the acquisition, matrix ion suppression was used up to 250 Da. The instrument was calibrated externally against a series of malto-oligosaccharides. The MS data were processed off-line using the Flex Analysis 3.0 software package (Bruker Daltonics).

Aldononitrile acetate analysis by GC/MS. Carbohydrate aldononitrile acetate derivatives of culture extracts were prepared and analyzed essentially by the method of Price (36). Samples were hydrolyzed in trifluoroacetic acid (2 M, 110°C, 30 min) on a reaction block. After the samples were cooled, the solvent was removed by evaporation, and aldononitrile acetates were prepared by treatment with hydroxylamine hydrochloride and acetic anhydride. The GC/MS analysis was performed on an Agilent (Santa Clara, CA) 6890N gas chromatograph interfaced with an Agilent 5973N mass-selective detector configured in electron impact (EI) mode and equipped with a Hewlett-Packard (Santa Clara, CA) 7683 series autoinjector. Chromatography was achieved on an Agilent J&W HP-5ms column (30 m by 0.25 mm) using helium as the carrier gas. The oven temperature was ramped over a linear gradient from 150 to 300°C at 10°C per min. Mass spectra were recorded in positive-ion mode over the *m/z* range of 60 to 550. Injector and detector/interface temperatures were 275 and 300°C, respectively. Data analysis was done off-line using HP Chemstation.

Production of T-2 toxin 3-glucoside. In order to isolate sufficient amounts of T-2 toxin 3-glucoside for nuclear magnetic resonance (NMR) analyses, larger cultures of *B. muscicola* were grown and fed greater amounts of T-2 toxin. Cultures were inoculated from a 2-day-old YM agar slant into two 50-ml Erlenmeyer flasks, each containing 20 ml G-YNB and grown in an incubator shaker at 25°C and 200 rpm. After 4 days, cultures were centrifuged, and each pellet was resuspended into 80 ml of fresh G-YNB and supplemented with 26.3 mg T-2 toxin dissolved in 200 μl methanol. The final concentration of methanol was less than 1% culture volume. Aliquots (1 ml) were removed daily and extracted with ethyl acetate (1 ml), and the extracts were analyzed with GC/MS to monitor the amount of T-2 toxin remaining in the cultures. Cultures were harvested after 4 days when GC/MS indicated that the T-2 toxin was less than 10% of the initial concentration.

The cultures (50 ml) were centrifuged (3,000 rpm, 10 min), and the supernatants were transferred to 50-ml conical tubes and extracted twice with equal volumes of ethyl acetate. Combined extracts were concentrated under a stream of nitrogen and applied to three silica gel 60 F-254 plates

TABLE 1 Bioconversion of T-2 toxin by yeasts in the *Trichomonascus* clade[a]

Species	NRRL strain designation	Acetylation to 3-acetyl T-2 toxin	Glycosylation to T-2 toxin 3-glucoside	Removal of the isovaleryl group to neosolaniol
Blastobotrys muscicola	Y-7993		X	
Blastobotrys robertii	Y-27775		X	
Blastobotrys proliferans	Y-17577	X		
Trichomonascus farinosus	Y-17593			
Blastobotrys nivea	Y-17581	X		
Blastobotrys capitulata	Y-17573			X
Blastobotrys arbuscula	Y-17585	X		
Trichomonascus apis	Y-48475			
Blastobotrys attinorum	Y-27639	X		
Blastobotrys parvus	Y-10004	X		
Trichomonascus petasosporus	YB-2092	X		X
Blastobotrys indianensis	YB-1950	X		X
Trichomonascus ciferrii	Y-10943	X		
Blastobotrys chiropterorum	Y-17071			
Blastobotrys terrestris	Y-17704	X		
Blastobotrys serpentis	Y-48249	X		
Blastobotrys adeninivorans	Y-17692	X		X
Blastobotrys raffinofermentans	Y-27150	X		X
Blastobotrys americana	Y-6884			
Blastobotrys peoriensis	YB-2290		X	
Blastobotrys mokoenaii	Y-27120	X		X
Blastobotrys malaysiensis	Y-6417	X		X
Blastobotrys illinoisensis	YB-1343	X		

[a] A capital letter X indicates that the yeast species was found to perform that change to T-2 toxin.

(20 by 20 cm; 0.25 mm thick; Merck) and separated by chromatography with dichloromethane-methanol (185:15). Bands were detected under short-wavelength UV light. The bands containing T-2 toxin glucoside ($R_f = 0.34$) and residual T-2 toxin ($R_f = 0.84$) were identified by removing a strip of one thin-layer chromatography (TLC) plate and treating it with 4-(*p*-nitrobenzyl)pyridine (NBP) and tetraethylenepentamine (TEPA) spray reagents, which give a blue band on a white background when reacted with compounds containing an epoxide moiety (45). The T-2 toxin glucoside was eluted from the silica gel with methanol and ethyl acetate (1:1) and evaporated under a stream of nitrogen.

NMR spectroscopy. Proton and carbon 13 NMR spectra were recorded with a Bruker AMX 500 spectrometer with CD_3OD as the solvent and tetramethylsilane as an internal standard.

Phytotoxicity. *Chlamydomonas reinhardtii* was used to assess the relative phytotoxicity (2, 30) of T-2 toxin and its three transformation products, neosolaniol (Fig. 1), 3-acetyl T-2 toxin (Fig. 1), and T-2 toxin 3-glucoside (Fig. 1). Duplicate 10-ml cultures were initiated with 1×10^5 cells/ml on a high-salt, high-acetate medium (17) containing 100 μM concentration of an individual trichothecene. The trichothecenes were dissolved in acetone with the final concentration of acetone in the cultures less than 1%. Culture doublings were calculated as follows: (log of the final density − log of the initial cell density)/log 2.

RESULTS

No changes were observed in T-2 toxin concentrations when stage 1 supernatants were incubated with T-2 toxin for up to 6 days. Stage 2 cultures of four species had no apparent metabolism of T-2 toxin after 6 days (Table 1). Three types of changes were observed in stage 2 cultures of the remaining species (Table 1 and Fig. 2): (i) acetylation to 3-acetyl T-2 toxin, (ii) removal of the isovaleryl group to neosolaniol, and (iii) decrease in T-2 toxin concentration. Three species, *Blastobotrys capitulata*, *Blastobotrys mokoenaii*, and *Blastobotrys malaysiensis*, converted T-2 toxin into neosolaniol by removal of the C-8 isovaleryl group (Table 1 and Fig. 2B). Neosolaniol was detected in the cultures of four additional species (Table 1). Fifteen species (Table 1) converted T-2 toxin into its 3-acetylated derivative (Fig. 2C), including three that also produced detectable amounts of neosolaniol and 3-acetylneosolaniol.

Three species, *Blastobotrys muscicola*, *Blastobotrys robertii*, and *Blastobotrys peoriensis*, had a decrease in T-2 toxin concentration in stage 2 G-YNB cultures, but no new metabolites were detected by GC/MS (Fig. 2D and 3). *B. muscicola* stage 2 YNB cultures (with glucose) had no remaining T-2 toxin detected after 4 days, while those cultures grown without additional glucose still had more than half of the T-2 toxin remaining at that point (Fig. 3). *B. muscicola* G-YNB stage 1 cultures that were fed T-2 toxin immediately after loop inoculation had an initial lag in T-2 toxin reduction, but only 15% remained after 4 days. The analysis by MALDI-TOF/MS indicated that T-2 toxin was converted by these three species to T-2 toxin glucoside (Fig. 4).

Purified T-2 toxin was analyzed by MALDI-TOF mass spectrometry and was apparent as a major $[M+Na]^+$ molecular adduct ion at *m/z* 489.1, plus a minor $[M+K]^+$ ion at *m/z* 505.1 (Fig. 4A). Following microbial incubation, new ion peaks are apparent at *m/z* 651.0 and 667.0, corresponding to the $[M+Na]^+$ and $[M+K]^+$ ions, respectively, for glucosylated T-2 toxin (Fig. 4B). The 162-Da mass difference between 651 and 489, and between 667 and 505, corresponds to the replacement of a single hydroxyl proton by a hexose motif. Minor $[M+Na-14]^+$ ion peaks (*m/z* 475.1 and 637.0) were also observed for the T-2 toxin and the glucosylated T-2 toxin, and a minor ion at *m/z* 609 is attributed to a small loss of a mono-*O*-acetyl group from the glucosylated T-2 toxin.

Confirmation of the identity of the hexose sugar was achieved by GC/MS analysis of aldononitrile acetate derivatives of culture

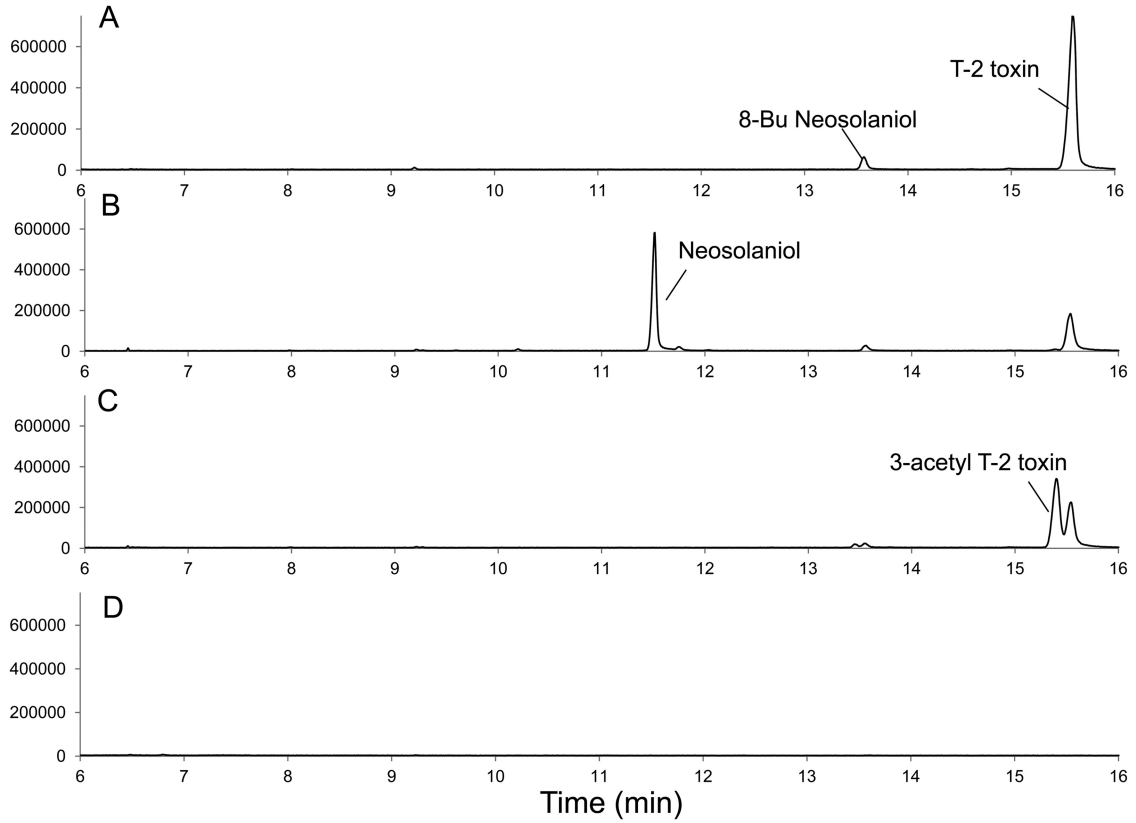

FIG 2 Representative GC/MS traces of culture extracts from feeding experiments on G-YNB. Time (in minutes) is shown on the *x* axes, and total ion current intensity is shown on the *y* axes of the graphs. (A) *B. muscicola* culture immediately after T-2 toxin was added, (B) *B. capitulata* culture 6 days after the culture was fed T-2 toxin; (C) *B. parvus* culture 6 days after the culture was fed T-2 toxin; (D) *B. muscicola* culture 6 days after the culture was fed T-2 toxin. 8-Bu, 8-butyryl.

extracts (Fig. 5). The samples were hydrolyzed by reflux with trifluoroacetic acid, and aldononitrile acetates were prepared as described previously (36). The glucosylated T-2 toxin gave rise to a single GC peak at 11.55 min (Fig. 5A) and gave MS ions characteristic of aldohexose aldononitrile acetates. This peak coeluted with a glucose aldononitrile acetate standard and was well separated from those of the mannosyl and galactosyl standards (Fig. 5B). Hence, the glycosyl modification to the T-2 toxin is assigned as a single *O*-linked glucose residue.

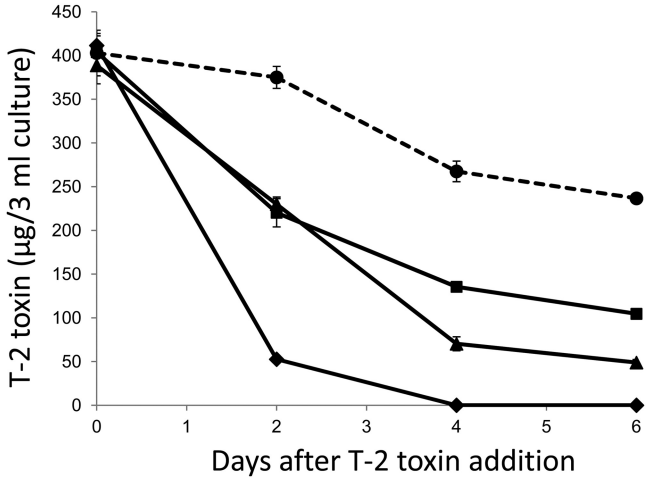

FIG 3 Time course (in days) of T-2 toxin metabolism by cultures of *B. muscicola* (diamonds), *B. robertii* (triangles), and *B. peoriensis* (squares) grown on G-YNB and cultures of *B. muscicola* grown on YNB (glucose-free medium) (circles) as measured by GC/MS. The graph shows average T-2 toxin concentrations ± standard deviations (error bars) from three cultures.

Larger-scale cultures were prepared in order to obtain sufficient amounts of material for NMR analyses. In one experiment, two *B. muscicola* 80-ml stage 2 G-YNB cultures fed a total of 52.6 mg of T-2 toxin and incubated for 4 days yielded, after TLC purification, 47.3 mg of T-2 toxin glucoside, a 66.8% yield on a molar basis.

Proton and ^{13}C NMR spectroscopy (Tables 2 and 3) confirmed that the *B. muscicola* transformation product of T-2 toxin was T-2 toxin 3-glucoside (Fig. 6 and Tables 2 and 3). Moreover, the anomeric signals for the glucosidyl group (H-26, 4.98 ppm, d, $J = 3.8$ Hz; C-26, 98.2 ppm) indicates that it is *O*-linked to the T-2 toxin by an axial (alpha-) glycosidic bond.

GC/MS analysis of *B. muscicola* cultures fed HT-2 toxin did not show metabolism of this trichothecene. However, no trichothecenes were detected with GC/MS 2 days after 4-deoxy T-2 toxin was fed to stage 2 cultures. MALDI-TOF MS analysis indicated that these *B. muscicola* G-YNB cultures had also converted 4-deoxy T-2 toxin into 4-deoxy T-2 toxin 3-glucoside. The 4-deoxy T-2 toxin ([M+Na]+ = *m/z* 431.178) was completely converted to glucosylated 4-deoxy T-2 toxin ([M+Na]+ = *m/z* 593.180) after 2 days.

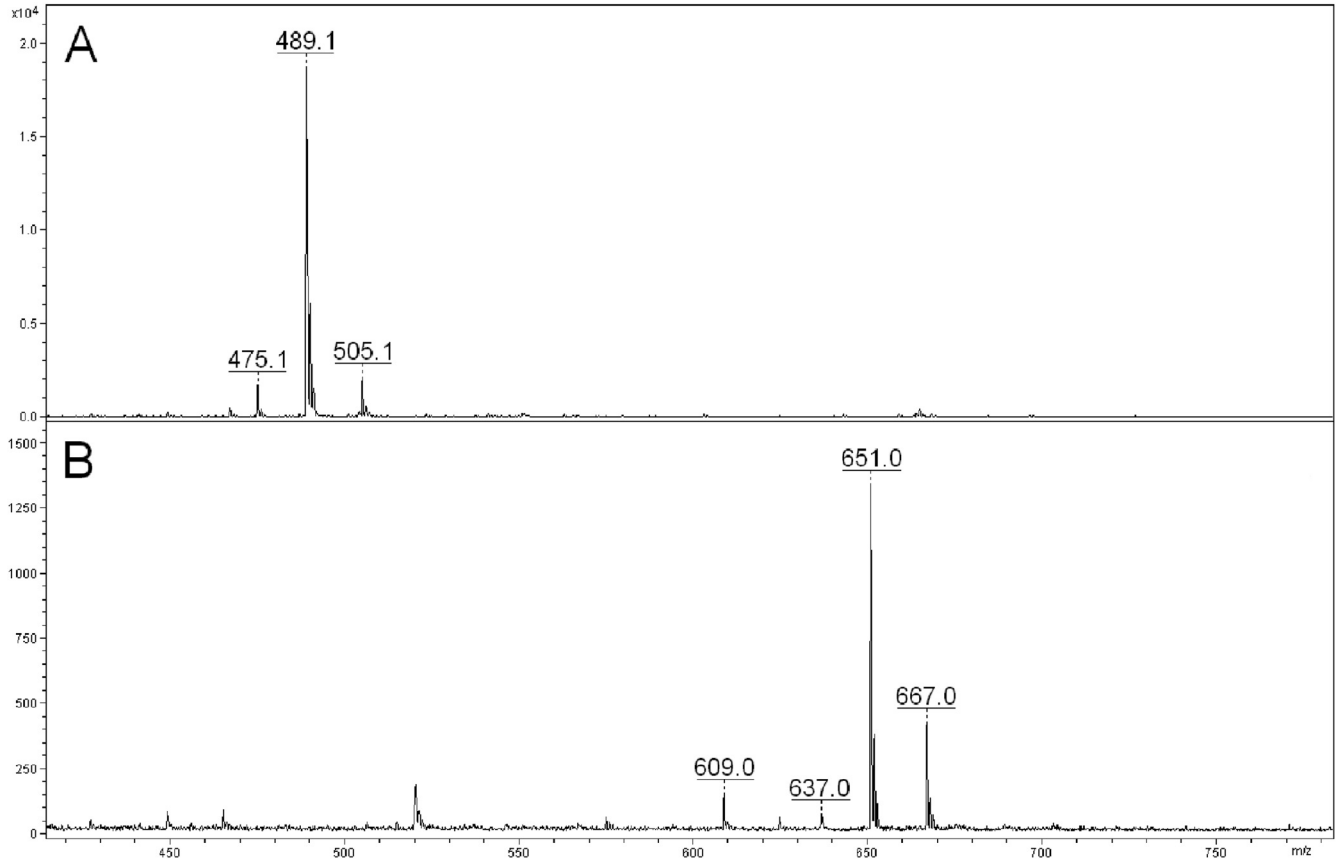

FIG 4 MALDI-TOF MS analysis. (A) T-2 toxin; (B) T-2 toxin 3-glucoside in *B. muscicola* culture extracted 6 days after the culture was fed T-2 toxin.

The relative phytotoxicity of T-2 toxin and three biotransformation products found in this study, neosolaniol, 3-acetyl T-2 toxin, and T-2 toxin 3-glucoside, were tested with *Chlamydomonas reinhardtii* cultures (2). T-2 toxin was quite phytotoxic at this concentration, and cultures treated with T-2 toxin had no increase in the number of cells after 6 days. In contrast, cultures treated with neosolaniol had 2.8 doublings, cultures treated with 3-acetyl T-2 toxin had 4.2 doublings, and cultures treated with T-2 toxin 3-glucoside had 5.5 doublings, compared to 5.1 doublings in cultures treated with acetone alone.

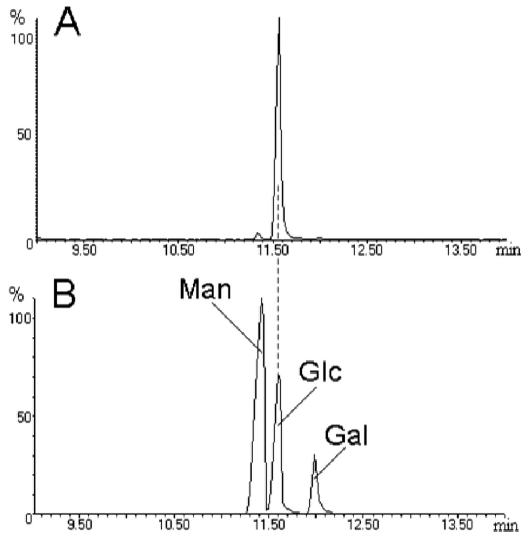

FIG 5 Analysis of sugar residues from T-2 toxin 3-glucoside.

DISCUSSION

Biotransformations are often detoxification or resistance mechanisms used by organisms for protection from the deleterious effects of toxins. One structural target of detoxification mechanisms is the trichothecene C-3 hydroxyl group. Changes to the C-3 hydroxyl group can decrease the toxicity of trichothecenes (2, 4, 21). Most *Fusarium* trichothecenes, including T-2 toxin, have a hydroxyl group at the C-3 position (Fig. 1), but their biosynthesis is via a series of intermediates that have a 3-acetyl group (31), which protect the toxin-producing organism from its own toxins. A number of other microorganisms, including *Aspergillus* and *Saccharomyces cerevisiae*, and non-trichothecene-producing *Fusarium* fungi have acetyltransferases that convert *Fusarium* trichothecenes into their less toxic 3-acetyl derivatives (1, 23, 46). In this study, 3-acetylation was the most common biotransformation of T-2 toxin, observed in 15 of the 23 yeast species that were tested.

Phylogenetic analysis of partial nuclear rRNA gene sequences from members of the *Trichomonascus* clade showed that the three T-2 toxin 3-glucoside-producing species occupy early branching positions in the tree (Fig. 7). *Blastobotrys muscicola* and *B. robertii* are sister species, whereas *B. peoriensis* is less closely related and is a sister species to *Blastobotrys americana*, which did not convert

TABLE 2 Proton NMR spectroscopy of T-2 toxin 3-glucoside (Fig. 6)

Position[a]	^{1}H shift(s) (ppm)	Mult, splitting (Hz)
1		
2	3.83	d, 4.7
3	4.34	dd, 3.2, 4.7
4	5.85	d, 3.2
5		
6		
7ab	2.38, 2.00	dd, 15.1, 5.8; d, 15.1
8	5.34	d, 5.6
9		
10	5.78	d, 5.9
11	4.46	d, 5.9
12		
13ab	3.05, 2.88	d, 3.8; d, 3.8
14	0.76	S
15ab	4.38, 4.13	d, 12.4; d, 12.4
16	1.75	S
17		
18ab	2.16, 2.16	M
19	2.07	M
20	0.98	d, 6.6
21	0.97	d, 6.6
22		
23*	2.10	S
24		
25*	2.05	S
26	4.98	d, 3.8
27	3.44	dd, 9.8, 3.8
28	3.72	t, 9.8
29	3.37	t, 9.5
30	3.59	ddd, 9.9, 4.8, 2.4
31ab	3.78, 3.70	dd, 11.9, 2.4; dd, 11.9, 4.9

[a] * indicates that shifts for positions 23 and 25 may be reversed.

TABLE 3 ^{13}C NMR spectroscopy of T-2 toxin 3-glucoside (Fig. 6)

Position[a]	^{13}C shift (ppm)	Mult
1		
2	76.4	CH
3	80.9	CH
4	80.7	CH
5	47.9	C
6	42.9	C
7	27.2	CH_2
8	68.0	CH
9	135.7	C
10	123.8	CH
11	67.2	CH
12	63.8	C
13	46.4	CH_2
14	5.7	CH_3
15	64.5	CH_2
16	19.0	CH_3
17	172.8	C
18	43.1	CH_2
19	25.5	CH
20	21.4	CH_3
21	21.3	CH_3
22**	171.0	C
23*	19.8	CH_3
24**	170.7	C
25*	19.4	CH_3
26	98.2	CH
27	72.0	CH
28	73.3	CH
29	70.1	CH
30	73.1	CH
31	60.9	CH_2

[a] * indicates that shifts for positions 23 and 25 may be reversed; ** indicates that shifts for positions 22 and 24 may be reversed.

T-2 to T-2 toxin 3-glucoside under the conditions tested. The seven species that convert T-2 toxin into neosolaniol are distributed among several subclades of the tree but nearly all represent sister species pairs. The 15 species that convert T-2 toxin into the 3-acetylated derivative are distributed throughout the tree.

T-2 toxin has two acetyl groups, at C-4 and C-15 (Fig. 1), both of which are reported targets of biotransformation. For example, a strain of *Curtobacterium* has been reported to rapidly convert T-2 toxin into HT-2 toxin and T-2 triol, the latter of which is significantly less toxic than T-2 toxin (47). A mixed enrichment culture of soil and freshwater bacteria was also reported to convert T-2 toxin to HT-2 toxin and T-2 triol (Fig. 1) (47). This type of deacetylation was not observed with any of the *Trichomonascus* clade species in this study.

Although deacetylation of T-2 toxin was not observed, strong isovaleryl esterase activity was found in three species, *B. capitulata*, *B. mokoenaii*, and *B. malaysiensis*, that converted T-2 toxin into neosolaniol. Small amounts of neosolaniol were formed by four other species (Table 1). Efficient conversion of T-2 toxin into neosolaniol has not been reported, but a minor degradation pathway was identified in a *Curtobacterium* strain (47) that led to neosolaniol and 4-deacetylneosolaniol. It is interesting to note that the 8-butyryl neosolaniol that was present at 5% in the T-2 toxin fed to cultures remained in cultures that had converted T-2 toxin to neosolaniol. Since these two compounds differ only in the substituent at C-8, the results suggest that these species have an acyl esterase that prefers isovaleryl to butyryl substrates.

The decrease in T-2 toxin concentrations observed in cultures of *B. muscicola*, *B. peoriensis*, and *B. robertii* was initially thought to be a result of binding of the trichothecene. Two types of trichothecene binding have been reported in other microorganisms, physical binding, such as binding in pockets on the surfaces of bacteria, and adsorption to polysaccharides in the cell walls of bacteria and yeasts (14, 15). Binding agents have been of interest as feed additives to effectively remove the bioavailability of the toxins, but they may remove only relatively low concentrations of T-2 toxin from contaminated material. For example, in feeding experiments with lactic acid bacteria, less than 20 μg/ml of T-2 toxin was removed from the cultures (14). In contrast, the three *Blastobotrys* species in

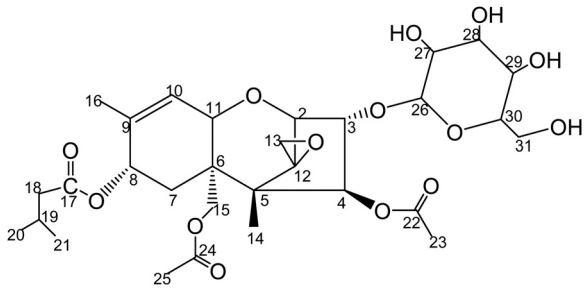

FIG 6 Structure of T-2 toxin 3-glucoside with the carbons numbered.

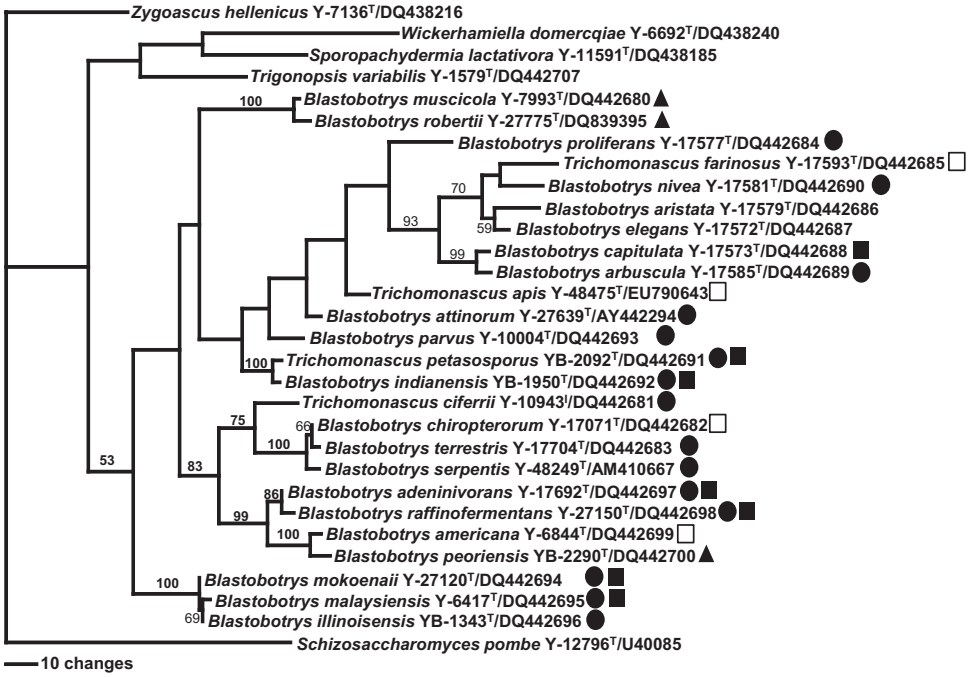

FIG 7 Phylogenetic relationships among species of *Trichomonascus* and its anamorphic genus *Blastobotrys* on the basis of maximum parsimony analysis of D1/D2 large-subunit (LSU) rRNA gene sequences; the phylogenetic tree was modified from that of Kurtzman and Robnett (27). Bootstrap values are for 1,000 replicates. The NRRL strain designations are shown. Type strains and isotype strains are indicated by superscript letters T and I, respectively, after the strain designation. GenBank accession numbers for the sequences analyzed are shown after slashes after the strains. Bioconversion of T-2 toxin is shown as follows: acetylation (black circle); glucosylation (black triangle); removal of isovaleryl (black square); no conversion (white square).

this study removed most of the T-2 toxin from cultures amended with approximately 140 μg/ml of the toxin, suggesting that these species produced a highly efficient binding agent or that they modified T-2 toxin in a way that was not detected by GC/MS. Indeed, MALDI-TOF analysis of extracts of these cultures indicated that the T-2 toxin was converted into T-2 toxin 3-glucoside.

Cultures of *B. muscicola* grown on stage 2 YNB without glucose had a decrease in T-2 toxin concentration but at a lower rate than cultures grown in the presence of glucose (Fig. 3). More than half of the T-2 toxin remained after 4 days, which suggests that the cells may have consumed any stored glucose reserves and were no longer able to form the glucose conjugate of T-2 toxin.

Glycosylation of other types of toxins or xenobiotics has been reported. For example, destruxin B, a phytotoxic peptide produced by *Alternaria brassicae*, is hydroxylated and glycosylated by *Brassica* species (34). Glycosylation of xenobiotics in plants is often paired with sequestration of the glycoside that is formed in the vacuole. Insect larvae can also detoxify plant flavonoids by glycosylation (38). While detoxification via glycosylation is not unusual in plant systems, it has not been frequently reported in microbes.

Trichothecene glycosides have been isolated from *Fusarium*-infected plant material (6, 7, 28). Trichothecene glycosides have been called masked mycotoxins because they may escape extraction or detection by methods that are commonly used to monitor mycotoxin contamination of food and feed (7). As noted previously, deoxynivalenol is a virulence factor in wheat head scab (37), and resistance to this disease has been correlated with the gene for a UDP-glucosyltransferase that converts deoxynivalenol into deoxynivalenol 3-glucoside (11, 29, 40), which is less phytotoxic (35). Although trichothecene glycosides may be less toxic, it is not known whether the sugar is removed in the digestive tracts of humans or animals. There is strong evidence that deoxynivalenol 3-glucoside may be hydrolyzed during digestion by gut bacteria to release the more toxic aglycone deoxynivalenol (8).

Studies on the toxicity and digestive fate of deoxynivalenol 3-glucoside have been facilitated by the availability of the compound. DON 3-glucoside has been isolated from wheat plants treated with pure DON (7) or prepared from DON by chemical synthesis (39). Briefly, DON is converted to 7,15-diacetyl DON 3-*O*-peracetylated glucoside, which is then treated to remove the acetyl groups from both the trichothecene and sugar moieties to yield DON 3-glucoside (39).

T-2 toxin and HT-2 toxin glucosides have recently been found in *Fusarium* culture material (12) and from *Fusarium*-infected plants (28) using advanced mass spectral techniques. Developing methods that can be used to measure these glucosides in cereal products or testing their animal toxicity or fate during digestion requires a relatively large supply of the compounds. The synthetic route used to produce DON 3-glucoside (39) is not practical for preparing T-2 toxin 3-glucoside because the final step in the synthesis, the removal of acetyl groups from the peracetylated sugar moiety, would also likely remove the acetyl and isovaleryl groups from the trichothecene portion of the molecule to produce T-2 tetraol 3-glucoside rather than the desired T-2 toxin 3-glucoside.

Although *B. muscicola* cultures converted T-2 toxin and 4-deoxy T-2 toxin to 3-glucosides, they did not metabolize HT-2 toxin. Preparation of HT-2 toxin glucoside from T-2 toxin glucoside may be possible with a mild hydrolysis of the C-4 acetyl group. Feeding studies with additional trichothecene substrates, purification and characterization of the *Blastobotrys* glucosyltrans-

ferase, and comparison of this enzyme with the plant glucosyltransferases that convert DON to DON 3-glucoside, may provide insight into protein structures, active sites, and kinetic properties that determine substrate specificity. This information may provide clues to engineer more-efficient trichothecene glucosyltransferase genes.

This study identified three types of bioconversion of T-2 toxin in this group of yeasts: 3-*O*-acetylation, removal of the C-8 isovaleryl group, and 3-*O*-glycosylation. The products of these transformations, 3-acetyl T-2 toxin, neosolaniol, and T-2 toxin 3-glucoside were all less phytotoxic than T-2 toxin in a *Chlamydomonas* assay.

This is the first report of an efficient microbial whole-cell catalytic method for preparing trichothecene glycosides. Three *Blastobotrys* species produced T-2 toxin 3-glucoside in quantities sufficient for further studies on the toxicology of these masked mycotoxins and for development of analytical methods for their detection. We did not determine the maximum amount of toxin that a culture could process or the concentration at which T-2 toxin is toxic to the yeast. Although producing sufficient quantities for toxicological studies of masked mycotoxins was beyond the scope of the present study, small scale-up experiments were done using approximately twice the concentration of T-2 toxin used in the initial screening. In one experiment, 47.3 mg of T-2 toxin 3-glucoside was purified from two 4-day-old 80-ml cultures of *B. muscicola* fed 52.6 mg of T-2 toxin, a 66.8% yield on a molar basis. While this is not the gram amounts required for toxicological testing, simply increasing the numbers of cultures may easily provide adequate amounts of the glucoside. Bioprospecting for microbes, especially those with other reported detoxification activities, is a promising means for finding new detoxification genes.

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