

土壌微生物によるs-トリアジン系除草剤の分解とその原位 置バイオレメディエーションへの応用

誌名	新潟大学農学部研究報告 = Bulletin of the Faculty of Agriculture, Niigata University
ISSN	03858634
著者名	原田,直樹
発行元	新潟大学農学部
巻/号	60巻1号
巻号補足	
掲載ページ	p. 1-7
発行年月	2007年8月

農林水産省 農林水産技術会議事務局筑波産学連携支援センター

Tsukuba Business-Academia Cooperation Support Center, Agriculture, Forestry and Fisheries Research Council
Secretariat



Biodegradation of *s*-Triazine Herbicides by Soil Microorganisms and Their Application for *In Situ* Bioremediation

Naoki HARADA*

(Received June 29, 2007)

Summary

Moderately persistent *s*-triazine compounds are widely used in agriculture for the control of various weeds. In this review, microbial degradation of chloro- and methylthio-*s*-triazines and a recent attempt to utilize isolated simazine-degrading bacteria for *in situ* bioremediation are described. As microorganisms capable of degrading chloro-*s*-triazines, atrazine-degrading *Pseudomonas* sp. strain ADP has been studied most extensively. Dechlorination of the *s*-triazine ring by the strain is mediated by atrazine chlorohydrolase (AtzA) and the subsequent degradation is associated with AtzBCDEF. A novel simazine-degrading β -proteobacterium strain CDB21 isolated by the author's group also possessed entire-set of genes of these enzymes (*atzABCDEF*). However, while *atzA* is located between ORF30 and *atzB* in strain ADP, ORF30 fused with *atzB* in strain CDB21. This indicates that localization of *atzA* in CDB21 differs from that in the pseudomonad, meaning that genetic variations regarding the degradation genes exist among microorganisms capable of degrading chloro-*s*-triazines. It is known that methylthio-*s*-triazines can be transformed to the corresponding hydroxy analogues via sulfur oxidation in aerobic and flooded soil. Recently, two types of bacteria that engage the process were isolated. One is *Bacillus cereus* strain JUN7 that can degrade methylthio-*s*-triazines to give the methylsulfinyl and hydroxy analogues under nutrient-rich conditions such as those with the Luria-Bertani medium. The other is *Rhodococcus* sp. strain FJ1117YT that is able to degrade methylthio-*s*-triazines supplied as the sole sulfur source. These strains were unable to degrade chloro-*s*-triazines. No gene encoding AtzA or TrzN (triazine hydrolase) was found in these strains, suggesting that they may have another metabolic system. Using a special charcoal material enriched with a simazine-degrading bacterial consortium involving strain CDB21, *in situ* bioremediation study was conducted in a golf course, where simazine is routinely applied for preservation of turf. As a result, the material was effective for preventing penetration of simazine into subsoils and aquatic environments nearby for approximately 2 years. This result suggests that use of adequate habitat materials is preferable to maintain population and activity of inoculant bacteria applied to soil environment.

Bull.Facul.Agric.Niigata Univ., 60:1-7, 2007

Key words : biodegradation, *in situ* bioremediation, simazine, simetryn, *s*-triazine

Recently, pollution of crops, soil and water with recalcitrant agricultural chemicals has become a major environmental issue that needs to be resolved. *S*-triazine compounds are very popular, and are widely used in agriculture for the control of various weeds. *S*-triazines are characterized by a symmetrical hexameric ring consisting of alternating carbon and nitrogen atoms, and are recognized as moderately persistent chemicals. In this review, microbial degradation of *s*-triazines is mainly described. In addition, a recent attempt to prevent environmental pollution by simazine, a typical chloro-*s*-triazine, using a special charcoal material enriched with a simazine-degrading consortium is presented.

Bacterial degradation of atrazine

In general, *s*-triazine herbicides are classified into three groups; chloro-, methylthio- and methoxy-*s*-triazines (Table I). In the *s*-triazine family, chloro-*s*-triazines such as atrazine and simazine are the most popular. In particular, atrazine is used globally to control annual grasses and broadleaf weeds in fields of the major crops, such as corn, sorghum, and sugar

cane.

Since atrazine sometimes contaminates ground water (Belluck *et al.*, 1991), environmental fate of the herbicide has been studied well. A number of Gram-negative (Yanze-Kontchou and Gshwind, 1994; de Souza *et al.*, 1996; Topp *et al.*, 2000b) and Gram-positive (Behki *et al.*, 1993; de Souza *et al.*, 1998; Topp *et al.*, 2000a; Piutti *et al.*, 2003; Satsuma, 2006) bacteria capable of degrading atrazine have been isolated. The catabolic pathways in the degradation of atrazine have been extensively studied in atrazine-degrading *Pseudomonas* sp. strain ADP. Six atrazine catabolic genes, *atzABCDEF*, were determined in the pseudomonad (Fig. 1) (de Souza *et al.*, 1996; Boundy-Mills *et al.*, 1997; Sadowsky *et al.*, 1998; Martinez *et al.*, 2001). In the first step of the degradation, dechlorination of the *s*-triazine ring is mediated by atrazine chlorohydrolase (AtzA) (de Souza *et al.*, 1996). In the case of *Nocardioide*s sp. strain C190, triazine hydrolase (TrzN) mediates the first step dechlorination of the degradation (Topp *et al.*, 2000a; Mulbry *et al.*, 2002).

Table 1. Structures and molecular weights of *s*-triazine herbicides

Compound	Structure			Molecular weights	Domestic sales 2003 ¹ (Tg or kL)
	X	R ₁	R ₂		
Atrazine	Cl	-CH ₂ CH ₃	-CH(CH ₃) ₂	215.7	51.36
Propazine	Cl	-CH(CH ₃) ₂	-CH(CH ₃) ₂	229.7	Withdrawn
Simazine	Cl	-CH ₂ CH ₃	-CH ₂ CH ₃	201.7	41.7
Terbuthylazine	Cl	-CH ₂ CH ₃	-C(CH ₃) ₃	229.7	Not registered
Ametryn	SCH ₃	-CH ₂ CH ₃	-CH(CH ₃) ₂	227.3	6.45
Desmetryn	SCH ₃	-CH ₃	-CH(CH ₃) ₂	213.3	Withdrawn
Dimethametryn	SCH ₃	-CH ₂ CH ₃	-CH(CH ₃)CH(CH ₃) ₂	255.4	15.28
Simetryn	SCH ₃	-CH ₂ CH ₃	-CH ₂ CH ₃	213.3	88.51
Prometryn	SCH ₃	-CH(CH ₃) ₂	-CH(CH ₃) ₂	241.4	18.31
Atraton	OCH ₃	-CH ₂ CH ₃	-CH(CH ₃) ₂	211.3	Not registered

¹ According to WebKis-Plus (<http://w-chemdb.nies.go.jp/>) offered by National Institute for Environmental Studies (accessed on June 19, 2007).

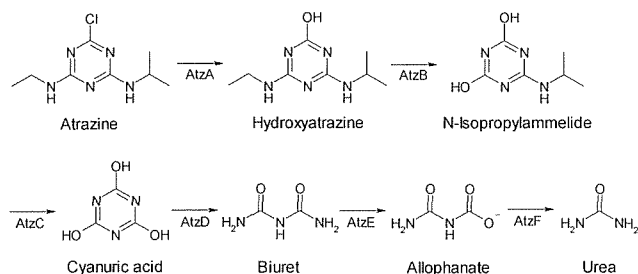


Fig. 1. Metabolic pathway of atrazine determined in *Pseudomonas* sp. strain ADP. Six atrazine catabolic genes, *atzABCDEF*, contribute to mineralization of atrazine (de Souza *et al.*, 1996 ; Boundy-Mills *et al.*, 1997 ; Sadowsky *et al.*, 1998; Martinez *et al.*, 2001).

Characteristics of simazine-degrading strain CDB21

Compared with atrazine, fewer reports regarding simazine-degrading bacteria have been available. Takagi and Yoshioka (2000) tried to enrich simazine-degrading bacteria using "soil-charcoal perfusion system", which is a rapid enrichment method involving use of porous charcoal. After a culture containing simazine as the sole carbon and nitrogen source was perfused in the system, a novel bacterial strain CDB21 was isolated from the enriched charcoal granules (Iwasaki *et al.*, 2007).

Strain CDB21 was Gram-negative, rod-shaped and motile by means of a single polar flagellum. Based on 16S rRNA sequence analysis, strain CDB21 was identified as a novel β -proteobacterium exhibiting 100% sequence identity with the uncultured bacterium HOCiCi25 (GenBank accession number AY328574). Phylogenetic affiliation based on the 16S

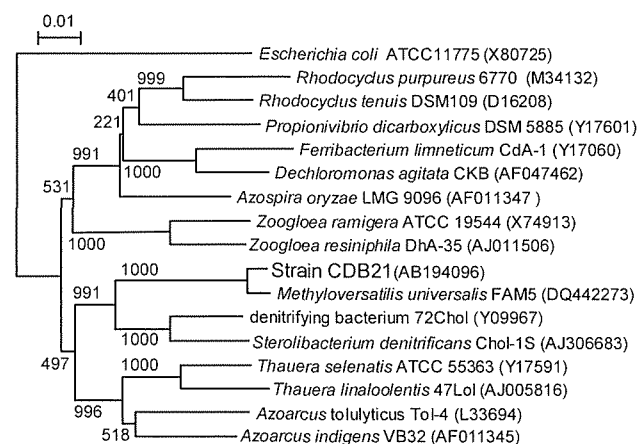


Fig. 2. Phylogenetic affiliation based on the 16S rRNA sequence data, showing the relationship of strain CDB21 to the most closely related bacteria and related representatives in the order *Rhodocyclales*. The GenBank accession numbers are provided in parentheses. The dendrogram was generated by the neighbor-joining method. The numbers at the branch points are bootstrap values based on 1,000 trials. The *Escherichia coli* sequence was used as an outgroup. Bar indicates 10 nucleotide substitutions per 1,000 nucleotide positions (Iwasaki *et al.*, 2007).

rRNA sequence data is shown in **Fig. 2**. Strain CDB21 can degrade simazine and other chlorinated *s*-triazine herbicides except propazine (**Fig. 3**).

PCR using primers that were specific for the genes of the atrazine-degrading enzymes of *Pseudomonas* sp. strain

ADP (*atzABCDEF*) showed that strain CDB21 also possessed the entire-set of genes of these enzymes (Iwasaki *et al.*, 2007). Nucleotide sequences of the *atzCDEF* genes of strain CDB21 were 100% identical with those of strain ADP. Sequence identity of the *atzA* genes was 99.7%. The 398-nucleotide upstream fragment of the *atzB* gene of strain CDB21 was 100% identical with ORF30 of strain ADP, and the 1,526-nucleotide downstream fragment showed 99.8% sequence similarity to the *atzB* gene of the pseudomonad.

In *Pseudomonas* sp. strain ADP, all the genes encoding atrazine-catabolic enzymes are located on a single catabolic plasmid, and *atzA* is located between ORF30 and *atzB* (Martinez *et al.*, 2001). In strain CDB21, its ORF30 fused with *atzB* (Fig. 4). This implies that localization of *atzA* in strain CDB21 differs from that in *Pseudomonas* sp. strain ADP.

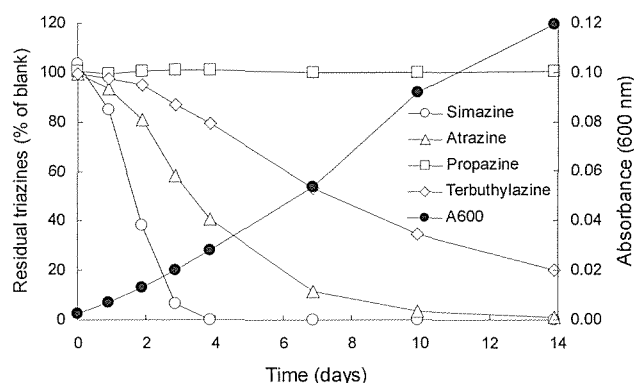


Fig. 3. Changes in the residual concentrations of s-triazines (simazine, atrazine, propazine, and terbuthylazine) in strain CDB21 culture supernatants. Residual concentrations of the s-triazines were expressed as the percentage of those in the blank culture. Growth of the cells was determined by measuring the absorbance at 600 nm. The details of the experiment conditions are described in Iwasaki *et al.* (2007).

ADP ORF30aa	L T G L K I T Y G T H E i *
ADP ORF30	CTGACAGGTC TAAAAATAACCTATGGAACACACGAAAttgga
CDB ORF30aa	L T G L K I T Y G T H E T L H R V S P A G H R G C R G H G A *
CDB <i>atzB</i>	362 CTGACAGGTC TAAAAATAACCTATGGAACACACGAAATTTACACCGGGTTTCACCAGCTGGTCACCGGGGATGTCGCGGGCACGGTGCTTAAT 455
CDB <i>atzBaa</i>	M E H T K L Y T G F H Q L V T G D V A G T V L N
	1 10 20
ADP <i>atzB</i>	atgaccaccactCTTTACACCGGGTTTCACCAGCTGGTCACCGGGGATGTCGCGGGCACGGTGCTTAAT
ADP <i>atzBaa</i>	m t t t L Y T G F H Q L V T G D V A G T V L N
	1 10 20

Fig. 4. A comparison of the boundary between the coding region and the transcribed but untranslated region of *atzB*. The 94-nucleotide coding region and upstream region of the *atzB* gene of strain CDB21 (nucleotide number 362 to 455) are aligned with the ORF30 downstream region and the *atzB* coding region of *Pseudomonas* sp. strain ADP. The deduced amino acids of ORF30 are displayed above the nucleotide sequences, and those of *atzB* are arranged below. The amino acids of AtzB are numbered sequentially from the amino terminal Met residue as 1 in both the bacteria, and the numbers are aligned below the amino acid residues. The nucleotides and amino acid residues of *Pseudomonas* sp. strain ADP, which do not match with those of strain CDB21, are provided in lowercase letters. The possible initiation codon of the *atzB* ORF of strain CDB21 and the possible Shine-Dalgarno sequence upstream of the start codon are underlined. The stop codons are represented by asterisks (Iwasaki *et al.*, 2007).

Between strain CDB21 and *Pseudomonas* sp. strain ADP, some codon substitutions occurred in the coding regions of *atzA* and *atzB*. Several amino acids in the amino terminal region of AtzB are exchanged as a result of the gene fusion of ORF30 and *atzB* in strain CDB21 (Fig. 4). These results mean that genetic variations regarding the degradation genes exist among microorganisms capable of degrading chloro-s-triazines.

Simetryn degradation via sulfur oxidation

In Asia, methylthio-s-triazines are being used in the control of broadleaf weeds in irrigated rice fields. However, microbial degradation of methylthio-s-triazines has not yet been studied enough. Cook and Hütter (1982) reported that three Gram-negative bacterial strains could utilize ametryn and/or prometryn as the sole sulfur source for growth. Aislabie *et al.* (2005) described that atrazine-degrading *Arthrobacter nicotinovorans* strain HIM could also degrade prometryn. In addition, the following enzymatic transformation of methylthio-s-triazines to the hydroxy analogues using bacterial species capable of degrading atrazine is known. Topp *et al.* (2000a) showed that methylthio-s-triazines were anaerobically hydrolyzed by whole cells or cell extracts of *Nocardioideis* sp. strain C190, and Seffernick *et al.* (2000) transformed ametryn to hydroxy ametryn using cell extracts obtained from *Clavibacter michiganensis* strain ATZ1. Shapir *et al.* (2005) reported that recombinant TrzN derived from *Arthrobacter aureus* strain TC1 could rapidly transform ametryn and methylsulfinyl ametryn to hydroxy ametryn.

In a metabolic study of methylthio-s-triazines in aerobic and flooded soil using ring and meththio-¹⁴C-labeled prometryn (Kaufman and Kearney, 1976), the labeled methylsulfinyl, methylsulfonyl and hydroxy analogues were produced. However, no available report regarding demonstration of sulfur oxidation during bacterial or enzymatic transformation of methylthio-s-triazines to the

hydroxy analogues has been available.

In the past few years, the author's group has investigated bacterial degradation of simetryn, since simetryn is one of the major herbicides used in paddy fields in Japan and it may cause algal growth inhibition in the agricultural basin (Kasai, 1999; Okamura *et al.*, 2002). First, we found a *Bacillus cereus* strain JUN7 from a Japanese soil sample, which can degrade simetryn to give the methylsulfinyl and hydroxy analogues under nutrient-rich conditions such as those with the Luria-Bertani (LB) medium (Harada *et al.*, 2006). The presence of the higher nutrient levels led to higher cell numbers and faster reduction in the simetryn concentrations (Fig. 5). The isolate did not grow in the minimum salt medium supplemented with simetryn as the sole carbon source, suggesting that the isolate transforms simetryn co-metabolically. As metabolites, methylsulfinyl and hydroxy analogues were determined by LCMS analysis. Strain JUN7 could decrease other methylthio-*s*-triazines such as dimethametryn and prometryn, but not chlorinated *s*-triazines (atrazine, simazine, and terbuthylazine) and methoxy-*s*-triazine (atraton) in 1/10 LB medium.

Rhodococcus sp. strain FJ1117YT that is able to degrade simetryn supplied as the sole sulfur source was also isolated (Fujii *et al.*, 2007). Three metabolites were found and identified as the methylsulfinyl, methylsulfonyl and hydroxyl

analogues (Fig. 6). Variations of the concentration of simetryn and its metabolites, and optical density at 660 nm in the culture medium of strain FJ1117YT are presented in Fig. 7. These results indicate that the methylthio group was progressively oxidized and hydrolyzed by strain FJ1117YT. The strain is also able to metabolize other methylthio-*s*-triazines such as ametryn, desmetryn, dimethametryn and prometryn through similar pathways.

In case of known chloro-*s*-triazine-degrading bacteria, AtzA or TrzN participates in the first metabolic step of the degradation. While AtzA enzyme only hydrolyzes the chloro group of *s*-triazine herbicides, TrzN does chloro-, methylthio- and methoxy-substituents. However, the genes encoding AtzA and TrzN were not found in the strains JUN7 and FJ1117YT, which are unable to degrade chloro-*s*-triazines, suggesting that they may have another metabolic system. Characterization of the enzymatic mechanism to metabolize methylthio-*s*-triazines in these strains is a future task.

Attempt to adapt strain CDB21 to *in situ* bioremediation

In situ bioremediation is considered to be a highly promising technology for the removal of environmental pollutants. Bioremediation using pollutant-degrading bacteria is generally divided into two approaches; biostimulation and bioaugmentation. Biostimulation is to modify environment of the contaminated soil by addition of various forms of limiting nutrients and to stimulate population and activity of existing degrading organisms. However, biostimulation is not always effective, especially when availability of the contaminant compounds to existing organisms is low. In such cases, bioaugmentation to introduce a group of natural microbial strains or a genetically engineered variant to achieve bioremediation is a good alternative, if isolated degrading

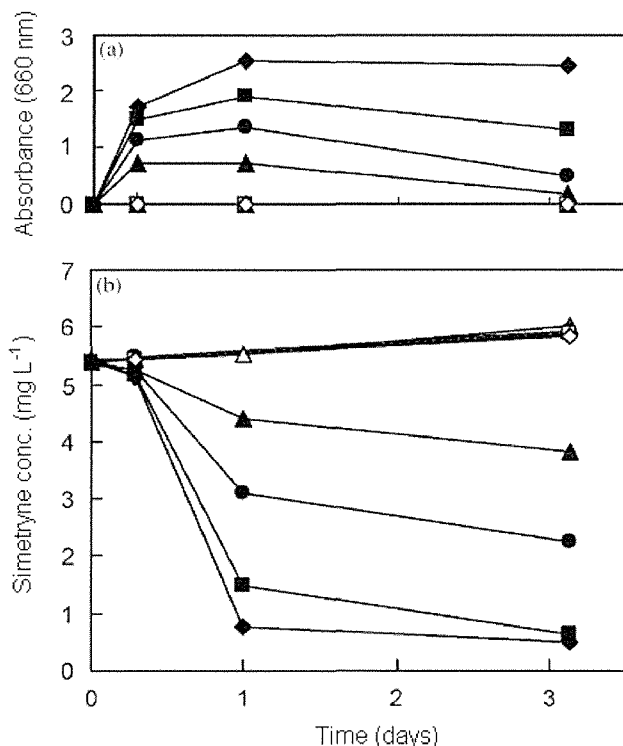


Fig. 5. Growth of strain JUN7 (a) and simetryn degradation by the isolate (b) in 1/10 (▲), 1/5 (●), 1/2 (■) and normal LB media (◆) supplemented with 5 mg L⁻¹ of simetryn. White-colored symbols mean the respective uninoculated controls (modified from Harada *et al.*, 2006).

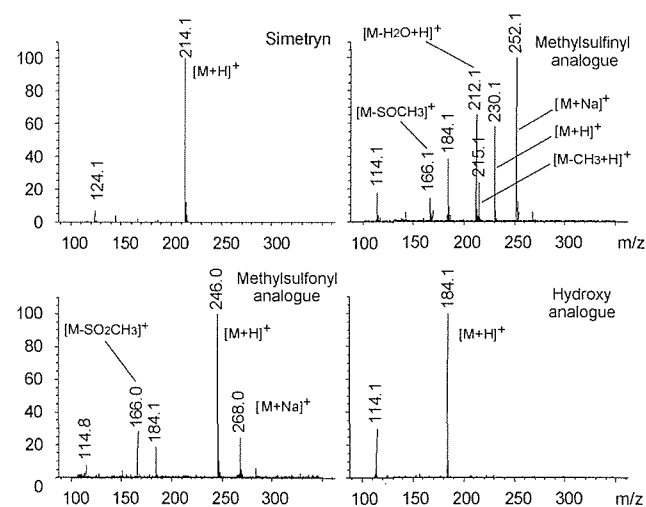


Fig. 6. Mass spectra of simetryn and its metabolites in the culture supernatant of strain FJ1117YT as recorded by LCMS (modified from Fujii *et al.*, 2007).

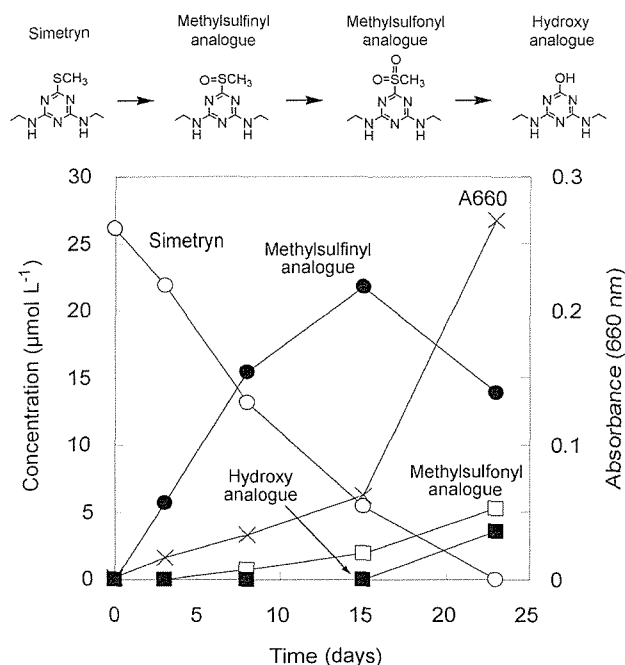


Fig. 7. Estimated metabolic pathway of simetryn by strain FJ1117YT, and variations of the concentration of simetryn and its metabolites, and optical density at 660 nm in the culture medium of strain FJ1117YT. The amounts of methylsulfinyl and hydroxyl analogues were calculated based on their molar absorbance coefficients relative to simetryn. The amounts of methylsulfonyl analogue were calculated on the initial substance-basis. The details of the experiment conditions are described in Fujii *et al.* (2007).

organisms are available.

To utilize isolated degrading bacteria as an inoculant for bioaugmentation to reduce soil contamination, it is necessary to avoid excessive decline in the inoculant population and activity following introduction into soil. Takagi and Yoshioka (1999) developed a special charcoal material enriched with a simazine-degrading bacterial consortium involving strain CDB21. They laid the material under turf of a golf course, and monitored changes in concentration of simazine in soil solution sampled under the charcoal layer and population of simazine-degrading bacteria in the charcoal (Takagi *et al.*, 2005). As shown in Fig. 8, simazine concentration in the soil solution sampled from the treatment plot were much lower than that from the control plot throughout the experiment, meaning that the material is effective for preventing penetration of simazine into subsoils and aquatic environments nearby for approximately 2 years. Population of strain CDB21 in the charcoal was maintained at a level of 10^5 cfu g^{-1} dry weight, following the initial decline observed in the first 120 days. Changes in bacterial community in the charcoal monitored by a PCR-DGGE method during the field study is presented in Fig. 9. Three species consisting of the simazine-degrading consortium enriched were detected in the

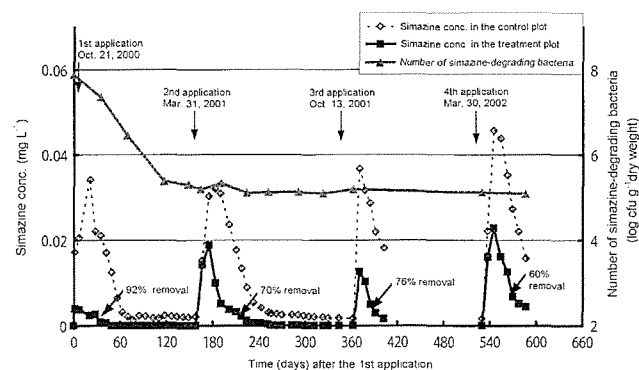


Fig. 8. Changes in concentration of simazine in the soil solutions sampled under the charcoal layer and population of simazine-degrading bacteria in the charcoal. In the control plot, a charcoal material without bacterial enrichment was laid in the same manner as in the treatment plot. Simazine application was performed 4 times (see arrows) during the experiment (Takagi *et al.*, 2005).

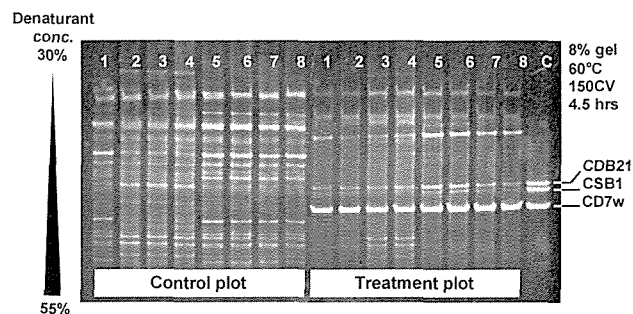


Fig. 9. Monitoring of changes in bacterial community in the charcoal enriched with a simazine-degrading bacterial consortium during the field *in situ* bioremediation study by PCR-DGGE. Sampling was performed on (1) Nov. 22, 2000, (2) Dec. 26, 2000, (3) Feb. 11, 2001, (4) Mar. 31, 2001, (5) May 2, 2001, (6) Oct. 13, 2001, (7) Mar. 31, 2002 and (8) May 25, 2002. Lane C is a result in the simazine-degrading bacterial consortium, consisting of strain CDB21, *Bradyrhizobium japonicum* CSB1 and *Arthrobacter* sp. strain CD7w (Takagi *et al.*, 2005).

treatment plot through the experiment, but not in the control plot in which a charcoal material without bacterial enrichment was laid in the same manner as in the treatment plot. These results suggest that use of adequate habitat materials such as charcoal is preferable to maintain population and activity of inoculated bacteria in soil environment.

REFERENCES

- Aislabie, J., A.K. Bej, J. Ryburn, N. Lloyd and A. Wilkins. 2005. Characterization of *Arthrobacter nicotinovorans* HIM, an atrazine-degrading bacterium, from agricultural soil New Zealand. *FEMS Microbiol. Ecol.*, **52** : 279-286.

- Behki, R., E. Topp, W. Dick and P. Germon. 1993. Metabolism of the herbicide atrazine by *Rhodococcus* strains. *Appl. Environ. Microbiol.*, **59** : 955-959.
- Belluck, D.A., S.L. Benjamin and T. Dawson. 1991. Groundwater contamination by atrazine and its metabolites: risk assessment, policy, and legal implications. pp. 254-273. In: *Pesticide transformation products: Fate and significance in the environment*. ACS Symposium Series 459. American Chemical Society, Washington, D.C.
- Boundy-Mills, K.L., M.L. de Souza, R.T. Mandelbaum, L.P. Wackett and M.J. Sadowsky. 1997. The *atzB* gene of *Pseudomonas* sp. strain ADP encodes the second enzyme of a novel atrazine degradation pathway. *Appl. Environ. Microbiol.*, **63** : 916-923.
- Cook, A.M. and R. Hütter. 1982. Ametryne and prometryne as sulfur sources for bacteria. *Appl. Environ. Microbiol.*, **43** : 781-786.
- de Souza, M.L., D. Newcombe, S. Alvey, D.E. Crowley, A. Hay, M.J. Sadowsky and L.P. Wackett. 1998. Molecular basis of a bacterial consortium: interspecies catabolism of atrazine. *Appl. Environ. Microbiol.*, **64** : 178-184.
- de Souza, M.L., M.J. Sadowsky and L.P. Wackett. 1996. Atrazine chlorohydrolase from *Pseudomonas* sp. strain ADP: gene sequence, enzyme purification, and protein characterization. *J. Bacteriol.*, **178** : 4894-4900.
- Fujii, K., K. Takagi, S. Hiradate, A. Iwasaki and N. Harada. 2007. Biodegradation of methylthio-s-triazines by *Rhodococcus* sp. strain FJ1117YT, and production of the corresponding methylsulfinyl, methylsulfonyl and hydroxy analogues. *Pest Manag. Sci.*, **63** : 254-260.
- Harada, N., K. Takagi, K. Fujii and A. Iwasaki. 2006. Transformation of methylthio-s-triazines via sulfur oxidation by strain JUN7, a *Bacillus cereus* species. *Soil Biol. Biochem.*, **38** : 2952-2957.
- Iwasaki, A. and K. Takagi, Y. Yoshioka, K. Fujii, Y. Kojima and N. Harada. 2007. Isolation and characterization of a novel simazine-degrading β -proteobacterium and detection of genes encoding s-triazine-degrading enzymes. *Pest Manag. Sci.*, **63** : 261-268.
- Kasai, F. 1999. Shifts in herbicide tolerance in paddy field periphyton following herbicide application. *Chemosphere*, **38** : 919-931.
- Kaufman, D.D. and P.C. Kearney. 1976. Microbial transformations in the soil. pp. 29-64. In: L.J. Audus (ed.). *Herbicides: physiology, biochemistry, ecology*, Vol. II. Academic press, London, New York, San Francisco.
- Martinez, B., J. Tomkins, L.P. Wackett, R. Wing and M.J. Sadowsky. 2001. Complete nucleotide sequence and organization of the atrazine catabolic plasmid pADP-1 from *Pseudomonas* sp. strain ADP. *J. Bacteriol.*, **183** : 5684-5697.
- Mulbry, W.W., H. Zhu, S.M. Nour and E. Topp. 2002. The triazine hydrolase gene *trzN* from *Nocardioides* sp. strain C190: Cloning and construction of gene-specific primers. *FEMS Microbiol. Lett.*, **206** : 75-79.
- Okamura, H., M. Piao, I. Aoyama, M. Sudo, T. Okubo and M. Nakamura. 2002. Algal growth inhibition by river water pollutants in the agricultural area around Lake Biwa, Japan. *Environ. Pollut.*, **117** : 411-419.
- Piutti, S., E. Semon, D. Landry, A. Hartmann, S. Dousset, E. Lichtfouse, E. Topp, G. Soulas and F. Martin-Laurent. 2003. Isolation and characterization of *Nocardioides* sp. SP12, an atrazine-degrading bacterial strain possessing the gene *trzN* from bulk- and maize rhizosphere soil. *FEMS Microbiol. Lett.*, **221** : 111-117.
- Sadowsky, M.J., Z. Tong, M. de Souza and L.P. Wackett. 1998. AtzC is a new member of the amidohydrolase protein superfamily and is homologous to other atrazine-metabolizing enzymes. *J. Bacteriol.*, **180** : 152-158.
- Satsuma, K. 2006. Characterisation of new strains of atrazine-degrading *Nocardioides* sp. isolated from Japanese riverbed sediment using naturally derived river ecosystem. *Pest Manag. Sci.*, **62** : 340-349.
- Seffernick, J.L., G. Johnson, M.J. Sadowsky and L.P. Wackett. 2000. Substrate specificity of atrazine chlorohydrolase and atrazine-catabolizing bacteria. *Appl. Environ. Microbiol.*, **66** : 4247-4252.
- Shapir, N., C. Rosendahl, G. Johnson, M. Andreina, M.J. Sadowsky and L.P. Wackett. 2005. Substrate specificity and colorimetric assay for recombinant TrzN derived from *Arthrobacter aureus* TC1. *Appl. Environ. Microbiol.*, **71** : 2214-2220.
- Takagi, K. and Y. Yoshioka. 1999. Bioremediation of recalcitrant pesticides in soil using charcoal with enrichments of pesticide decomposing bacteria. pp. 10-11. In: *Proceedings of Japan-UK workshop on bioremediation*.
- Takagi, K. and Y. Yoshioka. 2000. Development of a method for the rapid accumulation and isolation of recalcitrant-pesticide decomposing bacteria in soil using charcoal. pp. 69-70. In: *Pesticides, soil microbiology and sustainable agriculture. Abstracts and final program of the 3rd International Symposium on Environmental Aspects of Pesticide Microbiology*.
- Takagi, K., Y. Yoshioka, A. Iwasaki and N. Harada. 2005. *In situ* bioremediation of pesticide-contaminated soil by using charcoal enriched with consortia of degrading bacteria. *Journal of Resources and Environment*, **41** : 79-85 (in Japanese).
- Topp, E., W.M. Mulbry, H. Zhu, S.M. Nour and D. Cuppels. 2000a. Characterization of s-triazine herbicide metabolism by a *Nocardioides* sp. isolated from agricultural soils. *Appl. Environ. Microbiol.*, **66** : 3134-3141.
- Topp, E., H. Zhu, S.M. Nour, S. Houot, M. Lewis and D. Cuppels. 2000b. Characterization of an atrazine-degrading *Pseudaminobacter* sp. isolated from Canadian and French agricultural soils. *Appl. Environ. Microbiol.*, **66** : 2773-2782.
- Yanze-Kontchou, C. and N. Gschwind. 1994. Mineralization of the herbicide atrazine as a carbon source by *Pseudomonas* strain. *Appl. Environ. Microbiol.*, **60** : 4297-4302.

土壌微生物による s-トリアジン系除草剤の分解と その原位置バイオレメディエーションへの応用

原田直樹*

(平成19年6月29日受付)

要 約

環境中で中度の残留性が認められている s-triazine 化合物は、除草剤として農業上広く利用されている。本総説では、塩素化及びメチルチオ化 s-triazine 化合物の微生物分解に関するこれまでの知見を述べると共に、単離した simazine 分解菌を利用した原位置バイオレメディエーションの実施例について紹介する。塩素化 s-triazine 化合物の分解微生物としては atrazine 分解菌として単離された *Pseudomonas* sp. ADP 株が著名で、最も詳細に研究されている。ADP 株では、まず atrazine chlorohydrolase (AtzA) による脱塩素を初発反応として、その後 AtzBCDEF が順に関与して無機化に至る。これらの酵素遺伝子群の全部あるいは一部を有する塩素化 s-triazine 分解菌の単離例は多く、我々のグループが単離した新規 simazine 分解菌 CDB21 株でも *atzABCDEF* 遺伝子のすべてが見つかった。しかし、ADP 株では *atzA* が ORF30 と *atzB* の間に位置しているのに対して、CDB21 株では ORF30 が *atzB* とフューズしており、塩素化 s-triazine 分解遺伝子群の構造に多型があることが明らかになった。メチルチオ化 s-triazine 化合物では、好気及び湛水のいずれの条件においてもメチルチオ基の S 酸化を解して分解が進むことが古くから知られていたが、最近ようやくそのプロセスを担う微生物、*Bacillus cereus* JUN7 株と *Rhodococcus* sp. FJ1117YT 株が単離された。JUN7 株は Luria-Bertani 培地のような富栄養培地中で、また FJ1117YT 株はメチルチオ化 s-triazine 化合物を唯一の S 源として与えた無機培地中でメチルチオ基を S 酸化し、最終的に脱メチルチオ化物（水酸化物）を生成した。両株とも塩素化 s-triazine 化合物を分解せず、また *atzA* や *trzN* (triazine hydrolase 遺伝子) を持たないことから、未知の分解機構の存在が示唆される。また、CDB21 株を含む simazine 分解複合微生物系を集積させた特殊な木質炭化素材を用いて、芝草保全の為に定期的に simazine が散布されるゴルフ場において原位置バイオレメディエーション実証実験を行ったところ、本資材は約 2 年間に渡って simazine の下方浸透防止に有効であった。この結果は、土壌環境へ外部から微生物を導入する際にはその住処となりうる適当な資材の併用が好ましいことを示している。

新大農研報, 60:1-7, 2007

キーワード：微生物分解、原位置バイオレメディエーション、シマジン、シメトリン、s-トリアジン