

## Assimilation of Nitrogen from Nitrite and Trinitrotoluene in *Pseudomonas putida* JLR11

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***Pseudomonas putida* JLR11 releases nitrogen from the 2,4,6-trinitrotoluene (TNT) ring as nitrite or ammonium. These processes can occur simultaneously, as shown by the observation that a *nasB* mutant impaired in the reduction of nitrite to ammonium grew at a slower rate than the parental strain. Nitrogen from TNT is assimilated via the glutamine synthetase-glutamate synthase (GS-GOGAT) pathway, as evidenced by the inability of GOGAT mutants to use TNT. This pathway is also used to assimilate ammonium from reduced nitrate and nitrite. Three mutants that had insertions in *nitC*, *nasT*, and *cnmA*, which encode regulatory proteins, failed to grow on nitrite but grew on TNT, although slower than the wild type.**

Nitroaromatic compounds have multiple applications in the chemical, agrochemical, and pharmaceutical industries. A number of mono- and dinitroaromatic compounds have been shown to be mineralized by bacteria (8, 9, 11, 12, 21, 26, 27, 29, 30). However, no bacterium has been found to use 2,4,6-trinitrotoluene (TNT) as the sole carbon source, although some cases are known in which TNT can serve as a nitrogen source (1, 2, 4, 6, 27).

Metabolism of nitroaromatic compounds can involve monooxygenases that typically replace the nitro group with a hydroxyl group or dioxygenases that catalyze the initial attack of nonpolar nitroaromatic compounds, such as 2,4- and 2,6-dinitrotoluene (25, 26, 30). The attack is initiated at the nitro group farthest from any substituent, giving rise to the corresponding catechol and nitrite release. The nitro group on the aromatic ring could also be easily reduced by specific nitroreductases that add four electrons to produce the corresponding hydroxylamino derivative. In the case of 4-nitrobenzoate and 3-nitrophenol metabolism, the hydroxylamino group is removed as ammonium after rearrangement by lyases (7, 8, 17, 23). A number of mutases can also convert the aromatic hydroxylamino compound into an aminophenol via a Bamberger-like intramolecular rearrangement, with the subsequent release of ammonium (14).

In the case of TNT, nitrite has been proposed to be released after the formation of a hydride-Meisenheimer complex (10). Hughes and colleagues have described the partial reduction of nitro groups on the TNT ring to hydroxylamino, which may undergo a Bamberger rearrangement to produce a phenolic amine (16, 32).

*Pseudomonas putida* JLR11 with TNT as the sole nitrogen

source shows transient accumulation of nitrite in the culture medium (4). The present study was undertaken to elucidate the initial steps in the release of nitrogen from the TNT ring and the subsequent incorporation of ammonium into carbon skeletons. Our results support that TNT can suffer different attacks, releasing nitrite or ammonium, and that nitrogen from TNT is incorporated via the glutamine synthetase-glutamate synthase (GS-GOGAT) pathway.

**Isolation of mutants deficient in nitrite and TNT metabolism.** Random mini-Tn5 mutagenesis of *P. putida* JLR11 (Km<sup>r</sup>) with a mini-Tn5 transposon encoding tellurite resistance was carried out as described previously (28), and transconjugants were selected on M9 minimal medium with ammonium as the nitrogen source and glucose as the carbon source and supplemented with 50 µg of kanamycin/ml and 20 µg of tellurite/ml. Ten independent mutageneses were set up, and 13,600 transconjugants were screened. Growth of the transconjugants under aerobic conditions was tested in minimal medium with glucose as a C source and 2 mM nitrite as the N source. Five clones failed to grow with nitrite as the sole nitrogen source on minimal medium (Table 1) and were selected for further analysis. These mutants either failed to grow or grew slowly on TNT. The gene inactivated in each mutant strain was identified upon sequencing of the DNA adjacent to the mini-Tn5-Tel insertion. Table 1 indicates the inactivated open reading frame and the function of its corresponding gene product. In two of the mutants, the minitransposon was inserted into one of the genes that encode the subunits of GOGAT. In mutant 6.72, the knocked-out gene was *gltB* (3), which encoded the large subunit of GOGAT, whereas in mutant C42, the minitransposon was inserted in the *gltD* gene that encodes the minor subunit of GOGAT (3).

The fact that mutants in GOGAT failed to grow on nitrite and TNT suggested that the GS-GOGAT pathway is the main pathway for nitrogen assimilation when these nitrogen sources are used. In the other three mutants, the minitransposon interrupted a gene where the encoded protein exhibited a high

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TABLE 1. *Pseudomonas putida* JLR11 mini-Tn5-Tel mutants isolated as unable to grow on nitrite<sup>a</sup>

Strain	Growth on NH <sub>4</sub> <sup>+</sup>	Growth on NO <sub>2</sub> <sup>-</sup>	Growth on TNT	ORF <sup>b</sup> mutated	Function
Wild type	++	++	+		
C42	+/-	-	-	<i>gltD</i>	GOGAT minor subunit
6.72	+	-	-	<i>gltB</i>	GOGAT large subunit
9.46	++	-	+/-	<i>ntrC</i>	Master nitrogen metabolism transcriptional activator
10.51	++	-	+/-	<i>cnmA</i>	LysR family transcriptional regulator
36.35	++	-	+/-	<i>nasT</i>	Response regulator of <i>nasAB</i> operon

<sup>a</sup> Growth of the strains was tested on minimal medium with glucose and different nitrogen sources, namely, 10 mM NH<sub>4</sub>Cl, 2 mM nitrite, and 500 μM TNT. Growth was examined after 48 h of incubation at 30°C. ++, size of the colonies was ≥2 mm; +, colony size was <2 mm but >1 mm; -, no growth was observed. The open reading frame in which the mini-Tn5 transposon was inserted is indicated, as well as the function of the corresponding gene product in the wild-type strain.

<sup>b</sup> Open reading frame.

degree of similarity to gene regulators. In fact, in mutant 9.46, the mini-Tn5 transposon was inserted in the *ntrC* gene, which encodes the master regulator in nitrogen assimilation in a number of microorganisms (13, 19, 22, 31). In mutant 36.35, the knockout gene was homologous to *nasT* in *Azotobacter vinelandii*, whose gene product is a positive transcriptional regulator of the *nas* operon for nitrate and nitrite assimilation (9). In mutant 10.51, the mini-Tn5 transposon was inserted in a gene encoding a transcriptional regulator that exhibits homology with members of the LysR family (15). Since the exact role of the encoded protein is unknown, the gene was called *cnmA* for control nitrite metabolism gene *A*.

We constructed a genomic library of *P. putida* JLR11 in the pLAFR3 cosmid and identified cosmids bearing the inactivated gene in the mutants. In all cases the cosmid restored growth on nitrite and TNT to the mutant strain. This established a direct connection between the mutant's phenotype and its genotype. Figure 1 shows the genomic organization of the genes surrounding the transposon insertion in the genome of

*P. putida* JLR11, which was established by primer walking on the cosmid clone.

**Site-specific mutagenesis of the *nasB* gene of *P. putida* JLR11.** The *nasB* gene encodes assimilatory nitrite reductase in *Pseudomonas* (20, 24). Given that the mutagenesis procedure described above did not result in the isolation of *nasB* mutants, we decided to generate a mutant by site-specific inactivation. Using the appropriate primers, we amplified a 1,117-bp central fragment of the *nasB* gene of JLR11, and DNA was cloned in the pCHES1ΩGm (21) knockout plasmid to yield pAC1. The pAC1 plasmid was transferred by triparental mating to JLR11, and Gm<sup>r</sup> clones were selected. A random clone in which the *nasB* gene was inactivated after homologous recombination was selected, and the nature of the mutation was confirmed by Southern blotting. As expected, the *nasB* mutant grew with ammonium as the sole N source but did not grow at all with nitrite and exhibited slow growth with TNT.

These results suggest that in addition to a denitrase activity involved in nitrite release from TNT, the strain possesses another mechanism for assimilating nitrogen derived from TNT. A potential mechanism is the partial reduction of one of the nitro groups of TNT to a hydroxylamino moiety and the subsequent release of ammonium from the aromatic ring upon a Bamberger-like rearrangement. In support of this hypothesis, we have identified two nitroreductases in JLR11, PnrA and PnrB. The former exhibits TNT-specific nitroreduction, using NADPH to preferentially reduce the nitro group at position 4 to its corresponding 4-hydroxylamino-2,6-dinitrotoluene (A. Caballero, J. J. Lázaro, J. L. Ramos, and A. Esteve-Núñez, unpublished data). In this connection, it is worth mentioning that *Pseudomonas pseudoalcaligenes* JS52 uses nitrobenzene reductase to yield 4-hydroxylamino-2,6-dinitrotoluene. The fate of this product in vivo is not well known for JS52; although some authors have reported the formation of a yellow compound and the release of nitrite, no information on the in vivo or in vitro release of ammonium has been reported (5).

**Growth on liquid medium of different mutants with deficient nitrogen metabolism.** To obtain quantitative data on the behavior of mutants with different nitrogen sources, we determined the growth rates of the wild type and the mutants with glucose as the carbon source and one of the following nitrogen sources: 5 mM NH<sub>4</sub>Cl, 5 mM KNO<sub>3</sub>, 2 mM NaNO<sub>2</sub>, and TNT supplied above its limit of solubility in water (namely, 0.5 mM). With ammonium as an N source, the growth rate was similar in the wild-type strain, the three mutants affected in different regulatory genes, and the *nasB* mutant (generation time = 2.55

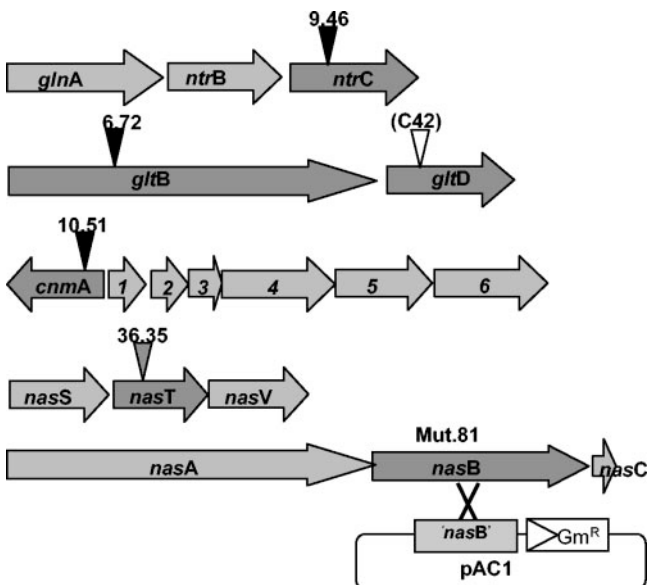


FIG. 1. Open reading frame maps indicating the position of the mini-Tn5-Tel insertion in JLR11 mutants deficient in growth with nitrite or TNT. The triangle indicates the transposon insertion site in each of the indicated mutants. The *nasB* mutant (mut.81) was generated by site-directed mutagenesis (21). (Note that the function of the products *orf1* to *orf6*, divergent with respect to *cnmA*, is unknown.)

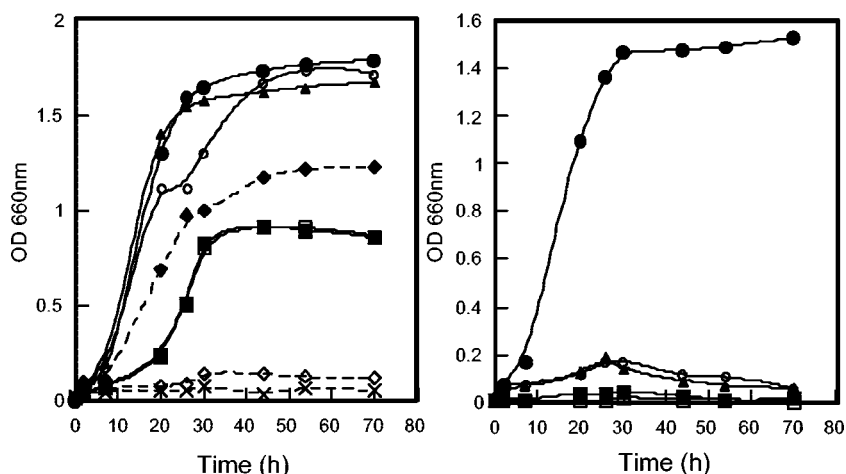


FIG. 2. Growth curves of *P. putida* JLR11 and its mutants in ammonium (left) and nitrite (right) as N sources. Bacterial cells were grown on minimal medium with glucose (0.5% [wt/vol]) as a C source at 30°C. Left panel: the continuous line indicates that an initial concentration of  $\text{NH}_4^+$  of 5 mM was used, and the discontinuous lines indicate that the initial  $\text{NH}_4^+$  concentration was 0.8 mM. Right panel: nitrite was used at a 2 mM concentration. Left and right panels: closed circles, wild type; closed squares, *gltB* mutant; open squares, *gltD* mutant; closed triangles, *ntrC* mutant; open circles, *nasB* mutant. Left panel, discontinuous line: closed diamonds, wild type; open diamonds, *gltB* mutant; crosses, *gltD* mutant.

$\pm 0.15$  h), and the cultures reached a turbidity of about 1.6 to 1.8 U at 660 nm (Fig. 2). In contrast, the mutants lacking GOGAT exhibited a long lag phase (i.e., 10 to 12 h); the growth rate in the exponential phase was slower than that of the wild type (doubling time =  $4.5 \pm 0.3$  h), and maximal turbidity of the culture was around 0.8 U (Fig. 2). When the ammonium concentration was lowered from 5 to 0.8 mM, the wild-type strain and the *nasB* mutant grew with a doubling time of about 6 h and the cultures reached a turbidity of about 0.8 to 1 at 660 nm. No growth at all was observed with either of the mutants in the GOGAT system under the low-ammonium condition (Fig. 2). None of the mutants in the GOGAT system or those exhibiting a knockout in the *nasB*, *nasT*, or *cnmA* gene grew significantly with nitrate or nitrite (Fig. 2). That the mutants failed to grow with nitrite is supported by the fact that they were unable to take up nitrite in resting-cell assays (not shown). Furthermore, when nitrate was present in the culture medium, the *nasB* mutant reduced it to nitrite, which accumulated up to a 350  $\mu\text{M}$  concentration in the culture medium, whereas the *ntrC*, *gltB*, *gltD*, and *nasT* mutants did not accumulate nitrite (data not shown). This series of results suggest that nitrogen from nitrate, nitrite, and ammonium (albeit at low concentrations) is preferentially assimilated via the GS-GOGAT pathway. Ammonium assimilation in the GOGAT mutants should take place preferentially via glutamate dehydrogenase, since a *gdh* gene is present in *P. putida* (26) (PP2080 or Q88L55 in TrEMBL), and this observation is in agreement with earlier reports on nitrogen metabolism in *Pseudomonas aeruginosa* (18).

Growth of the wild type and mutants was tested with glucose as a carbon source and TNT. While the GOGAT mutants failed to grow, the *ntrC*, *nasT*, and *nasB* mutants grew half as fast as the wild-type strain (doubling time, 23 to 29 h, compared to 15 h for the wild-type strain) and also reached a lower maximal turbidity. With the GOGAT mutants and the *nasB* mutant, a linear accumulation of nitrite (up to 30 to 45  $\mu\text{M}$ ) was observed, whereas nitrite accumulated only transiently in

the culture medium of the wild-type strain. Nitrite reductase activity was measured in cell extracts of the wild-type strain and the *nasB*, *ntrC*, and *nasT* mutants. Cells were grown in M9 minimal medium until the mid-log phase; the cells were then harvested, washed, and suspended in minimal medium with glucose and TNT or with glucose, ammonium, and TNT. After 3 h, nitrite reductase was measured in cell extracts. No nitrite reductase activity was found either in wild-type or mutants incubated in the presence of ammonium. However, nitrite reductase activity was found in cell extracts in the wild-type strain incubated only with TNT (128 mU/mg of protein) but not in extracts of *nasB* or the regulatory mutants, indicating that nitrite reductase is involved in the assimilation of nitrite released from TNT.

NtrC is a master regulator in nitrogen metabolism in many bacteria, although such mutants have not been described in pseudomonadaceae. In *P. putida* JLR11, as in other microbes, the induction of the operons involved in the use of different nitrogen sources, such as nitrate and nitrite, requires activation by NtrC. This is in agreement with the screening procedure we followed: isolation of mutants that did not grow on nitrite. Regarding the utilization of TNT as an N source, the mutant deficient in NtrC grew at a rate similar to that of the *nasB* mutant. This is probably due to the mutant's inability to use nitrite, since this metabolite accumulated with time in the culture medium, and resting cell assays showed that the NtrC mutant could not take up nitrite. In *A. vinelandii*, the *nasT* gene product has been proposed to act as an inducer of the nitrate/nitrite assimilatory pathway (9), and it is necessary for the induction of the *nas* operon in response to the presence of nitrate or nitrite in the culture medium. In this context, a *nasT* mutant of *P. putida* was impaired in the use of nitrate and nitrite, but in contrast with the NtrC mutant, it did not exhibit any limitation in the use of histidine and other amino acids as N sources (data not shown). Like the NtrC mutant, the *NasT* mutant grew at a slower rate than the wild-type strain with TNT, accumulated nitrite in the culture medium, and was

unable to consume nitrite in resting cell assays. The mutation in a gene encoding a LysR-like protein, designated *cnmA*, represents a new regulator of nitrogen metabolism of unknown function. Our results indicate that it is specifically involved in the control of nitrite metabolism, since no other phenotypic defect was found to be associated with this mutant, although its target is unknown at present.

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#### REFERENCES

- Boopathy, R., and C. F. Kulpa. 1992. Trinitrotoluene as a sole nitrogen source for a sulphate-reducing bacterium *Desulfovibrio* sp. (B strain) isolated from an anaerobic digester. *Curr. Microbiol.* **25**:235–241.
- Duque, E., A. Haidour, P. Godoy, and J. L. Ramos. 1993. Construction of a *Pseudomonas* hybrid strain that mineralizes 2,4,6-trinitrotoluene. *J. Bacteriol.* **175**:2278–2283.
- Eberl, L., A. Ammendola, M. H. Rothballer, M. Givskov, C. Sternberg, M. Kilstrop, K. H. Schleifer, and S. Molin. 2000. Inactivation of *glbB* abolishes expression of the assimilatory nitrate reductase gene (*nasB*) in *Pseudomonas putida* KT2442. *J. Bacteriol.* **182**:3368–3376.
- Esteve-Núñez, A., and J. L. Ramos. 1998. Metabolism of 2,4,6-trinitrotoluene by *Pseudomonas* sp. JLR11. *Environ. Sci. Technol.* **32**:3802–3808.
- Fiorella, P. D., and J. C. Spain. 1997. Transformation of 2,4,6-trinitrotoluene by *Pseudomonas pseudoalcaligenes* JS52. *Appl. Environ. Microbiol.* **63**:2007–2015.
- French, C. E., S. Nicklin, and N. C. Bruce. 1998. Aerobic degradation of 2,4,6-trinitrotoluene by *Enterobacter cloacae* PB2 and by pentaerythritol tetranitrate reductase. *Appl. Environ. Microbiol.* **64**:2864–2868.
- Groenewegen, P. E. J., P. Breeuwer, J. M. L. M. van Helvoort, A. A. M. Langenhoff, E. P. de Vries, and J. A. M. de Bont. 1992. Novel degradative pathway of 4-nitrobenzoate in *Comamonas acidovorans* NBA-10. *J. Gen. Microbiol.* **138**:1599–1605.
- Groenewegen, P. E. J., and J. A. M. de Bont. 1992. Degradation of 4-nitrobenzoate via 4-hydroxylaminobenzoate and 3,4-dihydroxybenzoate in *Comamonas acidovorans* NBA-10. *Arch. Microbiol.* **158**:381–386.
- Gutiérrez, J. C., F. Ramos, L. Ortner, and M. Tortolero. 1995. *nasST*, two genes involved in the induction of the assimilatory nitrite-nitrate reductase operon (*nasAB*) of *Azotobacter vinelandii*. *Mol. Microbiol.* **18**:579–591.
- Haidour, A., and J. L. Ramos. 1996. Identification of products resulting from the biological reduction of 2,4,6-trinitrotoluene, 2,4-dinitrotoluene, and 2,6-dinitrotoluene by *Pseudomonas* sp. *Environ. Sci. Technol.* **30**:2365–2370.
- Haigler, B. E., and J. C. Spain. 1993. Biodegradation of 4-nitrotoluene by *Pseudomonas* sp. strain 4NT. *Appl. Environ. Microbiol.* **59**:2239–2343.
- Haigler, B. E., W. H. Wallace, and J. C. Spain. 1994. Biodegradation of 2-nitrotoluene by *Pseudomonas* sp. strain JS42. *Appl. Environ. Microbiol.* **60**:3466–3469.
- He, L., E. Soupené, and S. Kustu. 1997. NtrC is required for control of *Klebsiella pneumoniae* NifL activity. *J. Bacteriol.* **179**:7446–7455.
- He, Z., L. J. Nadeau, and J. Spain. 2000. Characterization of hydroxylaminobenzene mutase from pNBZ139 cloned from *Pseudomonas pseudoalcaligenes* JS45. A highly associated SDS-stable enzyme catalyzing an intramolecular transfer of hydroxyl groups. *Eur. J. Biochem.* **267**:1110–1116.
- Henikoff, S., G. W. Haughn, J. M. Calvo, and J. C. Wallace. 1988. A large family of bacterial activator proteins. *Proc. Natl. Acad. Sci. USA* **85**:6602–6606.
- Hughes, J. B., C. Wang, K. Yesland, A. Richardson, R. Bhadra, G. Bennett, and F. Rudolph. 1998. Bamberger rearrangement during TNT metabolism by *Clostridium acetobutylicum*. *Environ. Sci. Technol.* **32**:494–500.
- Hughes, M. A., and P. A. Williams. 2001. Cloning and characterization of the *pnb* genes, encoding enzymes for 4-nitrobenzoate catabolism in *Pseudomonas putida* TW3. *J. Bacteriol.* **183**:1225–1232.
- Janssen, D. B., J. O. den Camp, P. J. Leenen, and C. van der Drift. 1980. The enzymes of the ammonia assimilation in *Pseudomonas aeruginosa*. *Arch. Microbiol.* **124**:197–203.
- Labes, M., V. Rastogi, R. Watson, and T. M. Finan. 1993. Symbiotic nitrogen fixation by a *nifA* deletion mutant of *Rhizobium meliloti*: the role of an unusual *ntrC* allele. *J. Bacteriol.* **175**:2662–2673.
- Lin, J. T., B. S. Goldman, and V. Stewart. 1993. Structures of genes *nasA* and *nasB*, encoding assimilatory nitrate and nitrite reductases in *Klebsiella pneumoniae* M5al. *J. Bacteriol.* **175**:2370–2378.
- Llamas, M. A., J. J. Rodríguez-Herva, R. E. W. Hancock, W. Bitter, J. Tommasen, and J. L. Ramos. 2003. Role of *Pseudomonas putida* *tol-oprL* gene products in uptake of solutes through the cytoplasmic membrane. *J. Bacteriol.* **185**:4707–4716.
- Masepohl, B., B. Kaiser, N. Isakovic, C. L. Richard, R. G. Kranz, and W. Klipp. 2001. Urea utilization in the phototrophic bacterium *Rhodospirillum rubrum* is regulated by the transcriptional activator NtrC. *J. Bacteriol.* **183**:637–643.
- Meulenberg, R., M. Pepi, and J. A. M. de Bont. 1996. Degradation of 3-nitrophenol by *Pseudomonas putida* B2 occurs via 1,2,4-benzenetriol. *Biodegradation* **7**:303–311.
- Nelson, K. E., C. Weinel, I. T. Paulsen, R. J. Dodson, H. Hilbert, V. A. P. Martins dos Santos, D. E. Fouts, S. R. Gill, M. Pop, M. Holmes, L. Brinkac, M. Beanan, R. T. DeBoy, S. Daugherty, J. Kolonay, R. Madupu, W. Nelson, O. White, J. Peterson, H. Khouri, I. Hance, P. Chris Lee, E. Holtzapfel, D. Scanlan, K. Tran, A. Moazzez, T. Utterback, M. Rizzo, K. Lee, D. Kosack, D. Moestl, H. Wedler, J. Lauber, D. Stjepandic, J. Hoheisel, M. Straetz, S. Heim, C. Kiewitz, J. Eisen, K. N. Timmis, A. Düsterhöft, B. Tümmeler, and C. M. Fraser. 2002. Complete genome sequence and comparative analysis of the metabolically versatile *Pseudomonas putida* KT2440. *Environ. Microbiol.* **4**:799–808.
- Nishino, S. F., G. C. Paoli, and J. C. Spain. 2000. Aerobic degradation of dinitrotoluenes and pathway for bacterial degradation of 2,6-dinitrotoluene. *Appl. Environ. Microbiol.* **66**:2139–2147.
- Nishino, S. F., and J. C. Spain. 2004. Catabolism of nitroaromatic compounds, p. 575–608. In J. L. Ramos (ed.), *Pseudomonas*, vol. III. Kluwer/Academic Plenum Publishers, London, United Kingdom.
- Pak, J. W., K. L. Knoke, D. R. Noguera, B. G. Fox, and G. H. Chambliss. 2000. Transformation of 2,4,6-trinitrotoluene by purified xenobiotic reductase B from *Pseudomonas fluorescens* I-C. *Appl. Environ. Microbiol.* **66**:4742–4750.
- Sánchez-Romero, J. M., R. Díaz-Orejas, and V. de Lorenzo. 1998. Resistance to tellurite as a selection marker for genetic manipulations of *Pseudomonas* strains. *Appl. Environ. Microbiol.* **64**:4040–4046.
- Sommerville, C. C., S. F. Nishino, and J. C. Spain. 1995. Purification and characterization of nitrobenzene nitroreductase from *Pseudomonas pseudoalcaligenes* JS52. *J. Bacteriol.* **177**:3837–3842.
- Spangoord, R. J., J. C. Spain, S. F. Nishino, and K. E. Mortelmans. 1991. Biodegradation of 2,4-dinitrotoluene by a *Pseudomonas* sp. *Appl. Environ. Microbiol.* **57**:3200–3205.
- Studholme, D. J., and R. Dixon. 2003. Domain architectures of  $\sigma^{54}$ -dependent transcriptional activators. *J. Bacteriol.* **185**:1757–1767.
- Watrous, M. M., S. Clark, R. Kutty, S. Huang, F. B. Rudolph, J. B. Hughes, and G. N. Bennett. 2003. 2,4,6-Trinitrotoluene reduction by an Fe-only hydrogenase in *Clostridium acetobutylicum*. *Appl. Environ. Microbiol.* **69**:1542–1547.