ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 1986, p. 570–575
0066-4804/86/040570-06$02.00/0

Vol. 29, No. 4

Trichodermin Esterase Activity and Trichodermin Resistance in *Mucor racemosus*

WILLIAM A. FONZI AND PAUL S. SYPHERD*

Department of Microbiology and Molecular Genetics, College of Medicine, University of California, Irvine, California 92717

Received 3 September 1985/Accepted 7 January 1986

***Mucor racemosus* exhibited inducible phenotypic resistance toward the protein synthesis inhibitor trichodermin. Induction of resistance was elicited by exposure to trichodermin or to cycloheximide. Both adapted and nonadapted cells took up [^{14}C]trichodermin from the medium. Trichodermin was found to be rapidly deacetylated to trichodermol upon entering the cell. Adapted cells deacetylated the drug more rapidly than nonadapted cells both in vivo and in vitro. The trichodermol resulting from deacetylation appeared in the medium, but the growth of adapting cells began well before the total conversion of trichodermin to trichodermol. Based on these data and the observation that trichodermol was a poor inhibitor of *Mucor*, adaptation appears to result from deacylation of the active antibiotic.**

Inducible phenotypic resistance has been observed in a number of cell types in response to a variety of growth inhibitory compounds. When the inhibitor mimics a normal cellular metabolite, phenotypic resistance can be effected by induction of the sensitive enzyme, as is seen in 2-thiazolealanine inhibition of histidine biosynthesis (17). However, it is less clear how adaptation to nonanalog inhibitors occurs. *Tetrahymena* spp. adapt to growth in the presence of cycloheximide, actinomycin D, colchicine, and several other compounds (8). *Rosa* spp. cell suspension cultures adapt to cycloheximide (6), and *Physarum polycephalum* exhibits phenotypic resistance to both trichodermin and anisomycin (10). In each of these cases an alteration in cellular permeability was suggested as a possible mechanism of resistance. Reduced permeability because of cell wall alterations appears to be responsible for the phenotypic resistance of *Candida* cells to amphotericin B (18).

The dimorphic fungus *Mucor racemosus* exhibits inducible phenotypic resistance to several diverse inhibitors (15). Although initially susceptible to cycloheximide, trichodermin, and amphotericin B, *M. racemosus* is only temporarily inhibited by these drugs (15). After a dose-dependent lag of 12 to 40 h, the entire population of cells resumes growth. Addition of fresh drug to the adapted cells does not cause growth inhibition, and growth in the absence of drug results in resensitization of the entire population of cells (15).

Perhaps even more interesting is the observation that *Mucor* cells adapted to growth in the presence of trichodermin acquire resistance to cycloheximide and amphotericin B (15). Likewise, cells adapted to growth in the presence of cycloheximide are cross-adapted to both trichodermin and amphotericin B (15). As a first step in understanding the phenomenon of phenotypic resistance and cross-adaptation in *M. racemosus*, we investigated the uptake and fate of radioactive trichodermin in adapted and nonadapted cells. Here we demonstrate that resistance to trichodermin is accompanied by an increase in the ability of the cells to metabolize the drug.

* Corresponding author.

MATERIALS AND METHODS

Organism and growth conditions. *Mucor racemosus* (*Mucor lusitanicus* ATCC 12168) was employed throughout these studies. The cells were grown in semidefined medium consisting of 2% glucose–0.5% Bacto-Peptone (pH 4.5; Difco Laboratories, Detroit, Mich.)–0.05% yeast nitrogen base without amino acids and ammonium sulfate (Difco). Cultures were grown at 28°C on a rotary shaker and sparged with water-saturated CO_2 at a flow rate of 1 volume per volume per min. Growth was monitored with a Klett-Summerson colorimeter equipped with a 540-nm filter.

Synthesis of [^{14}C]trichodermin. [^{14}C]trichodermin was synthesized by a modification of previously published methods (9, 24). Trichodermin (5 mg) was deacylated in 1.0 ml of ethanol mixed with 0.3 ml of 2 N NaOH and heated at 75°C for 30 min. The mixture was extracted three times with 1 volume of chloroform, and the combined chloroform extracts were washed and evaporated to dryness. Trichodermol was crystalized from the resulting syrup with petroleum ether, recrystalized from ethyl ether-hexane (1:1), and then dried in vacuo. The trichodermol was dissolved in 200 μl of dry pyridine and mixed with 500 μCi of [1-^{14}C]acetic anhydride (specific activity, 114 mCi/mmol; Amersham Corp., Arlington Heights, Ill.). Acylation was conducted at room temperature for 60 h. The solution was mixed with 1.0 ml of H_2O and extracted three times with 1 volume of petroleum ether. The trichodermin was purified from the extract by thin-layer chromatography (TLC) on silica gel 60 (EM Reagents) developed with chloroform-methanol (95:5) (21).

Uptake assays. Log-phase cells were collected by centrifugation, washed with 10 mM potassium phosphate (pH 5.5)–150 mM KCl, and suspended at a density of 2×10^6 cells per ml in the potassium phosphate-KCl buffer. [^{14}C]trichodermin (10 μM, 11.3 μCi/μmol) was added, and the samples were incubated at 28°C. After 15 min, 1 volume of ice-cold incubation buffer was added, and the cells were collected on glass fiber filters. The cells were washed three times with cold buffer. The filters were then dried and counted. Uptake was linear over this time period, and identical results were obtained if the cells were incubated in and washed with semidefined medium.

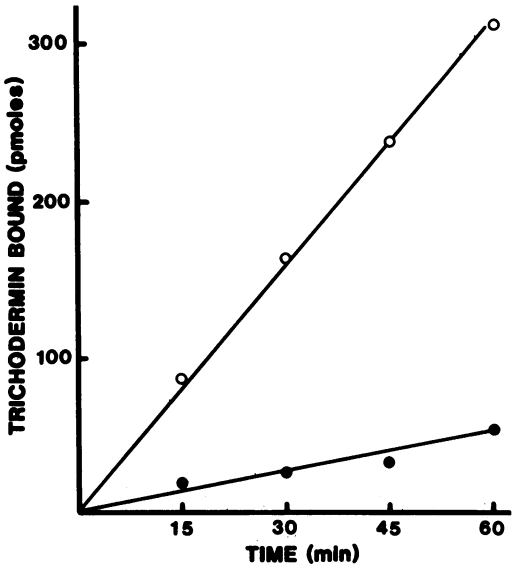

FIG. 1. Uptake of trichodermin by *M. racemosus*. Adapted (○) and nonadapted (●) cells were incubated with [^{14}C]trichodermin, and the amount of radioactivity associated with the cells was determined at the indicated time points.

Analysis of cell extracts. Following incubation under the standard uptake assay conditions, the cells were harvested by centrifugation for 15 s in a microfuge and washed twice with H_2O. The washed cells were extracted three times for 3 min at room temperature with 70% methanol–0.01 N HCl, and then the cells were removed by centrifugation for 2 min in a microfuge. A total of 90 to 95% of the label was extracted from the cells by this procedure. The combined extracts were lyophilized, and the resulting residue was dissolved in H_2O.

The extracts were analyzed by TLC on silica gel 60. Approximately 5,000 cpm of material was spotted on the plates, and the plates were developed with either acetic acid-ethanol-acetone (1:2:2) or chloroform-methanol (95:5) (21). Following chromatography, the plates were dried and sprayed with En^{3}Hance spray (New England Nuclear Corp., Boston, Mass.). Autoradiography was conducted at −70°C for 3 to 4 days.

Fate of trichodermol. Approximately 10^7 adapted or nonadapted cells were suspended in 1.0 ml of 10 mM potassium phosphate (pH 5.5)–150 mM KCl containing 100 μM trichodermin. The cell suspensions were incubated at 28°C with shaking and sparged with 100% CO_2. After 0, 15, or 60 min the samples were centrifuged for 30 s in a microfuge. The supernatant solution was removed and the cell pellet was washed twice with the potassium phosphate-KCl buffer before the cells were suspended in 1.0 ml of fresh buffer. The cell-free medium and the cell suspension were each extracted three times with 1 volume of diethyl ether. The extracts were evaporated with a stream of nitrogen, and the residue was dissolved in ethanol. The extracts were analyzed by TLC on silica gel 60 plates developed with chloroform-methanol (95:5). Material on the plates was visualized with *p*-anisaldehyde spray as described by Scott et al. (22).

Deacylation activity. The conditions used to measure the rate of deacylation of trichodermin by crude extracts were essentially those of Ohta et al. (19). Cells were suspended in 20 mM Tris (pH 7.5)–150 mM KCl and broken by vortexing with glass beads. The extract was centrifuged for 15 min at 10,000 × *g*, and the supernatant solution was used directly in the assays. A total of 20 to 200 μl of extract was assayed in a total volume of 0.5 ml containing 20 mM Tris (pH 7.5) 150 mM KCl, and 1.0 mM trichodermin (50 μCi/mmol). The samples were incubated at 37°C for 20 min and then were extracted three times with 1 volume of chloroform. The volatile water-soluble counts were taken as released acetate and were shown to parallel the increase in the amount of trichodermol in the chloroform extracts analyzed by TLC. The assay was linear with respect to time and to extract added. No derivatives of trichodermin other than trichodermol were detected on the TLC plates.

Protein determinations. Protein was determined by either the method of Lowry et al. (16) or by the method of Bradford (2).

Materials. Cycloheximide and nystatin were purchased from Sigma Chemical Co., St. Louis, Mo. Amphotericin B was from E. R. Squibb & Sons, Princeton, N.J. Trichodermin was the kind gift of W. O. Godtfredson, Leo Pharmaceutical Products, Ballerup, Denmark.

RESULTS

Trichodermin uptake by adapted cells. One mechanism that might account for the induced resistance of *M. racemosus* to trichodermin and the simultaneously induced cross-resistance to other drugs would be an alteration of the permeability of the cell. The uptake of [^{14}C]trichodermin by adapted and nonadapted cells was examined, and both were found to accumulate the drug (Fig. 1). Rather than exhibiting a decreased permeability, the adapted cells appeared to accumulate [^{14}C]trichodermin at a much higher rate than nonadapted cells. Radioactivity associated with the cells did not exchange with exogenous unlabeled drug, and 95% of the radioactivity was released from the cells by mechanical disruption (data not shown).

Intracellular fate of trichodermin. Results of the experiment described above suggest that trichodermin enters adapted cells. Therefore, interaction between trichodermin and the ribosomes is prevented either by physical segregation of drug and target site or by modification of either the drug or the target. To determine whether the trichodermin was being modified intracellularly, cells were extracted following incubation with [^{14}C]trichodermin as described above. The extract was fractionated by TLC and visualized by autoradiography (Fig. 2). The radioactivity was found to be present in multiple compounds, and most of the compounds were common to both adapted and nonadapted cells. Importantly, cells adapted to trichodermin exhibited no material which comigrated with authentic trichodermin. Nonadapted cells, however, did exhibit material with the same R_f as authentic trichodermin.

Based on these results it appears that trichodermin is rapidly modified following entry into the cell. It is not clear, however, whether the spots observed on the chromatogram are derivatives of trichodermin or represent the incorporation of [^{14}C]acetate following deacetylation of radioactive trichodermin. If the latter were true, then the same chromatographic pattern should be observed with extracts of cells incubated with [^{14}C]acetate. This is, indeed, the case (Fig. 3). Trichodermin-adapted cells generated the same radioactive products when incubated with either [^{14}C]trichodermin or [^{14}C]acetate. Although the pattern of spots on the chromatograph varied slightly from one analysis to another, identical patterns were always obtained when the extracts were chromotographed together.

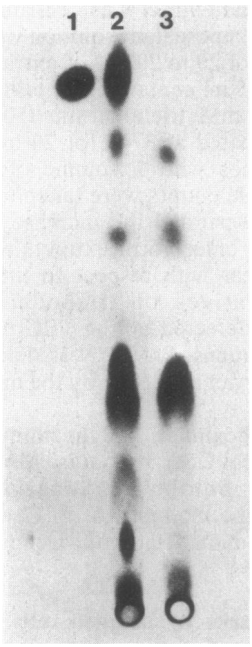

FIG. 2. Analysis of cell extracts for trichodermin. Following incubation with [^{14}C]trichodermin, cells were extracted with HCl-methanol as described in the text. The extracts were fractionated by ascending chromatography on silica gel, and the radioactive compounds were detected by autoradiography. Lane 1, authentic trichodermin; lane 2, extract from unadapted cells; lane 3, extract from adapted cells.

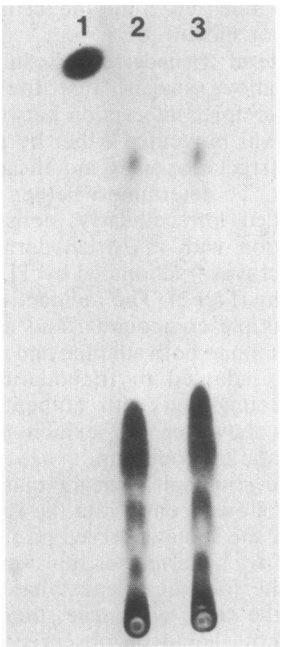

FIG. 3. Comparison of metabolic products of trichodermin and acetic acid. Trichodermin-adapted cells were incubated with either [^{14}C]trichodermin or [^{14}C]acetic acid and then harvested and extracted. The extracts were fractionated by ascending chromatography, and the radioactive compounds were detected by autoradiography. Lane 1, authentic trichodermin; lane 2, extract of cells incubated with [^{14}C]acetic acid; lane 3, extract of cells incubated with [^{14}C]trichodermin.

TABLE 1. Effect of acetic acid on the uptake of trichodermin

Acetic acid concn (mM)	Uptake rate[a]
0.0	313
0.01	361
0.10	338
1.00	332

[a] Rates are expressed as picomoles bound per minute per milligram of cell protein.

Since the uptake experiments measured only the movement of radioactivity into the cells and no trichodermin could be demonstrated in adapted cells, it was important to determine whether trichodermin was being deacetylated outside of the cell or after entering the cell. If trichodermin was being deacetylated externally, then the uptake of radioactivity by the cells should be competed by the addition of unlabeled acetate to the medium. Table 1 shows the results of such an experiment. Acetic acid at 100 times the trichodermin concentration had no effect on the uptake of radiolabeled trichodermin. In addition, the presence of 1 mM sodium arsenate, which inhibited acetic acid incorporation by 90%, had no effect on trichodermin uptake (data not shown). Therefore, it appears that trichodermin is deacetylated following entry into the cell.

Fate of trichodermol. Deacetylation of trichodermin yields trichodermol which the cells may further metabolize or excrete. The fate of the trichodermol was examined by incubating cells with trichodermin and then separately extracting the cells and the medium. The extracts were analyzed by TLC. The chromatograms in Fig. 4 demonstrate that both adapted and nonadapted cells excreted trichodermol into the medium. The amount of trichodermol in the medium increased with time, and adapted cells converted trichodermin to trichodermol at a much greater rate than nonadapted cells. No trichodermol could be detected in the cells; however, the trichodermol could have been removed when the cells were washed. We also could not

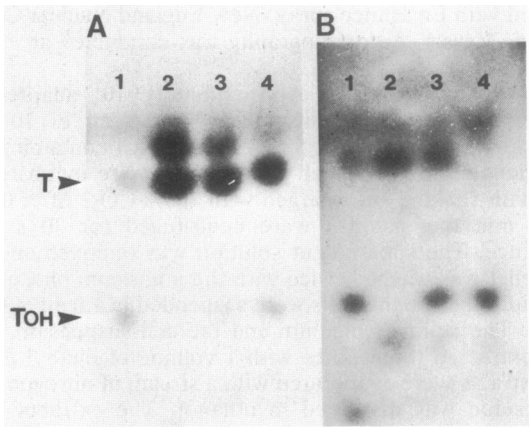

FIG. 4. Fate of trichodermol. Unadapted (A) or adapted cells (B) were incubated with trichodermin for 0 to 60 min. The cells and the incubation medium were then separated by centrifugation, and the medium was extracted with chloroform. The extracts were fractionated by ascending chromatography on silica gel. Compounds were visualized with *p*-anisaldehyde. Lane 1, authentic trichodermin (T) and trichodermol (TOH); lane 2, 0-min incubation; lane 3, 15-min incubation; lane 4, 60-min incubation.

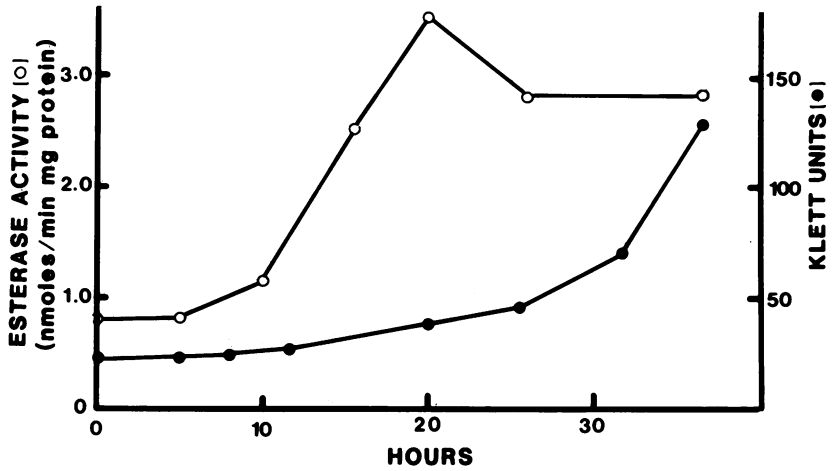

FIG. 5. Induction of trichodermin esterase activity. Trichodermin was added to exponentially growing cells at 0 h. At the indicated times the cells were harvested and assayed for trichodermin esterase activity (○). The growth of the cells was monitored with a Klett-Summerson colorimeter (●).

detect any new spots other than trichodermol in either the cells or the medium.

Trichodermin metabolism during adaptation. To detemine whether trichodermin esterase activity was induced prior to the resumption of cell growth, the esterase activity of cells undergoing adaptation was measured (Fig. 5). The esterase activity of the cells increased rapidly between 10 and 20 h after the addition of trichodermin to the medium. The increase in esterase activity preceded the resumption of significant cell growth by approximately 10 h.

In light of the preceding results it seemed reasonable to ask if all of the trichodermin must be converted to trichodermol before the cells can resume growth. This was not the case (Fig. 6). Although trichodermin was removed from the medium throughout the period of adaptation, almost 50% of the initial concentration of drug, a level inhibitory to nonadapted cells, was still present in the medium when the cells resumed growth. This is consistent with the observation that, once the cells are adapted, addition of fresh drug has no effect on cell growth (15). The loss of trichodermin from the medium was paralleled by the appearance of trichodermol, and no other derivatives were observed (data not shown). Taken together, these data suggest that resistance to trichodermin is effected intracellularly and that adaptation involves an increased ability to accomodate the drug within the cell.

Cross-adaptation. We examined the effect of other drugs that induce resistance in *M. racemosus* on the induction of trichodermin esterase activity. Table 2 lists the esterase activity of cells adapted to various drugs. Cycloheximide-adapted cells, which are also resistant to trichodermin (15), had a high level of trichodermin esterase activity. On the other hand, amphotericin-adapted cells, which are not resistant to trichodermin, contained uninduced levels of esterase activity.

DISCUSSION

The data reported in this study may provide an explanation for the phenotypic resistance to trichodermin exhibited

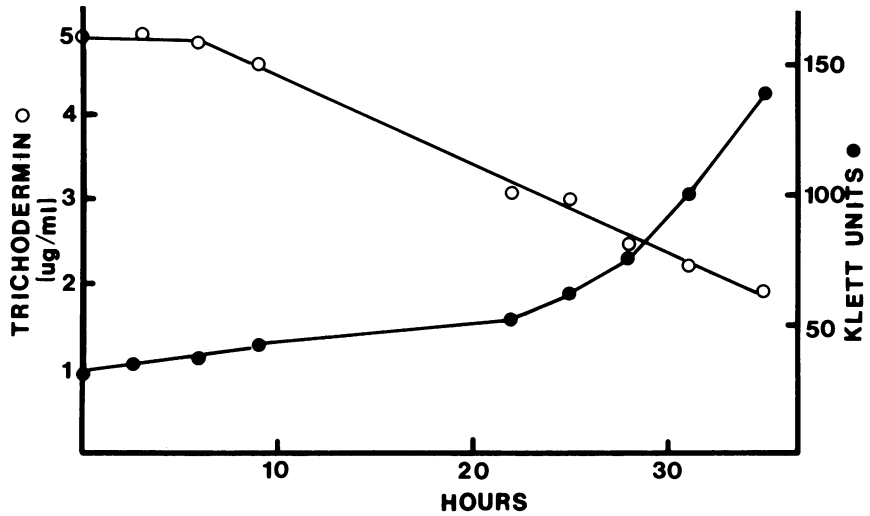

FIG. 6. Loss of trichodermin during adaptation. [^{14}C]trichodermin was added to exponentially growing cells at 0 h. At the indicated times the amount of radioactivity remaining in the medium was determined (○). The growth of the cells was monitored with a Klett-Summerson colorimeter (●).

TABLE 2. Esterase activity in nonadapted and adapted cells

Adapting drug	Esterase activity[a]
None	0.824
Amphotericin B	0.818
Cycloheximide	3.98
Trichodermin	2.86

[a] Activities are expressed as nanomoles of trichodermin deacetylated per minute per milligram of protein.

by *M. racemosus*. Central to the resistance mechanism is the ability of these cells to deacetylate trichodermin to the parent alcohol trichodermol. The deacetylation of trichodermin was demonstrated indirectly by the incorporation pattern produced from [^{14}C]trichodermin which coincided with the pattern of acetic acid incorporation (Fig. 3). Direct evidence was provided by the appearance of trichodermol in the medium of cells incubated with trichodermin (Fig. 4) and by the ability of cell extracts to convert trichodermin to trichodermol. The ability to deacetylate trichothecene derivatives is not unique to *M. racemosus*. The C-4 deacetylation of the trichothecane T-2 toxin to HT-2 toxin, analogous to the deacetylation of trichodermin to trichodermol, has been demonstrated for *Fusarium nivale* and *Fusarium solani* (26), rumen protozoa and bacteria (13), and a number of mammalian species (19). In mammalian species this transformation occurs primarily in the liver by nonspecific carboxyesterase (7, 19, 20). It is interesting, however, that *Mucor mucedo* apparently does not deacetylate the trichothecene anguidine at C-4 but, rather, acetylates the compound at C-3 (5).

The value of deacetylating trichodermin became apparent when we attempted to inhibit the growth of *M. racemosus* with trichodermol. We found only a modest reduction in growth rate, even at concentrations severalfold higher than the trichodermin concentration used in these studies (unpublished data). This is consistent with published observations indicating that trichodermol is a less effective inhibitor of protein synthesis than trichodermin (3, 11, 25) and has a lower binding affinity for ribosomes (24).

It is not, however, the ability to deacetylate trichodermin per se that effects resistance but, rather, the rate at which the cells deacetylate the drug. This is apparent from the observation that both nonadapted and adapted cells can convert trichodermin to trichodermol but differ in the rate of the conversion. The importance of the induced level of esterase activity to adaptation can be inferred from the results shown in Fig. 2. Trichodermin was detectable in nonadapted cells but could not be demonstrated in adapted cells. That is not to say that trichodermin is not present in adapted cells; however, we suggest that because of the increased esterase activity, the intracellular concentration of trichodermin is maintained at noninhibitory or only slightly inhibitory levels. In support of this notion is the observation that adapted cells initiated growth, even though trichodermin was still present in the medium (Fig. 6). Thus, adaptation is not merely the result of the complete conversion of trichodermin to the ineffectual trichodermol, which even nonadapted cells would eventually achieve, but to the increased intracellular level of the esterase.

Although adaptation can be accounted for on the basis of deacetylation, we cannot rule out the possibility that other ancillary alterations are occurring which facilitate growth in the presence of trichodermin or trichodermol. This is particularly true in light of the fact that trichothecenes may have other modes of action, in addition to inhibition of protein synthesis (4, 23). The isolation of esterase mutants should allow us to approach this question.

The more intriguing question, however, remains unanswered. How is cross-adaptation effected? Trichodermin induces resistance to both cycloheximide and amphotericin B (15). In seeking a common mechanism of resistance, it is not clear how induced esterase activity could also provide resistance to cycloheximide and amphotericin B since neither of these compounds contains ester groups. They do, however, contain hydroxyl groups which can be acylated. Carboxylesterases are capable of *trans*-esterification (14), and fungal acetylation of cycloheximide has been reported (12). But there need not be a common resistance mechanism. Cross-adaptation may result from the simultaneous induction of multiple resistance mechanisms. What may be common to all three drugs is that each is inactivated or kept at low intracellular levels by one or more modifications of the drug. Compounds such as trichodermin and cycloheximide may induce a broad range of specific and nonspecific enzymes capable of modification of diverse structural groups and compounds. Indeed, this is precisely the capability that is reflected by the well-documented proficiency of fungi in metabolizing numerous, diverse xenobiotics, and pesticides (1). This may be of clinical significance since many patients being treated for fungal infections are simultaneously receiving chemotherapy for other clinical problems, and these drugs may inadvertently induce fungal resistance to the antimycotic agent.

LITERATURE CITED

1. **Bollag, J. M.** 1982. Microbial transformations of pesticides, p. 125–168. *In* J. P. Rosazza (ed.), Microbial transformations of bioactive compounds. CRC Press, New York.
2. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72**:248–254.
3. **Carrasco, L., M. Barbacid, and D. Vazquez.** 1973. The trichodermin group of antibiotics, inhibitors of peptide bond formation by eukaryotic ribosomes. Biochim. Biophys. Acta **312**:368–376.
4. **Chiba, J., N. Nakano, N. Morooka, S. Makazawa, and Y. Watanabe.** 1972. Inhibitory effects of fusarenon-x, a sesquiterpene mycotoxin, on lipid synthesis and phosphate uptake in *Tetrahymena pyriformis*. Jpn. J. Med. Sci. Biol. **25**:291–296.
5. **Claridge, C. A., and H. Schmitz.** 1978. Microbial and chemical transformations of some 12,13-epoxytrichothec-9,10-enes. Appl. Environ. Microbiol. **36**:63–67.
6. **Davies, M. E., and C. P. Exworth.** 1973. Transient inhibition by cycloheximide of protein synthesis in cultured plant cell suspensions: a dose response paradox. Biochem. Biophys. Res. Commun. **50**:1075–1080.
7. **Ellison, R. A., and F. N. Kotsonis.** 1974. In vitro metabolism of T-2 toxin. Appl. Microbiol. **27**:423–424.
8. **Frankel, J.** 1970. An analysis of the recovery of *Tetrahymena* from effects of cycloheximide. J. Cell Physiol. **76**:55–64.
9. **Godtfredsen, W. O., and S. Vangedal.** 1965. Trichodermin, a sesquiterpene antibiotic. Acta Chem. Scand. **19**:1088–1102.
10. **Gorman, J. A.** 1977. Adaptation to trichodermin and anisomycin in *Physarum polycephalum*. J. Cell Physiol. **92**:447–456.
11. **Hernandez, F., and M. Cannon.** 1982. Inhibition of protein synthesis in *Saccharomyces cerevisiae* by the 12,13-epoxytrichothecens trichodermol, diacetoxyscirpenol and verrucarin A. Reversibility of the effects. J. Antibiot. **35**:875–881.
12. **Howe, R., and R. Moore.** 1968. Acetylation of cycloheximide by *Cunninghamella blakesleeana*. Experientia **24**:904.
13. **Kiessling, K., H. Pettersson, K. Sandholm, and M. Olsen.** 1984. Metabolism of aflatoxin, ochratoxin, zearalenone, and three trichothecenes by intact rumen fluid, rumen protozoa, and

rumen bacteria. Appl. Environ. Microbiol. **47**:1070–1073.
14. **Krisch, K.** 1971. Carboxylic ester hydrolases, p. 43–69. *In* P. D. Boyer (ed.), The enzymes. Academic Press, Inc., New York.
15. **Leathers, T. D., and P. S. Sypherd.** 1985. Inducible phenotypic multidrug resistance in the fungus *Mucor racemosus*. Antimicrob. Agents Chemother. **27**:892–896.
16. **Lowry, O., N. Rosebrough, A. Farr, and R. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
17. **Moyed, H. S.** 1964. Biochemicl mechanisms of drug resistance. Annu. Rev. Microbiol. **18**:347–366.
18. **Notario, V., E. F. Gale, D. Kerridge, and F. Wayman.** 1982. Phenotypic resistance to amphotericin B in *Candida albicans*: relationship to glucan metabolism. J. Gen. Microbiol. **128**: 761–777.
19. **Ohta, M., K. Ishii, and Y. Ueno.** 1977. Metabolism of trichothecene mycotoxins. I. Microsomal deacetylation of T-2 toxin in animal tissues. J. Biochem. **82**:1591–1598.
20. **Ohta, M., H. Matsumoto, K. Ishii, and Y. Ueno.** 1978. Metabolism of trichothecene mycotoxins. II. Substrate specificity of microsomal deacetylation of trichothecenes. J. Biochem. **84**: 697–706.
21. **Pathre, S. V., and C. J. Mirocha.** 1977. Assay methods for trichothecenes and review of their natural occurrence, p. 229–253. *In* J. V. Rodricks, C. W. Hesseltine, and M. A. Mehlman (ed.), Mycotoxins in human and animal health. Pathotox Publishing, Inc., Park Forest, Ill.
22. **Scott, P. M., J. W. Lawrence, and W. van Walbeek.** 1970. Detection of mycotoxins by thin-layer chromatography: application to screening of fungal extracts. Appl. Microbiol. **20**: 839–842.
23. **Ueno, Y.** 1977. Mode of action of trichothecenes. Pure Appl. Chem. **49**:1737–1745.
24. **Wei, C., I. M. Campbell, C. S. McLughlin, and M. H. Vaughan.** 1974. Binding of trichodermin to mammalian ribosomes and its inhibition by other 12,13-epoxytrichothecenes. Mol. Cell. Biochem. **3**:215–219.
25. **Wei, C., and C. S. McLaughlin.** 1974. Structure-function relationship in the 12,13-epoxytrichothecenes. Novel inhibitors of protein synthesis. Biochem. Biophys. Res. Commun. **57**: 838–844.
26. **Yoshizawa, T., and N. Morooka.** 1975. Comparative studies on microbial and chemical modifications of trichothecene mycotoxins. Appl. Microbiol. **30**:38–43.