

Minireview

Atrazine biodegradation in the lab and in the field: enzymatic activities and gene regulation

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Summary

Atrazine is an herbicide of the *s*-triazine family that is used primarily as a nitrogen source by degrading microorganisms. While many catabolic pathways for xenobiotics are subjected to catabolic repression by preferential carbon sources, atrazine utilization is repressed in the presence of preferential nitrogen sources. This phenomenon appears to restrict atrazine elimination in nitrogen-fertilized soils by indigenous organisms or in bioaugmentation approaches. The mechanisms of nitrogen control have been investigated in the model strain *Pseudomonas* sp. ADP. Expression of *atzA*, *atzB* and *atzC*, involved in the conversion of atrazine in cyanuric acid, is constitutive. The *atzDEF* operon, encoding the enzymes responsible for cyanuric acid mineralization, is a target for general nitrogen control. Regulation of *atzDEF* involves a complex interplay between the global regulatory elements of general nitrogen control and the pathway-specific LysR-type regulator AtzR. In addition, indirect evidence suggests that atrazine transport may also be a target for nitrogen regulation in this strain. The knowledge about regulatory mechanisms may allow the design of rational bioremediation strategies such as biostimulation using carbon sources or the use of mutant strains impaired in the assimilation of nitrogen sources for bioaugmentation.

Introduction

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine) is an effective herbicide used worldwide for

broad-leaf weed control. Because of its high mobility in soil and its massive application, atrazine has often been detected in surface and ground waters at concentrations well above the legal limits (Kolpin and Kalkhoff, 1993; Richards and Baker, 1993; Biradar and Rayburn, 1995; Hayes *et al.*, 2002; 2003; Tappe *et al.*, 2002). The high incidence of atrazine contamination, along with an increasing concern about the toxicological properties of atrazine, has prompted researchers to seek bioremediation options for atrazine-polluted sites (Allran and Karasov, 2001). Unlike most xenobiotics, atrazine and other *s*-triazines are used preferentially, and often exclusively, as nitrogen sources. The enzymology of the atrazine degradative pathways is now well understood (Ellis *et al.*, 2006). However, gene regulation has scarcely been studied and is emerging as a main factor that limits atrazine bioremediation in the field.

Atrazine-degrading microorganisms and pathways

Early atrazine-utilizing isolates partially metabolized the herbicide by means of dealkylation and deamination reactions that release the side-chains, but were unable to dehalogenate atrazine or cleave the triazine ring (Cook *et al.*, 1984; Mulbry, 1994; Nagy *et al.*, 1995; Shao *et al.*, 1995). However, since the early 1990s, an increasing number of bacteria capable of mineralizing atrazine have been isolated and characterized (Yanze-Kontchou and Gschwind, 1994; Mandelbaum *et al.*, 1995; Struthers *et al.*, 1998). Although atrazine is used primarily as a nitrogen source due to the fully oxidized state of the *s*-triazine ring carbon atoms (Mandelbaum *et al.*, 1995; Radosevich *et al.*, 1995; Struthers *et al.*, 1998; Topp *et al.*, 2000a), several organisms can metabolize the *N*-alkyl substituents as the sole carbon source (Shapir *et al.*, 2007).

Atrazine mineralization is achieved in two stages. Initially, the chlorine and *N*-alkyl side-chains are removed to yield cyanuric acid (2,4,6-trihydroxy-1,3,5-triazine). The enzymes involved in this stage often have broad substrate specificity and may perform a variety of reactions on different substrates (Shapir *et al.*, 2007). The *s*-triazine ring is then cleaved and subsequently

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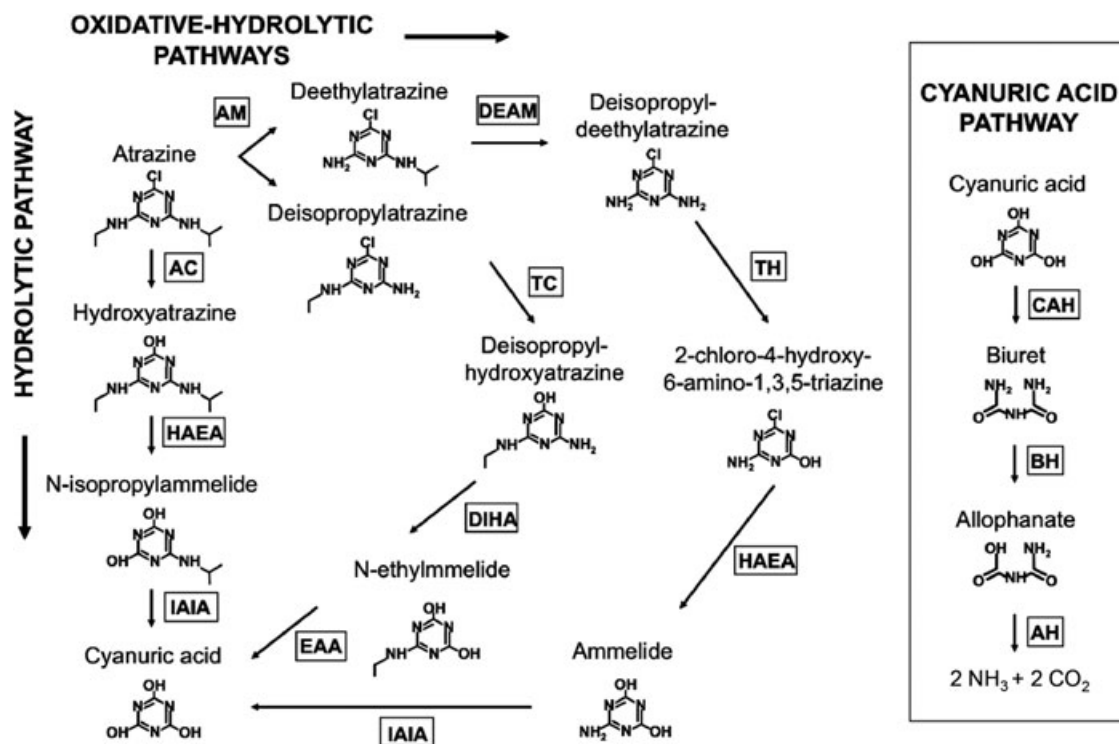


Fig. 1. Atrazine degradative pathways. Left: enzymatic steps catalysing the conversion of atrazine to the common intermediate cyanuric acid. Right: cyanuric acid degradative pathway. AC, atrazine chlorohydrolase; HAEA, hydroxyatrazine ethylaminohydrolase; IAIA, *N*-isopropylammelide isopropylamidohydrolase; TC, *s*-triazine chlorohydrolase; AM, atrazine monooxygenase; DEAM, deethylatrazine monooxygenase; DIHA, deisopropylhydroxyatrazine amidohydrolase; EAA, *N*-ethylammelide amidohydrolase; TH, *s*-triazine hydrolase; CAH, cyanuric acid hydrolase; BH, biuret hydrolase; AH, allophanate hydrolase.

converted to ammonium and carbon dioxide by a set of enzymes that have a much narrower substrate range. Based on the enzymatic activities compiled at the University of Minnesota Biocatalysis and Biodegradation Database (<http://umbbd.msi.umn.edu/>) (Ellis *et al.*, 2006), conversion of atrazine to cyanuric acid can be achieved via three distinct pathways, one of which is purely hydrolytic, while the other two are mixed oxidative-hydrolytic (Fig. 1). The hydrolytic pathway has been extensively characterized in *Pseudomonas* sp. ADP, and consists of three enzymatic steps catalysed by the gene products of *atzA*, *atzB* and *atzC* (Martinez *et al.*, 2001). *AtzA* is responsible for dechlorination of atrazine (de Souza *et al.*, 1996), and *AtzB* and *AtzC* catalyse the elimination of *N*-alkyl substituents from the *s*-triazine ring to yield cyanuric acid (Boundy-Mills *et al.*, 1997; Sadowsky *et al.*, 1998). The *atzA*, *atzB* and *atzC* genes have been shown to be widespread and plasmid-borne in multiple atrazine-degrading isolates from all continents (de Souza *et al.*, 1998a,b; Topp *et al.*, 2000a; Rousseaux *et al.*, 2001). In a few Gram-positive strains, *trzN* substitutes for *atzA*. *TrzN* is a broad substrate range hydrolase that performs dechlorination of atrazine and can remove multiple functional groups from a wide variety of *s*-triazines (Topp *et al.*, 2000b; Shapir *et al.*,

2005a). Oxidative-hydrolytic pathways involve the initial oxidative *N*-dealkylation of atrazine to deisopropylatrazine or deethylatrazine. The products of the above reactions may be dealkylated again to deisopropyldeethylatrazine, or may be subjected to hydrolytic dechlorination, deamination and/or dealkylation to yield cyanuric acid. Enzymes catalysing the oxidative reactions include *Rhodococcus* sp. N186/21 cytochrome P450 (Nagy *et al.*, 1995) and *AtrA* from *Rhodococcus* sp. TE1 (Shao *et al.*, 1995). The enzyme *TriA* displays a 98% identity with *AtzA*, but is active in deamination of atrazine derivatives rather than dehalogenation (Seffernick *et al.*, 2001). On the contrary, *TrzA* from *Rhodococcus corallinus* NRRLB-15444R and the broad-specificity hydrolase *AtzB* catalyse the dechlorination of dealkylated atrazine (Cook *et al.*, 1984; Mulbry, 1994; Seffernick *et al.*, 2002). Although a number of bacteria harbouring the complete set of genes encoding for the hydrolytic pathway have been identified, no single organism studied to date accomplishes all the enzymatic reactions required for atrazine conversion to cyanuric acid via an oxidative-hydrolytic pathway. Degradation may nevertheless be achieved by a consortium of organisms harbouring the appropriate combination of enzymes, as shown for deisopropylatrazine with a mixed

Table 1. Bacterial strains harbouring enzymatic activities involved in atrazine catabolism.

Strain	Enzymatic activities and genes	References
<i>Agrobacterium radiobacter</i> J14a	AC (<i>atzA</i>), HAEA (<i>atzB</i>), IAIA (<i>atzC</i>), CAH (<i>atzD</i>), BH (<i>atzE</i>), AH (<i>atzF</i>)	De Souza <i>et al.</i> (1998a); Struthers <i>et al.</i> (1998); Cheng <i>et al.</i> (2005)
<i>Alcaligenes</i> sp. SG1	AC (<i>atzA</i>), HAEA (<i>atzB</i>), IAIA (<i>atzC</i>) CAH (<i>trzD</i>), BH, ^a AH ^a	K.L. Boundy-Mills (unpublished); Cheng <i>et al.</i> (2005)
<i>Arthrobacter aurescens</i> TC1	AC (<i>trzM</i>), HAEA (<i>atzB</i>), IAIA (<i>atzC</i>)	Strong <i>et al.</i> (2002); Shapir <i>et al.</i> (2005a)
<i>Clavibacter michiganese</i> ATZ1	AC (<i>atzA</i>), HAEA (<i>atzB</i>)	de Souza <i>et al.</i> (1998a)
<i>Enterobacter cloacae</i> 99	CAH (<i>trzD</i>), BH, ^a AH (<i>trzF</i>)	Cook and Hutter (1981); Cook <i>et al.</i> (1985); Eaton and Karns (1991b); Cheng <i>et al.</i> (2005)
<i>Nocardioides</i> sp. C190	AC (<i>trzM</i>)	Topp <i>et al.</i> (2000b)
<i>Nocardia</i> sp.	AM, DEAM	Giardina <i>et al.</i> (1982); Giardi <i>et al.</i> (1985)
<i>Pseudoaminobacter</i> sp.	AC (<i>atzA</i>), HAEA (<i>atzB</i>), IAIA (<i>atzC</i>), CAH, ^a BH, ^a AH ^a	Topp <i>et al.</i> (2000a)
<i>Pseudomonas</i> sp. ADP	AC (<i>atzA</i>), HAEA (<i>atzB</i>), IAIA (<i>atzC</i>), CAH (<i>atzD</i>), BH (<i>atzE</i>), AH (<i>atzF</i>)	Mandelbaum <i>et al.</i> (1995); de Souza <i>et al.</i> (1996); Boundy-Mills <i>et al.</i> (1997); Martinez <i>et al.</i> (2001); Shapir <i>et al.</i> (2002); Fruchey <i>et al.</i> (2003); Shapir <i>et al.</i> (2005b)
<i>Pseudomonas</i> sp. CN1	IAIA (<i>atzC</i>), CAH, ^a BH, ^a AH ^a	de Souza <i>et al.</i> (1998a)
<i>Acidovorax avenae</i> ssp. <i>citrulli</i> (<i>Pseudomonas</i> sp. NRRLB-12227)	IAIA (<i>trzC</i>), CAH (<i>trzD</i>), BH, ^a AH, ^a TH (<i>triA</i>)	Cook and Hutter (1981); Cook <i>et al.</i> (1985); Eaton and Karns (1991a); Karns (1999); Seffernick <i>et al.</i> (2001); Seffernick <i>et al.</i> (2002)
<i>Pseudomonas huttiensis</i> NRRLB-12228	EAA, IAIA (<i>trzC</i>), CAH (<i>trzD</i>), BH, ^a AH ^a	Cook and Hutter (1981); Cook <i>et al.</i> (1984); Cook <i>et al.</i> (1985); Eaton and Karns (1991b)
<i>Ralstonia brasiliensis</i> M91-3	AC (<i>atzA</i>), HAEA (<i>atzB</i>), IAIA (<i>atzC</i>), CAH (<i>trzD</i>), BH, ^a AH ^a	Radosevich <i>et al.</i> (1995); de Souza <i>et al.</i> (1998a); Cheng <i>et al.</i> (2005)
<i>Ralstonia pickettii</i> D	CAH (<i>atzD</i>), BH (<i>atzE</i>), AH (<i>atzF</i>)	Cheng <i>et al.</i> (2005)
<i>Rhizobium</i> sp. PATR	AC (<i>atzA</i>)	Bouquard <i>et al.</i> (1997)
<i>Rhodococcus corallinus</i> NRRLB-15444R	TC (<i>trzA</i>), DIHA	Cook <i>et al.</i> (1984); Mulbry (1994)
<i>Rhodococcus</i> sp. NI86/21	AM (<i>thcB</i>)	Nagy <i>et al.</i> (1995)
<i>Rhodococcus</i> sp. TE1	AM (<i>atrA</i>)	Behki <i>et al.</i> (1993); Shao and Behki (1995)

a. Enzymatic activities are assumed based on experimental evidence of cyanuric acid mineralization by this strain.

When identified, the genes encoding the enzymatic activities are included in parentheses. Abbreviations used are the same as in Fig. 1.

culture of *R. corallinus* NRRLB-15444R and *Pseudomonas huttiensis* NRRLB-12228 (Cook *et al.*, 1984).

Cyanuric acid is a common intermediate of many *s*-triazine biodegradative pathways (Cook *et al.*, 1985) and its metabolization invariably occurs via hydrolytic cleavage of the *s*-triazine ring and consecutive hydrolysis of biuret and allophanate to produce ammonium and carbon dioxide (Cheng *et al.*, 2005). In the case of *Pseudomonas* sp. ADP and several other organisms (Martinez *et al.*, 2001; Cheng *et al.*, 2005), the enzymes responsible for these reactions are encoded by the *atzDEF* operon. Homologues to AtzD (TrzD) and AtzF (TrzF) with small differences in substrate affinity and specificity have been shown to perform the equivalent reactions in other bacteria (Karns, 1999; Shapir *et al.*, 2002; Fruchey *et al.*, 2003; Shapir *et al.*, 2005b). Some of the best-characterized atrazine-degrading bacterial strains, along with their enzymatic activities and genes, are listed in Table 1.

Effect of environmental nitrogen on atrazine degradation

The influence of nitrogen compounds on the efficiency of atrazine elimination has been a focus of research, since

most degrading strains use atrazine as a nitrogen source and agricultural soils are often rich in nitrogen due to routine fertilization. Numerous studies have established that nitrogen amendments have a negative effect on atrazine biodegradation by indigenous microbial populations in soils (Entry *et al.*, 1993; Alvey and Crowley, 1995; Abdelhafid *et al.*, 2000a,b; Guillén Garcés *et al.*, 2007). The effect of nitrogen sources on atrazine degradation has also been tested in pure cultures of several degrading strains (Bichat *et al.*, 1999; Gebendinger and Radosevich, 1999; García-González *et al.*, 2003). Atrazine degradation by *Agrobacterium radiobacter* J14a is not influenced by the simultaneous presence of ammonium, nitrate, urea or glycine in the growth medium (Bichat *et al.*, 1999). However, this may be exceptional, as this is one of the few bacterial strains known to use atrazine as a carbon and nitrogen source (Struthers *et al.*, 1998). In contrast, atrazine degradation by *Ralstonia* sp. M91-3 is inhibited by the presence of preferential nitrogen sources, such as ammonium, nitrate or urea, both in growing cells (Bichat *et al.*, 1999) and in washed cell suspensions previously grown in the presence of the indicated nitrogen sources (Radosevich *et al.*, 1995; Struthers *et al.*, 1998; Gebendinger and Radosevich, 1999).

Nitrogen control of atrazine degradation has been characterized extensively in *Pseudomonas* sp. ADP. Bichat and colleagues (1999) showed that this bacterial strain metabolizes atrazine rapidly in the presence of glycine, but significantly slower in the presence of ammonium, nitrate or urea. A later study (García-González *et al.*, 2003) demonstrated that atrazine degradation is inhibited in resting cell suspensions previously cultured in the presence of nitrogen sources that support fast growth of *Pseudomonas* sp. ADP, such as ammonium, urea, proline or nitrate. In contrast, cells acclimated in media containing a growth-limiting nitrogen source, such as serine, or a pathway metabolite, such as cyanuric acid, biuret or atrazine, efficiently eliminated atrazine in the resting cell assay. The presence of atrazine in growth media containing other nitrogen sources did not stimulate degradation, indicating that the pathway is not induced by its substrate. Nitrogen control of atrazine degradation requires assimilation of the repressing nitrogen source, since the inhibitory effect is suppressed in the presence of a glutamine synthetase inhibitor (García-González *et al.*, 2003).

The relevance of the negative effect of preferential nitrogen sources on atrazine degradation in cultures or cell suspensions to the bioremediation of contaminated soils has been addressed in *Pseudomonas* sp. ADP; García-González and colleagues (2003) showed that nitrate amendment inhibited atrazine mineralization in non-sterile soil microcosms bioaugmented with *Pseudomonas* sp. ADP. This effect is the result of nitrogen control, as evidenced by the fact that a mutant defective in nitrate assimilation effectively mineralized atrazine in the presence of nitrate in the soil microcosm assay. Inhibition of atrazine utilization by *Pseudomonas* sp. ADP mimics the results obtained with indigenous populations (Entry *et al.*, 1993; Alvey and Crowley, 1995; Abdelhafid *et al.*, 2000a,b; Guillén Garcés *et al.*, 2007), indicating that nitrogen control is likely a prevalent trait among atrazine-degrading strains that may limit degradation in soils with high nitrogen content. Indeed, the success of biostimulation strategies using carbon sources as amendments (Abdelhafid *et al.*, 2000a,b; Silva *et al.*, 2004) may reflect the shift in carbon/nitrogen balance caused by the added carbon, as this is the signal sensed by general nitrogen control (Ninfa and Atkinson, 2000; Commichau *et al.*, 2006).

Organization and regulation of the *Pseudomonas* sp. ADP atrazine catabolic genes

The *Pseudomonas* sp. ADP *atz* genes are harboured on the catabolic plasmid pADP-1. Two sets of genes can be distinguished with significant differences in organization and regulation. The genes *atzA*, *atzB* and *atzC*, encoding broad-specificity enzymes, are dispersed in an unstable region of the catabolic plasmid and flanked by insertion

sequence elements (Martinez *et al.*, 2001). Northern blot hybridization and real-time RT-PCR experiments showed that their transcription is constitutive (Martinez *et al.*, 2001; Devers *et al.*, 2004), and atrazine chlorohydrolase assays in crude extracts failed to reveal any post-transcriptional regulation (V. García-González, PhD thesis). New catabolic pathways normally exhibit unregulated or suboptimally regulated expression profiles, while in further evolved pathways, catabolic genes are assembled in operons whose expression responds to both substrate-specific and general physiological signals (Cases and de Lorenzo, 2001). Since atrazine was introduced in the environment about 50 years ago, it has been proposed that acquisition and evolution of *atzA*, *atzB* and *atzC* have taken place in recent times (Martinez *et al.*, 2001; Shapir *et al.*, 2007). In contrast, the three genes encoding narrow-specificity enzymes involved in the utilization of cyanuric acid are clustered in the *atzDEF* operon, which is located in a stable region of pADP-1 (Martinez *et al.*, 2001). Cyanuric acid is not only a common metabolite of multiple *s*-triazine degradation pathways, but also a compound that is naturally present in soils (Fruchey *et al.*, 2003). Cyanuric acid is used as a nitrogen source by multiple bacteria and supports fast growth of *Pseudomonas* sp. ADP (Neumann *et al.*, 2004). Due to the frequent occurrence of cyanuric acid in nature, the *atzDEF* genes may have assembled and evolved to achieve high specificity prior to the acquisition of *atzA*, *atzB* and *atzC* via transposition or recombination events.

Consistent with its early assembly and evolution, the *atzDEF* operon displays a complex and sophisticated regulation that integrates a specific response to the substrate of the pathway with a general response to the physiological status of the cell (Fig. 2). Expression of *atzDEF* is positively regulated in response to nitrogen limitation and cyanuric acid (García-González *et al.*, 2005). Transcription from the *atzDEF* promoter is strictly dependent on the product of the divergently transcribed gene, *atzR* (García-González *et al.*, 2005). AtzR is a LysR-type transcriptional regulator (LTTR) and, as most members of this family of proteins, simultaneously activates the expression of the catabolic genes and represses its own synthesis by binding to a single site with conserved structure located at the divergent promoter region (Schell, 1993; Porrúa *et al.*, 2007). Interestingly, AtzR can activate *atzDEF* expression in response to either nitrogen limitation or cyanuric acid, but also in response to both signals concurrently in a synergistic manner, which is an unusual feature for this type of regulators. Similarly to other LTTRs (Wang and Winans, 1995; Van Keulen *et al.*, 2003), the interaction of AtzR with its effector molecule, cyanuric acid, triggers the repositioning of AtzR at its binding site to achieve a conformation of the AtzR–DNA complexes that is competent for activation

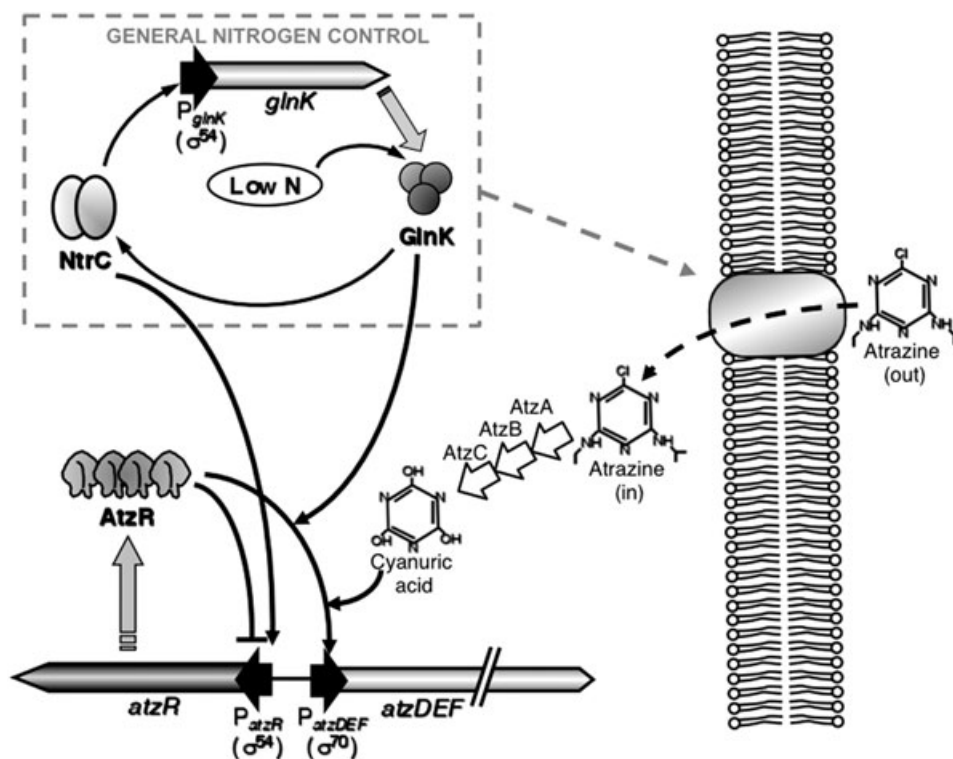


Fig. 2. Proposed regulatory circuit for the *Pseudomonas* sp. ADP atrazine degradative genes. Some elements of the general nitrogen control circuit, such as GlnD and NtrB, have been omitted for simplicity. Dashed lines denote hypothetical elements in the circuit for which experimental evidence is insufficient.

(Porrúa *et al.*, 2007). The nitrogen status modulates the activity of AtzR by two different mechanisms. First, transcription of *atzR* is a target for nitrogen regulation. In this case, the global regulator NtrC is responsible for activation of *PatzR* in response to nitrogen limitation (García-González *et al.*, 2005). NtrC is an archetypical activator of σ^{54} -RNA polymerase (Reitzer *et al.*, 1989; Su *et al.*, 1990; Rippe *et al.*, 1997), and *atzR* is expressed from a σ^{54} -dependent promoter, but unlike typical NtrC-regulated promoters, transcription activation does not require specific DNA binding by NtrC (Porrúa *et al.*, 2007; O. Porrúa, unpubl. results). Second, the ability of AtzR to activate the *PatzDEF* promoter is promoted in nitrogen-limiting conditions. Recent studies suggest that the signal transduction protein GlnK is the element that completes the regulatory circuit and communicates the nitrogen limitation signal to AtzR via protein–protein interaction (V. García-González, PhD thesis). Expression of GlnK is also subjected to positive control by NtrC in enterobacteria (Zimmer *et al.*, 2000) and in *Pseudomonas putida* KT2440 (Hervás *et al.*, 2008). Since *atzDEF* is transcribed from a σ^{70} -dependent promoter (García-González *et al.*, 2005), this circuit allows the general nitrogen control system to simultaneously regulate the expression of both the σ^{54} -dependent *PatzR* and the σ^{70} -dependent *PatzDEF*.

The shape of regulatory circuit for the *atzDEF* operon is a typical example of a feed-forward loop, in which a global regulatory element (NtrC) simultaneously controls the synthesis of the specific regulator (AtzR) and expression of the structural genes downstream (*atzDEF*). Feed-forward loop circuits are often found in nature because they permit not only the integration of the general and specific regulation, but also a response of the regulated genes that is proportional to the intensity of the stimuli (Cases and de Lorenzo, 2005). It should be mentioned that a very similar arrangement is found in the cascade regulation of the *Klebsiella pneumoniae* nitrogen fixation genes, where NtrC activates transcription of both *nifLA* and *glnK*, and GlnK abolishes formation of the inhibitory NifL–NifA complex, allowing *nifHDKTY* activation (Stips *et al.*, 2004).

Is atrazine transport a target for nitrogen control?

Nitrogen control of the cyanuric acid utilization operon *atzDEF* does not suffice to explain the inhibition of atrazine degradation by the presence of preferential nitrogen sources. Since expression of *atzA*, *atzB* and *atzC* is constitutive (Martinez *et al.*, 2001; Devers *et al.*, 2004), conversion of atrazine to cyanuric acid is expected to occur in any condition. Accordingly, growth in medium containing

ammonium did not affect atrazine elimination in cell-free extracts or toluene-permeabilized *Pseudomonas* sp. ADP cells (V. García-González, unpubl. results). This is in sharp contrast with the inhibitory effect of ammonium on atrazine utilization by whole cells (García-González *et al.*, 2003). Since disruption of the cell membrane eliminates nitrogen regulation, we believe that atrazine transport may also be a target for the nitrogen control system (Fig. 2). The existence of a specific transport system for atrazine was proposed first by Gebendinger and Radosevich (1999) *Ralstonia brasiliensis* M91-3, based on a major discrepancy between the atrazine degradation rates in cell extracts and whole cells. Transcriptomic studies have shown that transport systems for nitrogen sources are highly represented among the functions of the genes subjected to general nitrogen control in *Escherichia coli* and *P. putida* (Zimmer *et al.*, 2000; Hervás *et al.*, 2008). An atrazine transporter may have evolved from a pyrimidine transport system, due to the structural similarity between the *s*-triazine ring and the diazine ring in pyrimidines (Wackett *et al.*, 2002). Interestingly, synthesis of the cytosine permease CodB is subjected to general nitrogen control in both *P. putida* and *E. coli* (Zimmer *et al.*, 2000; Hervás *et al.*, 2008). Finally, the pADP-1 plasmid harbours genes encoding transport systems that may be suitable candidates for an atrazine transporter, such as *orf46*, annotated as a putative uracil/xanthine permease, or *orf94-95-96-97-98*, encoding a putative ABC-type transporter, which are located immediately downstream from and may be co-transcribed with *atzR* (Martinez *et al.*, 2001). A similar arrangement of genes encoding a transport system, clustered with *s*-triazine utilization genes, is observed at the melamine degradative plasmid pPDL12 of *Enterobacter chloacae* 99 (GenBank sequence Accession No. AF342826), and the atrazine utilization genes of *Arthrobacter aurescens* TC1 (Sajjaphan *et al.*, 2004).

Final remarks and future perspectives

While the catabolic potential of any microbial strain is dictated by its enzymatic complement, it is well known that most strains that perform well in a lab setting fail to behave similarly in natural conditions (Young and Burns, 1993). The reasons often mentioned are the suboptimal growth environmental conditions and competition with well-adapted indigenous organisms. A third factor may be the presence of environmental traits that repress the genes responsible for the catabolic activities. Recent findings point out that atrazine degradation in agricultural soils may be limited by the effect of nitrogen added as fertilizer on catabolic or transport gene expression (García-González *et al.*, 2003), and a complex regulatory circuit that may involve nitrogen control of atrazine utilization at three dif-

ferent levels has been described (Fig. 2). Based on the new knowledge on nitrogen control of atrazine biodegradation, rational strategies to overcome this limitation may be designed. Some of these strategies may include bio-stimulation using carbon sources in order to increase the carbon/nitrogen balance (Abdelhafid *et al.*, 2000a,b; Silva *et al.*, 2004), the application of inhibitors of nitrogen assimilation (García-González *et al.*, 2003), or the use of mutant strains impaired in the assimilation of nitrogen sources for bioaugmentation (García-González *et al.*, 2003).

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