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Evidence for taxonomic and functional drift of an atrazine-degrading culture in response to high atrazine input

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Abstract We evaluated the effects of variations in atrazine input on the evolution of a bacterial culture adapted to a low atrazine concentration. This initial culture (M3-K) was subjected to weekly subculturing in the presence of a high concentration of atrazine as the only N source (100 mg l^{-1}). After four subculturing, M3-K evolved to a new bacterial culture (M3) which exhibited a significant increase in the extent of atrazine mineralization in comparison with the initial culture. Molecular analyses of M3-K and M3 cultures by cloning, restriction analysis, and sequencing of the 16S rRNA genes revealed significant differences in culture structure and composition. M3-K culture comprised mainly *Actinobacteria* (40%), β-*Proteobacteria* (26%), and *Bacteroidetes* (16%). After exposure to a high atrazine concentration, the dominance of *Actinobacteria* decreased (14%), *Bacteroidetes* increased (27%), and β-*Proteobacteria* were replaced by γ-*Proteobacteria* (32%). Quantitative PCR revealed that the abundance of *atzB* and *atzC* genes relative to total bacteria decreased by a factor of 3–4 following the increase in atrazine concentration, while the relative abundance of *trzD* increased significantly (≈400 times). Presented study shows that variations in atrazine input drive both functional and compositional shifts in the atrazine-degrading bacterial culture.

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Introduction

In the environment, pollutants, such as pesticides used in agriculture to improve crop yield and quality, exert selective pressure on microbial life not only by ecotoxicity but also by promoting the growth of microbial populations able to use them as a nutrient source. While the toxic effect of pollutants leads to shifts in the relative abundance of sensitive and resistant populations, the metabolization of the pollutants by adapted microbial populations will lead to an increase in their relative abundance within the overall soil microflora (Van der Meer 2006). The genetic drift of soil microbial populations exposed to pollutants probably combines both pressures, resulting in significant changes in the functional diversity of the soil microbial community. The development of culture-independent molecular techniques based on soil DNA extraction and amplification by PCR-based approaches has made it possible to monitor the impact of pollutants on the soil microbial community. PCR products can be analyzed by cloning or genetic fingerprint. Genetic fingerprint methods consist in a rapid and simple electrophoretic analysis of the PCR products enabling the analysis of the genetic structure of the community (Ranjard et al. 2000). For example, amplified ribosomal DNA restriction analysis (Wang et al. 2008) or denaturing gradient gel electrophoresis (DGGE) (Chang et al. 2001; Seghers et al. 2003) of PCR-amplified 16S rRNA genes were used to monitor changes in the soil bacterial community structure following pesticides application. Methods including PCR cloning and characterization of

cloned sequences enable assessment of the diversity of a community in terms of the number of different populations and to the relative abundance of these populations. 16S rRNA cloning coupled to restriction fragment length polymorphism (RFLP) and partial sequencing was used to study bacterial community structure of the pesticide-contaminated site (Paul et al. 2006) and of the pesticide-degrading enrichment culture (Hussain et al. 2009).

Pesticides have also been shown to inhibit microbial activities (Accinelli et al. 2002). On the other hand, repeated application of pesticides, which is known to cause enhanced degradation (Barriuso and Houot 1996; Houot et al. 2000), was shown to promote the abundance of degrading populations estimated by quantitative PCR (qPCR), targeting functional genes (Baelum et al. 2006; Martin-Laurent et al. 2004). Several authors have shown that both the activity and the abundance of the pesticide-degrading community depended to a considerable extent on selective pressure exerted by pesticides (Baelum et al. 2008; Monard et al. 2010). Fournier et al. (1997) suggested that alternating high-low selective pressure (i.e., every other year) is a factor reducing the pesticide-degrading activity and modifying the functional diversity of the pesticide-degrading culture.

This study investigated the adaptability of a pesticide-degrading bacterial culture in response to high pesticide concentration. The pesticide used was the herbicide atrazine (2-chloro-4-ethylamine-6-isopropylamino-1,3,5 triazine). In the environment, this herbicide is degraded by adapted soil microbial populations, thereby providing an additional source of nitrogen and/or carbon. The *atz* and *trz* gene encoding enzymes responsible for degrading atrazine to completion (mineralization) have been characterized in numerous atrazine-degrading bacterial strains isolated worldwide (Boundy-Mills et al. 1997; De Souza et al. 1996; Martinez et al. 2001; Sadowsky et al. 1998). These genes are highly conserved, located on plasmids, and associated with insertion sequence elements, suggesting the possibility of diversity and evolution of atrazine degradation pathways in nature (De Souza et al. 1998b; Devers et al. 2007; Kolić et al. 2008).

In order to evaluate the impact of variations in atrazine input on the structural and functional changes occuring in the bacterial culture, we analyzed and compared two mixed atrazine-degrading cultures originating from agrochemical factory soil (Kolić et al. 2007; Udiković et al. 2003). The atrazine-mineralizing activity of the bacterial cultures was evaluated by radiorespirometry using ^{14}C ring-labeled atrazine. The structure and composition of the atrazine-degrading culture were analyzed by 16S rRNA gene cloning, screening of clones by PCR-RFLP, and sequencing using the DNA extracted from both cultures as a template. The relative abundance of the atrazine-degrading sequences was estimated by qPCR.

Material and methods

Atrazine-degrading bacterial cultures

Initial atrazine-degrading bacterial culture (M3-K) was isolated by continuous enrichment from a soil sample exposed to repeated spills of effluent from atrazine synthesis within the area around an agrochemical factory (Udiković et al. 2003). Briefly, continuous-flow unit was filled with soil filtrate followed by dosage of mineral salts (MS) medium containing atrazine (25 mg l^{-1}) and yeast extract (50 mg l^{-1}) at low dilution rate (D=0.1 h^{-1}) over a period of 2 months. In vitro evolution of M3-K to a new bacterial culture (M3) was driven by a high concentration of atrazine (100 mg l^{-1}) as the sole N source and sodium citrate (1 g l^{-1}) as the C source over a period of 30 days with weekly batch subculturing (Kolić et al. 2007). Three atrazine-degrading bacterial strains were isolated from this evolved culture (M3), and one catabolic strain was isolated from the initial M3-K culture.

Atrazine mineralization activity

The capacity of the bacterial cultures to mineralize ^{14}C ring-labeled atrazine was estimated by radiorespirometry in a liquid culture over a 4-day incubation period as described by Kolić et al. (2007). Bacterial cells were placed in a sterile respirometer with MS–citrate medium containing 30 mg l^{-1} of unlabeled atrazine and 52 Bq ml^{-1} of ring-labeled atrazine (Isotopchim, France, specific activity 910 MBq $mmol^{-1}$) (0.2 OD at 600 nm) and incubated at 20°C in the dark. The mineralization of atrazine was monitored by counting the $^{14}CO_2$, trapped in 5 ml of 0.2 M NaOH, using a scintillation counter (Packard). The parameters of the mineralization kinetics were determined by fitting a modified Gompertz model (Zwietering et al. 1990) to the mineralization curves using Sigma Plot 4.0. Statistical analysis was performed by subjecting the model parameters to a single factor analysis of variance followed by a Fisher procedure (n=3, P<0.01) (Statview©4.55, Abacus Concept, Inc.).

DNA extraction

The genomic DNA of mixed bacterial cultures was extracted using a QIAGEN Blood and Cell culture Midi kit according to the instructions (QIAGEN, France). The DNA was quantified at 260 nm using a Biophotometer (Eppendorf, Germany), and its quality was checked by electrophoresis on a 1% agarose gel.

Quantitative PCR assays

SYBR Green qPCR assays of the 16S rRNA, *atzA*, *B*, *C*, *D*, *E*, *F*, and *trzD*, *N* genes were performed using specific

primer pairs (Table 1). Quantitative PCR was carried out on an ABI Prism 7900 (Applied Biosystems) in a volume of 20 µl. The assay mixture contained 12.5 µl of SYBR Green PCR master mix (QuantiTect™ SYBR® Green PCR Kit; QIAGEN, France), 1 µM of each primer, and 5 µl of template DNA (10 ng) under the conditions previously described (Devers et al. 2004; Piutti et al. 2003). Three replicates were run for each gene target. Standard curves were obtained with serial dilutions of linearized plasmids containing the targeted genes (Devers et al. 2004; Piutti et al. 2003; Udiković-Kolić et al. 2010).

Cloning and restriction fragment length polymorphism analysis

Clone libraries were prepared from 16S rRNA gene sequences amplified from the genomic DNA of the bacterial cultures. PCR amplicons (~1.5 kb) were cloned using pGEM-T Easy Vector system (Promega); 114 (M3-K) and 74 (M3) PCR products of the recombinant clones (8.75 µl) were digested with tetrameric endonuclease *Alu*I (QBiogene). The digests were electrophoresed on 3% high-resolution agarose gel (QBiogene). The RFLP patterns obtained were compared and grouped into different RFLP profiles.

Sequencing and phylogenetic analysis

At least one member of each RFLP group as well as selected individual clones were sequenced using 926r primer (Table 1) on CEQ 2000-XL sequencer (Beckman Coulter) following the manufacturer's instructions. The 16S rDNA sequences were compared with those available in the GenBank by BLAST analysis. Sequences were aligned using ClustalX software. A neighbor-joining tree was constructed using NJ Plot. The 16S rDNA sequences of isolated catabolic active members of the initial M3-K and the evolved M3 culture (Kolić et al. 2007) were included in the neighbor-joining analysis to link taxonomic groups to atrazine-degrading activity.

Rarefaction and diversity analysis

Rarefaction curves were prepared using Analytic Rarefaction (version 2.1; Stratigraphy laboratory, University of Georgia). M3-K and M3 taxonomic diversity was assessed using the Shannon–Weiner index H', calculated as $H'=-\Sigma(p_i \ln p_i)$, where $p_i=n_i/N$, n_i is the number of clones in each phylotype, and N is the total number of clones, and using the reciprocal of Simpson's index ($1/D$) calculated as $1/D=1/\Sigma(p_i^2)$. The use of $1/D$ instead of the original formulation of Simpson's index ensures that an increase in the reciprocal index reflects an increase in diversity.

Nucleotide sequence accession numbers

The 16S rRNA gene sequences determined in this study were deposited in GenBank under accession numbers FJ756558-FJ756577.

Table 1 Sequence of the primer pairs used in this study

Target gene	Primer	Nucleotide sequence (5′ → 3′)	Reference
atzA	Af	ACGGGCGTCAATTCTATGAC	Devers et al. 2004
	Ar	CACCCACCTCACCATAGACC	
atzB	Bf	AGGGTGTTAGGTGGTGAAC	Devers et al. 2004
	Br	CACCACTGTGCTGTGGTAGA	
atzC	Cf	GCTCACATGCAGGTACTCCA	De Souza et al. 1998b
	Cr	TCCCCCAACTAAATCACAGC	
atzD	Df	TCCCACCTGACATCACAAAC	Devers et al. 2004
	Dr	GGGTCTCGAGGTTTGATTG	
atzE	Ef	GAGCCTCTGTCCGTAGATCG	Devers et al. 2004
	Er	GATGGCGTGTACCGTTTACC	
atzF	Ff	ACCAGCCCTTGAATCATCAG	Devers et al. 2004
	Fr	TATTGTCCCGATACCCAACG	
trzN	trzNf	CACCAGCACCTGTACGAAGG	Mulbry et al. 2002
	trzNr	GATTCGAACCATTCCAAACG	
trzD	trzDf	CCTCGCGTTCAAGGTCTACT	Karns 1999
	trzDr	TCGAAGCGATAACTGCATTG	
16S rDNA	16Sf	CTGGTAGTCCACGCCGTAAA	Devers et al. 2004
	16Sr	CGAATTAAACCACATGCTCCAC	
16S rDNA	926r	CCGTCAATTCMTTTRAGTTT	Chèneby et al. 2000

Results

Estimating the atrazine-mineralizing capacities of the bacterial cultures

The atrazine-mineralizing capacities of the initial M3-K and the evolved M3 bacterial culture were estimated by radiorespirometry using ^{14}C ring-labeled atrazine. The kinetics parameters estimated from the atrazine mineralization curves are presented in Table 2. M3-K and M3 cultures showed similar mineralization rates (μ_m). However, they differed significantly in their extents of atrazine mineralization (A). The evolved M3 culture had a significantly higher (A=71.3%) maximum mineralization extent than the initial M3-K culture (A=62.5%).

Assessing community structure changes by RFLP typing of the cloned 16S rDNA

RFLP screening of the M3-K and M3 libraries led to the identification of 39 different RFLP profiles out of 114 clones screened within the initial M3-K community library and of 20 profiles out of 74 clones within the evolved M3 community library. As can be seen from Fig. 1, M3-K and M3 showed clear differences in the distribution of RFLP profiles. Only four RFLP profiles were shared between M3-K and M3, and the dominant profiles observed in M3-K had disappeared from M3. The M3-K library was dominated by RFLP profile numbers 1, 2, 3, and 4, which accounted for 57% of the clone library, while the most abundant RFLP type numbers within the M3 library were 40, 41, and 12 accounting for 61% of the clone library.

Estimated richness

Rarefaction analysis, which plots the number of screened clones as a function of the number of RFLP profiles detected, showed that at the highest shared sample size (i.e., 74 clones), 29 RFLP profiles were observed for M3-K whereas only 20 RFLP profiles were observed for M3, and the 95% confidental intervals did not overlap (Fig. 2). This indicates the greater phylotype richness in the M3-K library as compared to M3. This was corroborated by the Shannon–Weiner (H') and the inverse of Simpson's index ($1/D$), which were higher for M3-K (H'=2.93; $1/D$= 10.90) than for M3 (H'=2.27; $1/D$= 6.15).

Assessment of the composition of M3-K and M3 cultures

Culture composition was evaluated by 16S rDNA sequencing. The phylogenetic tree (Fig. 3.) clustered M3-K and M3 16S rRNA gene sequences into three large and several smaller groups. The majority of the M3-K sequences were clustered within the *Actinobacteria* (40%), β-*Proteobacteria* (26%), and *Bacteroidetes* (16%). A smaller number of sequences were clustered within the α-*Proteobacteria* (3%). In contrast, after exposure of M3-K culture to high atrazine concentration, a shift from the dominant group of the β-subdivision to group of the γ-subdivision of the *Proteobacteria* (32%) was observed for M3 library. As observed for M3-K, the other bacterial groups dominating M3 library were *Bacteroidetes* (27%) and *Actinobacteria* (14%). *Firmicutes* and β-*Proteobacteria* were represented as small groups in that library (8% and 6%). In addition, the α-proteobacterial cluster comprised only cyanuric acid-degrading *Ochrobactrum* sp. CA1, isolated from M3 culture (Kolić et al. 2007), but this type of clones was not identified in the M3 library, which might be the consequence of the insufficient number of analyzed clones.

The *Actinobacteria* cluster consisted of *Arthrobacter* sp.-related sequences, among which were dominant profiles of the M3-K library (RFLP profile nos. 1 and 3) and of the M3 library (RFLP profile 12), branching with atrazine-degrading strains ATZ1 and ATZ2, previously isolated from the initial M3-K and the evolved M3 culture, respectively (94–98% similarity) (Kolić et al. 2007). The γ-proteobacterial cluster grouped the most abundant phylotype within the M3 library (RFLP profile 40), which showed significant identity to *Pseudomonas* sp. and the cyanuric acid-degrading strain CA2, previously isolated from the evolved M3 culture (Kolić et al. 2007). The β-proteobacterial cluster comprised the dominant phylotype of M3-K library (RFLP profile 2),

Table 2 Kinetic parameters of atrazine mineralization and calculated percentages of detected *atz* and *trz* gene copy numbers to 16S rRNA gene copy numbers

Culture	Atrazine mineralization		Atrazine-catabolic gene (% of 16S rDNA)			
	A (%)	μ_m (h^{-1})	*atzA*	*atzB*	*atzC*	*trzD*
Initial (M3-K)	62.5a (±2.4)	2.02a (±0.02)	3.28×10^{-4} ($\pm6.05\times10^{-5}$)	1.46 (±0.17)	2.09 (±0.52)	9.56×10^{-5} ($\pm4.99\times10^{-5}$)
Evolved (M3)	71.3b (±3.0)	2.22a (±0.30)	4.10×10^{-4} ($\pm1.31\times10^{-4}$)	0.36 (±0.06)	0.78 (±0.21)	3.88×10^{-2} (±0.01)

Values are means±standard error (n=3). For each parameter, values followed by the same letter do not differ significantly (n=3, P<0.01).
A, the maximum extent of mineralization; μ_m, the maximum mineralization rate

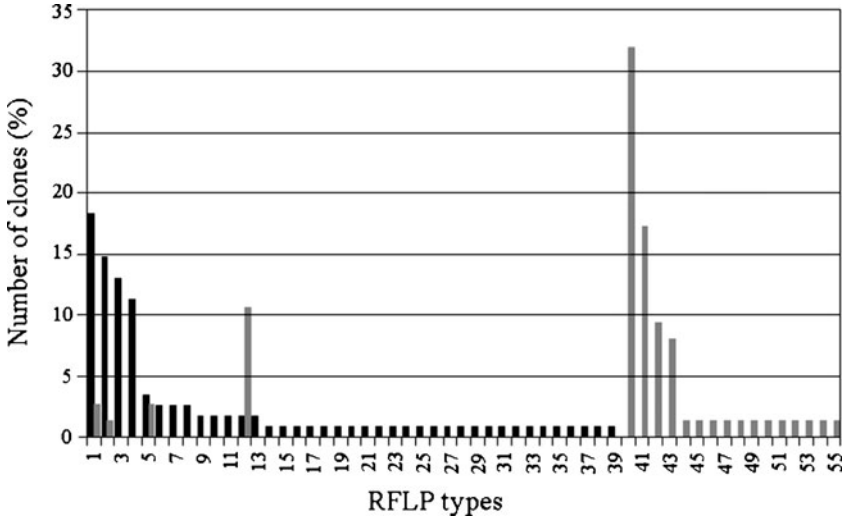

Fig. 1 Distribution of 16S rDNA RFLP families from the initial (M3-K, *black*) and evolved (M3, *gray*) atrazine-degrading culture

branching with *Achromobacter* sp. BP3 (94% similarity). The *Bacteroidetes* cluster grouped sequences of the dominant phylotype of M3 library (RFLP profile 41) and of M3-K library (RFLP type 4), matching *Sphingobacterium* sp. H168, with a similarity of 99% and 82%, respectively.

Relative abundance of atrazine-catabolic genes

The abundance of *atz* and *trz* genes encoding for atrazine-degrading enzymes in M3-K and M3 cultures was estimated by qPCR. Both M3-K and M3 cultures were shown to have a complex atrazine-degrading potential made up of a combination of *trzN*, *atzA*, *atzB*, *atzC*, and *trzD* sequences. Interestingly, both cultures contained *trzN* and *atzA* genes encoding for the enzymes responsible for the transformation of atrazine to hydroxyatrazine.

The relative abundance of atrazine degraders among the global bacterial community was calculated as *atz*/*trz* sequence copy numbers per copy number of 16S rRNA sequences (Table 2). This calculation was not performed for the gene *trzN* owing to its different amplification efficiency. The relative abundances of *atzB* and *atzC* genes were significantly lower in the M3 culture exposed to increased atrazine concentration (0.4% and 0.8%) than in the initial M3-K culture (1.5% and 2.1%). However, the M3 culture had a significantly higher relative abundance of *trzD* gene (0.04%) than M3-K (0.0001%). A similar, low relative abundance of the *atzA* gene was detected in both M3-K and M3 cultures.

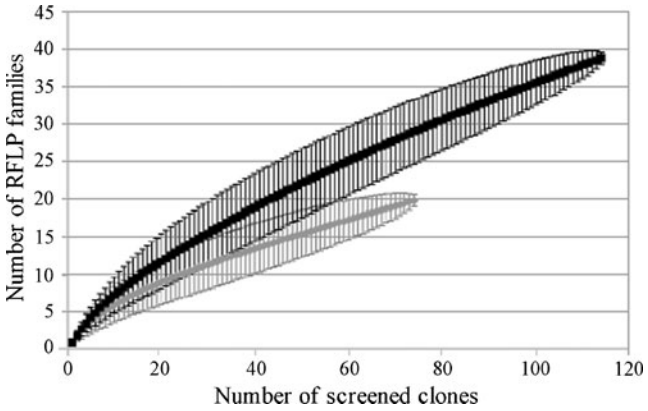

Fig. 2 Rarefaction curves of the observed diversity of 16S rDNA RFLP families in initial (M3-K, *black*) and evolved (M3, *gray*) atrazine-degrading culture. The *error bars* are 95% confidence intervals calculated from the variance of the number of phylotypes

Discussion

We studied the in vitro effects of variations in atrazine input on the evolution of a bacterial culture adapted to a low atrazine concentration. Subculturing of this initial culture (M3-K) over a 1-month period in the presence of a high atrazine concentration led to the selection of a newly evolved bacterial culture (M3) which exhibited a significant increase in the extent of atrazine mineralization in comparison with the initial culture. The taxonomic and functional composition of the initial and the evolved culture were studied to gain a better understanding of the processes involved in the bacterial community response to increased levels of atrazine contamination.

Genetic studies were conducted using DNA extraction and PCR. Although molecular methods such as DNA extraction, amplification, and cloning suffer from biases that can distort the community structure and composition, it was assumed that the potential biases had the same effect for the initial and the evolved cultures. Consequently, the drift in the taxonomic and functional composition of the atrazine-degrading culture during in vitro evolution could be estimated by comparing the initial and evolved culture. PCR-RFLP screening of the 16S rRNA gene clone libraries

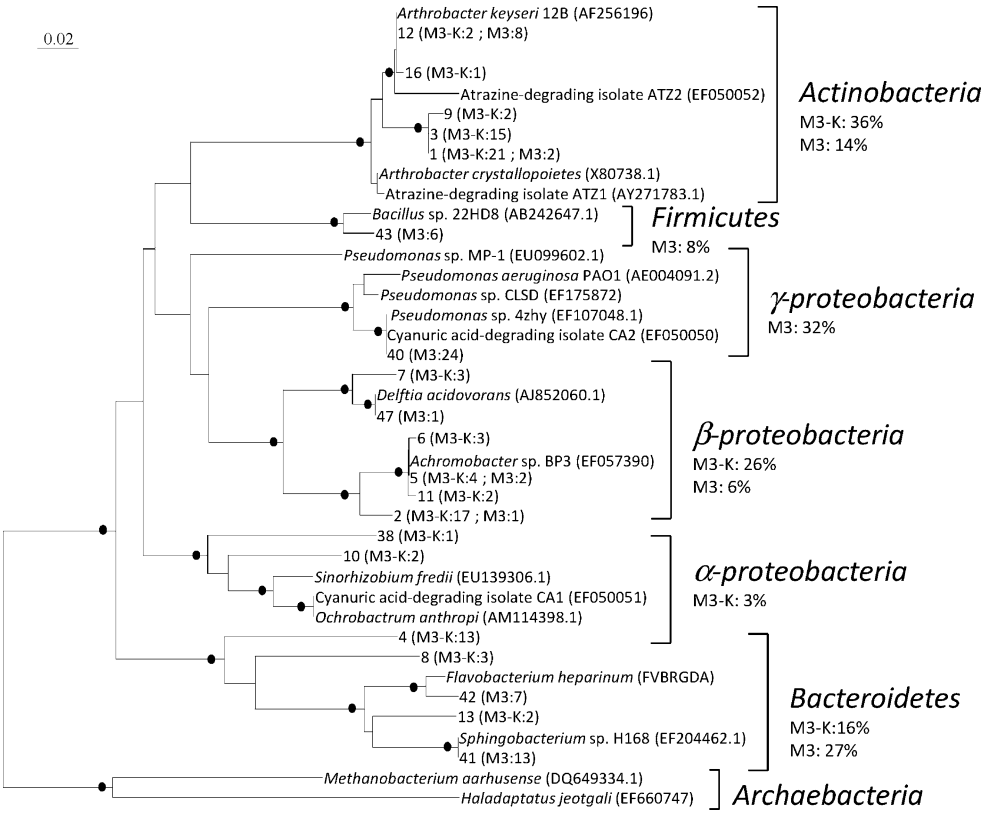

Fig. 3 16S rDNA phylogenetic (NJ) tree of the major RFLP families of initial (*M3-K*) and evolved (*M3*) atrazine-degrading culture and of related 16S rDNA sequences deposited in the GenBank database. Bootstrap values higher than 900 over 1,000 iterations are highlighted by a *black dot*. The number of clones for each atrazine-degrading culture is given in *brackets*. For each phylum, the relative abundance (in percent) is given for each atrazine-degrading culture

revealed the compositional shift of the evolved culture, exposed to a high atrazine input, as compared to the initial culture (Fig. 1). This shift may be due to the stimulation of particular degradative populations involved in different steps of atrazine degradation as well as to the toxicological impact on atrazine-sensitive populations. The toxicity of atrazine on photoluminescent bacteria was observed at a concentration of 20 mg l^{-1} (Gaggi et al. 1995; Kross et al. 1992), whereas with *Pseudomonas* sp. ADP, only a slight increase in atrazine toxicity was observed at increasing atrazine concentrations (from 50 to 200 mg l^{-1}) (Neumann et al. 2004).

As expected, both rarefaction curves and diversity indices indicated a lower diversity in the evolved culture as compared to the initial culture. It is possible that repeated exposure to a high concentration of atrazine as the sole N source exerted a strong selective pressure leading to a reduction in the taxonomic diversity of the newly evolved culture, where nitrogen is shared among culture members. Similar network of substrate sharing has been previously proposed for other pollutant-degrading communities (De Souza et al. 1998a; Pelz et al. 1999).

Clone distribution and sequence analysis showed that the relative abundance of the *Actinobacteria* (genus *Arthrobacter*) in the evolved culture was reduced from 40% to 14%. This conflicts with the identification of *Arthrobacter* sp. isolate ATZ2 as the numerically dominant culturable member of the M3 culture (Kolić et al. 2007). This observation further highlights that direct analyses based on DNA extraction provide a different image from approaches relying on the cultivation of microorganisms as these approaches are biased by the low culturability of most microbes. The comparative analysis of M3-K and M3 16S rRNA gene libraries further demonstrated that during the in vitro evolution experiment, the culture structure shifted from initially predominant sequences of the β-subdivision to sequences of the γ-subdivision of *Proteobacteria* in response to a high atrazine input. These *γ-Proteobacteria* sequences were closely related to *Pseudomonas* sp. isolate CA2, which has a confirmed capability of degrading and using cyanuric acid as a N source (Kolić et al. 2007). This observation suggests that the compositional drift in favor of *Pseudomonas* populations observed during in vitro evolution might be stimulated by the nitrogen-rich compound, cyanuric acid, which is the central intermediate of the atrazine-catabolic pathway. Similarly, Ghosh et al. (2009) showed the predominance of *Actinobacteria* in the microbial community that colonized beads containing a low atrazine concentration (20 mg atrazine kg^{-1}) buried in agricultural soil, whereas *γ-Proteobacteria* populations dominated the community colonizing beads containing a high atrazine concentration (200 mg atrazine kg^{-1}). Furthermore, the *Bacteroidetes* (genus *Sphingobacterium)* were shown to be one of the major bacterial groups in the evolved culture, suggesting that this group may have a specific metabolic capacity in some step(s) of atrazine degradation. However, although *Sphingo-*

bacterium sp. are known to degrade several pesticides, such as mefenacet and lindane (Pesce and Wunderlin 2004; Ye et al. 2004), so far as we are aware, the *Sphingobacterium* genus has not yet been shown to contain any atrazine degraders which is in accordance with previous investigations showing that strains belonging to *Sphingobacterium* genus were not culturable although they were detectable by molecular methods such as DGGE (El-Fantroussi 2000). Therefore, at this stage, the role of *Sphingobacterium* sp. within the evolved culture remains unknown.

Quantitative PCR analysis was applied to monitor possible shifts in the abundance of functional groups involved in atrazine degradation during the in vitro evolution experiment. Both the initial and evolved cultures harbored the *trzN–atzABC–trzD* gene combination. This combination is characterized by the presence of both *atzA* and *trzN* genes encoding for two enzymes transforming atrazine to hydroxyatrazine. This functional redundancy of the first step in atrazine catabolism may contribute to a better accomplishment of the atrazine-degrading function, and it was also detected in recently characterized atrazine-degrading culture enriched from highly polluted soil within the area around an agrochemical factory (Udiković-Kolić et al. 2010). However, recent findings based on a biogeographical approach suggest the prevalence of the gene *trzN* over *atzA* (Arbeli and Fuentes 2010). These results, therefore, suggest that the exclusion theory may not be applicable for *trzN* and *atzA* and that their role may be divergent depending on the study scale considered.

Presented study also showed that in the evolved culture, the relative abundances of *atzB* and *atzC* genes decreased by a factor of 3–4, while the relative abundance of *trzD* increased significantly (≈400 times) compared with the initial culture. These findings are in agreement with the taxonomic composition of the evolved culture characterized by a decreased abundance of *Arthrobacter* populations related to the isolate ATZ2 harboring the *trzN–atzBC* gene composition and a marked increase of *Pseudomonas* populations related to isolate CA2 harboring the *trzD* gene. The functional shift observed in the evolved culture in favor of bacterial populations harboring the *trzD* gene suggests that the opening of the *s*-triazine ring is crucial for functioning in the presence of a high atrazine concentration. The accumulation of cyanuric acid at significant concentration may cause a toxic effect. Furthermore, this enrichment in the *trzD* gene may contribute to the overall release of $^{14}CO_2$ from ^{14}C ring-labeled atrazine and thus may be responsible for the increased extent of atrazine mineralization observed with the evolved culture.

Overall results demonstrated that the in vitro high atrazine input induced a shift in the taxonomic and functional composition of an atrazine-degrading culture adapted to a low atrazine concentration. This study contributes to the description of the evolution of pesticide-degrading bacterial communities in response to environmental changes, such as pesticide contamination.

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