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Biotransformation of a Fungicide Ethaboxam by Soil Fungus *Cunninghamella elegans*

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Abstract Metabolism of a new fungicide ethaboxam by soil fungi was studied. Among the fungi tested, *Cunninghamella elegans* produced metabolites from ethaboxam, which were not found in the control experiments. M5, a major metabolite from ethaboxam, was firmly identified as *N*-deethylated ethaboxam by LC/MS/MS and NMR. *N*-Deethylated ethaboxam has been found as a single metabolite in *in vitro* metabolism with rat liver microsomes. M1 was proved to be 4-ethyl-2-(ethylamino)-1,3-thiazole-5-carboxamide (ETC) by comparing with the authentic compound. In addition, M2, M3, and M4, and M6 were tentatively identified by LC/MS/MS as hydroxylated and methoxylated ethaboxams, respectively. Production of the major metabolite, *N*-deethylated ethaboxam, by the fungus suggested that *C. elegans* would be an efficient eukaryotic microbial candidate for evaluating xenobiotic-driven mammalian risk assessment.

Key words: Ethaboxam, *Cunninghamella elegans*, metabolism, fungicide

Fungi play an important role in the metabolism of xenobiotic compounds in a soil environmental community [6, 7, 10, 16]. Microbial degradation provides the major means of detoxification of many classes of xenobiotics, such as pesticides. For example, *Rhizoctonia solani* [23], *Chaetomiun globosum* [25], and *Phanerochaete chrysosporium* [9] are able to metabolize the aromatic ring carbon of a herbicide alachlor. Moreover, studies on microbial metabolism have been used successfully as model systems to predict metabolic pathways in humans or to increase the efficiency of drugs used by metabolic activation [8]. A good example is the study of metabolism of naphthalene by *C. elegans*: The metabolism of naphthalene by crude microsomal preparations from *C. elegans* is very similar to the results obtained with hepatic microsomes and reconstituted enzyme systems that contain partially purified preparations of cytochrome P450 (CYP) [2]. Recently, Wang *et al.* [28] cloned and expressed the genes encoding cytochrome P450 from *Cunninghamella elegans*. Immunostaining experiments with three antibodies (CYP1A1, CYP2E1, and CYP3A1) against mammalian P450 enzymes and benzidine staining for hemoproteins showed positive results for the recombinant protein that was expressed in *E. coli*. They found that *C. elegans* CYP are clustered close to the cyp51 family by making a comparison with two other fungal CYPs.

Ethaboxam (*N*-[cyano(2-thienyl)methyl]-4-ethyl-2-(ethylamino)-1,3-thiazole-5-carboxamide) is a new fungicide that was discovered by LG Chemical Ltd., Korea, in 1993 [20]. This thiazole carboxamide was found to be very effective against *Oomycetes*, which are responsible for grape downy mildew (*Plasmoparra viticola*), potato late blight (*Phytophthora infestans*), tomato late blight (*P. infestant*), pepper phytophthora blight (*P. capsici*), cucumber downy mildew (*Pseudoperonospora cubensis*), and lettuce downy mildew (*Bremia lactucae*) by inhibiting mycellial growth after germination and nuclear migration [12, 13]. This fungicide was found to have good protective, curative, systemic, and persistent activity in the growth chamber tests. Furthermore, it was effective against several strains of *P. infestants* and *P. capsici*, which were resistant to a phenylamide fungicide, metalaxyl [12]. Foliar uptake of ethaboxam into cucumber plants was observed as low as 0.1±1.0% at 24 h after being sprayed [30]. Its solubility in water is approximately 12 mg/l, vapor pressure is 1.5×10^{-7}

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mmHg (25°C), and log P (*n*-octanol/water partition coefficient) is 2.86. Its half-life is 89 days at pH 4.0 and 46 days at pH 9.0 at room temperature [12]. No phytotoxicity was observed [13].

In the previous metabolic study in aerobic soil on ethaboxam, four compounds, *N*-[(Z)-amino(2-thienyl) methylidene]-4-ethyl-2-(ethylamino)-1,3-thiazole-5-carboxamide [AMETC], 4-ethyl-2-(ethylamine)-1,3-thiazole-5-carboxylic acid [ETCA], 4-ethyl-2-(ethylamino)-1,3-thiazole-5-carboxamide [ETC], and 4-ethyl-2-(ethylamino)-*N*-(2-thienylcarbonyl)-1,3-thiazole-5-carboxamide [ETTC], were found as metabolites [14]. The present study is the first report on the metabolism of a new fungicide ethaboxam by soil fungus *C. elegans*. This work was performed to elucidate the microbial metabolic pathway of the compound and to evaluate the *C. elegans* microbial model system for the mammalian hepatic microsomal metabolism of pesticides.

MATERIALS AND METHODS

Chemicals

Ethaboxam (purity 98.5%) was kindly provided by LG Chem Investment in Korea. Malt extract, peptone, yeast extract, xylose, lactose, and fructose were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Potato dextrose agar and potato dextrose broth (PDB) were purchased from Difco Laboratories (Detroit, MI, U.S.A.). Solvents used for HPLC analysis were of the HPLC grade (Duksan, Korea). All the other reagents and common chemicals were of reagent grade or better.

Soil Fungi and Culture Conditions

Cunninghamella elegans ATCC 36112, *Cunninghamella verticillata* VKPM F-430, *Mucor ramannianus* R-56, *Aspergillus niger* VKMF-1119, *Aspergillus niger* NRRL 599, *Fusarium moniliforme* 279, *Trichoderma viride* T-58, *Penicillium simplicissimum* KM-16, *Trichosporon cutaneum* ATCC 58094, and *Phanerochaete chrysosporium* BKMF-1767 were all kindly provided by Drs. Cerniglia and Parshikov at National Center for Toxicological Research in U.S. FDA (AR, U.S.A.). Potato dextrose medium was used to grow all of the fungi, except for *Phanerochaete chrysosporium* BKMF-1767 which was grown in the malt extract-peptone-yeast extract (MPY) broth.

Metabolism of Ethaboxam by Soil Fungi

After culture for 5 days at 28°C, the mycelia from a plate was transferred to a sterile blender cup containing 30 ml of sterile water for homogenizing (30 sec). Each portion (2 ml) of the blended mycelial suspension was inoculated to 100 ml of PDB in 250-ml Erlenmeyer flasks. The cultures were incubated at 28°C on a rotary shaker operating at 200 rpm. Ethaboxam (32.5 mg) in *N,N*-dimethylformamide (100 µl) was added to each flask. A culture without ethaboxam and a culture containing only media and ethaboxam in sterile flask were incubated as control experiments. Each culture was run in duplicate. After one week of incubation, fungi cultures (5 ml) were extracted with ethyl acetate (15 ml). The solvent was evaporated *in vacuo*, and the residue was dissolved in methanol (1 ml) and analyzed (20 µl) by HPLC. For further identification of metabolites, aliquots were analyzed by LC/MS/MS and/or NMR (Nuclear Magnetic Resonance Spectroscopy).

Analytical Methods

Ethaboxam and its metabolites were separated on a Zorbax Eclipse XDB C18 column (3.5 µm, 4.6×150 mm, Hewlett-Packard) using Hewlett Packard 1100 series HPLC (CA, U.S.A.). The mobile phase consisted of water and acetonitrile (0.1% acetic acid). The gradient was programmed linearly as follows; 1% acetonitrile at 0 min, 10% acetonitrile at 5 min, 30% acetonitrile at 15 min, 70% acetonitrile at 30 min, and 1% acetonitrile at 40 min. The flow rate was 1 ml/min and UV detection at 310 nm was performed.

LC/MS or LC/MS/MS was carried out by coupling HP 1100 HPLC system to a Quattro LC triple quadruple tandem

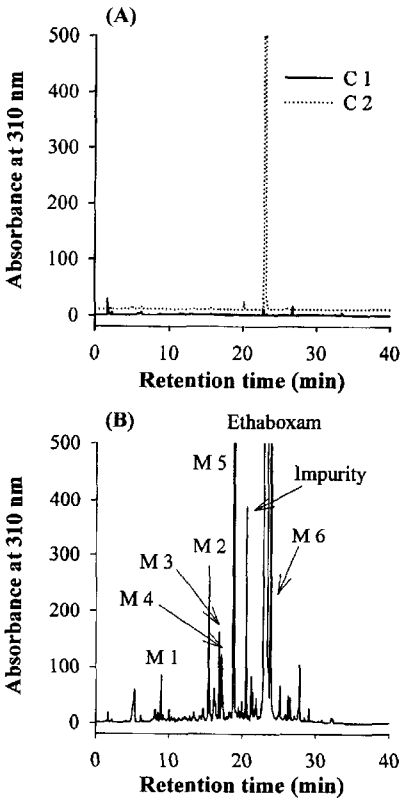


Fig. 1. HPLC elution profile of ethyl acetate-soluble extract of the incubation media.
(A) C1, *C. elegans* and media; C2, ethaboxam and media. (B) *C. elegans*, ethaboxam, and media.

mass spectrometer (Micromass, Manchester, U.K.) with electrospray ionization (ESI^+) mode. The source temperature, desolvation temperature, cone voltage, and capillary voltage were kept at 65°C, 270°C, 35 V, and 3.43 kV, respectively. The nebulizing gas and desolvation gas were ultra pure nitrogen set at 87 and 612 l/h, respectively. CID (collision induced dissociation) was carried out at 50.0 eV using nitrogen as the collision gas.

Structural Investigation of Ethaboxam and Its Metabolite by NMR

NMR measurements were performed on a Bruker Avance 400 spectrometer system (9.4 T) at a temperature of 298 K. The spectra of ^{1}H-NMR, ^{13}C-NMR, DEPT, COSY, HMQC, and HMBC were collected in $CDCl_3$ with TMS as an internal reference. For the ^{1}H-NMR analysis, 64 transients were acquired with a 1 sec relaxation delay by using 32 K data points. The 90° pulse was 9.7 μsec with a spectral width of 1,225 Hz. ^{13}C-NMR and DEPT spectra were obtained for a spectral width of 19,047 Hz, collecting 64 K data points. The 90° pulse was 9.8 μsec. Two-dimensional spectra were acquired with 2,048 data points for t_2 and 256 for t_1 increments. The delay time for long-ranged couplings of HMBC was 70 μsec.

Isolation and Characterization of Metabolite 5 (M5)

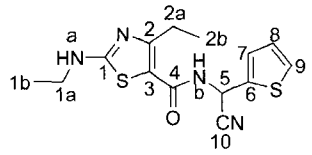
Culture broth (100 ml) was extracted twice with ethyl acetate (200 ml). After the solvent was evaporated *in vacuo*, the residue was dissolved in methanol, and purified with a semi-preparative HPLC equipped with C_{18} column (Alltech Econosphere C18 column, 10×250 mm, 10 μm) by using the mobile phase consisted of water and methanol. The gradient was programmed linearly as follow; 30% methanol at 0 min, 90% methanol at 20 min, 90% methanol at 25 min, and 30% acetonitrile at 30 min. The flow rate was 2 ml/min and UV detection was performed at 310 nm. Major metabolite M5, eluting at 19.1 min, was collected from many injections, pooled, dried, and analyzed by NMR.

Results and Discussion

Metabolism of Ethaboxam by *C. elegans*

Among ten strains of soil fungi tested, only *C. elegans* metabolized ethaboxam. Figure 1 illustrates an HPLC elution profile of the ethyl acetate-extractable compounds formed during a 7-day incubation of ethaboxam with *C. elegans*. Ethaboxam was metabolized to many compounds, including metabolites M1-M6. *C. elegans*, a zygomycete, was isolated for its ability to grow on crude oil as a sole source of carbon and energy [3]. The organism was observed to utilize the alkane fraction for growth. Other alkane-utilizing filamentous fungi and yeasts have been identified [29]. However, *C. elegans* distinguishes itself from the others by its remarkable ability to metabolize numerous structurally diverse compounds, such as polycyclic aromatic hydrocarbons

Table 1. NMR assignments of ethaboxam.



Assignment	of ^{13}C	CHn[a]	of ^{1}H (J_{Hz})[b]	HMBC	COSY
C2b	14.2	q	1.05(t, 7.5)	C2b/H2a	H2b/H2a
C1b	14.9	q	1.10(t, 7.21)	C1b/H1a	H1b/H1a
C2a	25.5	t	2.80(q, 7.5)	C2a/H2b	H2a/H2b
C1a	40.0	t	3.20(m)	C1a/H1b	H1a/H1b, H1a/NHa
C5	41.0	d	6.40(dd, 1.0, 8.3)	C5/H7	H5/H7, H5/Nha
C10	118.7	s	-	C10/H5	-
C8	128.2	d	6.92(dd 3.6, 5.2)	-	H8/H7, H8/H9
C9	128.3	d	7.39(m)	C9/H7	H9/H8, H9/H7
C7	128.4	d	7.16(dd, 1.0, 5.2)	C7/H9	H7/H8, H7/H9, H7/H5
C6	138.9	s	-	C6/H8, C6/H5, C6/H7, C6/H9	-
C3	162.5	s	-	C3/H2a	-
C4	162.6	s	-	C4/H5	-
C2	164.7	s	-	C2/H2a, 2/H2b	-
C1	170.2	s	-	C1/H1a	-
-NHa	-		7.11(m)		-
-NHb	-		7.91(d, 7.95)		-

[a]Data determined by DEPT.
[b]Data determined by HMQC.

(PAHs), drugs, and *N*-, *S*-, *O*-heterocyclic aromatic compound by both phase I and phase II metabolism [18, 24, 27, 31]. Therefore, *C. elegans* has been used as a eukaryotic microbial model of mammalian metabolism [15, 26]. Indeed, the presence of a mammalian type microsomal CYP enzyme system in this fungus for the xenobiotic metabolism has been recognized since the 1970s [29]. M5 in Fig. 1 was observed as a single metabolite in the metabolism of ethaboxam by rat microsomal fraction [17], again suggesting that *C. elegans* would have acted as a model system for mammalian metabolism.

Total Assignment of ^{1}H and ^{13}C Chemical Shift of Ethaboxam

There are 14 peaks in the ^{13}C-NMR spectrum of ethaboxam (Table 1). Two quartet, two triplet, four doublet, and six singlet carbons were observed in the DEPT spectra. Of the four doublet carbons, three (C8, C7, and C9 of thiophenyl group) were determined based on COSY, HMBC, and HMQC to be assigned at 128.2, 128.3, and 128.4 ppm, respectively. The remaining doublet should be C5. The singlet at 118.7 ppm was cyanide carbon. The other five singlet carbons could be assigned by the HMBC analysis. Two quartet and two triplet carbons of two ethyl groups were determined by careful examinations of C1a and C2a: Since C1a was attached to nitrogen directly, it was more downfield shifted than C2a. Therefore, the triplet peak at 40.0 ppm was assigned as C1a. Then, spin-spin couplings

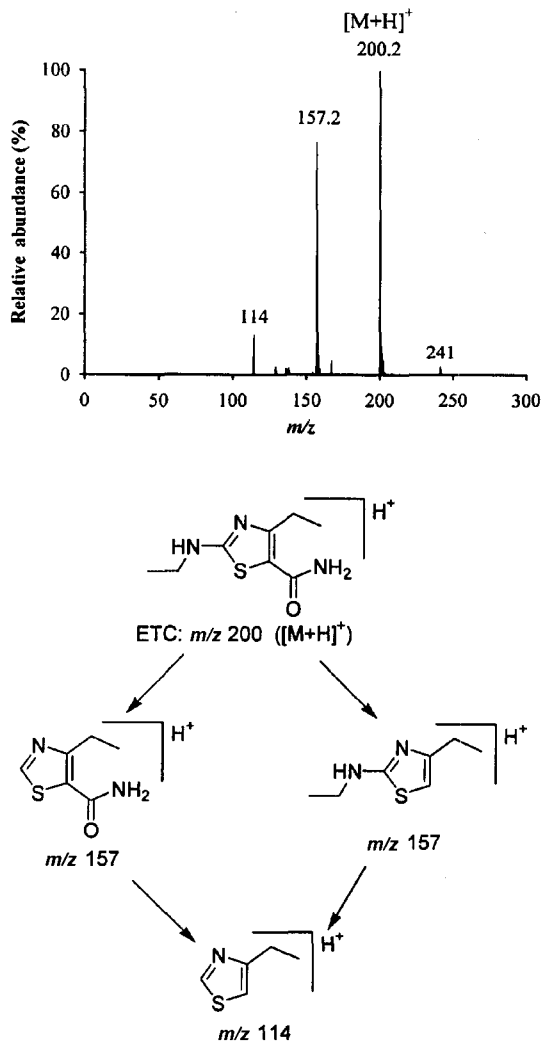


Fig. 2. LC/MS spectrum of M1 and fragmentation pathway by CID.

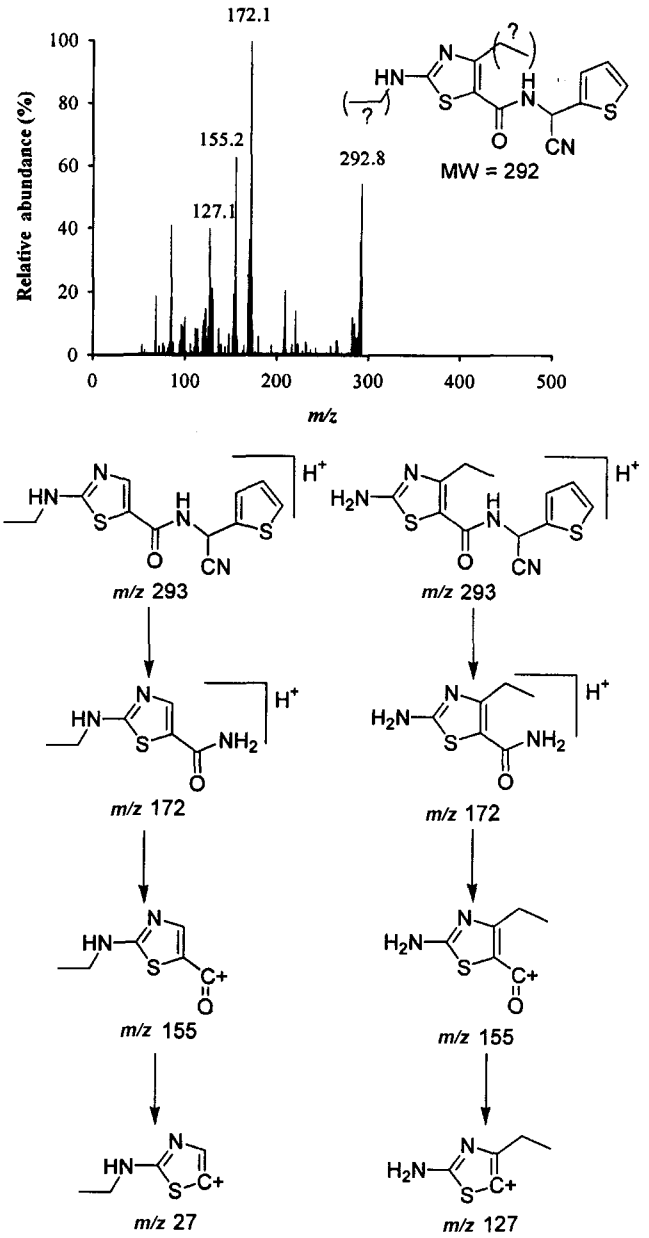


Fig. 3. LC/MS/MS spectrum of M5 and fragmentation pathway by CID.

by COSY gave information regarding the other peaks at 25.5, 14.2, and 14.9 ppm. There are two nitrogen protons in ethaboxam. NHb could be coupled with H5, so that the doublet proton at 7.91 ppm was assigned as NHb.

Identification of Ethaboxam Metabolites

M1 was identified as 4-ethyl-2-(ethylamino)-1,3-thiazole-5-carboxamide (ETC) by matching retention times and mass spectra with the authentic compound. A weak $[M+CH_3CN+H]^+$ solvent adduct ion at *m/z* 241 confirmed $[M+H]^+$ ion (Fig. 2) with other characteristic fragment ions at *m/z* 114 and 157, also supporting the correct structure. This metabolite was also observed previously from the aerobic soil metabolism of ethaboxam [14]. ETC must be an enzymatic hydrolysis product, since it was not observed in the reaction of ethaboxam with culture media used as a control experiment.

The mass spectrum of M5 has a strong solvent adduct $[M+CH_3CN+H]^+$ ion at *m/z* 334 and a base peak *m/z* 293 of $[M+H]^+$ ion, which suggested a deethylated form of ethaboxam (Fig. 3). Fragmentation of *m/z* 293 through CID resulted in characteristic daughter ions at *m/z* 127, 155, and 172, however, location of the position of deethylation could not be identified, since those ions could be produced from both forms of deethylated ethaboxam. Therefore, M5 was fractionated from the culture extract and analyzed by NMR (^{1}H-, ^{13}C-NMR, and DEPT135) for complete structural assignment. On the DEPT spectrum of isolated M5, C1b (14.9 ppm) and C1a (40.9 ppm) of ethaboxam disappeared, while C2a and C2b were still observed (Fig. 4). And multiplet of NHa changed to a singlet due to the loss of coupled ethyl group protons. These results indicate that M5 is *N*-deethylated ethaboxam. Such metabolic reactions were observed with various *N*-containing pesticides including dinitroaniline and triazine herbicides, and it appears to be formed through hydroxylation of *N*-methylene group by mixed-function oxidase (mfo, Fig. 6) [5, 21, 22].

M2, M3, and M4 showed a protonated molecular ion $[M+H]^+$ at *m/z* 337, with a characteristic fragment ion at *m/z* 319, suggesting a loss of hydroxyl group. Fragmentation of *m/z* 337 through CID gave characteristic ions at *m/z* 155, 183, 200, and 319, similar to those of M6. The structure of these metabolites was suggested to be hydroxylated ethaboxam (Fig. 5). This ring hydroxylation reaction by *C. elegans* has been observed in the metabolism of pyrene [4] and benzopyrene [1, 18], catalyzed by cytochrome P450 monooxygenase and epoxide hydroxylase reactions.

M6 showed a protonated molecular ion $[M+H]^+$ at *m/z* 350, with a weak solvent adduct $[M+CH_3CN+H]^+$ ion at *m/z* 392. A characteristic fragment ion at *m/z* 319 suggested a loss of methoxy group and fragmentation of *m/z* 350 through CID that gave characteristic daughter ions of methoxylated ethaboxam at *m/z* 155, 183, 200, and 319, which were already observed for M2, M3, and M4 (Fig. 5). The structure of M6 was suggested as methoxylated ethaboxam. This kind of methylation reaction catalyzed by methyl transferase has previously been reported in the metabolism of pentachlorophenol and chlorophenoxyacetic acid [11]. However, location of the position of hydroxylation or methoxylation still remains unresolved. Taken together, *C.*

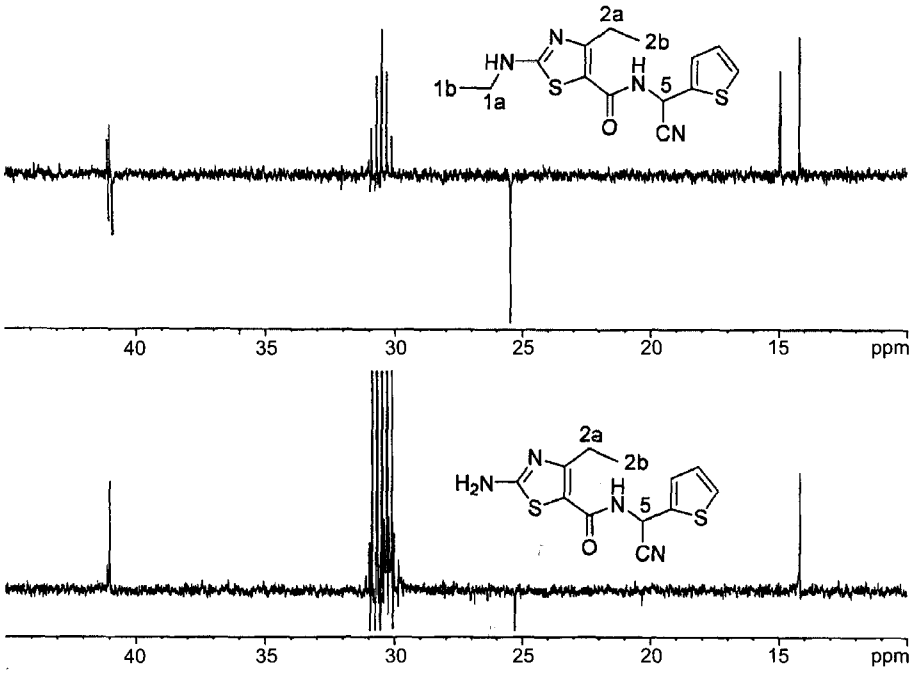


Fig. 4. DEPT spectra of ethaboxam (A) and M5 (B) (0–55 ppm).

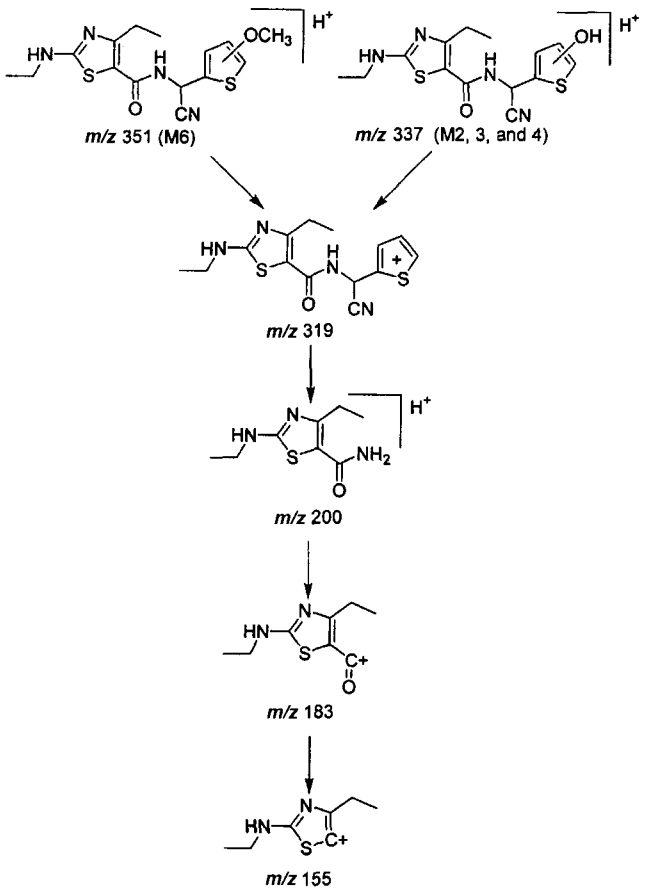


Fig. 5. Mass fragmentation scheme for M2, 3, 4, and M6.

elegans showed a diverse metabolic activity to biotransform a fungicide ethaboxam (Fig. 6). Considering the similarity to the metabolism of mammalian hepatic microsome, *C. elegans* could be an efficient microbial candidate to quickly deduce the mammalian metabolism for the xenobiotic compounds.

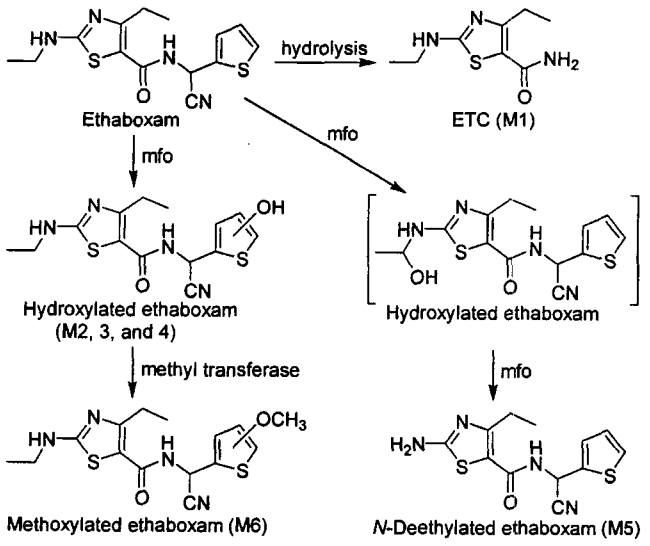


Fig. 6. Proposed biotransformation pathway of ethaboxam by soil fungus *C. elegans*.

Acknowledgments

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REFERENCES

1. Cerniglia, C. E. 1992. Biodegradation of polycyclic aromatic hydrocarbons. *Biodegradation* **3:** 351–368.
2. Cerniglia, C. E. and D. T. Gibson. 1978. Metabolism of naphthalene by cell extracts of *Cunninghamella elegans*. *Arch. Biochem. Biophy.* **186:** 121–127.
3. Cerniglia, C. E. and J. J. Perry. 1972. Crude oil degradation by microorganisms isolated from the marine environment. *Z. Allg. Mikrobiol.* **13:** 299–306.
4. Cerniglia, C. E., D. W. Kelly, J. P. Freeman, and D. W. Miller. 1986. Microbial metabolism of pyrene. *Chem. Biol. Interact.* **57:** 203–216.
5. Cha, C. J., D. R. Doerge, and C. E. Cerniglia. 2001. Biotransformation of malachite green by the fungus *Cunninghamella elegans*. *Appl. Environ. Microbiol.* **67:** 4358–4360.
6. Chung, N., I. S. Lee, H. S. Song, and W. G. Bang. 2000. Mechanisms used by white-rot fungus to degrade lignin and toxic chemicals. *J. Microbiol. Biotechnol.* **10:** 737–752.
7. Chung, N., G. Kang, G. H. Kim, I. S. Lee, and W. G. Bang. 2001. Effect of nutrient nitrogen on the degradation of pentachlorophenol by white rot fungus, *Phanerochaete chrysosporium*. *J. Microbiol. Biotechnol.* **11:** 704–708.
8. El Sayed, K. A., A. F. Halim, A. M. Zagholul, D. C. Dunbar, and J. D. McChesney. 2000. Transformation of jervine by *Cunninghamella elegans* ATCC 9245. *Phytochemistry* **55:** 19–22.
9. Ferrey, M. L., W. C. Koskinen, R. A. Blanchette, and T. A. Burnes. 1994. Mineralization of alachlor by lignin-degrading fungi. *Can. J. Microbiol.* **40:** 795–798.
10. Han, D. W., H. Suh, D. H. Lee, B. J. Park, K. Takatori, and J. C. Park. 2002. Detection of oleic acid biodegradation by fungi. *J. Microbiol. Biotechnol.* **12:** 514–517.
11. Hall, J. C., J. S. Wickenden, and K. Y. F. Yau. 2001. Biochemical conjugation of pesticides in plants and microorganisms: An overview of similarities and divergence, pp. 89–118. *In* J. C. Hall, R. E. Hoagland, and R. M. Zablotowicz (eds.), *Pesticide Biotransformation in Plants and Microorganisms*, ACS symposium series 777. American Chemical Society, Washington, DC, U.S.A.

12. Kim, D. S. 1997. Biological activity of LGC-30473: A novel oomycetes fungicide. *In*: *Proceedings of International Symposium on Fungicide Resistance and Development of New Fungicides*, pp. 7–64. Working Group on Fungicide Resistance, Korea.
13. Kim, D. S., H. C. Park, S. J. Chun, S. H. Yu, K. J. Choi, J. H. Oh, K. H. Shin, Y. J. Koh, B. S. Kim, Y. I. Hahm, and B. K. Chung. 1999. Field performance of a new fungicide ethaboxam against cucumber downy mildew, potato late blight and pepper phytophthora blight in Korea. *Plant Pathol. J.* **15:** 48–52.
14. Lee, Y. S. 1999. Aerobic soil metabolism of ethaboxam. MSc Thesis, Seoul National University, Seoul, Korea.
15. Moody, J. D., D. Zhang, T. M. Heinze, and C. E. Cerniglia. 2000. Transformation of amoxapine by *Cunninghamella elegans*. *Appl. Environ. Microbiol.* **66:** 3646–3649.
16. Oh, K. B., W. Mar, and I. M. Chang. 2001. Biodegradation of hydrocarbons by an organic solvent-tolerant fungus, *Cladosporium resinae* NK-1. *J. Microbiol. Biotechnol.* **11:** 56–60.
17. Park, M. K., H. G. Hur, K. H. Liu, Y. H. Lee, Y. S. Lee, and J. H. Kim. 2002. *In vitro* metabolism of ethaboxam by rat liver microsomes. *Agric. Chem. Biotechnol.* **45:** 94–98.
18. Pothuluri, J. V., F. E. Evans, T. M. Heinze, and C. E. Cerniglia. 1996. Formation of sulfate and glucoside conjugates of benzo[e]pyrene by *Cunninghamella elegans*. *Appl. Microbiol. Biotechnol.* **45:** 677–683.
19. Pothuluri, J. V., J. P. Freeman, and C. E. Cerniglia. 1999. Biotransformation of 1-nitrobenzo[e]pyrene by the fungus *Cunninghamella elegans*. *J. Ind. Microbiol. Biotechnol.* **22:** 52–58.
20. Ra, C. S., Y. S. Rew, and W. B. Cho. 1995. Synthesis and fungicidal activity of novel 2-aminothiazole carboxamide derivatives. *Korean J. Med. Chem.* **5:** 72–75.
21. Roberts, T. R. and D. H. Hutson. 1998. *Metabolic Pathways of Agrochemicals, Part 1: Herbicides and Plant Growth Regulators*, pp. 243–290, 605–609, and 623–656. The Royal Society of Chemistry, Cornwall, U.K.
22. Roberts, T. R. and D. H. Hutson. 1999. *Metabolic Pathways of Agrochemicals, Part 2: Insecticides and Fungicides*, pp. 451–454. The Royal Society of Chemistry, Cornwall, U.K.
23. Smith, A. E. and D. V. Phillips. 1975. Degradation of alachlor by *Rhizoctonia solani*. *Agronomy J.* **67:** 347–349.
24. Sutherland, J. B., J. P. Freeman, A. J. Williams, and J. Deck. 1999. Biotransformation of phthalazine by *Fusarium moniliforme* and *Cunninghamella elegans*. *Miologia* **91:** 114–116.
25. Tiedje, J. M. and M. L. Hagedorn. 1975. Degradation of alachlor by a soil fungus, *Chaetomium globosum*. *J. Agric. Food Chem.* **23:** 77–81.
26. Wackett, L. P. and D. T. Gibson. 1982. Metabolism of xenobiotic compounds by enzymes in cell extracts of the fungus *Cunninghamella elegans*. *Biochem. J.* **205:** 117–122.
27. Wackett, L. P. and P. C. D. Hersberger. 2001. *Biocatalysis and Biodegradation*, pp. 39–69. ASM Press, Washington, DC, U.S.A.
28. Wang, R. F., W. W. Cao, A. A. Khan, and C. E. Cerniglia. 2000. Cloning, sequencing, and expression in *Escherichia coli* of a cytochrome P450 gene from *Cunninghamella elegans*. *FEMS Microbiol. Lett.* **188:** 55–61.
29. Yadav, J. S. and J. C. Loper. 2000. Cloning and characterization of the cytochrome P450 oxidoreductase gene from the zygomycetes fungus *Cunninghamella*. *Biochem. Biophy. Res. Comm.* **268:** 345–353.
30. Yu, J. H., H. K. Park, K. Y. Cho, and J. H. Kim. 2001. Evaluation of foliar uptake of eight fungicides using a new measuring tool, congo red method. *Agric. Chem. Biotechnol.* **44:** 27–31.
31. Zhang, D., F. E. Evans, J. P. Freeman, Y. Yang, J. Deck, and C. E. Cerniglia. 1996. Formation of mammalian metabolites of cyclobenzaprine by the fungus, *Cunninghamella elegans*. *Chem. Biol. Interact.* **102:** 79–92.