Evolution of atrazine-degrading capabilities in the environment

Nikolina Udiković-Kolić, Colin Scott & Fabrice Martin-Laurent

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MINI-REVIEW

Evolution of atrazine-degrading capabilities in the environment

Nikolina Udiković-Kolić · Colin Scott · Fabrice Martin-Laurent

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Abstract Since their first introduction in the mid 1950s, man-made *s*-triazine herbicides such as atrazine have extensively been used in agriculture to control broadleaf weed growth in different crops, and thus contributed to improving crop yield and quality. Atrazine is the most widely used *s*-triazine herbicide for the control of weeds in crops such as corn and sorghum. Although atrazine was initially found to be slowly and partially biodegradable, predominantly by nonspecific P450 monoxygenases which do not sustain microbial growth, microorganisms gradually evolved as a result of repeated exposure, started using it as a growth substrate and eventually succeeded in mineralizing it. Within three decades, an entirely new hydrolase-dependent pathway for atrazine mineralization emerged and rapidly spread worldwide among genetically different bacteria. This review focuses on the enzymes involved in atrazine mineralization and their evolutionary histories, the genetic composition of microbial populations involved in atrazine degradation and the biotechnologies that have been developed, based on these systems, for the bioremediation of atrazine contamination in the environment.

Keywords Atrazine · Evolution · Biodegradation · Atrazine-degrading enzymes · *atz* · *trz* genes · Bioremediation

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Introduction

Increasing crop production in agriculture has been made easier by the use of effective chemical weed control agents, such as the *s*-triazine herbicides. Many *s*-triazine herbicides, such as atrazine, simazine, ametryn, prometryn and other related compounds were first synthesized in Switzerland by the Geigy laboratories in the 1950s (Cripps and Roberts 1978). Atrazine (2-chloro-4-ethylamine-6-isopropylamino-1,3,5 triazine) was registered in 1958 for grassy and broadleaf weed control in corn, sugarcane, sorghum and certain other crops. It was once the most widely used herbicide worldwide due to its low cost and high effectiveness.

The widespread use of atrazine resulted in its frequent detection in both surface and ground waters beyond authorized limits, leading to potential human exposure (Funari et al. 1989; Richards et al. 1996; Spliid and Koppen 1998). In addition, toxicological studies raised major concern as atrazine was postulated to be a possible carcinogen, an endocrine disrupter and a teratogen (Wiegand et al. 2001; MacLennan et al. 2002; Hayes et al. 2003). As a result, atrazine was banned in the European Union in 2003 (Bethsass and Colangelo 2006). However, it is still widely used around the world.

Microorganisms often respond to the input of xenobiotics into the environment by evolving mechanisms to use them as sources of nutrients and energy for their growth. As the structure of the herbicides based on a *s*-triazine ring differ from naturally occurring compounds (Esser et al. 1975), microorganisms slowly evolved enzymes and pathways capable of degrading them.

During the first 35 years of atrazine use, biodegradation in soils occurred very slowly: half-lives ranged from 2 months to more than 1 year (Harris 1967; Sheets 1970; Jones et al. 1982; Frank and Sirons 1985; Kruger et al. 1993). Degradation was also incomplete, leading to an accumulation of deethylatrazine (DEA) and deisopropylatrazine (DIA) as major metabolites.

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Very few ring cleavages were reported at the time and the detection of hydroxyatrazine was thought to be due to chemical hydrolysis (Skipper et al. 1967; Dao et al. 1979; Capriel and Haisch 1983).

The identification of several fungal and bacterial isolates able to transform atrazine by *N*-dealkylation of one or both side chains supported the idea that the previously observed oxidative dealkylation reactions were mediated biologically (Kaufman and Blake 1970; Giardina et al. 1982; Masaphy et al. 1993; Nagy et al. 1995a). It was later shown that dealkylation was catalyzed by a cytochrome P450 enzyme system, which acts as a non-specific oxidative catalyst and is involved in the metabolization of a range of structurally diverse herbicides (Behki and Khan 1986, 1994; Nagy et al. 1995a, b; Shao and Behki 1995). Such non-specific metabolization of atrazine accounts for the slow rates of atrazine biodegradation observed in soils where *s*-triazine herbicides are rarely applied (Fournier et al. 1997).

In the 1990s, evidence of microbial adaptation toward complete degradation (i.e., mineralization) of atrazine started to accumulate. There were a large number of reports on enhanced atrazine degradation in geographically distinct agricultural soils frequently treated with atrazine (Barriuso and Houot 1996; Ostrofsky et al. 1997; Pussemier et al. 1997; Vanderheyden et al. 1997; Houot et al. 2000; Hang et al. 2003; Krutz et al. 2008; Krutz et al. 2010). Experiments with ^{14}C ring-labelled atrazine showed that soil microbial communities were able to cleave the *s*-triazine ring and mineralize the molecule.

Enrichment culture and direct plating methods made it possible to isolate consortia (Mandelbaum et al. 1993; Assaf and Turco 1994a; Alvey and Crowley 1996; Yanze-Kontchou and Gschwind 1999; Udiković et al. 2003; Kolić et al. 2007) and pure bacterial strains that could mineralize and use atrazine as a carbon, energy and more frequently, nitrogen source (Yanze-Kontchou and Gschwind 1994; Mandelbaum et al. 1995; Radosevich et al. 1995; Struthers et al. 1998; Topp et al. 2000b; Rousseaux et al. 2001; Devers et al. 2007b). In response to repeated exposure, the soil microflora adapted to accelerated atrazine biodegradation, shifting from the commonly accepted dealkylation biodegradation pathway to a newly evolved hydrolytic mineralization pathway. That novel mineralization pathway, which produces only CO_2 and new microbial biomass without any other metabolites, was shown to have potential beneficial environmental consequences.

This review presents our current understanding of the enzymes involved in atrazine mineralization and their evolutionary histories, the genetic composition of the microbial populations involved in atrazine degradation and the biotechnologies recently developed, based on these systems, for remediation of atrazine contamination in the different compartments of the environment.

Hydrolytic atrazine mineralization pathway

The extensive efforts made to isolate microbial strains able to mineralize atrazine first remained unsuccessful for four decades. However, a mixed bacterial culture able to mineralize atrazine completely was isolated in the early 1990s (Mandelbaum et al. 1993). Shortly thereafter, several mixed and pure bacterial atrazine-mineralizing cultures were isolated. In all cases, mineralization proceeded via the formation of hydroxyatrazine (Yanze-Kontchou and Gschwind 1994; Mandelbaum et al. 1995; Radosevich et al. 1995; Struthers et al. 1998; de Souza et al. 1998a). This indicated that in direct response to the selection pressure induced by the use of atrazine, microorganisms had evolved a novel mechanism for degrading it so that they could use it as a growth substrate (i.e., C and/or N source).

The current understanding of that new pathway results from the detailed study of the genetic and enzymatic bases of atrazine degradation by pure bacterial cultures. The metabolic pathway for atrazine mineralization is composed of six successive hydrolyses: a dechlorination, two dealkylations, a ring cleavage, a biuret deamination, and an allophanate hydrolysis (Fig. 1).

The dechlorination of atrazine results from either AtzA (atrazine chlorohydrolase; Table 1) or TrzN (triazine hydrolase; Table 1) activity, and produces the nonherbicidal product hydroxyatrazine (Fig. 1). Both TrzN and AtzA are amidohydrolases; the X-ray structure of TrzN has been empirically solved (Seffernick et al. 2010) and a homology model for AtzA proposed and partly validated (Scott et al. 2009). Although both enzymes are metalloproteins that share the same general fold, TrzN uses the divalent cation Zn^{2+} more, while AtzA uses Fe^{2+}. Despite their similar physiological roles and structural folds, AtzA and TrzN differ significantly in their amino acid sequences (only 27 % identity), suggesting that the function evolved independently in the two enzymes. Furthermore, AtzA and TrzN display substantial differences in their substrate ranges: AtzA is restricted to the halohydrolysis of a range of *s*-triazine compounds, and TrzN hydrolyzes a range of leaving groups (e.g., $–OCH_3$, $–SCH_3$, –Cl, –F, –CN) from both triazines and pyrimidines (Table 1) (de Souza et al. 1996; Seffernick et al. 2000, 2002; Strong et al. 2002; Shapir et al. 2005a, 2006b).

Following dechlorination, hydroxyatrazine is transformed further to either *N*-ethylammelide or *N*-isopropylammelide by the hydrolytic removal of the *N*-isopropyl or *N*-ethyl side groups. While conversion of hydroxyatrazine to *N*-ethylammelide is catalyzed via an as-yet-unidentified enzyme (Topp et al. 2000a), its conversion to *N*-isopropylammelide is catalyzed by hydroxyatrazine hydrolase (AtzB; Table 1) (Boundy-Mills et al. 1997). AtzB was also shown to catalyze the hydrolytic deamination of *N*-ethylammelide

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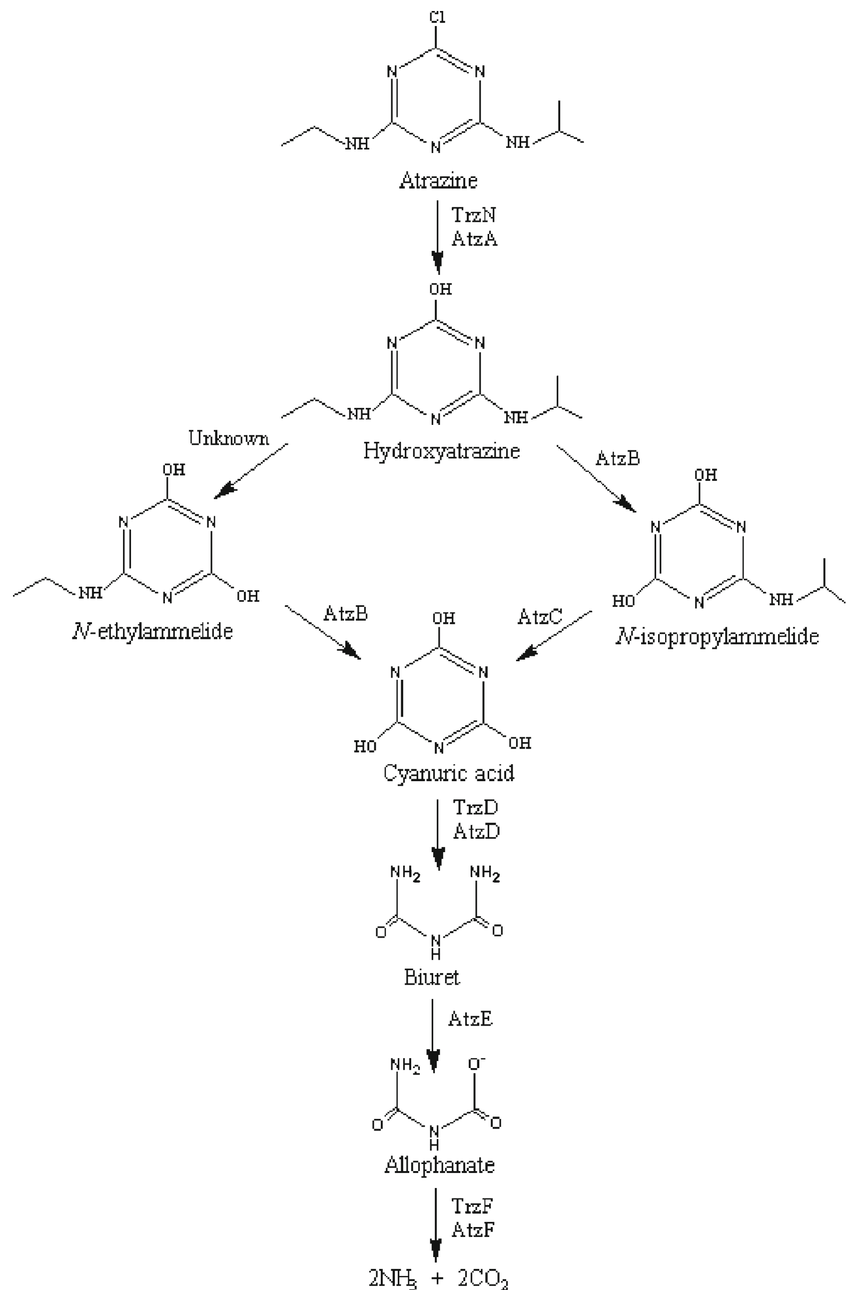

Fig. 1 Hydrolytic atrazine mineralization pathway

to cyanuric acid (Smith et al. 2005), a common intermediate in *s*-triazine catabolism (Cook 1987). However, *N*-isopropylammelide is converted to cyanuric acid and *N*-isopropylamine by *N*-isopropylammelide hydrolase (AtzC; Table 1) (Sadowsky et al. 1998). Ethyl- and isopropylamines released from the *s*-triazine ring by AtzB and AtzC can be used as carbon, nitrogen and/or energy sources for bacterial growth (de Souza et al. 1998a; Strong et al. 2002; Kolić et al. 2007).

AtzB and AtzC are zinc-dependent hydrolases from the amidohydrolase superfamily. The structure of AtzB was inferred by homology, while the X-ray structure for AtzC was empirically solved by Prof. Lawrence Wackett's group (PDB: 2QT3; unpublished data). Both enzymes have broad substrate range (Table 1) and remove a wide range of different *N*-alkyl substituents from the *s*-triazine ring (Sadowsky et al. 1998; Seffernick et al. 2007). Surprisingly, AtzB is also capable of dechlorination (Seffernick et al. 2007).

The lower atrazine degradation pathway is initiated by the cyanuric acid ring cleavage catalyzed *via* the enzyme cyanuric acid hydrolase (either AtzD or TrzD; Table 1), resulting in the formation of an unstable intermediate biuret carboxylate which spontaneously decomposes to biuret and CO_2 (Seffernick et al. 2012). These two enzymes are only 56 % similar, but both have very strict substrate specificity (Karns 1999; Fruchey et al. 2003). AtzD and TrzD are part of a family of proteins that also includes barbiturases;

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Table 1 Overview of the hydrolytic enzymes involved in atrazine catabolism

Catabolic step	Enzyme	Structure	Mechanistic details	Substrate range
Dechlorination	AtzA	Homology Model: amidohydrolase TIM barrel	Nucleophilic attack by Fe^{2+}-activated water, breakdown of tetrahedral intermediate facilitated by Ser331 and Asn328 (mutagenesis studies)	Chloro- and fluoro-*s*-triazines
	TrzN	X-ray structure: amidohydrolase TIM barrel	Nucleophilic attack by Zn^{2+}-activated water with Asp241 donating a proton to facilitate aromatic substitution	Hydrolysis of –Cl, –F, $–OCH_3$, $–SCH_3$, –SH and $–CF_3$ groups from a range of *s*-triazines and pyrimidines
Dealkylation	AtzB	Inferred: amidohydrolase TIM barrel	Nucleophilic attack by Zn^{2+} -activated water	Hydrolysis of an *N*-alkyl group from 2-hydroxy,4-*N*-alkyl, 6-*N*-alkyl *s*-triazines
				Dechlorination of 2-chloro,4-*N*-alkyl, 6-hydroxyl *s*-triazines
	AtzC	X-ray structure (unpublished): amidohydrolase TIM barrel	Nucleophilic attack by Zn^{2+} -activated water	Hydrolysis of an *N*-alkyl group from 2-hydroxy,4-*N*-alkyl, 6-*N*-alkyl *s*-triazines
Ring cleavage	AtzD	Unknown	Unknown	Cyanuric acid
				N-Methylisocyanuric acid
	TrzD	Unknown	Unknown	Cyanuric acid
				N-Methylisocyanuric acid
Biuret deamination	AtzE	Inferred: Amidase	Serine hydrolase with Ser–Ser–Lys catalytic triad (inferred from homology)	Biuret (not fully characterized)
Allophanate hydrolysis	AtzF	Inferred: Amidase	Serine hydrolase, catalytic triad consists of Lys91, Ser165 and Ser189 (mutagenesis studies)	Allophanate
				Malonamic acid
				Malonamide
				Biuret
	TrzF	Inferred: Amidase	Serine hydrolase, catalytic triad appears to consist of Lys91, Ser165 and Ser189 (by homology with AtzF)	Allophanate
				Malonamic acid
				Malonamide
				Biuret

however the structural fold to which these enzymes belong and the details of their catalytic mechanisms are not yet known (Seffernick et al. 2012).

Biuret is subsequently hydrolyzed by biuret hydrolase (AtzE; Table 1) to allophanate (Martinez et al. 2001; Cheng et al. 2005). Deamination of biuret releases ammonia which can support bacterial growth as nitrogen source. Unlike the other enzymes of the pathway, AtzE has not been produced in *Escherichia coli* for purification and further characterization. By homology AtzE is a member of the amidase family likely to share the broad structural and catalytic features of the family.

Allophanate hydrolase (AtzF or TrzF; Table 1) produces carbon dioxide and ammonia from allophanate (Martinez et al. 2001; Cheng et al. 2005; Shapir et al. 2005b, 2006a). Like AtzE, AtzF is a member of the amidase family. It has a relatively broad substrate range and can hydrolyze a number of compounds structurally related to allophanate, including biuret (presumably at a much lower rate than AtzE).

We can hypothesize that the pathways for atrazine catabolism were assembled recently, as they were not isolated prior to 1993. Indeed, some of the enzymatic activities, such as atrazine dechlorination, likely evolved after the introduction of atrazine into the environment. These enzymes therefore represent an excellent resource for studying the emergence of new enzymatic activities and the assembly of new catabolic pathways.

The four upper-pathway enzymes (TrzN, AtzA, AtzB, AtzC) probably evolved from previously existing members of the broader amidohydrolase family: they possess a common fold and a conserved reaction mechanism in which one or two divalent metals, coordinated by the enzymes, activate water for nucleophilic attack on the substrate (Sadowsky et al. 1998; Seffernick and Wackett 2001; Seffernick et al. 2002; Shapir et al. 2006b). We can reasonably assume that higher catalytic efficiency for the hydrolysis of *s*-triazine compounds occurred as a result of positive selection in the form of the growth advantage provided by the release of ammonia and carbon from the *N*-alkyl substituents and *s*-triazine ring (Copley 2009).

Interestingly, AtzA is known to be 98 % identical to melamine deaminase (TriA), differing by only nine amino acids. These closely related enzymes have been used as an excellent

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model system for studying the emergence of new enzyme activities. Like AtzB, TriA has low levels of dechlorinase activity, several orders of magnitude below its physiological deaminase activity; conversely, AtzA displays only dehalogenase activities. Recently, potential evolutionary trajectories were constructed between AtzA and TriA. The order in which the nine amino acid substitutions that separate the enzymes may have occurred in either enzyme, while maintaining significant catalytic activity, was dictated by epistatic interactions between three amino acids within the active site (at positions 331, 328 and 84) (Noor et al. 2012). The identities of the residues at positions 328 (an aspartate or an asparigine) and 331 (either a serine or a cysteine) were dictated by the catalytic roles of these residues in either deamination or dechlorination (Scott et al. 2010). The effect of a change at position 328 depended upon whether or not the residue at position 331 had been changed in a previous step of the trajectory (Noor et al. 2012). In addition to the co-dependent nature of the evolution of the reaction mechanism, trade-offs were measured between (1) the dechlorinase and deaminase activities, (2) physiological and promiscuous activities and (3) stability and activity.

In another recent study, Seffernick and coworkers defined a new protein family that contains the cyanuric hydrolases (i.e., AtzD) and barbiturate hydrolases, which catalyze analogous hydrolyses of cyanuric acid to carboxybutyrate (Seffernick et al. 2012) and barbituric acid to 3-oxo-3-ureidopropanoate (Soong et al. 2002). These enzymes are rare (found in only ~3 % of 6,423 surveyed genomes), and their rarity was suggested to be due to a low selection pressure for their catalytic activities as cyanuric and barbituric acids are relatively uncommon in nature.

AtzE and AtzF both appear to have evolved from the broader amidase family, which possesses a Ser–Ser–Lys catalytic triad and an αββα sandwich structure. Enzymes of that family are widespread and fulfill functions in central and secondary metabolism. AtzF may have evolved in a different physiological context as allophanate is also formed as an intermediate in urea decomposition in some prokaryotes via the action of urea carboxylase (Kanamori et al. 2004; Shapir et al. 2005b). An ancestor of AtzF may have had a role in the hydrolysis of allophanate formed from urea by urea carboxylase and been subsequently recruited into the atrazine catabolic pathway.

The genetics of atrazine degradation: pure microbial cultures vs. consortia

Pure cultures

Yanze-Kontchou and Gschwind (1994) were the first to report the isolation of a pure bacterial culture, *Pseudomonas* sp. YAYA6, capable of mineralizing atrazine and using it as a sole source of carbon for growth. In 1995, *Pseudomonas* sp. strain ADP and *Ralstonia* sp. M91-3 were also shown to grow on atrazine as a sole source of nitrogen and fully degrade it (Mandelbaum et al. 1995; Radosevich et al. 1995). Since these early reports, numerous phylogenetically divergent atrazine-degrading bacteria have been isolated all over the world, mainly from soils regularly exposed to atrazine or related *s*-triazines (Table 2). However, only a few of these isolates have been shown to mineralize atrazine.

The Gram-negative bacterium *Pseudomonas* sp. strain ADP had become a model of choice for dissecting the genetic and enzymatic basis of atrazine mineralization. This bacterium mineralizes atrazine in a six-step pathway involving the hydrolases encoded by *atzABCDEF,* located on a 108-kb plasmid pADP-1. *atzABC,* the first three genes forming the upper pathway, are located in the same region of the pADP-1 plasmid and separated by at least 7 kb long. These three genes are constitutively expressed (Martinez et al. 2001; Devers et al. 2004). It is noteworthy that the *atzABC* genes are flanked by insertion sequences and transposases, suggesting that they were acquired by horizontal gene transfer. In contrast, the lower pathway made of the *atzDEF* genes are organized in an operon placed under the regulation of *atzR*, a lys-R-type factor (Garcia-Gonzalez et al. 2005). These three genes are cotranscribed in response to nitrogen starvation and to the presence of cyanuric acid (Martinez et al. 2001; Devers et al. 2004; Garcia-Gonzalez et al. 2005).

There have been several reports of the isolation of Gram-negative bacteria able to mineralize atrazine, belonging to the genera *Agrobacterium*, *Alcaligenes*, *Ancylobacter*, *Chelatobacter*, *Pseudomonas*, *Pseudaminobacter* and *Ralstonia* (Table 2). These strains were shown to contain either the *atzABCDEF* genes, or *atzABC* genes in combination with the *trzD* gene which is functionally analogous to *atzD* (Karns 1999).

Topp et al. (2000a) reported the isolation of the first Gram-positive bacterium, *Nocardioides* sp. C190 that could grow on atrazine as a sole nitrogen source and degrade it to hydroxyatrazine and the end-product *N*-ethylammelide. The bacterium lacked *atz* genes and was found to harbour another catabolic gene, *trzN*, encoding for a chlorohydrolase (TrzN) (Mulbry et al. 2002) that has the same physiological function as AtzA but broader substrate specificity (Topp et al. 2000a). The second gene in the atrazine catabolic pathway of *Nocardioides* sp. C190, which transforms hydroxyatrazine to *N*-ethylammelide, remains unknown and it was suggested that TrzN could also catalyze this transformation.

Strong et al. (2002) isolated a second atrazine-degrading, Gram-positive bacterium, *Arthrobacter aurescens* TC1 that used atrazine as a sole carbon and nitrogen source and was capable of degrading 23 different *s*-triazine compounds. This bacterium transformed atrazine to cyanuric acid with a mixed *trz/atz* catabolic pathway made of *trzN* with *atzB*

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Table 2 Overview of some bacteria involved in atrazine catabolism

Strain	Order[a]	Origin	End product of atrazine degradation	Genes	Reference
Arthrobacter sp. DNS10	+ Actinomycetales	Agricultural black soil, China	Cyanuric acid	*trzN–atzBC*	(Zhang et al. 2011)
Arthrobacter sp. TES6	+ Actinomycetales	Agricultural soil, Egypt	Cyanuric acid	*trzN–atzBC*	(El Sebai et al. 2012)
Arthrobacter sp. T12B12	+ Actinomycetales	Soil with atrazine history, Colombia	ND	*trzN–atzBC*	(Arbeli and Fuentes, 2010)
Nocardioides sp. V3A16	+ Actinomycetales	Soil with atrazine history, Colombia	ND	*trzN–atzBC*	(Arbeli and Fuentes, 2010)
Nocardioides sp. C1S	+ Actinomycetales	Soil with atrazine history, Colombia	ND	*trzN–atzCDEF*	(Arbeli and Fuentes, 2010)
Ancylobacter sp. T10AII	− Rhizobiales	Soil with atrazine history, Colombia	ND	*atzABCDEF*	(Arbeli and Fuentes, 2010)
Agrobacterium tumefaciens ND4	− Rhizobiales	Atrazine-contaminated soil, USA	ND	*atzA*	(Siripattanakul et al. 2009)
Klebsiela ornithinolytica ND2	− Enterobacteriales	Atrazine-contaminated soil, USA	ND	*atzA*	(Siripattanakul et al. 2009)
Nocardioides sp. MTD22	+ Actinomycetales	Upland field soil, Japan	ND	*trzN*	(Yamazaki et al. 2008)
Nocardioides sp. AN3	+ Actinomycetales	Upland field soil, Japan	ND	*trzN*	(Yamazaki et al. 2008)
Arthrobacter sp. AD26	+ Actinomycetales	Industrial wastewater, China	ND	*trzN–atzBC*	(Li et al. 2008)
Agrobacterium sp. NEA-D	− Rhizobiales	Agricultural soil, France	CO_2	*atzABCDEF*	(Devers et al. 2007a)
Arthrobacter sp. 3A; 2B	+ Actinomycetales	Agrochemical factory soil, Croatia	Hydroxyatrazine	*trzN*	(Devers et al. 2007a)
Arthrobacter crystallopoietes Cit2	+ Actinomycetales	Agricultural soil, France	Cyanuric acid	*trzN–atzBC*	(Devers et al. 2007a; Rousseaux et al. 2001)
Nocardioides sp. 1D	+ Actinomycetales	Agrochemical factory soil, Croatia	Hydroxyatrazine	*trzN*	(Devers et al. 2007a)
Nocardioides sp. NEA-A	+ Actinomycetales	Agricultural soil, France	Cyanuric acid	*trzN–atzBC*	(Devers et al. 2007a)
Polaromonas sp. NEA-C	− Burkholderiales	Agricultural soil, France	Cyanuric acid	*trzN–atzBC*	(Devers et al. 2007a)
Sinorhizobium sp. NEA-B	− Rhizobiales	Agricultural soil, France	Cyanuric acid	*trzN–atzBC*	(Devers et al. 2007a)
Arthrobacter sp. AG1	+ Actinomycetales	Atrazine-contaminated soil, China	Cyanuric acid	*trzN–atzBC*	(Dai et al. 2007)
Arthrobacter sp. MCM B-436	+ Actinomycetales	Rhizospheric soil, India	Biuret	*trzN–atzABCD*	(Vaishampayan et al. 2007)
Arthrobacter sp. CMU6	+ Actinomycetales	Agricultural soil, USA	Cyanuric acid	*trzN–atzC*	(Vibber et al. 2007)
Nocardioides kribbensis CMU5	+ Actinomycetales	Agricultural soil, USA	Cyanuric acid	*trzN–atzBC*	(Vibber et al. 2007)
Nocardioides panacihumi	+ Actinomycetales	Agricultural soil, USA	Cyanuric acid	*trzN–atzC*	(Vibber et al. 2007)
Arthrobacter nicotinovorans HIM	+ Actinomycetales	Agricultural soil, New Zealand	Cyanuric acid	*atzABC*	(Aislabie et al. 2005)
Arthrobacter sp. AD1	+ Actinomycetales	Industrial wastewater, China	ND	*atzA*	(Cai et al. 2003)
Nocardioides sp. SP12	+ Actinomycetales	Agricultural soil, France	Cyanuric acid	*trzN–atzBC*	(Piutti et al. 2003)
Arthrobacter aurescens TC1	+ Actinomycetales	Spill site soil, USA	Cyanuric acid	*trzN–atzBC*	(Strong et al. 2002)

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Table 2 (continued)

Strain	Order[a]	Origin	End product of atrazine degradation	Genes	Reference
Chelatobacter heintzii Cit1	− Rhizobiales	Agricultural soil, France	CO_2	*atzABC–trzD*	(Rousseaux et al. 2001)
Chelatobacter heintzii Sal 1	− Rhizobiales	Agricultural soil, France	Hydroxyatrazine	*atzA*	(Rousseaux et al. 2001)
Stenotrophomonas maltophilia	− Xanthomonadales	Agricultural soil, France	Hydroxyatrazine	*atzA*	(Rousseaux et al. 2001)
Pseudaminobacter sp. 150	− Rhizobiales	Agricultural soil, Canada	Hydroxyatrazine	*atzAC*	(Topp et al. 2000b)
Nocardioides sp. C190	+ Actinomycetales	Agricultural soil, Canada	*N*-Ethylammelide	*trzN*	(Topp et al. 2000a)
Pseudaminobacter sp. C147	− Rhizobiales	Agricultural soil, Canada	CO_2	*atzABC*	(Topp et al. 2000b)
Clavibacter michiganese ATZ1	+ Actinomycetales	Agricultural soil, USA	*N*-Ethylammelide	*atzABC*	(de Souza et al. 1998a)
Isolate 38/38	ND	Atrazine-contaminated soil, USA	CO_2	*atzABC*	(de Souza et al. 1998b)
Agrobacterium radiobacter J14a	− Rhizobiales	Agricultural soil, USA	CO_2	*atzABCDEF*	(Cheng et al. 2005; de Souza et al. 1998b; Struthers et al. 1998)
Alcaligenes sp. SG1	− Burkholderiales	Industrial settling pond, USA	CO_2	*atzABC–trzD*	(Cheng et al. 2005; de Souza et al. 1998b)
Rhizobium sp. PATR	− Rhizobiales	Agricultural soil, France	Hydroxyatrazine	*atzA*	(Bouquard et al. 1997)
Ralstonia basilensis M91-3	− Burkholderiales	Agricultural soil, USA	CO_2	*atzABC–trzD*	(Cheng et al. 2005; de Souza et al. 1998b; Radosevich et al. 1995)
Pseudomonas sp. ADP	− Pseudomonadales	Herbicide spill site soil, USA	CO_2	*atzABCDEF*	(Mandelbaum et al. 1995)
Pseudomonas sp. YAYA6	− Pseudomonadales	Soil near atrazine production facility, Switzerland	CO_2	*atzA like*	(Yanze-Kontchou and Gschwind, 1994)

and *atzC* genes. Interestingly, Piutti et al. (2003) also reported the presence of that gene combination in *Nocardioides* sp. SP12. The genes are localized on a 380-kb plasmid, pTC1, and are not organized in an operon-like structure (Sajjaphan et al. 2004). Since these early reports of mixed *trz/atz* pathways, this *trzN–atzBC* gene combination has been found in various Gram-positive atrazine-degrading bacterial isolates (Table 2). These bacterial isolates only partially degrade atrazine, transforming it to cyanuric acid. More recently, Devers et al. (2007a) reported the presence of that gene combination in Gram-negative atrazine-degrading bacterial strains belonging to the *Polaromonas* and *Sinorhizobium* genera. Therefore, the mixed *trzN–atzBC* atrazine catabolic pathway seems to be widespread among Gram-positive and Gram-negative degrading strains.

In addition, several copies of atrazine-degrading genes have been found in several bacterial strains, suggesting a redundancy of the atrazine-degrading function. For example, *Arthrobacter aurescens* TC1 was shown to have six copies of *trzN* (Mongodin et al. 2006), *Nocardioides* sp. SP12 contains at least two copies of the *trzN, atzB* and *atzC* genes and *Agrobacterium* sp. NEA-D has two copies of the *atzB* gene (Devers et al. 2007a). We can hypothesize that this increase in the copy number of catabolic genes may protect bacteria from the loss of atrazine catabolic function in non-selective conditions or may also accelerate atrazine catabolism *via* a gene dosage effect.

Comparison of known atrazine-degrading strains also shows that there is considerable heterogeneity in the organization and location of the atrazine-catabolic genes within the genome. Catabolic genes have been found to be located: (1) on single plasmids differing in size according to the host (Piutti et al. 2003; Aislabie et al. 2005; Devers et al. 2007a), (2) on several plasmids varying in size (Topp et al. 2000b; Rousseaux et al. 2002; Devers et al. 2007a) or (3) occasionally on the bacterial chromosome (Cai et al. 2003; Devers et al. 2007a, b; Vaishampayan et al. 2007).

There is a significant diversity in the genera of bacteria capable of degrading atrazine, in the manner in which atrazine-degrading genes may be recruited and organized

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to create catabolic pathways and in the end-products of atrazine catabolism. This could perhaps be a feature of new metabolic pathways where an optimal genetic organization has yet to be established.

Microbial consortia

Microbial consortia, which are generally believed more common than pure cultures in nature, are more able to deal with a range of xenobiotics mainly by virtue of increased catabolic capabilities. Microbial consortia are considered more resilient, as their potential for hosting alternative metabolic pathways may contribute to their survival, thereby avoiding the loss of catabolic capability in adverse environmental conditions (Soulas 2003).

A few different atrazine-degrading bacterial consortia have been isolated and characterized with respect to the operating catabolic pathway(s) and establishment of the roles of individual members in atrazine mineralization. A common feature of these consortia is that they require the combined metabolic activities of two or more member species for complete atrazine mineralization. No individual species has a complete set of atrazine catabolism genes, but collectively the consortium possesses the complete metabolic capability. For example, de Souza et al. (1998a) were the first to demonstrate metabolic cooperation between two consortium members which mineralized atrazine by carrying out sequential steps of the degradation pathway that involved the *atzABC* genes (Table 3). The *Clavibacter* strain converted atrazine to *N*-ethylammelide and the *Pseudomonas* strain then dealkylated and mineralized the *s*-triazine ring.

Kolić et al. (2007) demonstrated that the complete atrazine degradation mechanism was distributed between at least four different bacterial species within a consortium enriched from soil at an agrochemical factory. Two *Arthrobacter* species, strain ATZ1 and strain ATZ2, were shown to participate in the upper pathway that converts atrazine to hydroxyatrazine, and then transforms it into *N*-isopropylammelide and finally to cyanuric acid. The first and the third steps of the pathway were carried out by both *Arthrobacter* strains as they contained the *trzN* and *atzC* genes, while the second step was only carried out by ATZ2 which harboured the *atzB* gene. Cyanuric acid was further degraded by *Ochrobactrum* sp. CA1 and *Pseudomonas* sp. CA2, two other members of the consortium, which both carried the *trzD* gene.

A similar example of cooperative metabolism was shown to occur within a complex eight-member atrazine-mineralizing community enriched from soil and using atrazine as the sole nitrogen source (Smith et al. 2005). In that community two upper atrazine-degrading pathways were shown to be operating, one proceeded through *N*-ethyammelide and the other through *N*-isopropylammelide as metabolic intermediates. *Nocardia* sp. initiated the degradation of atrazine by TrzN and subsequently converted the resulting hydroxyatrazine to *N*-ethylammelide by an unidentified gene product. Simultaneously, hydroxyatrazine was converted to *N*-isopropylammelide by AtzB of *Rhizobium* sp. Removal of the isopropylamine group was accomplished by all eight members of the consortium, which carried AtzC. However, the removal of the ethylamine group was uniquely mediated by *Rhizobium* sp., which was the only species in the enrichment culture that contained AtzB. The cyanuric acid generated in the upper pathways was further degraded by the other four strains of the consortium, all harbouring TrzD.

A number of other atrazine-degrading consortia have now been described (Table 3). The studies provide information on the catabolic genetic background and taxonomic diversity of consortia isolated from diverse geographic locations including arable soil, atrazine-manufacturing wastewater and riverbed sediment. A 16S rRNA gene cloning followed by restriction and sequence analysis showed the high diversity of the bacterial populations that formed degrading consortia, with one to four dominant RFLP families. For example, consortium V1, from atrazine-manufacturing wastewater, was dominated by *Pseudomonas* species whereas consortium Z4, from a spill-site soil, was dominated by *Arthrobacter* sp. and uncultivated species from the TM7 division suggesting that beside well-known atrazine-degrading *Arthrobacter* species, some yet uncultured microorganisms may play an important role in atrazine-degrading activity (Udiković-Kolić et al. 2010).

In addition, analysis of the arrangement and composition of atrazine degradation genes within consortia revealed various combinations of these genes (Table 3) and their localization on different-sized plasmids (Kolić et al. 2008; Udiković-Kolić et al. 2010). Interestingly, some of these gene combinations were characterized by functional redundancies of key steps of the upper and lower atrazine-catabolic pathway. For example, consortia Z4 and BSA38, originating from soil, were shown to contain two different genes, *atzA* and *trzN*, encoding enzymes that catalyze the first step of the upper pathway. Similarly, consortia Z3 and MSA15 contained two genes, *trzD* and *atzD*, coding for enzymes that catalyze the first step of the lower pathway (Martin-Laurent et al. 2006; Udiković-Kolić et al. 2010).

To our knowledge, these examples of functional redundancies were detected only in atrazine-degrading consortia. Although functional redundancy might not seem cost-effective from an evolutionary point of view, it may contribute to a better accomplishment of the atrazine-degrading function and to a better maintenance of the function among the soil microbial community. However, it is noteworthy that atrazine-degrading genes were present in less than 4 % of the bacterial populations forming the consortium, indicating that only a subset of the consortium can catabolize atrazine (Kolić et al. 2008; Udiković-Kolić et al. 2010).

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Table 3 Overview of some atrazine-degrading consortia

Mixed culture	Culture members	Origin	Gene composition	Reference
	Clavibacter michiganese ATZ1	Agricultural soil, USA	*atzABC*	(de Souza et al. 1998a)
	Pseudomonas sp. CN1			
	Two unidentified strains			
	Agrobacterium tumefaciens	Agricultural soil, USA	*trzN–atzBC–trzD*	(Smith et al. 2005)
	Caulobacter crescentus			
	Pseudomonas putida			
	Sphingomonas yaniokuyae			
	Nocardia sp.			
	Rhizobium sp.			
	Flavobacterium oryzihabitans			
	Variovax paradoxus			
M3	*Arthrobacter* sp. ATZ1	Agrochemical factory soil, Croatia	*trzN–atzABC–trzD*	(Kolić et al. 2007)
	Arthrobacter sp. ATZ2			
	Ochrobactrum sp. CA1			
	Pseudomonas sp. CA1			
V1	*Pseudomonas* sp.[a]	Industrial wastewater, Croatia	*trzN–atzBC–trzD*	(Kolić et al. 2007)
Z2	*Ochrobactrum* sp.[a]	Agrochemical factory soil, Croatia	*trzN–atzBC–trzD*	(Udiković-Kolić et al. 2010)
	Alcaligenes sp.[a]			
	Achromobacter sp.[a]			
	Flavobacterium sp.[a]			
	Arthrobacter sp.[a]			
Z3	*Ochrobactrum* [a]	Agrochemical factory soil, Croatia	*trzN–atzBCDEF– trzD*	(Udiković-Kolić et al. 2010)
	Rhizobium [a]			
	Hydrogenophaga [a]			
Z4	*Arthrobacter* [a]	Agrochemical factory soil, Croatia	*trzN–atzABC–trzD*	(Udiković-Kolić et al. 2010)
	Uncultured TM7[a]			
A14N	*Nocardioides* sp.	Riverbed sediment, Japan	*trzN–atzBC*	(Satsuma et al. 2006)
	Mycobacterium sp.			
	Leptospira sp.			
MSA15	*Variovax* sp.[a]	Maize rhizosphere soil, France	*trzN–atzABCDEF–trzD*	(Martin-Laurent et al. 2006)
	Burkholderia sp.[a]			
	Artrobacter sp.[a]			
BSA38	*Variovax* sp.[a]	Bulk soil, France	*trzN–atzBCDEF*	(Martin-Laurent et al. 2006)
	Burkholderia sp.[a]			
	Arthrobacter sp.[a]			
	Bosea sp.[a]			

[a] Dominant clones identified in 16S rDNA libraries of investigated consortia

Geographic and phylogenetic distribution of atrazine biodegradation ability

A large number of studies carried out since 1994 have reported the isolation of atrazine degraders belonging to diverse genera of Gram-negative and Gram-positive atrazine-degrading bacteria from soils in Europe, North and South America, Canada, North Africa, Asia and Australia (Table 2). There is very little genetic variation in the *atz* and *trz* genes from these different bacterial isolates, which have been found to share more than 98 % nucleotide sequence identity with the originally isolated *atz* and/or *trz* genes (de Souza et al. 1998b; Topp et al. 2000a; Arbeli and Fuentes 2010; Rousseaux et al. 2001; Piutti et al. 2003; Sajjaphan et al. 2004; Zhang et al. 2011). This observation suggests that horizontal gene transfer has been a major contributor to the geographic and phylogenetic distribution of the *atz* and *trz* genes.

The *atzA* gene has strictly been found in Gram-negative bacteria, distributed among the Alpha- (Bouquard et al. 1997;

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Struthers et al. 1998; Topp et al. 2000b; Rousseaux et al. 2001; Devers et al. 2007b; Arbeli and Fuentes 2010), Beta- (de Souza et al. 1998b; Devers et al. 2007a), and Gamma-proteobacteria (Mandelbaum et al. 1995; Rousseaux et al. 2001; Singh et al. 2004). Conversely, until recently (2007), the *trzN* gene was restricted to Gram-positive genera (Table 2). The dominance of each of these genes in particular geographic locations seems to be based upon which bacterial genera are more competitive in each environment, and on the fact that specific soil characteristics rather than geographic distances affect the observed microbial biogeography (Arbeli and Fuentes 2010).

A comparison of kinetic parameters (K_m and V_{max}) and of the *s*-triazine substrate specificities of enzymes TrzN and AtzA showed that TrzN had a higher specificity for atrazine, a higher atrazine degradation rate and a much broader substrate range than AtzA (de Souza et al. 1996; Topp et al. 2000a; Shapir et al. 2007). The observed superior performance characteristics of TrzN might be responsible for the prevalence of the gene *trzN* over its *atzA* analogue in the environment and/or for generating cultivation bias that end in more frequent isolation of bacteria that carry this gene (Arbeli and Fuentes 2010). As the *trzN* gene appears to be more competitive than *atzA*, the persistence of *atzA* in bacterial populations may be a consequence of the lack of horizontal gene transfer of *trzN* from Gram-positive genera to Gram-negative ones.

The recent isolation of *trzN*-containing *Polaromonas* and *Sinorhizobium* species (Devers et al. 2007a) suggests that such a transfer has now occurred (at least twice), and it will be interesting to read about the changes in the frequencies of *atzA* vs. *trzN* in Gram-negative bacteria in light of the apparently superior fitness benefit provided by *trzN*.

Mechanisms involved in atrazine-degrading gene transfer and plasticity

As mentioned earlier, atrazine-degrading genes are mainly reported to be located on plasmids. It is noteworthy that *atz* genes were first described on pADP-1, a large 108-kb plasmid isolated from *Pseudomonas* sp. ADP, found to be self-transmissible under laboratory conditions (Mandelbaum et al. 1995; Martinez et al. 2001). Using an *Agrobacterium tumefaciens* St96-4 recombinant strain harbouring pADP1::Tn5, Devers et al. (2004) showed that horizontal gene transfer of atrazine-degrading genes to soil microflora occurred at a transfer frequency of 10^{-4} per donor. However, the dispersion of atrazine-degrading genes does not seem to be restricted to horizontal gene transfer by bacterial conjugation since Ghosh et al. (2008) showed that *trzN* amplicons could be obtained by PCR using viral DNA fractions purified from MC-induced bead communities. This observation suggests that bacteriophage-mediated lysogeny could be a prevalent mechanism for dispersal of atrazine-degrading genes among the soil microflora.

In addition to being plasmid-borne, atrazine catabolic genes are almost invariably packaged with flanking insertion sequence (IS) elements (Shapir et al. 2007). Devers et al. (2007a) showed that among 17 atrazine-degrading strains they screened, most of the atrazine-degrading genes of the upper pathway colocalized with the IS*1071* probe. In addition, the isolation of transconjugants *Variovorax* sp. MD1 and MD2 revealed that the *atzAB* cassette moved from a plasmidic to a chromosomal location in a unique rearrangement event by homologous recombination mediated by IS*1071* (Devers et al. 2007b). In an in vitro evolution experiment performed on *Pseudomonas* sp. ADP, IS elements were proved responsible for the duplication of the *atzB* gene which confers a selective advantage to the newly evolved population under atrazine selection pressure (Devers et al. 2008). Conversely, Changey et al. (2011) showed that under cyanuric acid selection pressure, homologous recombination mediated by IS*Pps1* led to the selective loss of a 47-kb fragment containing the *atzABC* genes. The loss of that region containing three constitutively expressed functional genes thereby constituting a genetic burden under cyanuric acid selection pressure was responsible for the gain in fitness of the newly evolved population. These different studies underline the plasticity of the atrazine-degrading potential mediated by ISs and suggest that ISs not only favour the expansion of the degrading genetic potential thanks to dispersion and duplication events but may also contribute to its reduction thanks to deletion events. Evidence for expansion and reduction of the atrazine-degrading genetic background as a result of high atrazine input has also recently been shown to occur within the atrazine-degrading bacterial consortium leading to its increased fitness under selective conditions (Udiković-Kolić et al. 2011). Unfortunately, despite the attempts by different research teams worldwide to isolate and characterize atrazine degraders, relatively little is known about the evolutionary history of plasmids harbouring *atz/trz* genes. Indeed up to now only pADP-1 and pTC1 have been fully sequenced (Martinez et al. 2001; Mongodin et al. 2006) and a few other plasmid sequences are partially known (Topp et al. 2000a; Strong et al. 2002). However, they are not sufficient to allow for a full description of the processes involved in the evolution of those plasmids or of their dispersion among soil microflora worldwide.

Bioremediation

The use of atrazine catabolism in the bioremediation of atrazine has been a major focus for applied research since

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the discovery of biotic atrazine degradation. Field-scale trials have been conducted for a number of different approaches.

Biostimulation, enhancing the growth and performance of native microorganisms *via* the addition of exogenous energy and nutrient sources (Scow and Hicks 2005; Kannisery and Sims 2011) has been used to increase the rate of atrazine degradation. As early as 1973, Hance achieved substantial enhancements in atrazine degradation by supplementing soil samples with inorganic salts (Hance 1973). More rational, targeted approaches have also been successful, whereby the nutrient composition of the soil can be adjusted to 'encourage' the specific degradation of the herbicide. For example, there are several reports where an increase in soil carbon *via* the addition of simple or complex carbohydrates, thus creating a nitrogen-limited environment and stimulating the degradation of atrazine as a nitrogen source (McCormick and Hiltbold 1966; Assaf and Turco 1994b; Wagner and Chahal 1996; Abdelhafid et al. 2000).

Bioaugmentation, that is, the addition of non-indigenous atrazine-degrading bacterial populations to accelerate the degradation rate of atrazine residues, has also been applied at different sites. In 1997, Grigg and co-workers reported the treatment of atrazine-contaminated soil from the Purdue Agricultural Experiment Station in Indiana using ex situ bioaugmentation, where they achieved a 20-fold increase in the initial rate of atrazine degradation recorded before bioaugmentation (Grigg et al. 1997). In other studies, Runes et al. (2001) reported the successful use of bioaugmentation which led to the degradation of atrazine in simulated wetland sediment. In another microcosm study, Monard et al. (2008) showed that bioaugmentation with *Pseudomonas* sp. strain ADP and *Chelatobacter heintzii* led to an increase in the rate of atrazine degradation in arable soils.

There are, however, limitations to the use of living microorganisms for bioremediation. For example, temperature or pH extremes, the presence of toxins or the absence of nutrients, salinity, and high substrate concentrations can limit bacterial growth and replication. In addition to these limitations, the impact of the inoculation of living microorganisms on the environment but also on animal and human health should not be ignored, and Europe regulations on the use of living microorganisms are strict. In order to avoid these difficulties, several attempts have been made to use dead cells or cell-free enzymes. As an alternative to traditional bioaugementation using living microorganisms, Strong et al. (2000) achieved bioremediation with a killed and stabilized transgenic *E. coli* at field scale (Strong et al. 2000). More recently, Scott et al. (2010) reported from a field trial conducted in Australia that the inoculation of cell-free TrzN (as a DNA-free cell lysate) into a 1.5-megaliter dam reservoir contaminated with atrazine led to its removal.

The activity of degrading-enzymes can be improved by immobilizing or entrapping killed bacteria and enzymes. Indeed, purified AtzA and non-viable atrazine-degrading bacteria were encapsulated in sol and silica gels, respectively, and then used for treating atrazine-contaminated water at the bench scale (Kauffmann and Mandelbaum 1998; Reátegui et al. 2012). The entrapped remediants were successfully used for extended periods, and for multiple rounds of remediation.

Phytoremediation is also a potential technology that could be used to decontaminate atrazine-polluted soils, and to that end transgenic tobacco plants carrying the *atzA* gene have been produced that exhibit enhanced tolerance to atrazine (Wang et al. 2005, 2010). The screening of a library of *Arabidopsis thaliana* mutants led to the isolation of a mutant able to phytoaccumulate atrazine when exposed to sucrose amendment (Sulmon et al. 2007). These observations carried out under laboratory conditions were further confirmed by applying sucrose to plants grown on atrazine-polluted soil, which increased plant tolerance and xenobiotic absorption. That procedure thus appears as potentially useful for phytoremediation.

Conclusions

s-Triazine compounds were known to be co-metabolically degraded for more than 30 years, until the first report of atrazine mineralization in the early 1990´s. Although we cannot rule out that microbes evolved earlier, before becoming able to grow on those compounds, since then the existence of atrazine-degaders was evidenced in different arable soils regularly exposed to these herbicides. Those microbes are now dispersed worldwide and the *atz/trz* genes coding for the enzymes responsible for the mineralization of *s*-triazine are extremely conserved, which shows that their emergence is recent and that their evolution is only starting. The atrazine-degrading potential appears to be highly versatile and sensitive to rapid evolution under changes in selection pressure mainly driven by atrazine exposure. The adaptation of the soil microflora to the mineralization of *s*-triazine compounds is a fantastatic model for highlighting the incredible capacity of environmental microbes to use xenobiotic compounds as nutrient and energy sources. Such a capacity contributes to building up knowledge about the understanding of functional communities that provide an ecosystemic soil filtration service and to developing innovative bioremediation strategies. However it raises questions as to the genericity of the processes observed. In particular, is the time required to get microbial adaptation to pesticide mineralization always that long? This question is of prime interest considering the recent evolution of water quality guidelines and the general concern about the impact of agriculture on the quality of our environment. We could hypothesize that in a near future, firms that develop new

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pesticides might make a point of taking this question into account to favour the development of compounds easily degradable by soil microorganisms and thus avoid their dispersal across the environment.

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References

Abdelhafid R, Houot S, Barriuso E (2000) How increasing availabilities of carbon and nitrogen affect atrazine behaviour in soils. Biol Fertil Soils 30:333–340

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

Alvey S, Crowley DE (1996) Survival and activity of an atrazine-mineralizing bacterial consortium in rhizosphere soil. Environ Sci Technol 30:1596–1603

Arbeli Z, Fuentes C (2010) Prevalence of the gene *trzN* and biogeographic patterns among atrazine-degrading bacteria isolated from 13 Colombian agricultural soils. FEMS Microbiol Ecol 73: 611–623

Assaf NA, Turco RF (1994a) Accelerated biodegradation by a microbial consortium is possible in culture and soil. Biodegradation 5:29–35

Assaf NA, Turco RF (1994b) Influence of carbon and nitrogen application on the mineralization of atrazine and its metabolites in soil. Pest Sci 41:41–47

Barriuso E, Houot S (1996) Rapid mineralisation of *s*-triazine ring of atrazine in soils in relation to soil management. Soil Biol Biochem 28:1341–1348

Behki RM, Khan SU (1986) Degradation by *Pseudomonas*: *N*-dealkylation and dehalogenation of atrazine and its metabolites. J Agr Food Chem 34:746–749

Behki RM, Khan SU (1994) Degradation of atrazine, propazine, and simazine by *Rhodococcus* strain B-30. J Agr Food Chem 42:1237–1241

Bethsass J, Colangelo A (2006) European Union bans atrazine, while the United States negotiates continued use. Int J Occup Env Heal 12:260–267

Boundy-Mills KL, de Souza ML, Mandelbaum RT, Wackett LP, Sadowsky MJ (1997) The *atz*B gene of *Pseudomonas* sp. strain ADP encodes the second enzyme of a novel atrazine degradation pathway. Appl Environ Microbiol 63:916–923

Bouquard C, Ouazzani J, Prome JC, Michel-Briand Y, Plésiat P (1997) Dechlorination of atrazine by a *Rhizobium* sp. isolate. Appl Environ Microbiol 63:862–866

Cai B, Han Y, Liu B, Ren Y, Jiang S (2003) Isolation and characterization of an atrazine-degrading bacterium from industrial wastewater in China. Lett Appl Microbiol 36:272–276

Capriel P, Haisch A (1983) Persistance of atrazine and its metabolites in soil after a single herbicide application. Z Pflanz Bodenkund 146:474–480

Changey F, Devers-Lamrani M, Rouard N, Martin-Laurent F (2011) *In vitro* evolution of an atrazine-degrading population under cyanuric acid selection pressure: evidence for selective loss of a 47 kb region on the plasmid pADP1 containing the *atzA, B* and *C* genes. Gene 490:18v25

Cheng G, Shapir N, Sadowsky MJ, Wackett LP (2005) Allophanate hydrolase, not urease, functions in bacterial cyanuric acid metabolism. Appl Environ Microbiol 71:4437–4445

Cook AM (1987) Biodegradation of *s*-triazine xenobiotics. FEMS Microbiol Rev 46:93–116

Copley SD (2009) Evolution of efficient pathways for degradation of anthropogenic chemicals. Nat Chem Biol 5:560–567

Cripps RE, Roberts TR (1978) Microbial degradation of herbicides. In: Hill IR, Wright SJL (eds) Pesticide microbiology: microbiological aspects of pesticide behaviour in the environment. Academic Press, New York, pp 669–720

Dai XZ, Jiang JD, Gu LF, Pan RQ, Li SP (2007) Study on the atrazine-degrading genes in *Arthrobacter* sp. AG1. Chin J Biotechn 23:789–793

Dao TH, Lavy TL, Sorensen RC (1979) Atrazine degradation and residue distribution in soil. Soil Sci Soc Am J 43:1129–1134

de Souza ML, Sadowsky MJ, Wackett LP (1996) Atrazine chlorohydrolase from *Pseudomonas* sp. strain ADP: gene sequence, enzyme purification, and protein characterization. J Bacteriol 178:4894–4900

de Souza ML, Newcombe D, Alvey S, Crowley DE, Hay A, Sadowsky MJ, Wackett LP (1998a) Molecular basis of a bacterial consortium: interspecies catabolism of atrazine. Appl Environ Microbiol 64:178–184

de Souza ML, Seffernick J, Martinez B, Sadowsky MJ, Wackett LP (1998b) The atrazine catabolism genes *atzABC* are widespread and highly conserved. J Bacteriol 180:1951–1954

Devers M, Soulas G, Martin-Laurent F (2004) Real-time reverse transcription PCR analysis of expression of atrazine catabolism genes in two bacterial strains isolated from soil. J Microbiol Meth 56:3–15

Devers M, Azhari NE, Kolic NU, Martin-Laurent F (2007a) Detection and organization of atrazine-degrading genetic potential of seventeen bacterial isolates belonging to divergent taxa indicate a recent common origin of their catabolic functions. FEMS Microbiol Lett 273:78–86

Devers M, Rouard N, Martin-Laurent F (2007b) Genetic rearrangement of the *atzAB* atrazine-degrading gene cassette from pADP1::Tn5 to the chromosome of *Variovorax* sp. MD1 and MD2. Gene 392:1–6

Devers M, Rouard N, Martin-Laurent F (2008) Fitness drift of an atrazine-degrading population under atrazine selection pressure. Environ Microbiol 10:676–684

El Sebai T, Devers-Lamrani M, Changey F, Rouard N, Martin-Laurent F (2012) Evidence of atrazine mineralization in a soil from the Nile Delta: isolation of *Arthrobacter* sp TES6, an atrazine-degrading strain. Int Biodeter Biodegr 65:1249–1255

Esser HO, Dupuis G, Ebert E, Marco GJ, Vogel C (1975) *s*-Triazines. In: Kearney PC, Kaufman DJ (eds) Herbicides, chemistry, degradation and mode of action. Marcel Dekker, New York, pp 129–208

Fournier J, Soulas G, Parekh N (1997) Main microbial mechanisms of pesticide degradation in soils. In: Tarradellas J (ed) Soil ecotoxicology. Lewis Publishers CRC, New York, pp 85–116

Frank R, Sirons GJ (1985) Dissipation of atrazine residues from soils. B Environ Contam Tox 34:541–548

Fruchey I, Shapir N, Sadowsky MJ, Wackett LP (2003) On the origins of cyanuric acid hydrolase: purification, substrates, and prevalence of AtzD from *Pseudomonas* sp. strain ADP. Appl Environ Microbiol 69:3653–3657

Funari E, Acquafresca G, Arca F, Baldi M, Bastianetti J, Cappelli A, Carli G, Carniel A, Chierici S, Fanuzzi A, Ferraro P, Lopez A, Mattioni R, Narese M, Peretti A, Salamana M, Zapponi G (1989) Preliminary-report on the atrazine and

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molinate water-supply contamination in Italy. Chemosphere 18:2339–2343

Garcia-Gonzalez V, Govantes F, Porrua O, Santero E (2005) Regulation of the *Pseudomonas* sp. strain ADP cyanuric acid degradation operon. J Bacteriol 187:155–167

Ghosh D, Roy K, Williamson KE, White DC, Wommack KE, Sublette KL, Radosevich M (2008) Prevalence of lysogeny among soil bacteria and presence of 16S rRNA and *trzN* genes in viral-community DNA. Appl Environ Microbiol 74:495–502

Giardina MC, Giardi MT, Filacchioni G (1982) Atrazine metabolism by *Nocardia*: elucidation of initial pathway and synthesis of potential metabolites. Agr Biol Chem 46:1439–1445

Grigg BC, Assaf NA, Turco RF (1997) Removal of atrazine contamination in soil and liquid systems using bioaugmentation. Pest Sci 50:211–220

Hance RJ (1973) The effect of nutrients on the decomposition of the herbicides atrazine and linuron incubated with soil. Pest Sci 4:817–822

Hang S, Barriuso E, Houot S (2003) Behavior of C-14-atrazine in Argentinean topsoils under different cropping managements. J Environ Qual 32:2216–2222

Harris CI (1967) Fate of 2-chloro-*s*-triazine herbicides in soil. J Agr Food Chem 15:157–162

Hayes T, Haston K, Tsui M, Hoang A, Haeffele C, Vonk A (2003) Atrazine-induced hermaphroditism at 0.1 ppb in American leopard frogs (*Rana pipiens*): laboratory and field evidence. Environ Health Persp 111:568–575

Houot S, Topp E, Yassir A, Soulas G (2000) Dependence of accelerated degradation of atrazine on soil pH in French and Canadian soils. Soil Biol Biochem 32:615–625

Jones TW, Kemp WM, Stevenson JC, Means JC (1982) Degradation of atrazine in estuarine water sediment systems and soils. J Environ Qual 11:632–638

Kanamori T, Kanou N, Atomi H, Imanaka T (2004) Enzymatic characterization of a prokaryotic urea carboxylase. J Bacteriol 186:2532–2539

Kannisery RG, Sims GK (2011) Biostimulation for the enhanced degradation of herbicides in soil. Appl Environ Soil Sci 2011: 10 pages

Karns JS (1999) Gene sequence and properties of an *s*-triazine ring cleavage enzyme from *Pseudomonas* sp. strain NRRLB-12227. Appl Environ Microbiol 65:3512–3517

Kauffmann C, Mandelbaum RT (1998) Entrapment of atrazine chlorohydrolase in sol–gel glass matrix. J Biotechnol 62:169–176

Kaufman DD, Blake J (1970) Degradation of atrazine by soil fungi. Soil Biol Biochem 2:73–80

Kolić NU, Martin-Laurent F, Devers M, Petrić I, Kolar AB, Hršak D (2008) Genetic potential, diversity and activity of an atrazine-degrading community enriched from a herbicide factory effluent. J Appl Microbiol 105:1334–1343

Kolić NU, Hršak D, Begonja Kolar A, Petrić I, Stipičević S, Soulas G, Martin-Laurent F (2007) Combined metabolic activity within an atrazine-mineralizing community enriched from agrochemical factory soil. Int Biodeter Biodegr 60:299–307

Kruger EL, Somasundaram L, Kanwar RS, Coats JR (1993) Persistence and degradation of [C-14] atrazine and [C-14] deisopropylatrazine as affected by soil depth and moisture conditions. Environ Toxicol Chem 12:1959–1967

Krutz LJ, Shaner DL, Accinelli C, Zablotowicz RM, Henry WB (2008) Atrazine dissipation in *s*-triazine-adapted and nonadapted soil from Colorado and Mississippi: implications of enhanced degradation on atrazine fate and transport parameters. J Environ Qual 37:848–857

Krutz LJ, Shaner DL, Zablotowicz RM (2010) Enhanced degradation and soil depth effects on the fate of atrazine and major metabolites in Colorado and Mississippi soils. J Environ Qual 39:1369–1377

Li QY, Li Y, Zhu XK, Cai BL (2008) Isolation and characterization of atrazine-degrading *Arthrobacter* sp. AD26 and use of this strain in bioremediation of contaminated soil. J Environ Sci 20:1226–1230

MacLennan PA, Delzell E, Sathiakumar N, Myers SL, Cheng H, Grizzle W, Chen VW, Wu XC (2002) Cancer incidence among triazine herbicide manufacturing workers. J Occup Environ Med 44:1048–1058

Mandelbaum RT, Wackett LP, Allan DL (1993) Mineralization of the *s*-triazine ring of atrazine by stable bacterial mixed cultures. Appl Environ Microbiol 59:1695–1701

Mandelbaum RT, Allan DL, Wackett LP (1995) Isolation and characterization of a *Pseudomonas* sp. that mineralizes the *s*-triazine herbicide atrazine. Appl Environ Microbiol 61:1451–1457

Martin-Laurent F, Barres B, Wagschal I, Piutti S, Devers M, Soulas G, Philippot L (2006) Impact of the maize rhizosphere on the genetic structure, the diversity and the atrazine-degrading gene composition of cultivable atrazine-degrading communities. Plant Soil 282:99–115

Martinez B, Tomkins J, Wackett LP, Wing R, Sadowsky MJ (2001) Complete nucleotide sequence and organization of the atrazine catabolic plasmid pADP-1 from *Pseudomonas* sp. strain ADP. J Bacteriol 183:5684–5697

Masaphy S, Levanon D, Vaya J, Henis Y (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

McCormick LL, Hiltbold AE (1966) Microbiological decomposition of atrazine and diuron is soil. Weeds 14:77–82

Monard C, Martin-Laurent F, Vecchiato C, Francez AJ, Vandenkoornhuyse P, Binet F (2008) Combined effect of bioaugmentation and bioturbation on atrazine degradation in soil. Soil Biol Biochem 40:2253–2259

Mongodin EF, Shapir N, Daugherty SC, Deboy RT, Emerson JB, Shvartzbeyn A, Radune D, Vamathevan J, Riggs F, Grinberg V, Khouri H, Wackett LP, Nelson KE, Sadowsky MJ (2006) Secrets of soil survival revealed by the genome sequence of *Arthrobacter aurescens* TC1. Plos Genet 2:2094–2106

Mulbry WW, Zhu H, Nour SM, Topp E (2002) The triazine hydrolase gene *trzN* from *Nocardioides* sp. strain C190: Cloning and construction of gene-specific primers. FEMS Microbiol Lett 206: 75–79

Nagy I, Compernolle F, Ghys K, Vanderleyden J, Demot R (1995a) 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. Appl Environ Microbiol 61:2056–2060

Nagy I, Verheijen S, Schrijver A, Damme J, Proost P, Schoofs G, Vanderleyden J, Mot R (1995b) Characterization of the *Rhodococcus* sp. NI86/21 gene encoding alcohol: N, N′-dimethyl-4-nitrosoaniline oxidoreductase inducible by atrazine and thiocarbamate herbicides. Arch Microbiol 163:439–446

Noor S, Taylor MC, Russell RJ, Jeriin LS, Jackson CJ, Oakeshott JG, Scott C (2012) Intramolecular epistasis and the evolution of a new enzymatic function. PLoS One. doi:10.1371/journal.pone.0039822

Ostrofsky EB, Traina SJ, Tuovinen OH (1997) Variation in atrazine mineralization rates in relation to agricultural management practice. J Environ Qual 26:647–657

Piutti S, Semon E, Landry D, Hartmann A, Dousset S, Lichtfouse E, Topp E, Soulas G, Martin-Laurent F (2003) Isolation and characterisation of *Nocardioides* sp. SP12, an atrazine-

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degrading bacterial strain possessing the gene *trzN* from bulk- and maize rhizosphere soil. FEMS Microbiol Lett 221:111–117

Pussemier L, Goux S, Vanderheyden V, Debongnie P, Tresinie I, Foucart G (1997) Rapid dissipation of atrazine in soils taken from various maize fields. Weed Res 37:171–179

Radosevich M, Traina SJ, Hao YL, Tuovinen OH (1995) Degradation and mineralization of atrazine by a soil bacterial isolate. Appl Environ Microbiol 61:297–302

Reátegui E, Reynolds E, Kasinkas L, Aggarwal A, Sadowsky M, Aksan A, Wackett L (2012) Silica gel-encapsuled AtzA biocatalyst for atrazine biodegradation. Appl Microbiol Biotechnol. doi:10.1007/s00253-011-3821-2

Richards RP, Baker DB, Creamer NL, Kramer JW, Ewing DE, Merryfield BJ, Wallrabenstein LK (1996) Well water quality, well vulnerability, and agricultural contamination in the midwestern United States. J Environ Qual 25:930–930

Rousseaux S, Hartmann A, Soulas G (2001) Isolation and characterisation of new Gram-negative and Gram-positive atrazine degrading bacteria from different French soils. FEMS Microbiol Ecol 36:211–222

Rousseaux S, Soulas G, Hartmann A (2002) Plasmid localisation of atrazine-degrading genes in newly described *Chelatobacter* and *Arthrobacter* strains. FEMS Microbiol Ecol 41:69–75

Runes HB, Jenkins JJ, Bottomley PJ (2001) Atrazine degradation by bioaugmented sediment from constructed wetlands. Appl Microbiol Biotech 57:427–432

Sadowsky MJ, Tong ZK, de Souza M, Wackett LP (1998) AtzC is a new member of the amidohydrolase protein superfamily and is homologous to other atrazine-metabolizing enzymes. J Bacteriol 180:152–158

Sajjaphan K, Shapir N, Wackett LP, Palmer M, Blackmon B, Tomkins J, Sadowsky MJ (2004) *Arthrobacter aurescens* TC1 atrazine catabolism genes *trzN*, *atzB*, and *atzC* are linked on a 160-kilobase region and are functional in *Escherichia coli*. Appl Environ Microbiol 70:4402–4407

Satsuma K, Kameshiro M, Hayashi O, Sato K, Kato Y (2006) Characterization of a *Nocardioides*-based, atrazine-mineralizing microbial colony isolated from Japanese riverbed sediment. J Pest Sci 31:420–423

Scott C, Jackson CJ, Coppin CW, Mourant RG, Hilton ME, Sutherland TD, Russell RJ, Oakeshott JG (2009) Catalytic improvement end evolution of atrazine chlorohydrolase. Appl Environ Microbiol 75:2184–2191

Scott C, Lewis SE, Milla R, Taylor MC, Rodgers AJW, Dumsday G, Brodie JE, Oakeshott JG, Russell RJ (2010) A free-enzyme catalyst for the bioremediation of environmental atrazine contamination. J Environ Manage 91:2075–2078

Scow KM, Hicks KA (2005) Natural attenuation and enhanced bioremediation of organic contaminants in groundwater. Curr Opin Biotechnol 16:246–253

Seffernick J, Erickson JS, Cameron S, Cho S, Dodge AG, Richman J, Sadowsky J, Wackett LP (2012) Defining the cyanuric acid hydrolase (AtzD)/barbiturase protein family: sequences and reactions. J Bacteriol. doi:10.1128/JB.00791-12

Seffernick JL, Johnson G, Sadowsky MJ, Wackett LP (2000) Substrate specificity of atrazine chlorohydrolase and atrazine-catabolizing bacteria. Appl Environ Microbiol 66:4247–4252

Seffernick JL, Wackett LP (2001) Rapid evolution of bacterial catabolic enzymes: a case study with atrazine chlorohydrolase. Biochemistry 40:12747–12753

Seffernick JL, McTavish H, Osborne JP, de Souza ML, Sadowsky MJ, Wackett LP (2002) Atrazine chlorohydrolase from *Pseudomonas* sp. strain ADP is a metalloenzyme. Biochemistry 41: 14430–14437

Seffernick JL, Aleem A, Osborne JP, Johnson G, Sadowsky MJ, Wackett LP (2007) Hydroxyatrazine *N*-ethylaminohydrolase (AtzB): an amidohydrolase superfamily enzyme catalyzing deamination and dechlorination. J Bacteriol 189:6989–6997

Seffernick JL, Reynolds E, Fedorov AA, Fedorov E, Almo SC, Sadowsky MJ, Wackett LP (2010) X-ray structure and mutational analysis of the atrazine chlorohydrolase TrzN. J Biol Chem 285:30606–30614

Shao ZQ, Behki R (1995) Cloning of the genes for degradation of the herbicides EPTC (*S*-ethyl dipropylthiocarbamate) and atrazine from *Rhodococcus* sp. strain TE1. Appl Environ Microbiol 61:2061–2065

Shapir N, Rosendahl C, Johnson G, Andreina M, Sadowsky MJ, Wackett LP (2005a) Substrate specificity and colorimetric assay for recombinant TrzN derived from *Arthrobacter aurescens* TC1. Appl Environ Microbiol 71:2214–2220

Shapir N, Sadowsky MJ, Wackett LP (2005b) Purification and characterization of allophanate hydrolase (AtzF) from *Pseudomonas* sp. strain ADP. J Bacteriol 187:3731–3738

Shapir N, Cheng G, Sadowsky MJ, Wackett LP (2006a) Purification and characterization of TrzF: biuret hydrolysis by allophanate hydrolase supports growth. Appl Environ Microbiol 72: 2491–2495

Shapir N, Pedersen C, Gil O, Strong L, Seffernick J, Sadowsky MJ, Wackett LP (2006b) TrzN from *Arthrobacter aurescens* TC1 is a zinc amidohydrolase. J Bacteriol 188:5859–5864

Shapir N, Mongodin EF, Sadowsky J, Daughetry SC, Nelson KE, Wackett L (2007) Evolution of catabolic pathways: genomic insights into microbial *s*-triazine metabolism. J Bacteriol 189:674–682

Sheets TJ (1970) Persistence of triazine herbicides in soils. Residue Rev 32:287–310

Singh P, Suri CR, Cameotra SS (2004) Isolation of a member of *Acinetobacter* species involved in atrazine degradation. Biochem Bioph Res Co 317:697–702

Siripattanakul S, Wirojanagud W, McEvoy J, Limpiyakorn T, Khan E (2009) Atrazine degradation by stable mixed cultures enriched from agricultural soil and their characterization. J Appl Microbiol 106:986–992

Skipper HD, Gilmour CM, Furtick WR (1967) Microbial versus chemical degradation of atrazine in soils. Soil Sci Soc Am Pro 31:653–656

Smith D, Alvey S, Crowley DE (2005) Cooperative catabolic pathways within an atrazine-degrading enrichment culture isolated from soil. FEMS Microbiol Ecol 53:265–273

Soong CL, Ogawa J, Sakuradani E, Shimizu S (2002) Barbiturase, a novel zinc-containing amidohydrolase involved in oxidative pyrimidine metabolism. J Biol Chem 277:7051–7058

Soulas G (2003) Pesticide degradation in soils. In: Encyclopedia of environmental microbiology. John Wiley and Sons, Oxford, pp 2385–2402

Spliid NH, Koppen B (1998) Occurrence of pesticides in Danish shallow ground water. Chemosphere 37:1307–1316

Strong LC, McTavish H, Sadowsky MJ, Wackett LP (2000) Field-scale remediation of atrazine-contaminated soil using recombinant *Escherichia coli* expressing atrazine chlorohydrolase. Environ Microbiol 2:91–98

Strong LC, Rosendahl C, Johnson G, Sadowsky MJ, Wackett LP (2002) *Arthrobacter aurescens* TC1 metabolizes diverse *s*-triazine ring compounds. Appl Environ Microbiol 68: 5973–5980

Struthers JK, Jayachandran K, Moorman TB (1998) Biodegradation of atrazine by *Agrobacterium radiobacter* J14a and use of this strain in bioremediation of contaminated soil. Appl Environ Microbiol 64:3368–3375

Sulmon C, Gouesbet G, Binet F, Martin-Laurent F, El Amrani A, Couée I (2007) Sucrose amendment enhances phytoaccumulation of the herbicide atrazine in *Arabidopsis thaliana*. Env Poll 145:507–515

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Topp E, Mulbry WM, Zhu H, Nour SM, Cuppels D (2000a) Characterization of *s*-triazine herbicide metabolism by a *Nocardioides* sp. isolated from agricultural soils. Appl Environ Microbiol 66:3134–3141

Topp E, Zhu H, Nour SM, Houot S, Lewis M, Cuppels D (2000b) Characterization of an atrazine-degrading *Pseudaminobacter* sp. isolated from Canadian and French agricultural soils. Appl Environ Microbiol 66:2773–2782

Udiković-Kolić N, Devers-Lamrani M, Petrić I, Hršak D, Martin-Laurent F (2011) Evidence for taxonomic and functional drift of an atrazine-degrading culture in response to high atrazine input. Appl Microbiol Biotech 90:1547–1554

Udiković-Kolić N, Hršak D, Devers M, Klepac-Ceraj V, Petrić I, Martin-Laurent F (2010) Taxonomic and functional diversity of atrazine-degrading bacterial communities enriched from agro-chemical factory soil. J Appl Microbiol 109:355–367

Udiković N, Hršak D, Mendaš G, Filipčić D (2003) Enrichment and characterization of atrazine degrading bacterial communities. Food Technol Biotech 41:211–217

Vaishampayan PA, Kanekar PP, Dhakephalkar PK (2007) Isolation and characterization of *Arthrobacter* sp. strain MCM B-436, an atrazine-degrading bacterium, from rhizospheric soil. Int Biodeter Biodegr 60:273–278

Vanderheyden V, Debongnie P, Pussemier L (1997) Accelerated degradation and mineralization of atrazine in surface and subsurface soil materials. Pestic Sci 49:237–242

Vibber L, Pressler M, Colores G (2007) Isolation and characterization of novel atrazine-degrading microorganisms from an agricultural soil. Appl Microbiol Biot 75:921–928

Wagner GH, Chahal KS (1996) Decomposition of carbon-14 labelled atrazine in soil samples from Sanborn field. Soil Sci Soc Am J 30:752–754

Wang L, Samac DA, Shapir N, Wackett LP, Vance CP, Olszewski NE, Sadowsky MJ (2005) Biodegradation of atrazine in transgenic plants expressing a modified bacterial atrazine chlorohydrolase (*atzA*) gene. Plant Biotechnol 3:475–486

Wang HZ, Chen XW, Xing XG, Hao XH, Chen DF (2010) Transgenic tobacco plants expressing atzA exhibit resistance and strong ability to degrade atrazine. Plant Cell Rep 29:1391–1399

Wiegand C, Krause E, Steinberg C, Pflugmacher S (2001) Toxicokinetics of atrazine in embryos of the zebrafish (*Danio rerio*). Ecotox Environ Saf 49:199–205

Yamazaki K, Fujii K, Iwasaki A, Takagi K, Satsuma K, Harada N, Uchimura T (2008) Different substrate specificities of two triazine hydrolases (TrzNs) from *Nocardioides* species. FEMS Microbiol Lett 286:171–177

Yanze-Kontchou C, Gschwind N (1994) Mineralization of the herbicide atrazine as a carbon source by a *Pseudomonas* strain. Appl Environ Microbiol 60:4297–4302

Yanze-Kontchou C, Gschwind N (1999) Biodegradation of *s*-triazine compounds by a stable mixed bacterial community. Ecotox Environ Safe 43:47–56

Zhang Y, Jiang Z, Cao B, Hu M, Wang ZG, Dong XN (2011) Metabolic ability and gene characteristics of *Arthrobacter* sp. strain DNS10, the sole atrazine-degrading strain in a consortium isolated from black soil. Int Biodeter Biodegr 65:1140–1144