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Biodegradation of Chloroacetamide Herbicides by *Paracoccus* sp. FLY-8 in Vitro

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ABSTRACT: A butachlor-degrading strain, designated FLY-8, was isolated from rice field soil and was identified as *Paracoccus* sp. Strain FLY-8 could degrade and utilize six chloroacetamide herbicides as carbon sources for growth, and the degradation rates followed the order alachlor > acetochlor > propisochlor > butachlor > pretilachlor > metolachlor. The influence of molecular structure of the chloroacetamide herbicides on the microbial degradation rate was first analyzed; the results indicated that the substitutions of alkoxymethyl side chain with alkoxyethyl side chain greatly reduced the degradation efficiencies; the length of amide nitrogen's alkoxymethyl significantly affected the biodegradability of these herbicides: the longer the alkyl was, the slower the degradation efficiencies occurred. The phenyl alkyl substituents have no obvious influence on the degradation efficiency. The pathway of butachlor complete mineralization was elucidated on the basis of the results of metabolite identification and enzyme assays. Butachlor was degraded to alachlor by partial C-dealkylation and then converted to 2-chloro-*N*-(2,6-dimethylphenyl) acetamide by N-dealkylation, which subsequently transformed to 2,6-diethylaniline, which was further degraded via the metabolites aniline and catechol, and catechol was oxidized through an ortho-cleavage pathway. This study highlights an important potential use of strain FLY-8 for the in situ bioremediation of chloroacetamide herbicides and their metabolite-contaminated environment.

KEYWORDS: chloroacetamide herbicide, biodegradation, *Paracoccus* sp. FLY-8, molecular structure, degradation pathway

INTRODUCTION

Chloroacetamide herbicides are among the most important class of preemergence herbicides used in corn, cotton, soybean, and many other crops for control of annual grass and broadleaf weeds. The most commonly used chloroacetamide herbicides in the world are acetochlor [2-chloro-*N*-(ethoxymethyl)-*N*-(2-ethyl-6-methyl-phenyl)-acetamide], butachlor [*N*-(butoxymethyl)-2-chloro-*N*-(2,6-diethylphenyl)acetamide], metolachlor [2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl)acetamide], alachlor [*N*-(methoxymethyl)-2-chloro-*N*-(2,6-diethylphenyl)acetamide], and pretilachlor [2-chloro-*N*-(2,6-diethylphenyl)-*N*-(2-propoxyethyl)acetamide]. Chloroacetamide herbicides persist for a long time in soil, and the residues consistently injure subsequent rotation crops, especially in sandy soils with low organic matter.[1] Because of the widespread use of these herbicides and their characteristics, residues of these herbicides and their degradation metabolites have been frequently detected in ground and surface waters.[2,3] Several studies demonstrated that these herbicides are highly toxic to some aquatic organisms[4] and are carcinogenic in rats: acetochlor and alachlor cause tumors in the nasal turbinates, butachlor causes stomach tumors, and metolachlor causes liver tumors.[5] Thus, there is great concern about the behavior and fate of chloroacetamide herbicides and their degradation metabolites in the environment.

In general, chloroacetamide herbicides in the environment are degraded by both abiotic and biotic pathways, including photo-oxidation, chemical hydrolysis, and biodegradation. Several studies have demonstrated that biodegradation is the most important factor in the fate of chloroacetamide herbicides in the environment, and the chemical hydrolysis and photo-oxidation of chloroacetamide herbicides are of minor importance at typical pH values in soil and water.[6] Many micro-organisms capable of degrading chloroacetamide herbicides have been isolated, and the metabolites have also been identified.[7−12] Chakraborty and Bhattacharyya found degradation of butachlor by *Fusarium solani* resulted in the production of at least 30−32 metabolites and the main degradative pathways involved dechlorination, hydroxylation, dehydrogenation, debutoxymethylation, C-dealkylation, N-dealkylation, O-dealkylation, and cyclization.[8] However, reported microbial metabolism of chloroacetamide herbicides resulted in partial biodegradation and generally proceeded through cometabolism, leading to the accumulation of their metabolites, which contaminated the soil and surface and groundwaters. Chloroacetanilide herbicides share the same molecular core of 2-chloroacetanilide and differ only in the type and arrangement of substitutions in the amide nitrogen and phenyl ring. Up to now, the influence of the molecular structure of chloroacetanilide herbicides on their biodegradability has not been studied.

The objective of this study was to screen isolates that were capable of completely mineralizing chloroacetamide herbicides and to elucidate the pathway of butachlor mineralization. Moreover, the influence of molecular structure of the chloroacetamide herbicides on their biodegradability was first analyzed. The pathway of butachlor mineralization was also elucidated in this paper.

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MATERIALS AND METHODS

Chemicals and Media. Alachlor (95.5%), acetochlor (95.5%), pretilachlor (96.5%), butachlor (96.5%), propisochlor (96.5%), and metolachlor (97.5%) were kindly provided by Binnong Technology Co., Ltd., Shandong Province, China. The standards of the six chloroacetamide herbicides were purchased from Shanghai Pesticide Research Institute. HPLC-grade methanol was purchased from Sigma-Aldrich (St. Louis, MO). All other chemicals and reagents were of analytical grade and available commercially. Stock solutions of different chloroacetamide herbicides (2%, w/v) were prepared with methanol each month, sterilized by membrane filtration (pore size = 0.22 μm), and stored in dark bottles at 20 °C prior to use.

Luria−Bertani (LB) medium contained (L^{-1}) 10.0 g of tryptone, 5.0 g of yeast extract, and 10.0 g of NaCl, pH 7.0. MSM consisted of the following components: 1.0 g of $(NH_4)_2SO_4$, 1.0 g of NaCl, 1.5 g of K_2HPO_4, 0.5 g of KH_2PO_4, and 0.2 g of $MgSO_4 \cdot 7H_2O$ per liter of water, pH 7.0; for solid medium, 15.0 g/L agar was added. Medium was sterilized at 121 °C for 30 min.

Isolation and Identification of Chloroacetamide Herbicide-Degrading Bacteria. To isolate butachlor-degrading bacteria, a conventional enrichment culture was carried out according to the method of Wang et al.[13] Soil samples used as initial inoculants for enrichment were collected from the surface layer (0−10 cm) of a rice field in Jiangsu Province, China, which had been sprayed with butachlor and acetochlor for more than 5 years. About 1.0 g of the soil sample was added to a 250 mL Erlenmeyer flask containing 50 mL of MSM with the addition of 100 mg/L butachlor as the carbon source and incubated at 30 °C on a rotary shaker at 150 rpm for about 5 days. Five milliliters of the enrichment culture was transferred into another 50 mL fresh enrichment medium and incubated for another 5 days. After five rounds of transfer, the enrichment culture was diluted and spread on LB agar plates. Colonies that grew on the plates were picked up, purified by repeated streaking, and tested for the ability to degrade chloroacetamide herbicides (see below). The isolates were characterized and identified by morphological, physiological, and biochemical characteristics as well as 16S rRNA gene sequence analysis according to the method described by Guo et al.[14]

Growth and Degradation Experiments. The isolates were pregrown in LB (30 °C, 150 rpm) for 12 h. Cells were harvested by centrifugation (2084*g*, 15 min) and washed twice with fresh MSM. After the cell density had been adjusted to about 1.0×10^8 cfu/mL, an inoculum (1%, v/v) was inoculated into 100 mL of MSM with the addition of 100 mg/L different chloroacetamide herbicides as the carbon source in a 250 mL Erlenmeyer flask. All cultures were incubated aerobically at 30 °C and 150 rpm on a rotary shaker. At 24 h intervals, 5 mL samples were taken from the cultures, bacterial growth was monitored by measuring the colony forming units (cfu/mL), and degradation of different chloroacetamide herbicides was determined by high-performance liquid chromatography (HPLC) as described below. Each treatment was performed in three replicates; control experiments without inoculation and/or without substrate were carried out under the same conditions.

Aromatic Substrate Range. To study the ability to degrade and utilize some aromatic substrates for growth, the MSM was supplemented with different aromatic substrates (toluene, aniline, salicylic acid, protocatechuate, chlorobenzene, catechol, *m*-hydroquinone, nitrobenzene, *p*-hydroquinone, 2,6-diethylaniline, 2,6-xylidine, 3,5-xylidine, and gentisic acid; final concentrations, 50−100 mg/L) as carbon source. The degradation of different aromatic substrates in the cultures was qualitatively determined by UV scan (the wavelength range from 200 to 400 nm), and bacterial growth was monitored by measuring the optical density of the culture at 610 nm.

Chemical Analysis. The culture samples were centrifuged at 12000*g* for 10 min, and the supernatant was partitioned with an equal volume of hexane/ethyl acetate (1:1, 5 mL) after saturation with sodium chloride. The organic phase was passed through anhydrous sodium sulfate and evaporated to dryness using a stream of nitrogen at room temperature and redissolved in methanol. Samples in methanol were then filtered through a 0.22 μm Millipore membrane filiter. The chloroacetamide herbicides were analyzed by HPLC. The separation column (i.d., 4.6 mm; length, 25 cm) was filled with Kromasil 100-5C18. The mobile phase was methanol/water (75:25, v/v), and the flow rate was 1.0 mL/min. The injection volume was 20 μL, and the column elution was monitored by measuring at 230 nm with a UV-900 wavelength absorbance detector. The concentrations of chloroacetamide herbicides were calculated on the basis of a peak area from calibration curve.

To determine the metabolites produced during butachlor degradation, gas chromatography−mass spectrometry (GC-MS) analyses were performed in electron ionization (EI) mode (70 eV) with a Finnigan gas chromatograph equipped with an MS detector. Gas chromatography was conducted using a RTX-5MS column (15 m × 0.25 mm × 0.25 μm, Restek Corp.). The column temperature was programmed from 50 °C (1.5 min hold) to 220 °C at 20 °C/min and held for 1 min and finally increased to 260 °C at 50 °C/min and held at 260 °C for 10 min; helium was used as the carrier gas at a constant flow of 1.0 mL/min. The samples were analyzed in split mode (1:20) at an injection temperature of 220 °C and an EI source temperature of 250 °C and scanned in the mass range from m/z 30 to 650.

Assays of Enzymatic Activities. The isolate was grown at 30 °C in MSM with 100 mg/L butachlor, 2,6-diethylaniline, or glucose as carbon source; the cells were harvested at maximum growth by centrifugation at 5000*g*, washed twice with 20 mM phosphate buffer (pH 7.0), resuspended in the same buffer, and then adjusted to a cell density (optical density at 600 nm) of 5.0 (resting cells). For cell wet weight determination, the cell suspension was centrifuged, and the pellet was used to determine the cell wet weight by balance. Aniline-dependent oxygen uptake rate was measured in the buffer (3.0 mL) containing 0.6 mL of the resting cells at 30 °C after an endogenous rate was estimated. The activities of aniline dioxygenase were assayed with an oxygen electrode (YSI) according to the method of Liu et al.[15] One unit of aniline dioxygenase was defined as the amount of cells consuming 1 μmol of oxygen per minute.

To prepare the cell-free extract, the suspended cells were sonicated (Auto Science, UH-650B ultrasonic processor, 30% intensity) for 5 min and centrifuged at 12000*g* for 20 min at 4 °C. The clear supernatant was used as crude cell extract. Protein concentration was quantified according to the method of Bradford et al.[16] The activities of catechol 1,2-dioxygenase and catechol 2,3-dioxygenase were assayed according to the method of Liu et al.[15] One unit of catechol oxygenase was defined as the amount of protein needed for oxidation of 1 μmol of catechol per minute.

RESULTS AND DISCUSSION

Strain Isolation and Identification. After five rounds of transfers, the enrichment culture could degrade about 85% of 50 mg/L butachlor within 5 days. A total of 11 pure cultures were obtained from the enrichment samples. The 11 strains were inoculated individually into LB and MSM media with the addition of 100 mg/L butachlor. The results indicated that 2 of the 11 strains could not degrade butachlor in either LB or MSM medium; 7 of the 11 strains could grow and degrade butachlor only in LB medium. Only 2 strains were able to grow in both conditions and degrade butachlor. One strain, designated FLY-8, was selected for further study due to its broad-spectrum substrate specificity. This strain was able to degrade about 25−95% of the initially added 100 mg/L alachlor, acetochlor,

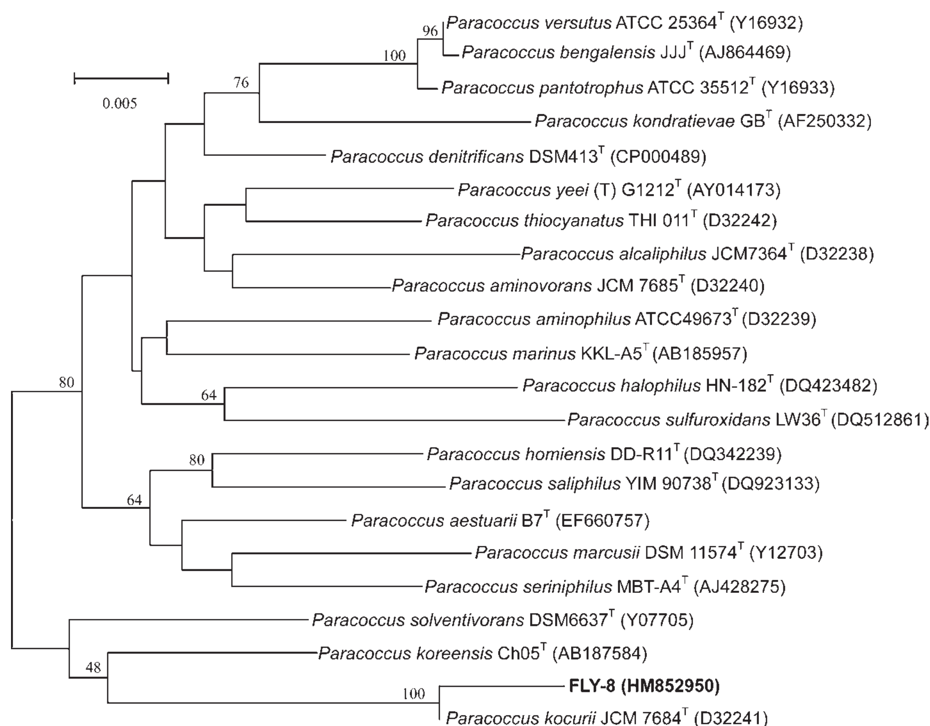

Figure 1. Phylogenetic tree based on the 16S rRNA gene sequences of strain FLY-8 and related species. The GenBank accession number for each microorganism used in the analysis is shown after the species name. The scale bar indicates 0.005 substitutions per nucleotide position. Bootstrap values obtained with 1000 resamplings are indicated as percentages at all branches.

pretilachlor, butachlor, metolachlor, and propisochlor in MSM medium within 5 days of incubation and utilized these herbicides as carbon sources for growth.

Strain FLY-8 was a non-spore-forming, Gram-negative, non-motile, and rod-shaped bacterium. Colonies grown on LB agar were circular, convex, and pale yellow. Growth was observed over a temperature range of 15−35 °C (optimum 25−30 °C), a salinity range of 0−2% NaCl (optimum 0.5% NaCl), and a pH range of 6.0−8.0 (optimum 7.0). Biochemically, it showed positive results for oxidase, catalase, and nitrate reduction and a negative results for urease. The DNA G+C content is 69.5 mol %. Phylogenetic analysis of the 16S rDNA gene sequences revealed that strain FLY-8 grouped among *Paracoccus* species and formed a subclade with *Paracoccus kocurii* JCM 7684^T (similarity = 99.4%) with a high bootstrap value of 100% (Figure 1). Thus, on the basis of the results of phenotypic, genotypic, and phylogenetic properties, strain FLY-8 was identified as a *Paracoccus* sp. The 16S rRNA gene sequence (1375 bp) of the isolate was deposited in GenBank with the accession no. HM 852950.

Degradation and Utilization of Different Chloroacetamide Herbicides by Strain FLY-8. The growth of strain FLY-8 on MSM supplemented with different chloroacetamide herbicides and its ability to degrade these herbicides are shown in Tables 1 and 2. When the initial concentration of different chloroacetamide herbicides was 100 mg/L, 98.7% alachlor, 88.2% acetochlor, 78.3% propisochlor, 65.2% butachlor, 35.9% pretilachlor, and 24.1% metolachlor were degraded by strain FLY-8, respectively, after 5 days of incubation at 30 °C (Table 1); correspondingly, the cell densities were increased from about 1.4×10^6 to 1.4×10^7, 1.2×10^7, 1.1×10^7, 9.5×10^6, 5.1×10^6, and 3.9×10^6 cfu/mL, respectively (Table 2). No significant change in chloroacetamide herbicide concentration was observed in cultures that were not inoculated with strain FLY-8, and no growth was observed for strain FLY-8 when it was inoculated into the culture without the addition of chloroacetamide herbicides. Thus, we concluded that strain FLY-8 was able to degrade the six chloroacetamide herbicides used in this study and utilized these herbicides as carbon sources for growth; the degradation rates followed the order alachlor > acetochlor > propisochlor > butachlor > pretilachlor > metolachlor.

The six chloroacetamide herbicides could be classified into two groups by the type and size of the amide nitrogen's alkoxyalkyl substituent. Group A herbicides, including alachlor, acetochlor, propisochlor, and butachlor, have an alkoxymethyl side chain on the amide nitrogen; and group B herbicides, including pretilachlor and metolachlor, have an alkoxyethyl side chain on the amide nitrogen. Our studies showed that the herbicides in group B were degraded significantly more slowly than those in group A, suggesting that the substitution of an alkoxymethyl side chain with an alkoxyethyl side chain greatly reduced the degradation efficiencies. Comparison of the degradation rates within group A suggested a possible negative correlation between the length of amide nitrogen's alkoxymethyl and the biodegradability of these herbicides. Alachlor, acetochlor, propisochlor, and butachlor have methoxymethyl, ethoxymethyl, isopropoxymethyl, and butoxymethyl, respectively, and the degradation rates followed the order alachlor > acetochlor > propisochlor > butachlor; the longer the alkyl on the amide nitrogen was, the slower were the degradation efficiencies. The explanation may lie in that the increase in length of the alkoxymethyl substituents on the amide nitrogen would exert greater steric hindrance to the enzyme−substrate interaction. Friedman et al. found that the number and length of phenyl alkyl substituents significantly affected the biodegradability of

Table 1. Residue Concentrations of Different Chloroacetamide Herbicides in MSM When Inoculated with Strain FLY-8

herbicide		residue concn[a] (mg/L)					
		0 days	1 day	2 days	3 days	4 days	5 days
alachlor	inoculated	98.49 (4.12) a	97.33 (3.07) a	85.29 (4.22) b	55.25 (4.53) e	9.90 (4.22) e	1.32 (4.94) f
	control	100.84 (3.19)	98.14 (3.08)	100.84 (1.81)	97.84 (1.76)	97.60 (1.54)	95.11 (3.54)
acetochlor	inoculated	100.01 (3.16) a	97.36 (4.08) a	91.99 (3.56) ab	66.93 (3.96) d	24.56 (3.60) d	11.85 (4.79) e
	control	100.77 (3.15)	95.57 (3.03)	101.17 (1.97)	98.17 (1.70)	97.67 (2.04)	96.40 (3.65)
propisochlor	inoculated	101.81 (4.95) a	96.88 (4.61) a	94.89 (4.60) a	79.42 (4.82) c	56.12 (3.60) c	22.14 (4.39) d
	control	100.51 (3.04)	98.04 (3.26)	101.27 (2.90)	98.20 (1.65)	98.27 (1.70)	96.11 (4.16)
butachlor	inoculated	100.49 (4.95) a	96.99 (4.49) a	93.65 (4.31) ab	83.41 (4.72) bc	55.24 (4.05) c	35.02 (4.00) c
	control	100.04 (3.01)	97.70 (3.08)	98.27 (1.65)	97.10 (4.70)	95.61 (3.25)	95.44 (3.60)
pretilachlor	inoculated	100.40 (3.38) a	97.68 (4.57) a	96.04 (3.90) a	88.31 (4.82) ab	78.34 (3.62) b	64.37 (4.51) b
	control	100.37 (3.81)	98.71 (4.06)	96.61 (3.80)	99.77 (2.35)	97.81 (1.50)	97.61 (2.14)
metolachlor	inoculated	101.77 (0.57) a	100.41 (3.49) a	95.34 (3.54) a	92.27 (3.01) a	88.01 (4.60) a	77.21 (4.63) a
	control	100.64 (3.13)	99.71 (4.76)	98.94 (4.32)	97.67 (4.76)	101.54 (3.69)	99.19 (2.59)

[a] The data presented are means of three replicates with standard deviation (in parentheses), which was within 5% of the mean. Different letters indicate significant differences ($p < 0.05$, LSD test), considering effects of chloroacetamide herbicide type and time.

Table 2. Growth of Strain FLY-8 in MSM Supplemented with Different Chloroacetamide Herbicides (Final Concentration = 100 mg/L)

herbicide	growth[a] (10^6 cfu/mL)					
	0 days	1 day	2 days	3 days	4 days	5 days
alachlor	1.36 (0.17) a	1.31 (0.14) a	3.22 (0.22) a	5.37 (0.58) a	8.40 (0.64) a	14.20 (0.73) a
acetochlor	1.50 (0.27) a	1.33 (0.22) a	1.99 (0.29) b	4.08 (0.35) b	8.17 (0.68) a	12.17 (1.25) b
propisochlor	1.30 (0.19) a	1.31 (0.23) a	1.38 (0.33) c	3.89 (0.25) b	7.08 (0.95) b	11.28 (0.70) b
butachlor	1.41 (0.17) a	1.29 (0.24) a	1.37 (0.38) c	3.76 (0.45) b	5.55 (0.55) c	9.71 (0.82) c
pretilachlor	1.57 (0.27) a	1.38 (0.21) a	1.25 (0.14) c	2.60 (0.34) c	3.40 (0.38) d	5.12 (0.30) d
metolachlor	1.26 (0.22) a	1.25 (0.14) a	1.18 (0.23) c	1.64 (0.25) d	2.71 (0.29) d	3.71 (0.40) e
control	1.44 (0.26) a	1.27 (0.31) a	1.24 (0.30) c	0.81 (0.20) e	0.56 (0.15) e	0.35 (0.09) f

[a] The data presented are means of three replicates with standard deviation (in parentheses), which was within 5% of the mean. Different letters indicate significant differences ($p < 0.05$, LSD test), considering effects of chloroacetamide herbicide type and time. Control, without addition of herbicide.

chloroacetanilide herbicides by anodic Fenton treatment; for example, acetochlor and metolachlor (2-methyl, 6-ethylphenyl substituents)[17] degraded more rapidly than alachlor and butachlor (2,6-diethylphenyl substituents). However, in our study, the phenylalkyl substituent seems to have no obvious influence on the degradation efficiency of chloroacetanilide herbicides by strain FLY-8.

Aromatic Substrate Range. Strain FLY-8 degraded and utilized toluene, aniline, 2,6-diethylaniline, salicylic acid, protocatechuate, catechol, *m*-hydroquinone, *p*-hydroquinone, and 2,6-xylidine as carbon source for growth. However, it did not degrade and grow on several other aromatic substrates, including 3,5-xylidine, nitrobenzene, chlorobenzene, and gentisic acid (data not shown).

Metabolites of Butachlor Degradation by Strain FLY-8. The degradation products of butachlor in the culture medium extracts were identified by GC-MS. The extracted base peak chromatograms of the sample of 5 days incubation are shown in Figure 2(1). GC-MS analysis of the sample gave four peaks at retention times (RT) of 4.16, 5.27, 6.07, and 7.30 min representing metabolites D, C, B, and A, respectively [Figure 2(2)]. The four peaks subsequently disappeared after 7 days of incubation, indicating that these metabolites were finally degraded.

Each of the four peaks was indentified according to its mass spectra and the NIST library identification program. Compound A had the same retention time as the butachlor standard (RT = 7.30 min). The mass spectral data also demonstrated that compound A was butachlor [Figure 2(3)]. Compound B with RT = 6.07 min showed a molecular ion at m/z 269 ($[M]^+$). On the basis of the molecular weight and the characteristic fragment ion peaks, compound B was identified as alachlor [2-chloro-*N*-(2,6-dimethylphenyl)-*N*-(methoxymethyl)acetamide] [Figure 2(4)]. Compound C with RT = 5.27 min showed a molecular ion at m/z 225 $[M]^+$, which corresponds to the N-dealkylation of butachlor (or alachlor). Therefore, it was proposed to be

2-chloro-*N*-(2,6-dimethylphenyl)acetamide [Figure 2(5)]. Compound D with RT = 4.16 min showed a molecular ion at m/z 149 $[M]^+$, which corresponded to the loss of a chloroethanamide group from 2-chloro-*N*-(2,6-diethylphenyl)acetamide. It was identified as 2,6-diethylaniline [Figure 2(6)].

The results of degradation experiments and metabolite identification indicated that stain FLY-8 could utilize butachlor (and the other five chloroacetamide herbicides tested) as a carbon source for growth in MSM medium and completely mineralized butachlor. Thus, in light of its broad-spectrum substrate specificities and complete mineralization of chloroacetamide herbicides, strain FLY-8 has the potential to be applied to the removal of chloroacetamide herbicides and their metabolite residues from the environment.

Enzyme Activity in Cell or Cell-free Extract. The aniline dioxygenase, catechol 1,2-dioxygenase, and catechol 2,3-dioxygenase activities of the cellular lysates obtained from strain FLY-8 cells grown on butachlor, 2,6-diethylaniline, or glucose were determined (Table 3). The results showed that strain FLY-8 showed a high level of aniline dioxygenase and catechol 1,2-dioxygenase when the cells were grown on 100 mg/L butachlor or 2,6-diethylaniline, as compared with control cells (grown on glucose). However, no catechol 2,3-dioxygenase was detected under the same culture conditions. These results suggest that aniline

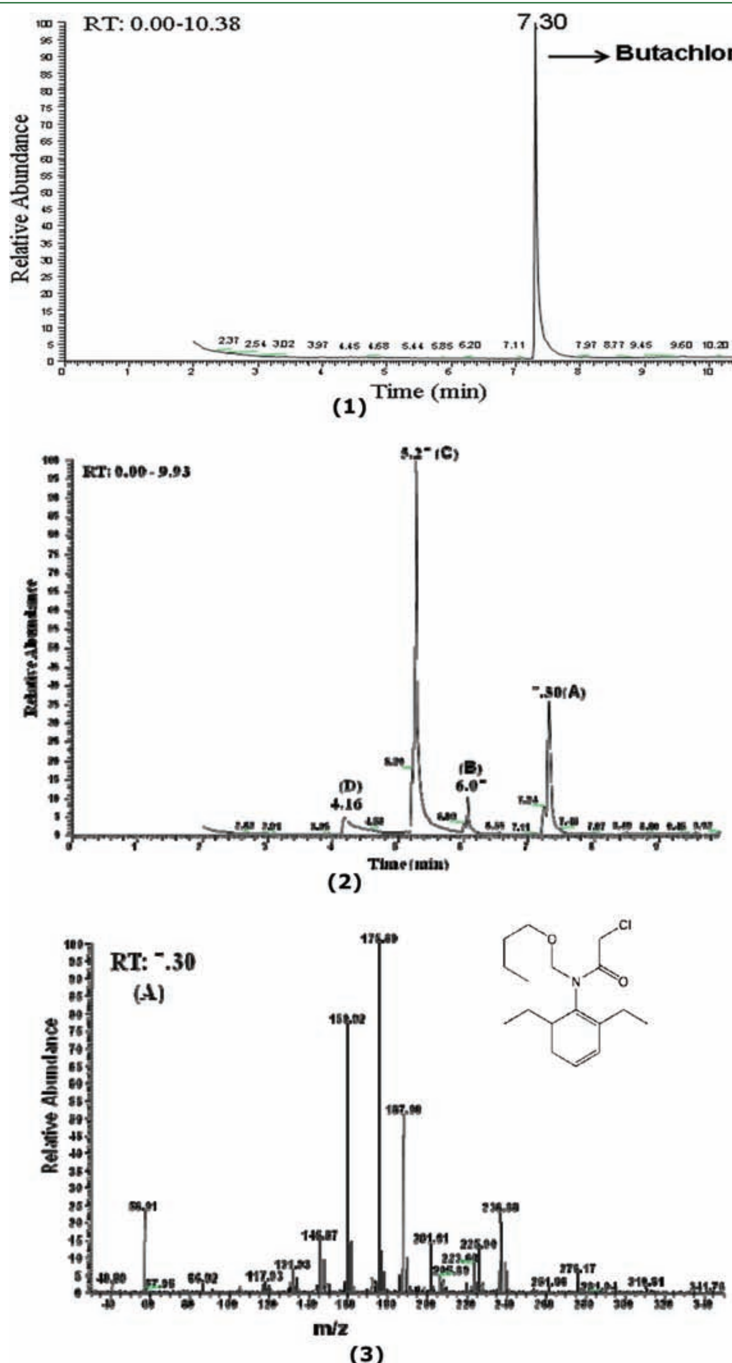

Figure 2. Continued

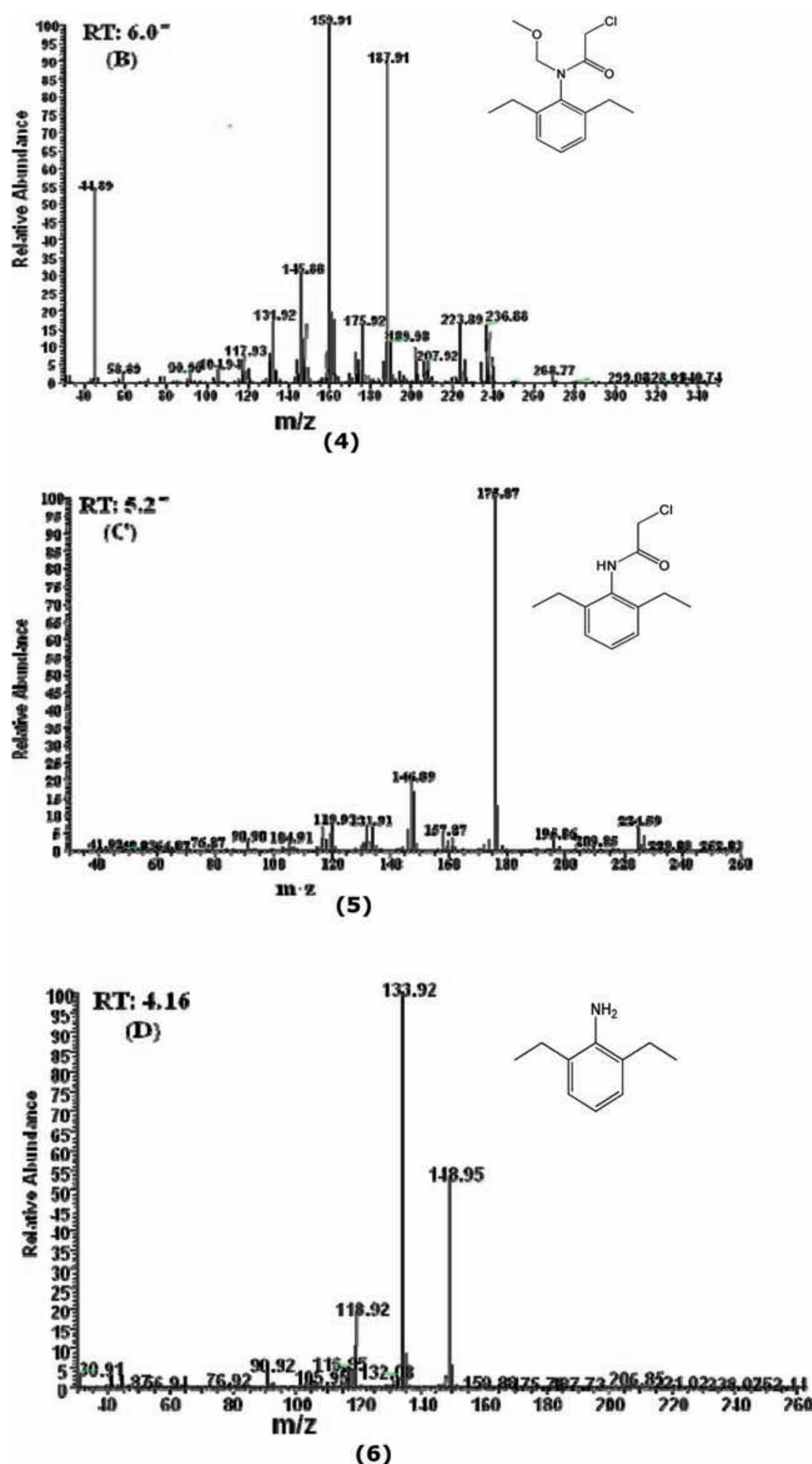

Figure 2. Gas chromatogram of the extract obtained from the culture at 0 days (1) and after 5 days of incubation (2); mass spectra for the peak with RT value of 7.3 min (3); mass spectra for the peak with RT value of 6.07 min (4); mass spectra for the peak with RT value of 5.27 min (5); mass spectra for the peak with RT value of 4.16 (6).

dioxygenase and catechol 1,2-dioxygenase are critical enzymes in the multistep biodegradation of butachlor by *Paracoccus* sp. FLY-8.

On the basis of the results of metabolite identification and enzymatic studies, the plausible complete mineralization pathway of butachlor was proposed (Figure 3). In strain FLY-8, the first step of butachlor degradation was to generate alachlor by partial C-dealkylation followed by conversion to 2-chloro-*N*-(2,6-dimethylphenyl)acetamide by N-dealkylation. 2-Chloro-*N*-(2,6-diethylphenyl)acetamide was subsequently transformed to 2,6-diethylaniline.The fact that the aniline dioxygenase and catechol 1, 2-dioxygenase were induced by butachlor and 2, 6-diethylaniline indicated that the 2,6-diethylaniline was further

Table 3. Specific Activities of Enzymes in the Cell or Cell-free Extract of Strain FLY-8 Cultured in MSM with Different Carbon Sources

growth substrate	aniline dioxygenase (U/mg wet cell)	catechol 1,2-dioxygenase (U/mg protein)	catechol 2,3-dioxygenase (U/mg protein)
butachlor	0.28 ± 0.05	0.45 ± 0.08	–[a]
2,6-diethylaniline	0.42 ± 0.09	0.67 ± 0.09	–
glucose	W[b]	W	–

[a] –, no activity was detected. [b] W, very weak activity.

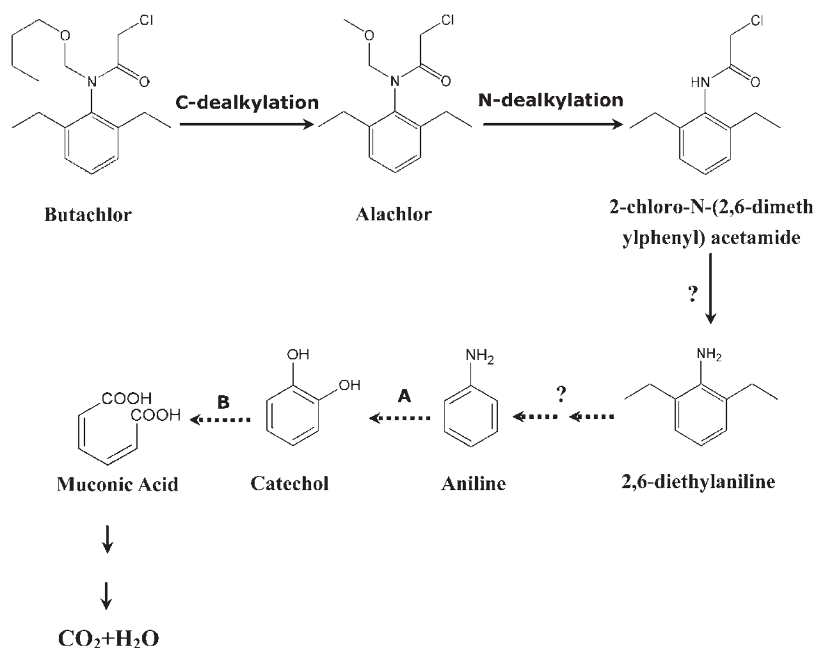

Figure 3. Proposed metabolic pathways of butachlor by strain FLY-8: A, catalyzed by aniline dioxygenase; B, catalyzed by catechol 1,2-dioxygenase.

degraded via the metabolite aniline and catechol and that catechol was oxidized through an ortho-cleavage pathway.

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Author Contributions

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