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MICROBIAL TRANSFORMATION OF ZEARALENONE, I. FORMATION OF ZEARALENONE-4-*O*-β-GLUCOSIDE

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Zearalenone is a natural mycotoxin produced by a number of *Fusarium* species, particularly *F. roseum* (*graminearum*) and *F. tricinctum* (1-4). This compound has been associated with a hyperestrogenic syndrome causing serious problems when fed to many classes of livestock (6,7). The diseased animals show signs of genital disorders involving vulvovaginitis, edematous uterus, and overian atrophy (7). In addition, it was reported (5-9) that zearalenone and some of its derivatives have anabolic effects and are currently used as growth promoters. The screening of 150 microorganisms for transformation of zearalenone showed several metabolites including α- and β-zearalenone, and β-zearalanol. The present communication describes studies related to the bioconversion of zearalenone to zearalenone glucoside by *Thamnidium elegans* and *Mucor bainieri*.

Small-scale experiments with 150 fungi showed that only two cultures produced a unique metabolite that had a low Rf value that was obtained in considerable yields by *T. elegans* (60%) and *M. baineiri* (30%). Large-scale fermentations with *T. elegans* were carried out to obtain sufficient quantities of the metabolite for structure elucidation. The metabolite was identified as zearalenone-4-*O*-β-glucoside on the basis of ir, nmr, and mass spectral analysis.

After this report was submitted for publication, Kamimura (10) reported the structure of zearalenone-4-*O*-β-glucoside produced through microbial conversion of zearalenone by a species of *Rhizopus*, and the spectral data supporting the structure can be found in that reference.

EXPERIMENTAL

Melting points were determined on a Fisher-Jones hot plate apparatus and are uncorrected. Ir spectra were taken with a Perkin-Elmer 281 spectrophotometer using nujol discs. High resolution mass spectra were determined on an LKB 9000 GC mass spectrometer. Nmr were taken in $CDCl_3$ or acetone-d_6 using TMS as an internal standard. ^1H nmr and C^{13} nmr were taken on a 300 MHz Nicolet NT-300-W3 spectrometer.

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Tlc was performed on 0.25 mm Si gel HF-254 plastic plates (E. Merck, Darmstadt), and chromatograms were developed with toluene-MeOH-Me_2CO (5:2:2) or $CHCl_3$-MeOH (9:1). The metabolite was detected under uv light and visualized by spraying with 1% ceric ammonium sulfate reagent [1% $Ce(NH_4)_4(SO_4)_4$ in H_3PO_4] or Fast blue salt reagent (23) then heated with a heat gun. Column chromatography was performed on Si gel 60, 70-230 mesh, and the sample was applied to the column by preadsorption from MeOH solution. A $CHCl_3$/MeOH solvent system was used for elution.

T. elegans NRRL 1613 and *M. bainieri* NRRL 2988 were obtained from the Northern Regional Research Laboratories, Peoria, Illinois, and the cultures were maintained on potato dextrose agar (PDA, BBL) slants and stored at 4°. Preliminary screening for biotransformation products was carried out on a gyratory shaker using the standard two-stage fermentation protocol (24,25) and operating at 250 rpm (27°) in Erlenmeyer flasks (125 ml) containing 25 ml medium consisting of 20 g of glucose, 5 g of yeast extract, 5 g, neopeptone, 5 g NaCl, and 5 g K_2HPO_4 in 1000 ml of H_2O. The media was adjusted to pH 7 before sterilization by autoclaving for 15 min. Stage I cultures were initiated by pipetting an aqueous suspension (spores, mycelia, or both) from a slant of either *T. elegans* or *M. bainieri* into 25 ml media contained in 125-ml Erlenmeyer flasks, incubated for 48 h, then 5 ml of this culture was used to inoculate fresh media to result in stage II cultures. Twenty-four hours later, zearalenone (100 mg dissolved in 0.60 ml of DMF) was distributed equally among 20 flasks. Culture controls consisted of fermentation blanks in which organisms were grown under identical conditions but without the substrate.

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