Biotransformations of Fenoxaprop-ethyl by Fluorescent *Pseudomonas* Strains

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Fenoxaprop-ethyl (FE) transformation by pure cultures of four *Pseudomonas* strains was studied using ^{14}C-labeled herbicide, labeled in either the dioxyphenyl (DOP) or the chlorophenyl (CP) ring. Resting cells rapidly hydrolyzed FE to fenoxaprop acid (FA), but cleavage of the ether bond proceeded slowly. Degradation of FE by *P. fluorescens* strains RA-2 and UA5-40 cultured in tryptic soy broth (TSB) or minimal media with glucose (MMG) or propionate (MMP) was assessed. TSB cultures completely hydrolyzed FE to FA within 5 days. Polar metabolites (predominantly glycylcysteine and cysteine conjugates arising from FE or FA), an unidentified metabolite, and 6-chloro-2,3-dihydrobenzoxazol-2-one (CDHB) accumulated in TSB cultures treated with ^{14}C-CP-labeled FE during an 11-day study, whereas 2-(4-hydroxyphenoxy)propionic acid accumulated in ^{14}C-DOP-labeled FE−TSB cultures. FE transformation by MMP cultures was limited to ester hydrolysis of FE to FA. Hydrolysis of FE to FA was never detected in RA-2 MMG cultures and was low in UA5-40 MMG cultures. Cleavage of the benzoxazolyloxyphenyl ether bond occurred in MMG cultures of both strains; that is, 50% of ^{14}C-CP-labeled FE was recovered as CDHB, and hydroquinone accumulated in MMG ^{14}C-DOP-labeled FE cultures. No mineralization of FE to CO_2 was observed, regardless of the ^{14}C label or growth media used as substrate. Strains *of P. fluorescens* can promote significant cometabolic transformations of FE and may contribute to the dissipation of FE in the environment.

Keywords: *Herbicide; biodegradation; pseudomonad; microbial metabolism; aryloxyphenoxy propionate*

INTRODUCTION

Fenoxaprop-ethyl [(±)-ethyl 2-[4-[(6-chloro-2-benzoxazolyl)oxy]phenoxy]propanoate] (FE) is commonly used for postemergence control of various annual grass weeds in soybeans, rice, wheat, and turf (WSSA, 1994). The metabolism of FE in various plant species has been studied, and its degradation pathway is relatively well-defined (Hall and Stephenson, 1995; Yaacoby et al., 1991; Hoagland et al., 1996; Edwards and Cole, 1996). Also, the fate of FE has been studied in soils from several geographical areas (Köcher et al., 1982; Smith and Aubin, 1990; Toole and Crosby, 1989). In these studies, FE was rapidly hydrolyzed to fenoxaprop-acid (FA). This hydrolysis occurred more rapidly in moist nonsterile soils, suggesting microbial degradation (Köcher et al., 1982; Smith and Aubin, 1990). The metabolite FA is phytotoxic and can inhibit seed germination of various grass weeds (Bieringer et al., 1982). FA undergoes further degradation in soils, forming metabolites such as 6-chloro-2,3-dihydrobenzoxazol-2-one (CDHB), 4-[(6-chloro-2-benzoxazolyl)oxy]phenetole, and 4-[(6-chloro-2-benzoxazolyl)oxy]phenol (Smith, 1985). Studies using ^{14}C-labeled FE showed that some metabolites are incorporated into soil organic matter and microbial biomass, with some mineralization to CO_2 (Köcher et al., 1982; Smith and Aubin, 1990). FE is also subject to photodegradation yielding CDHB, 2-(4-hydroxyphenoxy)propanoic acid (HPP), and ethyl 2-(4-hydroxyphenoxy)propanoate (Toole and Crosby, 1989).

Very limited information exists on specific microorganisms that are capable of transforming FE in soils. Studies using a mixed microbial consortium showed that FE can be utilized as a sole carbon and nitrogen source (Gennari et al., 1995). FA and CDHB were the metabolites identified in these studies. Among bacteria, the genus *Pseudomonas* has a wide spectrum of metabolic and cometabolic transformation capability for various xenobiotics, including herbicides (Häggbloom, 1992; Houghton and Shanley, 1994). Previous research from our laboratory has indicated the potential for cleavage of the ether linkage of the diphenyl ether herbicides fluorodifen [2,4′-dinitro-4-(trifluoromethyl)diphenyl ether] (Zablotowicz et al., 1994) and acifluorfen [5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoic acid] (Zablotowicz et al., 1997) by strains of such bacteria. We hypothesized that glutathione conjugation might also be a mechanism for bacterial transformation of FE, especially among fluorescent pseudomonads. Strains of *P. fluorescens* and *P. putida* also possess esterase activity on diverse substrates, for example, *p*-nitrophenyl butyrate and fluorescein diacetate (Zablotowicz et al., 1996). Our objectives in these studies were to evaluate pure cultures of fluorescent pseudomonad strains and their cell-free enzyme preparations for ability to degrade FE. We also sought to assess the interaction of nutrition and growth status on FE transformation.

MATERIALS AND METHODS

Bacterial Strains. *P. fluorescens* strains BD4-13, RA-2, and UA5-40 and *P. putida* strain M-17 have been previously described (Zablotowicz et al., 1995; Hoagland and Zablotowicz,

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Table 1. R_f Values for FE and Metabolites in Five TLC Solvent Systems

	solvent systems				
compound[a]	(A) toluene/ethyl acetate/ acetic acid/water, 50:50:1:0.5 (v/v/v/v)	(B) chloroform/ methanol/water, 65:25:1 (v/v/v)	(C) butanol/acetic acid/water, 12:3:5 (v/v/v)	(D) butanone/acetic acid/water, 10:1:1 (v/v/v)	(E) benzene/acetone, 10:1 (v/v)
FE	0.94	0.98	0.98	0.97	0.89
FA	0.14	0.53	0.83	0.97	0.00
CDHB	0.55	0.98	0.93	0.93	0.48
HPP	0.14	0.24	0.81	nd[b]	0.09
HQ	0.68	0.86	nd	nd	nd
CDHB−GSH	0.00	nd	0.39	0.08	0.00
CDHB−GlyCys	0.00	nd	0.51	0.39	nd
CDHB−Cys	0.00	nd	0.69	0.62	nd

[a] FE, fenoxaprop-ethyl; FA, fenoxaprop acid; CDHB, 6-chloro-2,3-dihydrobenzoxazol-2-one; HPP, 2-(4-hydroxyphenoxy)propionate; HQ, hydroquinone; CDHB−GSH, *S*-(6-chlorobenzoxazole-2-yl)glutathione; CDHB−GlyCys, *S*-(6-chlorobenzoxazole-2-yl)glycylcysteine; CDHB−Cys, *S*-(6-chlorobenzoxazole-2-yl)cysteine. [b] nd, not determined.

1995). These cultures were maintained as frozen glycerol and tryptic soy agar stocks. Prior to use in these experiments, subcultures of these stocks were assessed for purity on selective media (Gould et al., 1985) and dilute tryptic soy agar.

Chemicals. All chemicals and solvents were of analytical grade or higher purity. FE, ^{14}C-labeled dioxyphenyl, and chlorophenyl (sp. act. = 997.2 and 1107 MBq g^{-1}, respectively) were supplied by AgrEvo, Frankfurt, Germany. The high-purity unlabeled metabolite standards of FA (HOE 088406), CDHB (HOE 054014), and HPP (HOE 096918) were also provided by AgrEvo. These products were used without further purification. Glutathione (GSH), glycylcysteine (Gly-Cys), cysteine (Cys), and equine glutathione *S*-transferase (GST) were obtained from Sigma Chemical Co. (St. Louis, MO). Conjugates derived from FE or FA [the GSH conjugate [*S*-(6-chlorobenzoxazole-2-yl)glutathione = CDHB−GSH], the Gly-Cys conjugate [*S*-(6-chlorobenzoxazole-2-yl)glycylcysteine = CDHB−GlyCys], and the Cys conjugate [*S*-(6-chlorobenzoxazole-2-yl)cysteine = CDHB−Cys]] were produced via alkaline-mediated synthesis as described elsewhere (Breaux et al., 1987). The glutathione conjugate was also synthesized enzymatically using equine GST.

Analysis. Thin-layer chromatography (TLC) was used for analysis of FE and metabolites. In TLC analysis, aliquots (10−50 μL) were mechanically spotted (40 °C) with a multispotter (Analytical Instruments, Inc., Baltimore, MD) on silica gel plates with fluorescent indicator (20 × 20 cm, 250 μm; Whatman, Clifton, NJ). Plates were developed 10 cm in several solvent systems using Camag twin-trough chambers (Camag Scientific Inc., Wilmington, NC). R_f values for parent herbicide and metabolites in five solvent systems are summarized in Table 1. Resolution of metabolites by TLC analysis of ^{14}C-DOP-labeled FE was superior in the chloroform/methanol/water (65:25:1, v/v/v) solvent (B); that is, there was better distinction between FA and HPP, and the zone for hydroquinone (HQ) was more clearly defined. Radiological TLC (RAD-TLC) chromatograms were analyzed and quantitated by integration of peak areas using a Bioscan System 200 imaging scanner (Bioscan, Washington, DC).

Experiment I: Fenoxaprop-ethyl Transformations by *Pseudomonas* Cell Suspensions and Cell-Free Extracts. The bacterial strains were grown on tryptic soy broth (TSB) for 48 h, and cells were harvested by centrifugation (10 min, 6000*g*, 10 °C). The cells were washed three times in potassium phosphate buffer (KP_i, 50 mM, pH 7.6) and resuspended in KP_i to a final optical density (OD) of 8.0 at 660 nm. Cell suspensions (2.0 mL; three replicates) were placed in sterile 25 mL Corex centrifuge tubes and treated with a methanol solution of either ^{14}C-CP- or ^{14}C-DOP-labeled FE to attain a final concentration of 9.0 μM (i.e., 3.2 or 3.6 kBq mL^{-1}, respectively). Cells were incubated on a rotary shaker (100 rpm, 30 °C). Aliquots were removed at various times, and the reactions were terminated by addition of 3 volumes of acetone. Cells were disrupted and extracted in a sonication bath (Branson model 5200, Branson Ultrasonics Corp, Danbury, CT; 20 min, 30 °C). The acetone extracts were clarified by centrifugation (14000*g*, 10 min). Recovery and analysis of FE and metabolites were achieved by RAD-TLC using a toluene/ethyl acetate/acetic acid/water (50:50:1:0.5, v/v/v/v) solvent system (A) (Tal et al., 1993). An additional study was conducted with cell suspensions of UA5-40 incubated with either 9.0 or 45.0 μM ^{14}C-CP-labeled FE (3.6 and 18.0 kBq mL^{-1}). Cells were incubated as above for 92 h. Studies were also performed using crude cell-free extracts (CFE) of *P. fluorescens* strains RA-2 and UA5-40. CFEs were prepared from washed cells that were disrupted by sonication and from which structural cell wall material had been removed by centrifugation (22000*g*, 20 min). This yielded extracts of ~5 mg mL^{-1} protein in 50 mM KP_i. CFEs (400 μL) were treated with ^{14}C-CP labeled FE, 0.45 nmol with or without 1.0 μmol of GSH (in KP_i). Final concentrations of 4 mg mL^{-1} protein, 9.0 μM FE, and 0 or 2 mM GSH in a final volume of 0.5 mL were incubated at 100 rpm at 30 °C. Aliquots were removed at 2.5 and 5 h, terminated (3 volumes of acetone), and analyzed using the butanol/acetic acid/water (12:3:5, v/v/v) and butanone/acetic acid/water (10:1:1, v/v/v) solvent systems (C and D, respectively), as well as solvent system A (see Table 1).

Experiment II: Degradation of FE by *P. fluorescens* Strains Grown under Various Nutrient Regimes. *P. fluorescens* strains RA-2 and UA5-40 were evaluated for growth on potential metabolites of FE: CDHB, ethanol, HPP, HQ, and propionate (250 mg L^{-1}) compared to unamended Novick's mineral salts buffer (Novick and Alexander, 1985). Erlenmeyer flasks (50 mL) containing 20 mL of media were inoculated with washed cell suspensions of these strains (300 μL, initial OD_{660nm} = 0.04) and incubated on a rotary shaker (30 °C, 100 rpm). These incubations were conducted in triplicate, including uninoculated controls for each substrate. Growth was monitored daily by determining OD_{660nm}. Uninoculated HQ control solutions exhibited a purple coloration consistent with oxidation of HQ. Corrections for this color development were preformed by subtraction of control optical density values from those of inoculated treatments.

Transformation of FE by strains RA-2 and UA5-40 was assessed in the three nutritional regimes: TSB and Novick's mineral salts media with either sodium propionate or glucose (2.5 g L^{-1}), containing either ^{14}C-CP- (7.2 kBq mL^{-1}) or ^{14}C-DOP-labeled (7.9 kBq mL^{-1}) FE at 20 μM. Sterile Corex centrifuge tubes (25 mL) containing 1.5 mL of the above media were inoculated with 15 μL of washed cells (initial OD_{660nm} = 0.04) and placed on a rotary shaker (30 °C, 100 rpm). Incubations were conducted in triplicate, including appropriate uninoculated controls. Aliquots were removed at 2, 5, 9, and 11 days after inoculation, extracted, and analyzed by RAD-TLC, as described above, using solvent systems A and B. ^{14}C-CP-labeled FE treatments were also developed with solvent C. Recovery of radioactivity was determined using liquid scintillation counting (LSC) (Packard-Tri-Carb 4000, Packard Instruments Co., Meriden, CT) after mixing with Ecolume scintillation cocktail (ICN, Costa Mesa, CA). Growth and changes in pH were assessed under similar regimes using unlabeled FE (20 μM). Erlenmeyer flasks (50 mL) containing

Table 2. Metabolism of FE[a] (9 μM, Chlorophenyl Label) by Resting Cell Suspensions of Four *Pseudomonas* Strains after a 48-h Incubation

strain	FE, nmol mL^{-1}	FA, nmol mL^{-1}	CDHB, nmol mL^{-1}
UA5-40	1.2 ± 0.02[b]	7.2 ± 0.05	0.6 ± 0.03
BD4-13	0.8 ± 0.01	7.9 ± 0.03	0.3 ± 0.02
RA-2	0.9 ± 0.05	7.7 ± 0.05	0.4 ± 0.02
M-17	0.2 ± 0.02	8.7 ± 0.04	0.1 ± 0.02
control	9.00	nd[c]	nd

[a] FE, fenoxaprop-ethyl; FA, fenoxaprop acid; CDHB, 6-chloro-2,3-dihydrobenzoxazol-2-one. [b] Mean and standard deviation of three replicates. [c] nd, not determined.

9.0 mL of the appropriate media were inoculated with 75 μL of washed cells (initial OD_{660nm} = 0.04) and incubated on a rotary shaker (30 °C, 100 rpm). Growth was determined by measuring increased absorbance (660 nm) at 2 and 5 days; pH was also determined at 5 days.

Experiment III: Mineralization Studies of ^{14}C-Dioxyphenyl-Labeled FE. The potential of RA-2 and UA5-40 to mineralize ^{14}C-DOP-labeled FE was assessed using modifications of a method described by Lehmicke et al. (1979). Briefly, sterile shell vials (1 dram) were filled with 750 μL of TSB or Novick's mineral salts media. The Novick's medium was either unamended or contained glucose, sodium propionate (2.5 g L^{-1}), or HQ (0.5 g L^{-1}). The final concentration of ^{14}C-labeled FE (7.9 kBq mL^{-1}) in the TSB and Novick's solutions was 20 μM. Because FE was initially dissolved in ethanol, all final solutions contained ethanol at a final concentration of 1.5%. Vials were inoculated with 10 μL of inoculum, as described above, and sealed with sterile polyurethane foam plugs. Shell vials were placed in 20-mL scintillation vials containing 1.0 mL of 1 N sodium hydroxide (NaOH), tightly sealed with aluminum foil-lined caps, and incubated on a rotary shaker (50 rpm, 30 °C). Shell vials were removed daily and placed in scintillation vials containing fresh NaOH. Hionic-Fluor scintillation cocktail (Packard Instrument Co., Meriden, CT) was added to the vials containing the sampled NaOH, and radioactivity was determined by LSC. Each incubation was conducted in five replicates sampled daily over a 7-day period. Cells exposed to ^{14}C-DOP-labeled FE were extracted with acetone and analyzed for FE and metabolites using solvent system B.

RESULTS AND DISCUSSION

Fenoxaprop-ethyl Transformations by *Pseudomonas* Cell Suspensions and Cell-Free Extracts. All *Pseudomonas* strains rapidly hydrolyzed FE to FA (Table 2). After a 48-h incubation, 82−96% of the FE was hydrolyzed to FA, with little or no additional FA formed over a 96-h incubation (data not shown). Cells incubated with ^{14}C-CP-labeled FE slowly accumulated CDHB, with only 1.1−6.7% of the radioactivity recovered as this metabolite during a 48-h incubation. Cells incubated with ^{14}C-DOP-labeled FE formed a similar amount of FA and also accumulated HPP (R_f = 0.13 in solvent system A) equivalent to CDHB formation (data not shown). Increasing FE concentration from 9.0 to 45.0 μM in suspensions of UA5-40 resulted in a similar degree of FE hydrolysis to FA and subsequent CDHB formation (Figure 1). Microbial degradation of FE to FA and CDHB in soils (Smith, 1985; Smith and Aubin, 1990) and in mixed cultures of microorganisms (Gennari et al., 1995) has been reported. The present studies are the first to demonstrate degradation of FE to CDHB by pure cultures of microorganisms.

CFEs of RA-2 and UA5-40 hydrolyzed 73−85% of 9.0 μM FE to FA after a 5-h incubation, regardless of exogenous GSH (Table 3). No GSH conjugates were formed by CFE of these strains. No FA and only small amounts of GSH conjugates were observed in the buffer with GSH control treatments. However, the positive control (equine GST + GSH) formed >80% CDHB−GSH conjugate (R_f = 0.00 in solvent A, R_f = 0.39 in solvent C, and R_f = 0.08 in solvent D) with some accumulation of FA during a 5-h incubation. Spontaneous nonenzymatic GSH conjugation with FE has been reported in several plant species (Tal et al., 1995). Our results corroborated this phenomenon, that is, a low level of spontaneous GSH conjugation in the GSH (no enzyme) control. High protein in the CFE extracts could have sequestered either FE or FA, thereby inhibiting GSH conjugation in these bacterial preparations. However, the fact that esterase activity was observed without GSH conjugation in CFEs suggests that FE-GST activity is most likely lacking in these strains.

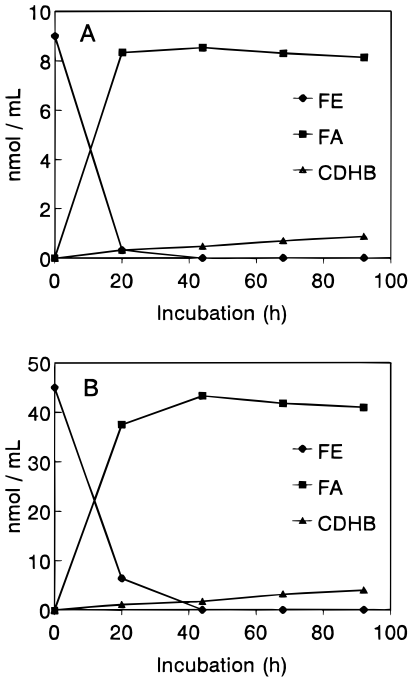

Figure 1. Transformation of FE at 9.0 μM (A) and 45.0 μM (B) to FA and CDHB by cell suspensions of *P. fluorescens* strain UA5-40 over a 96-h time course. Data points represent means of three to four replications.

Table 3. Catabolism of ^{14}C-FE[a] (9 μM, Chlorophenyl Label) by Cell-Free Extracts of *P. fluorescens* RA-2 and UA5-40 in the Presence or Absence of Glutathione (2 mM) during a 5-h Incubation

	% ^{14}C recovered		
treatment	FE	FA	glutathione conjugate
equine GST + GSH	0[b]	16.8 ± 2.1	83.2 ± 2.1
buffer − GSH	100	0	0
buffer + GSH	97.8 ± 2.2	0	2.2 ± 2.2
UA5-40 − GSH	21.8 ± 2.3	78.2 ± 2.3	0
UA5-40 + GSH	28.4 ± 7.7	71.6 ± 7.7	0
RA-2 − GSH	14.8 ± 2.7	85.2 ± 2.7	0
RA-2 + GSH	18.6 ± 4.2	81.4 ± 4.2	0

[a] FE, fenoxaprop-ethyl; FA, fenoxaprop acid; GST, glutathione *S*-tranferase; GSH, glutathione. [b] Mean of three replicates (butanol/acetic acid/water, solvent C).

Degradation of FE by *P. fluorescens* Strains Grown under Various Nutrient Regimes. Both *P. fluorescens* strains RA-2 and UA5-40 were capable of growth on minimal medium amended with several products of FE catabolism, that is, ethanol, propionate, and HQ (Table 4). CDHB and HPP were unable to serve

Table 4. Growth of Two *P. fluorescens* Strains on Five Degradation Products (250 mg L^{-1}) of FE[a] during a 48-h Incubation

	maximum growth (OD_{660nm})	
substrate	RA-2	UA5-40
CDHB	0.092 ± 0.002[b]	0.038 ± 0.002
ethanol	0.261 ± 0.008	0.203 ± 0.010
hydroquinone	0.195 ± 0.012	0.148 ± 0.003
2-(4-hydroxyphenoxy)propionate	0.076 ± 0.001	0.052 ± 0.001
propionate	0.210 ± 0.004	0.158 ± 0.012
control (mineral salts)	0.074 ± 0.004	0.052 ± 0.001

[a] FE, fenoxaprop-ethyl; CDHB, 6-chloro-2,3-dihydrobenzoxazol-2-one. [b] Mean and standard deviation of three replicates.

as the sole carbon source for either strain. Nutrient source had a significant effect on bacterial growth and metabolism of FE (Tables 5−8). The most rapid cell growth and highest cell yields were observed on TSB, with intermediate yields on MMG, and the lowest yields on MMP (data not shown). TSB cultures of both strains exhibited nearly complete hydrolysis of FE to FA or other metabolites within 5 days (Tables 5−8). Polar metabolites (CDHB−GSH and CDHB−GlyCys) were observed early in the study (4% at 2 days) in TSB cultures of both strains treated with ^{14}C-CP-labeled FE (Tables 5 and 6). Accumulation of glutathione-derived polar metabolites increased during the incubation, with 18% of the initial radioactivity recovered as GlyCys and Cys conjugates at the end of the study (11 days). There was a concomitant decrease in FA over this period. TSB cultures treated with ^{14}C-CP-labeled FE accumulated moderate amounts of CDHB (5−7% at 9 and 11 days) and an unidentified metabolite [R_f = 0.30 in solvent A, comigrates with CDHB in solvent B, R_f = 0.98, and R_f = 0.10 in the benzene/acetone (10:1) solvent E]. TSB cultures treated with ^{14}C-DOP-labeled FE accumulated HPP (R_f = 0.18 in solvent A, R_f = 0.54 in solvent B), also with a concomitant decrease in FA. TSB cultures of RA-2 accumulated two minor unidentified metabolites that accounted for about 7 and 3% of the initial radioactivity, respectively (R_f = 0.06 and 0.00 in solvent A and R_f = 0.13 and 0.00 in B). A nearly stoichiometric formation of metabolites derived from FA (37−51%) was observed with TSB cultures of RA-2 when both ^{14}C labels of FE were examined. However, in TSB cultures of UA5-40, 53% of the ^{14}C-CP label and 80% of the ^{14}C-DOP label were recovered as FA.

Carbon source had a significant effect on FE degradation when both strains were grown on minimal medium. On MMP, UA5-40 completely hydrolyzed FE to FA within 5 days, whereas in cultures of RA-2, 24 and 37% of the initial FE of the ^{14}C-CP and ^{14}C-DOP labels, respectively, remained as FE after 11 days (Tables 5−8). Apparently, propionate (and ethanol from the FE stocks)

Table 5. Influence of Nutrient Regime on the Metabolism of ^{14}C-Chlorophenyl-Labeled FE[a] (Percent Recovery) by *P. fluorescens* Strain UA5-40 during an 11-Day Incubation

days after inoculation	FE	FA	polar metabolites	unidentified R_f = 0.30	CDHB
		Tryptic Soy Broth (TSB)			
2	7.0 ± 12.0[b]	88.8 ± 10.0	3.8 ± 2.0	nd[c]	nd
5	nd	90.7 ± 1.0	8.3 ± 0.3	nd	<1.0
9	nd	69.2 ± 11.7	14.9 ± 4.0	10.8 ± 5.4	5.1 ± 2.2
11	nd	53.4 ± 8.5	18.6 ± 3.8	21.1 ± 5.4	6.9 ± 1.4
		Mineral Salts−Glucose Broth (MMG)			
2	80.2 ± 13.0	17.8 ± 11.0	nd	nd	2.0 ± 0.1
5	74.3 ± 2.6	1.5 ± 2.3	nd	nd	24.0 ± 4.6
9	53.6 ± 12.0	nd	nd	nd	46.4 ± 12.0
11	54.1 ± 6.0	nd	nd	nd	46.0 ± 6.0
		Mineral Salts−Propionate Broth (MMP)			
2	59.2 ± 2.1	40.8 ± 2.1	nd	nd	nd
5	nd	100	nd	nd	nd
9	nd	98.3 ± 1.4	nd	nd	1.7 ± 1.4
11	nd	97.4 ± 1.0	nd	nd	2.3 ± 1.0

[a] FE, fenoxaprop-ethyl; FA, fenoxaprop acid; CDHB, 6-chloro-2,3-dihydrobenzoxazol-2-one. [b] Means and standard deviation of three replicates. [c] nd, not determined.

Table 6. Influence of Nutrient Regime on the Metabolism of ^{14}C-Chlorophenyl-Labeled FE[a] (Percent Recovery) by *P. fluorescens* Strain RA-2 during an 11-Day Incubation

days after inoculation	FE	FA	polar metabolites	unidentified R_f = 0.30	CDHB
		Tryptic Soy Broth (TSB)			
2	37.0 ± 2.5[b]	59.0 ± 2.5	4.0 ± 10	nd[c]	nd
5	1.0 ± 1.6	91.0 ± 1.7	7.9 ± 0.5	nd	nd
9	0.9 ± 1.4	75.2 ± 6.3	12.3 ± 1.4	6.8 ± 4.7	4.9 ± 1.7
11	nd	63.1 ± 11.0	18.4 ± 1.7	12.7 ± 7.9	5.5 ± 1.8
		Mineral Salts−Glucose Broth (MMG)			
2	82.4 ± 12.0	nd	nd	nd	17.6 ± 12.0
5	48.4 ± 6.6	nd	nd	nd	51.0 ± 6.6
9	38.0 ± 4.0	nd	nd	nd	62.0 ± 4.0
11	36.7 ± 1.1	nd	nd	nd	63.3 ± 1.1
		Mineral Salts−Propionate Broth (MMP)			
2	91.1 ± 2.2	8.9 ± 2.2	nd	nd	nd
5	82.3 ± 2.0	17.7 ± 2.0	nd	nd	nd
9	45.3 ± 17.0	53.9 ± 16.0	nd	nd	0.8 ± 1.3
11	23.9 ± 19.8	73.2 ± 16.0	1.9 ± 1.7	nd	1.0 ± 1.6

[a] FE, fenoxaprop-ethyl; FA, fenoxaprop acid; CDHB, 6-chloro-2,3-dihydrobenzoxazol-2-one. [b] Means of three replicates ± standard deviation. [c] nd, not determined.

Table 7. Influence of Nutrient Regime on the Metabolism of ^{14}C-Dioxyphenyl-Labeled FE[a] (Percent Recovery) by *P. fluorescens* Strain UA5-40 during an 11-Day Incubation

days after inoculation	FE	FA	2-(4-hydroxyphenoxy)propionate	other metabolites	hydroquinone
		Tryptic Soy Broth (TSB)			
2	22.2 ± 14.0[b]	77.8 ± 14.0	nd[c]	nd	nd
5	nd	96.6 ± 2.8	3.4 ± 2.8	nd	nd
9	nd	84.7 ± 1.3	15.3 ± 1.3	nd	nd
11	nd	79.6 ± 2.2	20.3 ± 2.2	nd	nd
		Mineral Salts−Glucose Broth (MMG)			
2	80.8 ± 11.0	17.1 ± 11.0	nd	nd	19.2 ± 11.0
5	68.9 ± 3.1	4.3 ± 0.5	nd	nd	26.8 ± 2.6
9	44.9 ± 14.1	nd	nd	nd	55.1 ± 14.1
11	54.2 ± 6.9	nd	nd	nd	45.8 ± 6.9
		Mineral Salts− Propionate Broth (MMP)			
2	65.3 ± 1.5	34.7 ± 1.5	nd	nd	nd
5	nd	100	nd	nd	nd
9	nd	98.1 ± 1.7	nd	1.9 ± 1.7	nd
11	nd	95.8 ± 3.7	nd	4.2 ± 3.7	nd

[a] FE, fenoxaprop-ethyl; FA, fenoxaprop acid. [b] Means of three replicates ± standard deviation. [c] nd, not determined.

Table 8. Influence of Nutrient Regime on the Metabolism of ^{14}C-Dioxyphenyl-Labeled FE[a] (Percent Recovery) by *P. fluorescens* Strain RA-2 during an 11-Day Incubation

days after inoculation	FE	FA	2-(4-hydroxyphenoxy)propionate	other metabolites	hydroquinone
		Tryptic Soy Broth			
2	49.2 ± 18.0[b]	50.8 ± 18.0	nd[c]	nd	nd
5	nd	98.5 ± 2.5	nd	1.5 ± 2.5	nd
9	nd	65.3 ± 16.9	31.5 ± 14.0	3.2 ± 2.9	nd
11	nd	48.4 ± 4.0	37.5 ± 1.4	14.1 ± 3.0	nd
		Mineral Salts−Glucose Broth (MMG)			
2	71.2 ± 16.0	nd	nd	nd	28.8 ± 16.0
5	54.7 ± 8.4	nd	nd	nd	45.1 ± 8.4
9	54.3 ± 3.5	nd	nd	nd	45.7 ± 3.5
11	48.5 ± 9.6	nd	nd	nd	51.5 ± 9.6
		Mineral Salts−Propionate Broth (MMP)			
2	89.2 ± 1.4	10.8 ± 1.4	nd	nd	nd
5	79.2 ± 5.0	20.8 ± 5.0	nd	nd	nd
9	47.8 ± 16.6	52.2 ± 16.6	nd	nd	nd
11	37.1 ± 18.8	62.9 ± 18.8	nd	nd	nd

[a] FE, fenoxaprop-ethyl; FA, fenoxaprop acid. [b] Means of three replicates ± standard deviation. [c] nd, not determined.

was unable to support cometabolic transformations of FA by these *P. fluorescens* strains. Further transformation of FA was minimal in MMP cultures of both strains.

In MMG cultures of both strains, 40−50% of the initial FE remained after 11 days, with FA only transiently observed in MMG cultures of UA5-40. Both strains grown on MMG media with ^{14}C-CP-labeled FE accumulated CDHB without accumulation of polar metabolites (sulfhydryl conjugates) or the unidentified metabolite observed in TSB cultures. A more rapid accumulation of CDHB was observed with RA-2, with 46 and 63% of the radioactivity recovered as CDHB in UA5-40 and RA-2 MMG cultures, respectively, after 11 days. No HPP was observed in cultures growing on MMG media with ^{14}C-DOP-labeled FE, but HQ was observed instead of HPP. A nearly stoichiometric accumulation of CDHB and HQ was noted, suggesting that benzoxazolyloxyphenyl ether bond cleavage occurred. The fact that HQ, rather than HPP, accumulated is evidence for phenoxypropionate ether bond cleavage. In MMG-grown cells, the broth pH decreased to ~3.5−4.0 following 5 days of growth, whereas the pH of TSB and MMP cultures was slightly alkaline (8.5). Acidity may inhibit esterase activity, because optimal pH is typically in the neutral to slightly alkaline range, depending on substrate and enzyme source. Effects of pH on the hydrolysis of the herbicide diclofop-methyl using purified enzymes and soils has been evaluated (Gaynor, 1992). Lipase-mediated hydrolysis of diclofop-methyl was unaffected by pH, but the greatest esterase hydrolytic activity occurred at pH 7. Exposure of FA, CDHB−GSH, and CDHB−Cys to strong acid (1 N, 80 °C) can result in hydrolysis to CDHB (Tal et al., 1993). Acidification (pH 2.0) of aqueous samples containing FE prior to solid-phase extraction resulted in inefficient extraction of FE from water (Balinova, 1996). The speculated cause was hydrolysis of the ethyl ester; however, we did not observe this phenomenon in our studies. Decomposition of FE can occur under both alkaline and acidic conditions (WSSA, 1994). However, spontaneous chemical degradation of FE under moderate acidic pH values has not been reported in the literature. In controlled laboratory studies, rapid nonenzymatic phenyl ether cleavage of FE occurred at pH values of 3.6 and below, but not at pH 4.6−8.6 (Hall et al., 1998). It is also possible that oxidative cleavage of FE at the benzoxazolyloxyphenyl and phenoxypropionate ether bonds was supported by glucose in the absence of amino acids, which are present in TSB (TSB also contains 2.5 g L^{-1} dextrose).

Mineralization Studies of Dioxyphenyl-Labeled FE. Little or no (<1.0%) mineralization of ^{14}C-DOP-labeled FE by either strain was observed over an 11-day incubation, regardless of carbon substrate. At termination, the cell suspensions were extracted with acetone, and the recovery of FE and metabolites was assessed (Table 9). Cells treated with ethanol alone (1.5%) exhibited no metabolism of FE. Cells grown on

Table 9. Effect of Carbon Source on the Metabolism of ^{14}C-Dioxyphenyl-Labeled FE[a] by Two *P. fluorescens* Strains, 11 Days after Inoculation under Low Oxygen Tension (Mineralization Study)[b]

	% recovery (initial concn = 9.0 μM)			
treatment	R_f = 0.13[c]	HPP	FA	FE
UA5-40				
glucose	nd[d]	nd	100	nd
hydroquinone	nd	nd	100	nd
propionate	1.1 ± 2.2	3.5 ± 4.0	95.4 ± 5.7	nd
TSB	4.4 ± 0.7	71.8 ± 2.6	23.2 ± 2.3	0.6 ± 1.3
ethanol	nd	nd	0.9 ± 1.3	99.1 ± 1.3
RA-2				
glucose	nd	2.7 ± 3.4	97.3 ± 3.4	nd
hydroquinone	nd	4.5 ± 3.1	95.5 ± 3.1	nd
propionate	1.2 ± 2.3	7.3 ± 2.7	91.6 ± 3.8	nd
TSB	5.9 ± 1.1	69.9 ± 3.2	25.6 ± 1.3	nd
ethanol	nd	nd	nd	100

[a] HPP, 2-(4-hydroxyphenoxy)propionate; FA, fenoxaprop acid; FE, fenoxaprop-ethyl; TSB, tryptic soy broth. [b] Means of five replicates ± standard deviation. [c] Solvent B (chloroform/methanol/water; 65:25:1). [d] nd, not determined.

glucose, HQ, propionate, and TSB completely converted FE to FA and other metabolites. Under these conditions, UA5-40 did not further metabolize FA with either glucose or HQ, but propionate supported minimal metabolism to HPP and other metabolites (4%). TSB-grown UA5-40 cells converted >75% of FE to HPP and another, unidentified, metabolite (R_f = 0.13). In RA-2, 3−7% of the initial FE was recovered as HPP in glucose, HQ, and propionate. As with UA5-40, 75% of the radioactivity from TSB-grown RA-2 cells was recovered, predominantly as HPP (70%) and a component with an R_f = 0.13 in solvent system B in low abundance.

There is a disparity in FA metabolism in glucose-grown cells in this and the previous experiment. In this study, there was less oxygen availabe due to the experimental system's lower medium-to-air ratio and lower orbital shaking speed. Measurement of pH was not achieved due to the small volume of cells (1.0 mL unit^{-1}). TSB-grown cells in this study also provided more complete degradation of FA than in the previous experiment. These studies, along with previous data, indicate that these reactions are cometabolic transformations that require exogenous carbon sources. Although both strains can utilize ethanol, propionate, and HQ as sole carbon sources, these substrates did not support cleavage of the ether bond of FA. Because no mineralization was observed, it is concluded that growing cells on either propionate or HQ did not induce enzymes that could metabolize the ^{14}C-DOP-labeled ring moiety of FE.

SUMMARY AND CONCLUSIONS

Fenoxaprop-ethyl has been shown to be cometabolically transformed by several fluorescent *Pseudomonas* strains. This is the first study to demonstrate degradation of FE by pure bacterial cultures. As with many cometabolic transformations, the ability to catabolize FE is affected by culture nutrition and physiological status. We have found various metabolites of FE formed by these *P. fluorescens* strains, as summarized in Figure 2. The initial transformation of FE via hydrolysis of the ethyl ester group is relatively rapid, as demonstrated here using resting cells, crude CFEs, and actively growing cells on certain media. Cleavage of the benzoxazolyloxyphenyl ether bond is the degradation

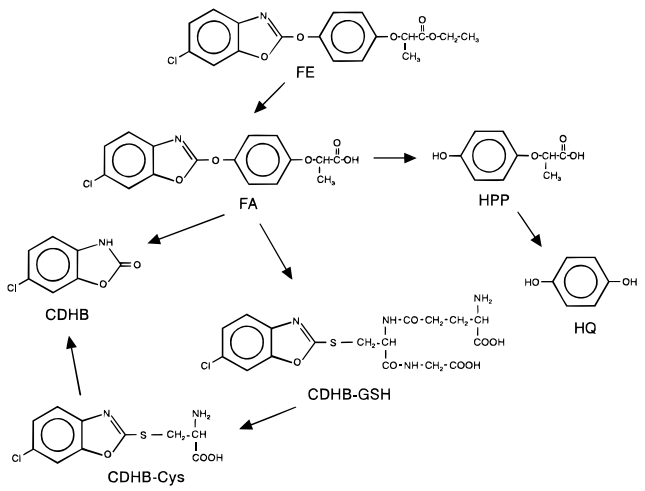

Figure 2. Chemical relationships of the metabolites arising from the biotransformation of FE by pure cultures of some fluorescent pseudomonads. CDHB, 6-chloro-2,3-dihydrobenzoxazol-2-one; CDHB−Cys, *S*-(6-chlorobenzoxazol-2-yl)-cysteine; CDHB−GSH, *S*-(6-chlorobenzoxazole-2-yl)glutathione; FA, fenoxaprop acid; FE, fenoxaprop-ethyl; HPP, 2-(4-hydroxyphenoxy)propionate; HQ, hydroquinone.

step of greatest environmental significance and is highly dependent on nutritional status. Accumulation of polar metabolites (CDHB−GSH, CDHB−GlyCys, and CDHB−Cys conjugates) prior to accumulation of CDHB (or the unidentified metabolite) on rich media (TSB) indicates that GSH conjugation is the mechanism of benzoxazolyloxyphenyl ether bond cleavage. This is verified by the nearly stoichiometric accumulation of HPP. However, observing CDHB and HQ production in MMG cultures without accumulation of polar conjugates may also indicate the potential for oxidative phenoxypropionate ether bond cleavage, especially because HPP was not observed in MMG cultures. Failure to detect conjugation of FE with GSH in crude enzyme extract preparations, plus the slow accumulation of polar conjugates in TSB cultures, indicates a potential for nonenzymatic GSH conjugation of FE in these *P. fluorescens* strains. This nonenzymatic conjugation is similar to the proposed pathway in FE-tolerant plants (Tal et al., 1995), rather than via enzymatic GSH conjugation by purified wheat GST (Edwards and Cole, 1996). Oxidative cleavage of diphenyl ether compounds has been demonstrated by several bacterial species, for example, diphenyl ether by *Pseudomonas cepacia* (Pfeiffer et al., 1989), mono- and dihalogenated diphenyl ethers by *Sphingomonas* sp. (Schmidt et al., 1992, 1993), and phenoxybenzoates by a *Pseudomonas* sp. (Topp and Akhtar, 1991). Fluorescent pseudomonads such as these used in our studies comprise a dominant component of the rhizosphere bacterial community and are also relatively common soil bacteria. Due to their relative abundance in these habitats, fluorescent pseudomonads may contribute significantly to the degradation of FE in terrestrial environments.

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