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Transformation of 2,4,6-trinitrotoluene (TNT) by *Raoultella terrigena*

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Abstract

Manufacture of nitroorganic explosives generates toxic wastes leading to contamination of soils and waters, especially groundwater. For that reason bacteria living in environments highly contaminated with 2,4,6-trinitrotoluene (TNT) and other nitroorganic compounds were investigated for their capacity for TNT degradation. One isolate, *Raoultella terrigena* strain HB, removed TNT at concentrations between 10 and 100 mg l^{-1} completely from culture supernatants under optimum aerobic conditions within several hours. Only low concentrations of nutrient supplements were needed for the cometabolic transformation process. Radioactivity measurements with ring-labelled ^{14}C–TNT detected about 10–20% of the initial radioactivity in the culture supernatant and the residual 80–90% as water-insoluble organic compounds in the cellular pellet. HPLC analysis identified aminodinitrotoluenes (2-ADNT, 4-ADNT) and diaminonitrotoluenes (2,4-DANT) as the metabolites which remained soluble in the culture medium and azoxy-dimers as the main products in the cell extracts. Hence, the new isolate could be useful for the removal of TNT from contaminated waters.

Abbreviations: ADNT – aminodinitrotoluene; DANT – diaminonitrotoluene; TN-4,4′-azo – 2,2′,6,6′-tetranitro-4,4′-azotoluene; TN-2,2′-azoxy – 4,4′,6,6′-tetranitro-2,2′-azoxytoluene; TN-4,4′-azoxy – 2,2′,6,6′-tetranitro-4,4′-azoxytoluene; TNT – 2,4,6-trinitrotoluene

Introduction

2,4,6-Trinitrotoluene (TNT) is a nitroaromatic explosive that has been released into soil and water ecosystems mainly due to its massive use during the two World Wars. As a result, many sites used for TNT production have become seriously contaminated with the explosive and related compounds (Fuller et al. 2004; Lewis et al. 2004). Typical explosive-contaminated sites may contain up to 10,000 mg kg^{-1} TNT in soils and up to 100 mg l^{-1} in water. TNT and its metabolites exhibit a high toxic and mutagenic potential on prokaryotes and eukaryotes (Spanggord et al. 1995; Honeycutt et al. 1996; Lachance et al. 1999). It has been estimated that nearly 3,200 sites in Germany require environmental cleanup (Preuß & Haas 1987; Preuß & Eitelberg 1999). Biological based remediation strategies are promising economical and ecological alternatives for existing physical/chemical technologies.

The extensive research on biodegradation of TNT by bacteria and fungi has been summarized in several recent reviews (Fritsche et al. 2000; Hawari et al. 2000; Lenke et al. 2000; Rosser et al. 2001; van Aken & Agathos 2001; Heiss & Knackmus 2002; Zhao et al. 2004). The presence of three electron-withdrawing nitro groups in TNT

introduces steric constraints and confers a high electron deficiency to the aromatic ring. The molecule is thus regarded as resistant to oxidative microbial degradation, although some bacteria can use the nitro-groups of TNT as a nitrogen source. Only low mineralization rates have been reported for bacteria in contrast to several ligninolytic fungi. Instead of oxidation, many bacteria catalyze the reduction of one or two nitro groups of TNT to monoaminodinitrotoluenes (ADNT) and diaminonitrotoluenes (DANT) under both aerobic and anaerobic conditions. The electron transfer is mediated by oxygen-insensitive cytoplasmatic nitroreductases (Pak et al. 2000; Kim & Song 2005). Reactive nitroso- and hydroxylamino intermediates can further react to condensated azoxy-dimers and acetyl derivates of TNT. Under strictly anaerobic conditions ADNT is further reduced to 2,4,6-triaminotoluene (TAT) which is highly reactive, and can polymerize or irreversibly bind to soil (Thiele et al. 2002).

The reductive reactions are the basis of several treatment processes for the bioremediation of TNT contaminated soils (Lenke et al. 2000; Fuller et al. 2004; Lewis et al. 2004). No comparable biological approach exists for contaminated aquatic environments. In this study we describe a new isolate *Raoultella terrigena* strain HB originating from a contaminated former explosive production site, as a vehicle to remove TNT from contaminated waters.

Materials and methods

Isolation of TNT-degrading microorganisms

Water and soil samples were collected from abandoned TNT production sites in Germany (Hallschlag/Rhineland-Palatinate, Moschwig/Saxony-Anhalt). Microorganisms were enriched in 0.1×Standard I nutrient broth (Merck, Darmstadt, Germany, No. 107882) supplemented with TNT (10 mg l^{-1}). From these cultures single colonies were obtained on Standard I nutrient agar (Merck, Darmstadt, Germany, No. 107881) containing TNT (10 mg l^{-1}) and further characterized. TNT used for degradation experiments originated from a solid sample collected at a former explosives production site in Germany; it was recrystallized from absolute ethanol. In the resulting product no impurites could be detected by GC and HPLC. The identity was confirmed by determination of the melting point, retention times, UV-spectrum and EI-mass spectrum against a reference sample of TNT (Dynamit Nobel, Troisdorf, Germany).

Identification of the isolates

Isolates of different morphological colony appearance were further differentiated by macrorestriction and plasmid analysis as described by Claus et al. (1995). Identification was done by sequencing of the 16S rDNA genes amplified by polymerase chain reaction (PCR). Primers used were Eubak 338F (ACTCCTACGGGAGGCAG) and C 1992R (CCACGGGCGGTGTGTAC). The PCR amplificates were sequenced by Genterprise Inc. (Mainz, Germany).

Analytical methods

Reversed-phase high-performance liquid chromatography (HPLC) was used to separate TNT and other nitroorganic compounds. Before HPLC analyses all samples were centrifuged (16,000×g, 5 min) and filtrated (membrane pore size: 0.2 μm). The following HPLC-DAD equipment was used (all parts Gynkotek, Germany): pump M 480, on-line degasser, autosampler Gina 50, diode array detector UVD 340 S, column oven STH. The separation was performed on a Nucleodur column 100–3 C_{18}ec 250×4 mm (Macherey-Nagel, Düren, Germany) using a multistep methanol/water gradient: 1. step: 0 → 10 min, 40 → 50% methanol; 2. step: 10 → 35 min, isocratic 50% methanol; 3. step: 35 → 80 min, 50 → 85% methanol at a flow rate of 0.4 ml min^{-1} and a column temperature of 20 °C. The injection volume was 50 μl, the detection wavelength was set to 230 nm. For the separation of the three azo- and azoxy-derivatives a Nucleosil column 120–3 C_{18}ec 250×4 mm (Macherey-Nagel, Düren, Germany) and an acetonitrile/water gradient was used (0 → 60 min, 40 → 85% acetonitrile). The flow rate and the column temperature were identical to the first method. The following analytical standards were used: 2-amino-4,6-dinitrotoluene (2-ADNT), 4-amino-2,6-dinitrotoluene (4-ADNT), 2,4-diamino-6-nitrotoluene (2,4-DANT), (LGC Promochem, Wesel, Germany); 3,5-dinitroaniline (3,5-DNA),

2-nitroaniline (2-NA), 1,3,5-trinitrobenzene (TNB) (Sigma-Aldrich, Taufkirchen, Germany); 1,3-dinitrobenzene (1,3-DNB), 2,4-dinitrotoluene (2,4-DNT) (Merck, Darmstadt, Germany); hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), 2,4,6-trinitrotoluene (TNT) (Dynamit Nobel, Troisdorf, Germany); 2,2′,6,6′-tetranitro-4,4′-azotoluene (TN-4,4′-azo) (AccuStandard, New Haven, USA); 4,4′,6,6′-tetranitro-2,2′-azoxytoluene (TN-2,2′-azoxy), 2,2′,6,6′-tetranitro-4,4′-azoxytoluene (TN-4,4′-azoxy) (synthesis according to Sitzmann 1974).

Samples of ^{14}C-activity were analyzed by HPLC-UV with a flow-through scintillation counter (Radio-HPLC) on a Merck Hitachi model LaChrom 7000 series equipped with an UV-detector set at 254 nm and a Synergi 4u MAX-RP 80A 250×4.6 mm (Phenomenex) column. The radioactivity was measured with an EG&G Berthold model LB 507 B apparatus using a measuring cell YG 150U 4. A binary gradient elution program was used consisting of water (A) and methanol (B): 1. step: 0 → 50 min, 25 → 100% B; 2. step: 50 → 60 min, isocratic 100% B; 3. step: 60 → 70 min, 100 → 25% B; 4. step: 70 → 80 min, isocratic 25% B. The flow was kept constant at 0.7 ml min^{-1}.

For rapid estimations of microbial TNT degradation a spectrophotometric method was adapted from Oh et al. (2000). Nitrite in the culture fluids was measured spectrophotometrically as described by Kim et al. (2002).

TNT degradation experiments

The mineral salt medium of Kalafut et al. (1998) was used to study the TNT degrading capabilities of the bacterial isolates. TNT was added to the mineral salt medium in concentrations of 10, 50 and 100 mg l^{-1} before autoclaving (15 min, 121 °C; traces of TNB were sometimes identified after this treatment). For experiments with solid media, mineral salt media were prepared with agarose (12 g l^{-1}). The influence of nutrient supplements was tested at increasing concentrations (0–3.0%) of Standard I nutrient broth or glucose (0–1.0%) in the minimal salt medium.

In order to simulate more natural conditions, the mineral salt media were also prepared with contaminated water and soil extracts from the original site of the microbial isolates. The soil extracts were obtained by shaking a 10% (w/v) aqueous soil suspension for 2 h (200 rpm) before autoclaving (20 min, 121 °C). Water insoluble materials were removed by centrifugation (10,000×*g*, 30 min) and the supernatant was passed through a paper filter (Schleicher and Schuell, Dassel, Germany, No. 589).

The media were prepared either in volumes of 50 ml and 100 ml in Erlenmeyer flasks or 3 ml in 16 ml screw cap tubes.

Cells used for inocula were precultured in Standard I nutrient broth for 16 h at 30 °C on a shaker. The cells were either used directly or washed twice in mineral salt media before inoculation into mineral salt media. The cultures were incubated up to 7 days at 30 °C on a rotary shaker (200 rpm).

At regular intervals, aliquots were taken from the cultures to determine bacterial growth and TNT elimination. Growth of the cultures was followed by counting the colony forming units (cfu) on Standard I nutrient agar and by measuring the optical density at 600 nm. TNT and its metabolites in the culture supernatants were determined after centrifugation of the aliquots at 16,000×*g* for 5 min.

At the end of the experiments, cells and water-insoluble materials were separated by centrifugation at 40,000×*g* for 30 min. The resulting pellet was washed twice with phosphate buffered saline (pH 7.4). The pellet was extracted with acetonitrile for 16 h at 30 °C and centrifuged as above. The resulting fractions (supernatant, washings, acetonitrile extract) were analysed by HPLC.

A separate set of experiments was performed with resting cells: 1 ml samples of a preculture grown in Standard I nutrient broth were centrifuged (16,000×g, 5 min) and the cell pellets resuspended in the same volume of minimal salt medium containing TNT (100 mg l^{-1}). After incubation at 30 °C the assays were centrifuged (16,000×*g*, 5 min) at the indicated intervals and the supernatants analysed by HPLC. Cells inactivated by autoclaving served as controls.

Mass balance of ^{14}C-TNT

[Ring U–^{14}C]TNT (2.2 mCi $mmol^{-1}$) was synthesised as described by Kröger and Fels (2000). Radioactivity was determined by liquid scintillation counting (1414 Wallac WinSpectral Liquid Scintillation Counter, Turku, Finland, and

TriCarb Liquid Scintillation Analyzer, Model 2500TR, Packard Canberra) with Rotiszint eco plus (Roth, Karlsruhe, Germany, No. 0016.2) as cocktail.

Minimal salt media were prepared with non-radioactive TNT (100 mg l^{-1}) and up to 2×10^6 dpm ml^{-1} [ring U-^{14}C]TNT. The nutrient media (100 ml) were inoculated with 500 μl of a preculture in Standard I nutrient broth and incubated for up to 7 days at 30 °C on a rotary shaker (200 rpm). Cells and water-insoluble sediments were separated by centrifugation at 40,000×*g* for 30 min and washed twice with phosphate buffered saline (pH 7.4). Radioactivity in the different fractions (culture supernatant, washings, cells, water-insoluble sediment) was determined after mixing 100 μl sample with 4 ml of Rotiszint eco plus. C^{14}-radioactivity was also measured in the acetonitrile extract of the sediment (obtained as above). Unextractable ^{14}C remaining in the pellet was determined by combusting to $^{14}CO_2$ in a biological oxidizer (Zinsser OX 500).

Results

Isolation and characterization of TNT transforming bacteria

Microorganisms were isolated from water and soil samples of two former ammunition production plants in Germany. Bacteria from these contaminated environments were isolated on Standard I nutrient agar in the presence of TNT (10 mg l^{-1}). One isolate which transformed TNT most efficiently was identified by the sequence of its 16S rDNA (700 nucleotides sequenced) as *Raoultella terrigena*. Strain HB (DSMZ No. 16101) consists of Gram-negative, oxidase-negative, catalase-positive, non-motile rods. It grows well at temperatures between 4 °C and 40 °C with an optimum at 30 °C (data not shown). Growth at low temperatures is a hallmark of the new described genus *Raoultella*, which is facultative anaerobic, having both a respiratory and a fermentative type of metabolism (Drancourt et al. 2001).

Growth in the presence of TNT

On minimal salt agar supplemented with TNT (100 mg l^{-1}), *R. terrigena* strain HB produced brownish pigments within and around the growth zone (Figure 1). Growth in liquid media was determined by colony counts and optical density. After a lag time of 24 h, the colony forming units (cfu) in the minimal salt medium with TNT increased rapidly and reached the same level as in the medium without TNT (Figure 2). The significant increase of the optical density of cells grown in the presence of TNT is not a result of higher cell densities, but obviously attributed to altered spectroscopic properties of the cells and the culture media by accumulation of coloured TNT metabolites, similar to those observed on solid agar media. Determination of the optical density is thus not an appropriate parameter for estimating bacterial growth in dependence of TNT.

Cometabolic transformation

R. terrigena strain HB did not grow with TNT as the only source of carbon and energy and no TNT transformation was observed without an additional nutrient source. However, microbial growth and transformation of TNT occurred even at low nutrient concentrations in the minimal salt media (Table 1). At concentrations of ≥0.05% Standard I nutrient broth or glucose in the minimal salt media, TNT was completely removed from the culture broth within 7 days of incubation.

When the inocula were taken directly from a preculture grown in Standard I nutrient broth without further cell washings, the nutrients supplied by this complex medium were sufficient to

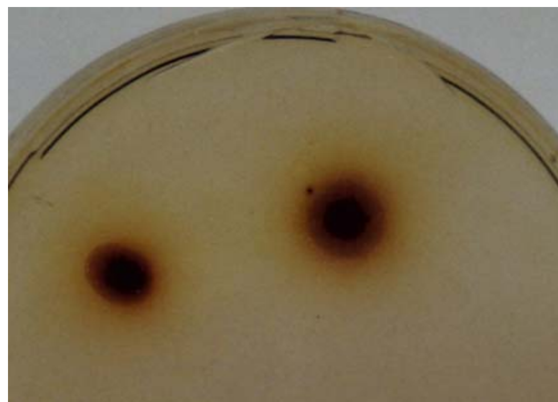

Figure 1. Colonies of *R. terrigena* strain HB on solid minimal salt media containing TNT (100 mg l^{-1}); Dark (brownish) pigments accumulated within and around the growth zones.

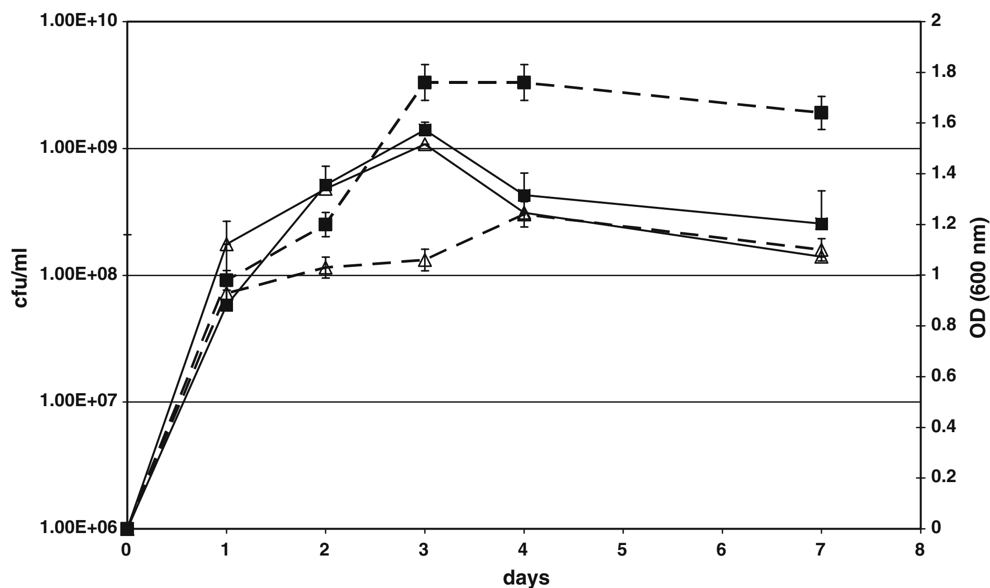

Figure 2. Growth of *R. terrigena* strain HB in liquid minimal salt media without (Δ) and with (■) 100 mg TNT l^{-1}; solid line (colony counts); dotted line (optical density).

promote bacterial TNT transformation (Table 2). Under the same conditions TNT was removed from original surface waters or soil extracts contaminated with a mixture of nitroorganic compounds (Table 2).

With pregrown (resting) cells, the cometabolic transformation of TNT occurred within a few hours of incubation (Figure 3). No reaction was observed with cells which have been inactivated by autoclaving, indicating an active transformation process.

Table 1. Influence of nutrient concentrations on bacterial TNT elimination*

Nutrient	Concentration in minimal salt medium (%)	TNT (mg l^{-1}) in cultures of *R. terrigena* HB
Glucose	0.000	89
	0.003	88
	0.006	40
	0.012	37
	0.050	< 1.0
Standard I nutrient broth	0.000	87
	0.004	86
	0.008	85
	0.016	22
	0.032	< 1.0

*TNT in the culture supernatants was determined after incubation for 7 days at 30 °C.
Data are the means of triplicate determinations. The variability between replicate samples within experiments was less than 10%.

Metabolites

At the standard concentration of 0.3% glucose in the mineral salt media, TNT could not be detected in growing cultures *R. terrigena* strain HB already after 4 h of growth (Table 3). 4-Amino-2,6-dinitrotoluene (4-ADNT) was the main product identified in the culture supernatants of resting or growing cells (Tables 2 and 3; Figure 3). Within the first 4 h of incubation, intermediately also some azoxy dimers were identified (Table 3). The nitrite concentrations in the supernatants increased slightly during the time of incubation, especially at lower glucose concentrations, however no denitrated metabolites of TNT were identified by HPLC. The main transformation products found in the cell extracts were 4-ADNT and TN-4,4′-azoxy dimers (Table 3).

Besides nitrite concentrations, there is some more bias in the amounts and nature of metabolites formed at different nutrient supplements. For instance 2,4-diamino-6-nitrotoluene (2,4-DANT) was only detected at high glucose concentrations, whereas more condensated products were found in cells grown at low glucose concentrations.

Table 2. TNT elimination by *R. terrigena* strain HB in contaminated surface waters and soil extracts*

Sample	Inoculum	2,4,6-TNT (μmol)	4-ADNT (μmol)	2-ADNT (μmol)
Mineral salt medium	Sterile control	352.0	0.0	0.0
	R. terrigena HB	0.0	15.2	4.8
Surface water (Moschwig)	Sterile control	30.0	7.2	6.5
	R. terrigena HB	0.0	12.1	3.4
Surface water (Hallschlag)	Sterile control	39.0	3.5	4.1
	R. terrigena HB	0.0	9.5	2.8
Soil extract (Moschwig)	Sterile control	293.0	26.0	40.4
	R. terrigena HB	0.0	40.4	21.6
Soil extract (Hallschlag)	Sterile control	363.0	23.1	30.4
	R. terrigena HB	0.0	53.1	34.3

*Bacterial inocula were taken directly from precultures grown in Standard I nutrient broth. The water samples and soil extracts were supplemented with mineral salts (without glucose) and incubated for 7 days at 30 °C. Only the culture supernatants were analysed for TNT and metabolites in these experiments. Data are the means of triplicate determinations. The variability between replicate samples within experiments was less than 10%.

Mass balance of ^{14}C-TNT

The radioactivity derived from ring-labelled ^{14}C-TNT was determined in the different fractions obtained at the end of the experiments (Table 4). In the supernatants about 30% of the initial radioactivity was found after 3 days, and 15% after 6 days of incubation. The residual radioactivity was essentially found in the brown-coloured cell fraction, which presented the main pool of radioactivity after prolonged incubation. A maximum of 61% of the cell-bound radioactivity could

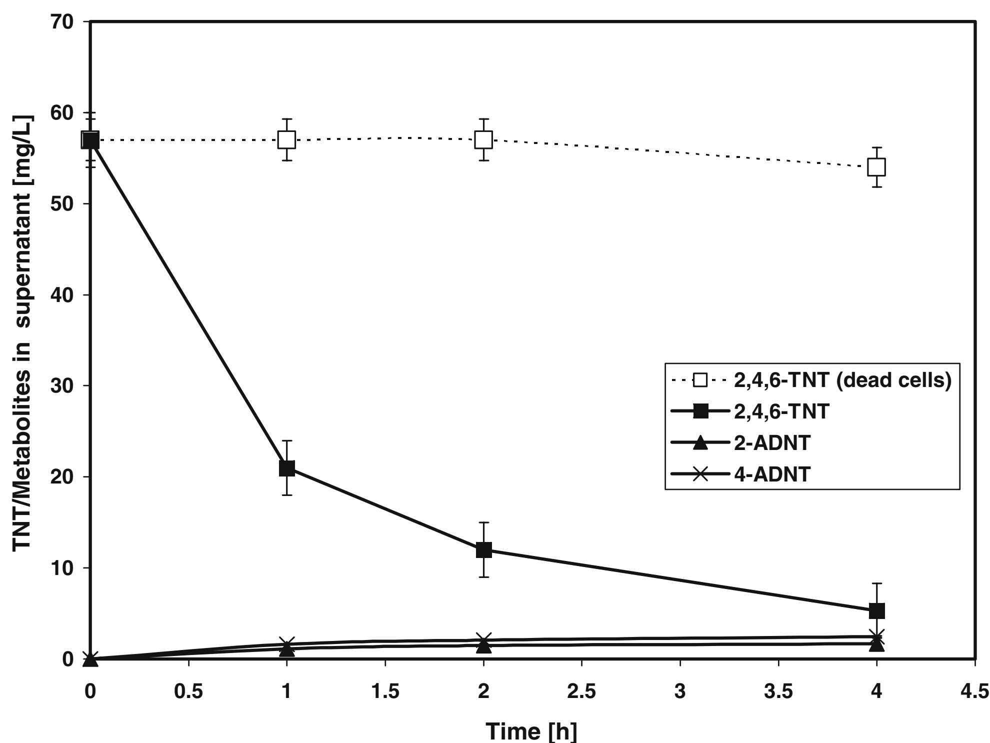

Figure 3. Removal of TNT from the liquid minimal salt medium by resting cells of *R. terrigena* strain HB; autoclaved cells (□) served as controls.

Table 3. Transformation products of TNT identified in cultures of *R. terrigena* strain HB

Compounds (μmol)								
Incubation [h]	2,4,6-TNT	2-ADNT	4-ADNT	2,4-DANT	TN-2,2′-azoxy	TN-4,4′-azoxy	TN-4,4′-azo	Nitrite
Sterile control	128	0.0	0.0	0.0	0.0	0.0	0.0	2.32
4 supernatant	0.0/0.0	4.5/4.3	12.7/10.4	1.5/0.0	0.0/2.5*	4.9/4.9*	0.0/0.0	3.8/2.6
24 supernatant	0.0/0.0	0.0/1.7	3.3/10.9	3.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	2.6/4.9
48 supernatant	0.0/0.0	0.0/0.0	4.3/6.1	5.2/0.0	0.0/0.0	0.0/0.0	0.0/0.0	2.0/4.9
72 supernatant	0.0/0.0	0.0/0.0	2.0/5.0	4.2/0.0	0.0/0.0	0.0/0.0	0.0/0.0	5.5/5.8
72 cell extract	0.0/0.0	0.1/0.3	0.3/5.3	0.1/0.0	0.7/1.7*	30.2/64.1*	1.9/n.d.*	n.d.

*Numbers mean TNT equivalents, taking account that 2 mol TNT form 1 mol azoxy dimer; n.d.: not determined; 0.0: below detection limit of HPLC analysis; the results before and behind the slash are given for 3 g l^{-1} and 0.3 g l^{-1} glucose in the mineral salt media, respectively. Data are the means of triplicate determinations. The variability between replicate samples within experiments was less than 10%.

be extracted with acetonitrile. No volatile $^{14}CO_2$ could be detected in repeated and different experimental designs (data not shown).

Discussion

The toxicity and the risk associated with nitroorganic explosives necessitate the development of cost-effective bioremediation technologies for their removal. As mineralization of TNT is insignificant, bioremediation technologies such as composting and bioslurry processes have been developed for soil environments (Lenke et al. 2000; Fuller et al. 2004; Lewis et al 2004). However, such strategies are not applicable for contaminated waters.

In this study we describe a new isolate *R. terrigena* strain HB, which grows in the presence of 100 mg TNT l^{-1}, a concentration which is toxic to many microorganisms (Fuller & Manning 1997). The growth was coincident with a disappearance of TNT from the culture media within a few hours incubation under optimum aerobic conditions. Already low nutrient concentrations ($\geq$0.05% glucose) were sufficient to promote growth and TNT removal by *R. terrigena* strain HB. The need for nutrient supplementation and lack of $^{14}CO_2$ production from ring-labelled TNT clearly indicates a cometabolic process. This is further confirmed by the effective TNT transformation by resting cells.

Table 4. ^{14}C-balance

% of initial radioactivity*		
Fraction	3 days	6 days
Supernatant	30.8	15.2
Cell pellet	67.9[a]	90.0[b]
Washings	2.5	1.3
Sum	101.1	106.5

Radioactivity in the different fractions was determined after 3 and 6 days incubation at 30 °C in minimal salt media (with glucose). Data present the mean values of triplicate determinations. The variability between replicate samples within experiments was less than 10%.
*Sterile control contained 5.22×10^5 DPM ml^{-1} ring-labelled ^{14}C-TNT.
[a]determined as suspension, [b]determined after combustion.

The efficiency of TNT removal under more *in situ* – like conditions, was demonstrated in experiments with water and soil samples originating from contaminated sites which contained a complex mixture of nitroorganic compounds.

The ^{14}C-balance revealed that about 15% of the initial radioactivity remained in the culture supernatants, whereas up to 90% were found in the cell pellet. At low glucose concentrations aminodinitrotoluenes (ADNTs) were identified in the culture supernatants, whereas under high glucose conditions diaminonitrotoluenes (DANTs) were found instead. This may be explained by an increased availability of reduction equivalents for nitro-group reduction. A maximum of 60% of the cell-bound radioactivity could be extracted with acetonitrile. Tetranitroazoxy-dimers were the main compounds identified in these extracts. We suppose that the differences in the condensation products found at the two glucose concentrations are rather related to variable biomass production and extraction efficiency than to physiological

properties. The appearance of nitrite indicates a limited release of nitrogen from the aromatic ring which may occur through dimerization reactions (Pak et al. 2000). We cannot exclude the existence of further metabolites, e.g. more polar or polymeric compounds which were not solvent extractable and/or tightly bound to cell components.

Our finding that the main fraction of TNT metabolites is cell-associated, deviates from most other reports, where the main fraction of transformation products remained in the supernatant in the form of ADNTs (Kalafut et al. 1998; Kim et al 2002; Zhao et al. 2004). In experiments with a mixed culture from a sewage plant more than 60% of the initial radioactivity was found in the supernatant under aerobic conditions. The dominating transformation product was 4-ADNT, accompanied by small amounts of 2-ADNT and TN-4,4′-azoxy (Kröger et al. 2004). Under anerobic conditions more than 95% of the initial TNT concentration was converted to reduced products and was available in solution.

Similar to our study a strain of *Pseudomonas aeruginosa* MX accumulated 71% of the initial ^{14}C-TNT in the cell pellet leaving 21% in the supernatant. In the latter fraction 2-ADNT was the main metabolite and TN-2,2′-azoxy accumulated in the cells (Oh et al. 2003). In the same study it was shown that the relation of monomeric and dimeric metabolites is strongly influenced by the pH. At pH 5.0–6.0 the monomers dominated, whereas a pH $\geq$ 7.0 favoured the formation of azoxy dimers. This is very close to our experiments, which were conducted at pH 7.1. In contrast to the study of Oh et al. (2003) we identified not the *ortho*- but the *para*-reduction products (4-ADNT, TN-4,4′-azoxy) as the main metabolites.

As *R. terrigena* strain HB grows rapidly at low temperatures and different redox conditions it is a promising candidate for the detoxification of TNT-contaminated waters under *in-situ* conditions. The metabolites associated with the cell fraction can be removed together with the biomass, e.g. by filtration or flocculation.

Conclusions

The accumulation of TNT metabolites within the bacterial cells offers the opportunity to clean up contaminated waters after separation of the biomass. *R. terrigena* strain HB removed TNT also under *in situ*-like conditions, i.e., in media with surface water and soil extracts contaminated with various nitroorganic compounds. As our new isolate is well adapted to different environmental conditions, e.g. low and high temperature, nutrient and redox states, *R. terrigena* strain HB may be a superior candidate for field applications, at least as long as biotransformation and not biomineralization is the state of art.

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