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A Single Cytochrome P-450 System Is Involved in Degradation of the Herbicides EPTC (*S*-Ethyl Dipropylthiocarbamate) and Atrazine by *Rhodococcus* sp. Strain NI86/21

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During atrazine degradation by *Rhodococcus* sp. strain NI86/21, N-dealkylated metabolites and an hydroxyisopropyl derivative are produced. The cytochrome P-450 system that is involved in degradation of thiocarbamate herbicides by strain NI86/21 (I. Nagy, G. Schoofs, F. Compernolle, P. Proost, J. Vanderleyden, and R. De Mot, J. Bacteriol. 177:676–687, 1995) is also required for atrazine degradation. Atrazine-degrading activity was conferred on the atrazine-negative strains, mutant FAJ2027 of *Rhodococcus* sp. strain NI86/21 and *Rhodococcus erythropolis* SQ1, upon transformation with the genes encoding the cytochrome P-450 system.

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine) is a major herbicide of the *s*-triazine family which has been heavily used throughout the world, especially in corn-growing areas (47). Currently, there is an increasing concern regarding its use, since several reports have shown contamination of surface waters and groundwaters with the herbicide and phytotoxic degradation products, such as deethylatrazine (2-chloro-4-amino-6-isopropylamine-1,3,5-triazine) and deisopropylatrazine (2-chloro-4-ethylamino-6-amino-1,3,5-triazine) (9, 26, 29, 35, 37, 44, 46, 50, 53). Removal of triazines from contaminated wastewaters by biological treatment is usually inefficient (33, 36). The apparent recalcitrance of these compounds to biodegradation is in line with the observations that, until recently, no microorganism capable of complete atrazine mineralization had been identified and that microbial consortia of unknown complexity are required to effect this mineralization (3, 31). A limited number of microorganisms carrying out specific steps in the degradation of *s*-triazines have been isolated from contaminated soils or wastewater (reviewed in references 10 and 22). Recently, a number of strains capable of mineralization of the atrazine ring were isolated (30, 49, 56). However, the enzymatic systems involved have not yet been characterized.

The major degradation products generated by white rot fungi such as *Phanerochaete chrysosporium* (38) and *Pleurotus pulmonarius* (32) are N-dealkylated products. The metabolites produced by some mycorrhizal fungi such as *Hymenoscyphus ericae* have not yet been characterized (18). Bacterial isolates capable of *s*-triazine dealkylation have been identified as *Pseudomonas* spp. (6) or as nocardioform actinomycetes, namely *Nocardia* sp. (23–25) and *Rhodococcus* spp. (5, 7). The *Nocardia* strain was also capable of further deamination (24). The dealkylated metabolites, unlike the parent dialkyl triazines, were dechlorinated and subsequently deaminated by *Rhodococcus corallinus* NRRL B-15444R (13, 14). Such dehalogenating activity was also reported for two *Pseudomonas* strains (6). Recently, the dechlorinating enzyme, *s*-triazine hydrolase, of *R. corallinus* was characterized (39) and the sequence of the corresponding structural gene was determined (51).

A mixed culture of *R. corallinus* and *Pseudomonas* sp. strain NRRL B-12228 was capable of complete metabolism of deethylsimazine (2-chloro-4-amino-6-ethylamino-1,3,5-triazine) (13). *Pseudomonas* sp. strain NRRL B-12228 and the related strain NRRL B-12227 can use the dechlorinated and (partially) dealkylated *s*-triazine ring as a nitrogen source (12). For these strains, the degradative pathway of the *s*-triazine ring has been elucidated (11, 28) and DNA fragments carrying the genes required for ring cleavage were cloned (19, 20).

Although dealkylation is a major pathway for atrazine biodegradation in soils (22), no bacterial or fungal enzymes involved in this initiating step have yet been identified. It was shown that the 77-kb plasmid of *Rhodococcus* sp. strain TE1, which confers the ability to degrade thiocarbamate herbicides such as EPTC (*S*-ethyl dipropylthiocarbamate) (54), also enables N dealkylation of atrazine and related *s*-triazines by this strain (5). N dealkylation also constitutes a crucial step in the bacterial catabolism of EPTC (17). Recently, we identified the *thcB* gene, encoding a novel type of cytochrome P-450 that is essential for N dealkylation of EPTC by *Rhodococcus* sp. strain NI86/21 (42). The genes *thcC* and *thcD* for the associated electron-supplying system, consisting of a 2Fe-2S ferredoxin (ThcC) and ferredoxin reductase (ThcD), were located downstream of *thcB*. Since we observed that strain NI86/21 also degrades atrazine, we investigated the possibility that this cytochrome P-450 system could also be involved in dealkylation of *s*-triazine herbicides.

Atrazine (99% purity) was obtained from North-Hungarian Chemical Works (Sajobábony, Hungary). Analytical standards of deethylatrazine and deisopropylatrazine were a gift of J. Dingwall (Ciba-Geigy, Basel, Switzerland). Hydroxyisopropylatrazine [2-chloro-4-ethylamino-6-(1-hydroxyprop-2-yl)amino-1,3,5-triazine] was kindly provided by S. Masaphy (Migal Technological Center, Kiryat Shmona, Israel). Samples of culture supernatants were extracted with an equal volume of ethyl acetate for gas chromatographic analysis (GC). Samples (5 μl) containing butylate [*S*-ethyl bis(2-methylpropyl)thiocarbamate] (40 μg/ml) as an internal standard were injected at a 20:1 split ratio. The HP-5890 gas chromatograph, equipped with an

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HP-5MS column (30 m by 0.25 mm; cross-linked 5% phenylmethyl silicone; 0.25-μm film thickness), was operated in a two-step temperature gradient (60°C, 1 min; increases of 20°C/min, 6 min; 180°C, 7 min; increases of 20°C/min, 4 min; and 260°C, 4 min). The injector and flame ionization detection temperatures were 275 and 300°C, respectively. The inlet pressure was 105 kPa. Mass spectrometry (MS) (direct insertion probe and GC-MS) was carried out on an HP-59827A quadrupole mass spectrometer, with both electron impact at 70 eV and positive chemical ionization with methane as the reagent gas. Samples for GC-MS were injected on a Chrompack CPSil5 silicone-coated capillary column (length, 25 m; inner diameter, 0.32 mm; film thickness, 1 μm; and He gas flow, 2 ml/min). Following column injection at 150°C, the temperature was raised at 8°C/min to 270°C. The *Rhodococcus* strains used in this study were wild-type strain NI86/21 and its EPTC-minus mutant FAJ2027 (lacking cytochrome P-450 ThcB) previously isolated by chemical mutagenesis (42). Plasmids pFAJ2350 (containing the *thcB* gene) and pFAJ2309 (carrying the *thcBCD* cluster) (42) were introduced into FAJ2027 or *Rhodococcus erythropolis* SQ1 (48) by electroporation (16). pFAJ2308 is similar to pFAJ2309 but contains the complete small *orf5* gene downstream of *thcD* (*Eco*RV-*Hin*dIII fragment), which is truncated in pFAJ2309 (*Eco*RV-*Kpn*I fragment). *orf5* is not required for EPTC degradation (42). Because no transformant of FAJ2027 that contained pFAJ2309 was obtained, FAJ2027(pFAJ2308) was used in this study. Curing of these pDA71 derivatives from FAJ2027 cells recovered after degradation experiments was accomplished by repeated subculturing in Luria-Bertani (LB) medium lacking chloramphenicol (48). The loss of the plasmid was confirmed by hybridization, and the herbicide-degrading abilities of the cured strains were determined in order to avoid false conclusions based on eventual reversion of the mutation in FAJ2027. Biodegradation of atrazine by *Rhodococcus* strains at 30°C was monitored in shake flasks with basal salt medium (BSM) (41) containing various amounts of the herbicide as the sole carbon source. Atrazine concentrations above 55 mg/liter (255 μM) were not used, because the herbicide was no longer completely soluble. For some experiments, NH_4NO_3 was omitted or fructose (1 g/liter) was added. The inoculum consisted of washed *Rhodococcus* cells grown aerobically for 48 h in LB medium at 30°C. For experiments to detect degradation in the presence of fructose, cells grown in BSM with 1 g of fructose per liter were used. In most experiments, a high initial cell density (20 g of washed cells per liter) was used. Some of the degradation experiments were also carried out with a 30-fold-reduced initial cell density (optical density at 600 nm [OD_{600}], 0.19). In uninoculated controls, no loss of atrazine was detectable. Two-dimensional protein electrophoresis of atrazine-grown cells was done as described by De Mot and Vanderleyden (15). Cytochrome P-450 quantification of cell extracts was based on CO difference spectra and used an extinction coefficient of 91 $mM^{-1} \cdot cm^{-1}$ at 452 nm (34). The bicinchoninic acid method was used for determination of protein concentrations (52).

Involvement of the thiocarbamate-inducible cytochrome P-450 system in atrazine degradation by *Rhodococcus* sp. strain NI86/21. Decomposition of atrazine by *Rhodococcus* sp. strain NI86/21 was monitored in BSM medium (Table 1). At a high cell density, degradation of atrazine proceeded quickly. When ammonium nitrate was omitted from the medium, a more extensive conversion was obtained after 4 days, although the initial rate of atrazine degradation was lower. In the presence of the readily metabolized fructose carbon source, the rate of atrazine breakdown was further increased. Under these conditions, atrazine at about 250 μM was degraded within 48 h (Fig. 1). Atrazine degradation in BSM with a low initial cell density (OD_{600}, 0.19) was quite slow, and no significant increase in the turbidity of the culture occurred, which indicated little cell growth (data not shown). Supplementing the medium with fructose, however, sustained growth, with concomitant degradation of the triazine herbicide (Table 1). This result indicated that the atrazine breakdown by strain NI86/21 is not repressed by the presence of an easily assimilated carbon source. Similarly, atrazine degradation by *Rhodococcus* strains TE1 (5) and B30 (7) was not affected by glucose or glycerol. In general, the metabolism of rhodococci is much less sensitive to catabolite repression than that of pseudomonads and often the presence of an easily degradable substrate enables accelerated breakdown of other, more recalcitrant compounds (55).

Previously, we isolated a chemical mutant of *Rhodococcus* sp. strain NI86/21, FAJ2027, which had lost the ability to degrade thiocarbamates such as EPTC (42). This defect was restored by introducing the *thcB* gene of strain NI86/21, which encodes a novel type of cytochrome P-450 enzyme (42).

TABLE 1. Degradation of atrazine by *Rhodococcus* sp. strain NI86/21 and acquisition of atrazine-degrading ability by mutant FAJ2027 and *R. erythropolis* SQ1 upon introduction of the thiocarbamate degradation genes of strain NI86/21

Strain	Medium[a]	Residual atrazine (%)[b] after:			
		24 h	48 h	72 h	96 h
NI86/21	BSM	48 ± 2	18 ± 1	11 ± 2	10 ± 2
	BSM (−N)	60 ± 2	23 ± 2	4 ± 1	0
	BSM (+C)	11 ± 1	0	ND[c]	ND
	BSM (+C)[d]	43 ± 1	14 ± 2	ND	10 ± 2
FAJ2027(pFAJ2350)	BSM	ND	63 ± 3	ND	40 ± 3
FAJ2027(pFAJ2308)	BSM	ND	20 ± 3	ND	7 ± 2
	BSM (+C)[d]	42 ± 3	10 ± 2	0	ND
SQ1(pFAJ2309)	BSM (+C)	100 ± 1	86 ± 2	69 ± 2	64 ± 1

[a] BSM, basal salt medium; BSM (−N), BSM without NH_4NO_3; BSM (+C), BSM with fructose (1 g/liter).
[b] Values are the means ± standard deviations for three replicates. The initial concentration was 220 μM, unless indicated otherwise.
[c] ND, not determined.
[d] The initial cell density (20 g/liter) was lowered 30-fold (OD_{600}, 0.19). The atrazine concentration was 135 μM.

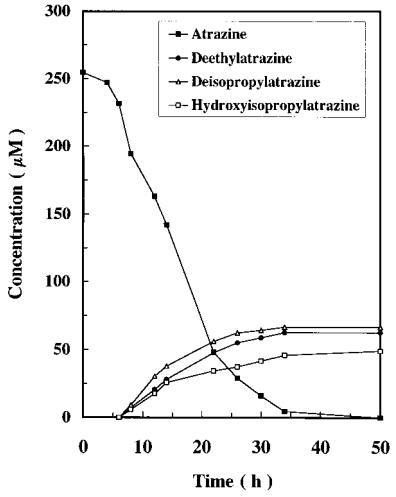

FIG. 1. Degradation of atrazine and accumulation of metabolites by *Rhodococcus* sp. strain NI86/21 cells growing in BSM with fructose. The cell density (OD_{600}) increased from 0.19 to 1.20 after 50 h.

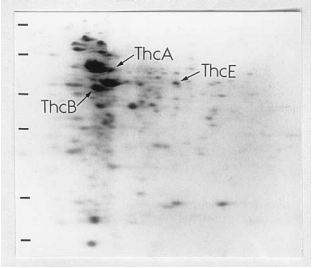

FIG. 2. Two-dimensional protein electrophoresis of total proteins extracted from *Rhodococcus* sp. strain NI86/21 grown in BSM with atrazine and fructose. The positions of induced proteins that have been identified in thiocarbamate-grown cells are indicated by arrows: aldehyde dehydrogenase (ThcA), cytochrome P-450 (ThcB), and *N*,*N*′-dimethyl-4-nitrosoaniline-dependent alcohol oxidoreductase (ThcE). The positions of the molecular weight markers (in thousands, from top to bottom: 97.4, 66.2, 45.0, 31.0, 21.5, and 14.4) are indicated by the dashes on the left. The pH gradient extends from left (acidic) to right (basic). The proteins were stained with Coomassie brilliant blue.

FAJ2027 was also not able to degrade atrazine. This ability was regained upon transformation of this mutant with plasmid pFAJ2350 containing the *thcB* gene, although the decomposition rate (about 2% of residual atrazine after 2 weeks of incubation) was lower than that in the wild-type strain (data not shown). Atrazine degradation was restored to the wild-type level when plasmid pFAJ2308, which also carries *thcC* (encoding the 2Fe-2S ferredoxin, rhodocoxin) and *thcD* (encoding rhodocoxin reductase), was introduced into mutant FAJ2027. Furthermore, the *thcBCD* cluster also conferred an atrazine-degrading phenotype on *R. erythropolis* SQ1 (Table 1). Compared with degradation of atrazine by strain NI86/21 and the complemented mutant, that by strain SQ1(pFAJ2309) was much slower. A similar observation was made previously for EPTC degradation and was attributed to a decreased expression level (about 18-fold lower) of cytochrome P-450 in this SQ1 transformant (42). The current data provide genetic evidence that the same cytochrome P-450 system is involved in biodegradation of both thiocarbamates and atrazine by *Rhodococcus* sp. strain NI86/21. Using two-dimensional protein electrophoresis, we confirmed that the thiocarbamate-inducible cytochrome P-450 enzyme of this strain, ThcB, is also induced by atrazine (Fig. 2). ThcB is not detectable in cells grown with acetate, glycerol, or fructose as a carbon source (42). Two other proteins that are induced by thiocarbamates, namely, the aldehyde dehydrogenase ThcA (42) and the *N*,*N*′-dimethyl-4-nitrosoaniline-dependent alcohol oxidoreductase ThcE (43), also accumulated during atrazine degradation. A fourth thiocarbamate-inducible protein with an M_r of 30,000 (42) was not detected in atrazine-degrading cells. The level of cytochrome P-450 in these cells (~90 pmol [4.4 μg] per mg of protein) was about half of the level in EPTC-grown cells. Our results are consistent with the observation that several *Rhodococcus* isolates that degrade EPTC can also metabolize atrazine (5). Furthermore, the metabolisms of both EPTC and atrazine by *Rhodococcus* sp. strain TE1 are associated with the same plasmid (5, 54). In *Rhodococcus* sp. strain NI86/21, the cytochrome P-450 system that mediates degradation of the two types of herbicides is not plasmid borne (42).

Identification of atrazine metabolites. Three metabolites accumulating during biodegradation of atrazine by *Rhodococcus* sp. strain NI86/21 were identified. GCs of the medium extracts revealed major peaks at 10.8, 11.1, and 17.5 min coinciding with the retention times of authentic standards of, respectively, deisopropylatrazine, deethylatrazine, and hydroxyisopropylatrazine. Chemical ionization-MS analysis using a direct insertion probe confirmed that the three compounds were the main constituents of the metabolite mixture. Furthermore, electron impact and chemical ionization mass spectra acquired via GC-MS analysis were identical to those for the authentic materials. The time course of atrazine degradation by *Rhodococcus* sp. strain NI86/21 at a high cell density with concurrent accumulation of the major degradation products is shown in Fig. 1. These data show that both N deethylation and N deisopropylation of atrazine are carried out by strain NI86/21. In addition, β-hydroxylation of the isopropyl side chain occurred. Recently, we observed that β-hydroxylation of the *S*-propyl substituent of vernolate (*S*-propyl dipropylthiocarbamate) constitutes a major degradation route in *Rhodococcus* sp. strain NI86/21 (40). The metabolites of atrazine were apparently not further degraded by *Rhodococcus* sp. strain NI86/21, and there was no indication of dechlorination or ring cleavage. The same proportion of metabolites was produced under the various conditions listed in Table 1 (data not shown). Similar metabolite profiles were also detected during incubation with the atrazine-degrading transformants of mutant FAJ2027 and *R. erythropolis* SQ1. Our data show that both monodealkylation reactions of atrazine are carried out at comparable rates by *Rhodococcus* sp. strain NI86/21. *Rhodococcus* sp. strain TE1 (5) and *Rhodococcus* sp. strain B30 (7) each showed a preferential deethylation of atrazine. Most likely, a related cytochrome P-450 system is also involved in atrazine conversion by strains TE1 and B30. For these strains, the reactions were shown to depend on the presence of oxygen (5, 7). The β-hydroxylated product generated by *Rhodococcus* sp. strain NI86/21 was recently also identified as a major metabolite during atrazine degradation by the fungus *P. pulmonarius* (32). A related hydroxyisopropyl metabolite was produced by *Rhodococcus* sp. strain B30 from propazine [2-chloro-4,6-bis (isopropylamino)-1,3,5-triazine] but was not reported with atrazine (7). However, the relative amount of this metabolite was not estimated. Growth of *Rhodococcus* sp. strain NI86/21 was supported in minimal medium with atrazine as a carbon source but not as a nitrogen source, indicating that following N dealkylation the side chains are assimilated and the *s*-triazine ring is left undegraded, as is the case for strains B30 and TE1 (5, 7).

Cytochrome P-450-mediated degradation of thiocarbamate and *s*-triazine herbicides. The metabolites generated by *Rhodococcus* sp. strain NI86/21 are consistent with the involvement of a cytochrome P-450 system in atrazine degradation, as inferred from genetic data (as described above). Studies on metabolism of atrazine in vertebrates (1) and plants (21) indicated that detoxification in these systems also involves N-dealkylation reactions that may be catalyzed by cytochrome P-450 enzymes. Indeed, carbon hydroxylation and heteroatom dealkylation represent typical cytochrome P-450-mediated reactions (27). N dealkylation is believed to result from a hydroxylation of the C-N carbon (α position), followed by decomposition of the unstable intermediate into the N-dealkylated product and the alkyl fragment as an aldehyde or ketone (45). Thus, acetaldehyde and acetone would be released from atrazine by N deethylation and N deisopropylation, respectively. N dealkylation is also the major route of thiocarbamate degradation in *Rhodococcus* sp. strain NI86/21, generating alkyl aldehydes (such as propionaldehyde from EPTC). The aldehyde dehydrogenase (42) and the alcohol:*N*,*N*′-dimethyl-4-nitrosoaniline oxidoreductase (43) that likely are involved in the further assimilation of the aldehydes are also induced by atrazine. On the other hand, some rhodococci display acetone-metabolizing activity (2). Apparently, strain NI86/21 is capable of degrading structurally different herbicides by the use of a cytochrome

P-450 with relaxed substrate specificity. Further experiments using a reconstituted system will be directed at determining the substrate spectrum of this enzyme. Other xenobiotics may be identified as substrates for the *Rhodococcus* sp. strain NI86/21 cytochrome P-450. Recently, it was shown that the same plasmid of *Rhodococcus* sp. strain TE1 that is required for degradation of both *s*-triazines and thiocarbamates also is essential for ring hydroxylation of *N*-methylcarbamate insecticides (8). By using the cloned genes of *Rhodococcus* sp. strain NI86/21, it should be possible to elucidate whether the cytochrome P-450 system which degrades thiocarbamates and *s*-triazines also catalyzes the biotransformation of *N*-methylcarbamates or whether another plasmid-encoded enzyme modifies these insecticides. It is not yet clear whether the nonspecific oxidative lignin-decomposing system which enables white rot fungi to degrade a variety of environmental pollutants, including halogenated aromatic compounds (4), may also be involved in atrazine degradation (32, 38).

Although degradation of triazines by rhodococci is limited, dealkylation may facilitate such further biodegradation steps as dechlorination and ring breakdown by other bacteria. We have now identified the genes for an atrazine dealkylating system in *Rhodococcus* sp. strain NI86/21. Recently, a dechlorinating enzyme (39) and the corresponding gene (51) involved in the further breakdown of dealkylated triazines by *R. corallinus* were characterized. This knowledge, together with the availability of triazine ring-cleaving *Pseudomonas* isolates such as NRRL B-12228 and NRRL B-12227, creates new perspectives for the engineering of strains with a potential for accelerated degradation of triazine herbicides.

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REFERENCES

1. **Adams, N. H., P. E. Levi, and E. Hodgson.** 1990. In vitro studies of the metabolism of atrazine, simazine, and terbutryn in several vertebrate species. J. Agric. Food Chem. **38:**1411–1417.
2. **Ashraf, W., A. Mihdhir, and J. C. Murrell.** 1994. Bacterial oxidation of propane. FEMS Microbiol. Lett. **122:**1–6.
3. **Assaf, N. A., and R. F. Turco.** 1994. Accelerated biodegradation of atrazine by a microbial consortium is possible in culture and soil. Biodegradation **5:**29–35.
4. **Barr, D., and S. D. Aust.** 1994. Mechanisms white rot fungi use to degrade pollutants. Environ. Sci. Technol. **28:**78A–87A.
5. **Behki, R., E. Topp, W. Dick, and P. Germon.** 1993. Metabolism of the herbicide atrazine by *Rhodococcus* strains. Appl. Environ. Microbiol. **59:** 1955–1959.
6. **Behki, R. M., and S. Khan.** 1986. Degradation of atrazine by *Pseudomonas*: N-dealkylation and dehalogenation of atrazine and its metabolites. J. Agric. Food Chem. **34:**746–749.
7. **Behki, R. M., and S. Khan.** 1994. Degradation of atrazine, propazine, and simazine by *Rhodococcus* strain B-30. J. Agric. Food Chem. **42:**1237–1241.
8. **Behki, R. M., E. E. Topp, and B. A. Blackwell.** 1994. Ring hydroxylation of N-methylcarbamate insecticides by *Rhodococcus* TE1. J. Agric. Food Chem. **42:**1375–1378.
9. **Buser, H.** 1990. Atrazine and other *s*-triazine herbicides in lakes and in rain in Switzerland. Environ. Sci. Technol. **24:**1049–1058.
10. **Cook, A. M.** 1987. Biodegradation of *s*-triazine xenobiotics. FEMS Microbiol. Rev. **46:**93–116.
11. **Cook, A. M., P. Beilstein, H. Grossenbacher, and R. Hütter.** 1985. Ring cleavage and degradative pathway of cyanuric acid in bacteria. Biochem. J. **231:**25–30.
12. **Cook, A. M., and R. Hütter.** 1981. *s*-Triazines as nitrogen sources for bacteria. J. Agric. Food Chem. **29:**1135–1143.
13. **Cook, A. M., and R. Hütter.** 1984. Deethylsimazine: bacterial dechlorination, deamination, and complete degradation. J. Agric. Food Chem. **32:**581–585.
14. **Cook, A. M., and R. Hütter.** 1986. Ring dechlorination of deethylsimazine by hydrolases from *Rhodococcus corallinus*. FEMS Microbiol. Lett. **34:**335–338.
15. **De Mot, R., and J. Vanderleyden.** 1989. Application of two-dimensional protein analysis for strain fingerprinting and mutant analysis of *Azospirillum* species. Can. J. Microbiol. **35:**960–967.
16. **Desomer, J., P. Dhaese, and M. Van Montagu.** 1990. Transformation of *Rhodococcus fascians* by high-voltage electroporation and development of *R. fascians* cloning vectors. Appl. Environ. Microbiol. **56:**2818–2825.
17. **Dick, W. A., R. O. Ankumah, G. McClung, and N. Abou-Assaf.** 1990. Enhanced degradation of *S*-ethyl *N*,*N*-dipropylcarbamothioate in soil and by an isolated soil microorganism. ACS Symp. Ser. **426:**98–112.
18. **Donnelly, P. K., J. A. Entry, and D. L. Crawford.** 1993. Degradation of atrazine and 2,4-dichlorophenoxyacetic acid by mycorrhizal fungi at three nitrogen concentrations in vitro. Appl. Environ. Microbiol. **59:**2642–2647.
19. **Eaton, R. W., and J. S. Karns.** 1991. Cloning and analysis of *s*-triazine catabolic genes from *Pseudomonas* sp. strain NRRLB-12227. J. Bacteriol. **173:**1215–1222.
20. **Eaton, R. W., and J. S. Karns.** 1991. Cloning and comparison of the DNA encoding ammelide aminohydrolase and cyanuric acid amidohydrolase from three *s*-triazine-degrading bacterial strains. J. Bacteriol. **173:**1363–1366.
21. **Edwards, R., and W. J. Owen.** 1989. The comparative metabolism of the *s*-triazine herbicides atrazine and terbutryne in suspension cultures of potato and wheat. Pestic. Biochem. Physiol. **34:**246–254.
22. **Erickson, L. E., and K. H. Lee.** 1989. Degradation of atrazine and related *s*-triazines. Crit. Rev. Environ. Control **19:**1–14.
23. **Giardi, M. T., M. C. Giardina, and G. Filacchioni.** 1985. Chemical and biological degradation of primary metabolites of atrazine by a *Nocardia* strain. Agric. Biol. Chem. **49:**1551–1558.
24. **Giardina, M. C., M. T. Giardi, and G. Filacchioni.** 1980. 4-Amino-2-chloro-1,3,5-triazine: a new metabolite of atrazine by a soil bacterium. Agric. Biol. Chem. **44:**2067–2072.
25. **Giardina, M. C., M. T. Giardi, and G. Filacchioni.** 1982. Atrazine metabolism by *Nocardia*: elucidation of initial pathway and synthesis of potential metabolites. Agric. Biol. Chem. **46:**1439–1445.
26. **Goodrich, J. A., B. W. Lykins, Jr., and R. M. Clark.** 1991. Drinking water from agriculturally contaminated groundwater. J. Environ. Qual. **20:**707–717.
27. **Guengerich, F. P.** 1990. Enzymatic oxidation of xenobiotic chemicals. Crit. Rev. Biochem. Mol. Biol. **25:**97–153.
28. **Jutzi, K., A. M. Cook, and R. Hütter.** 1982. The degradative pathway of the *s*-triazine melamine. Biochem. J. **208:**679–684.
29. **Koterba, M. T., W. S. L. Banks, and R. J. Shedlock.** 1993. Pesticides in shallow groundwater in the Delmarva peninsula. J. Environ. Qual. **22:**500–518.
30. **Mandelbaum, R. T., D. L. Allan, and L. P. Wackett.** 1995. Isolation and characterization of a *Pseudomonas* sp. that mineralizes the *s*-triazine herbicide atrazine. Appl. Environ. Microbiol. **61:**1451–1457.
31. **Mandelbaum, R. T., L. P. Wackett, and D. L. Allan.** 1993. Mineralization of the *s*-triazine ring of atrazine by stable bacterial mixed cultures. Appl. Environ. Microbiol. **59:**1695–1701.
32. **Masaphy, S., D. Levanon, J. Vaya, and Y. Henis.** 1993. Isolation and characterization of a novel atrazine metabolite produced by the fungus *Pleurotus pulmonarius*, 2-chloro-4-ethylamino-6-(1-hydroxyisopropyl)amino-1,3,5-triazine. Appl. Environ. Microbiol. **59:**4342–4346.
33. **Masten, S. J., and S. H. R. Davies.** 1994. The use of ozonation to degrade organic contaminants in wastewaters. Environ. Sci. Technol. **28:**180A–185A.
34. **Matsuoka, T., S. Miyakoshi, K. Tanzawa, K. Nakahara, M. Hosobuchi, and N. Serizawa.** 1989. Purification and characterization of cytochrome $P\text{-}450_{sca}$ from *Streptomyces carbophilus*. ML-236B (compactin) induces a cytochrome $P\text{-}450_{sca}$ in *Streptomyces carbophilus* that hydroxylates ML-236B to pravastatin sodium (CS-514), a tissue-selective inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme-A reductase. Eur. J. Biochem. **184:**707–713.
35. **McMahon, P. B., F. H. Chapelle, and M. L. Jagucki.** 1992. Atrazine mineralization potential of alluvial-aquifer sediments under aerobic conditions. Environ. Sci. Technol. **26:**1556–1559.
36. **Meakins, N. C., J. M. Bubb, and J. N. Lester.** 1994. The behaviour of the *s*-triazine herbicides, atrazine and simazine, during primary and secondary biological waste water treatment. Chemosphere **28:**1611–1622.
37. **Mills, M. S., and E. M. Thurman.** 1994. Preferential dealkylation reactions of *s*-triazine herbicides in the unsaturated zone. Environ. Sci. Technol. **28:** 600–605.
38. **Mougin, C., C. Laugero, M. Asther, J. Dubroca, P. Frasse, and M. Asther.** 1994. Biotransformation of the herbicide atrazine by the white rot fungus *Phanerochaete chrysosporium*. Appl. Environ. Microbiol. **60:**705–708.
39. **Mulbry, W. W.** 1994. Purification and characterization of an inducible *s*-triazine hydrolase from *Rhodococcus corallinus* NRRL B-15444R. Appl. Environ. Microbiol. **60:**613–618.
40. **Nagy, I., F. Compernolle, and R. De Mot.** Unpublished data.
41. **Nagy, I., J. Nagy, J. Matyas, and M. Kecskés.** 1987. Decomposition of EPTC by soil microbes in two soils, p. 525–530. *In* Proceedings of the British Crop

Protection Conference—Weeds, vol. 1. Lavenham Press Ltd., Lavenham, United Kingdom.
42. **Nagy, I., G. Schoofs, F. Compernolle, P. Proost, J. Vanderleyden, and R. De Mot.** 1995. Degradation of the thiocarbamate herbicide EPTC (*S*-ethyl dipropylcarbamothioate) and biosafening by *Rhodococcus* sp. strain NI86/21 involve an inducible cytochrome P-450 system and aldehyde dehydrogenase. J. Bacteriol. **177:**676–687.
43. **Nagy, I., S. Verheijen, A. De Schrijver, J. Van Damme, P. Proost, G. Schoofs, J. Vanderleyden, and R. De Mot.** Arch. Microbiol., in press.
44. **Nair, D. R., and J. L. Schnoor.** 1992. Effect of two electron acceptors on atrazine mineralization rates in soil. Environ. Sci. Technol. **26:**2298–2300.
45. **Okazaki, O., and F. P. Guengerich.** 1993. Evidence for specific base catalysis in *N*-dealkylation reactions catalyzed by cytochrome P450 and chloroperoxidase. J. Biol. Chem. **268:**1546–1552.
46. **Pereira, W. E., and C. E. Rostad.** 1990. Occurrence, distributions, and transport of herbicides and their degradation products in the lower Mississippi river and its tributaries. Environ. Sci. Technol. **24:**1990.
47. **Phillips, M., and J. McDougall.** 1993. Triazine herbicides, p. 10–11. *In* Wood-McKenzie report, part 1.
48. **Quan, S., and E. R. Dabbs.** 1993. Nocardioform arsenic resistance plasmid characterization and improved *Rhodococcus* cloning vectors. Plasmid **29:**74–79.
49. **Radosevich, M., S. J. Traina, Y.-L. Hao, and O. H. Tuovinen.** 1995. Degradation and mineralization of atrazine by a soil bacterial isolate. Appl. Environ. Microbiol. **61:**297–302.
50. **Schottler, S. P., S. J. Eisenreich, and P. D. Capel.** 1994. Atrazine, alachlor, and cyanazine in a large agricultural river system. Environ. Sci. Technol. **28:**1078–1089.
51. **Seffens, W., and W. W. Mulbry.** Unpublished data.
52. **Smith, P. K., R. I. Krohn, G. T. Hermanson, A. K. Mallia, F. H. Gartner, M. D. Provenzano, E. K. Fujimoto, N. M. Goeke, B. J. Olson, and D. C. Klenk.** 1985. Measurement of protein using bicinchoninic acid. Anal. Biochem. **150:**76–85.
53. **Spalding, R. F., D. D. Snow, D. A. Cassada, and M. E. Burbach.** 1994. Study of pesticide occurrence in two closely spaced lakes in northeastern Nebraska. J. Environ. Qual. **23:**571–578.
54. **Tam, A. C., R. M. Behki, and S. V. Khan.** 1987. Isolation and characterization of an EPTC-degrading *Arthrobacter* strain and evidence for plasmid-associated EPTC degradation. Appl. Environ. Microbiol. **53:**1088–1093.
55. **Warhurst, A. M., and C. A. Fewson.** 1994. Biotransformations catalyzed by the genus *Rhodococcus*. Crit. Rev. Biotechnol. **14:**29–73.
56. **Yanze-Kontchou, C., and N. Gschwind.** 1994. Mineralization of the herbicide atrazine as a carbon source by a *Pseudomonas* strain. Appl. Environ. Microbiol. **60:**4297–4302.