Arch Microbiol (1992) 158: 149–154

Archives of
Microbiology

Catabolism of 2,6-dinitrophenol by *Alcaligenes eutrophus* JMP 134 and JMP 222

S. Ecker[1], T. Widmann[1], H. Lenke[1], O. Dickel[1], P. Fischer[2], C. Bruhn[1], and H.-J. Knackmuss[1]

[1] Institut für Mikrobiologie and [2] Institut für Organische Chemie, Biochemie und Isotopenforschung der Universität Stuttgart, W-7000 Stuttgart 1, Federal Republic of Germany

Received August 29, 1991/Accepted December 4, 1991

Abstract. Both *Alcaligenes eutrophus* JMP 134 and its plasmid-free derivative *Alcaligenes eutrophus* JMP 222 utilize 2,6-dinitrophenol as sole source of carbon, energy, and nitrogen. In the presence of ammonia resting cells of these strains release two mol of nitrite per mol of 2,6-dinitrophenol. *Alcaligenes eutrophus* JMP 222-α1D, a mutant of strain JMP 222 obtained by transposon (Tn5) mutagenesis, is able to use 2,6-dinitrophenol as nitrogen source but not as source of carbon and energy. Resting cells of this mutant liberate only one mol of nitrite per mol of 2,6-dinitrophenol. A single metabolite was detected by high-pressure liquid chromatography and identified as 2-hydroxy-5-nitropenta-2,4-dienoic acid from the mass spectrum, the ^{1}H-, and ^{13}C-NMR spectra. Strain JMP 222-α1S, a spontaneous mutant of strain JMP 222-α1D, accumulates 4-nitropyrogallol which was identified as the initial metabolite of 2,6-dinitrophenol degradation.

Key words: 2–Hydroxy 5–nitropenta–2,4–dienoic acid – Nitrite elimination – 4–Nitropyrogallol

Nitroaromatic compounds are important building blocks for the synthesis of dyestuffs, explosives, and pharmaceuticals. In agriculture nitrosubstituted aromatic compounds are used as herbicides and insecticides. Due to their widespread use, nitroaromatic compounds are found in wastewaters, rivers, ground water, soil, and in the atmosphere (Hallas and Alexander 1983; Zoeteman et al. 1980; Kearney and Kaufman 1975; Grosjean 1985). In virtue of their well-known toxicity for man and animals, their occurrence in the environment causes serious problems. Therefore numerous investigations were focussed on the isolation of microorganisms which are able to partly degrade or to mineralize these toxic xenobiotics. Detailed knowledge already exists of the mechanisms of degradation of mononitrophenols (Spain et al. 1979; Zeyer and Kearney 1984; Zeyer et al. 1986; Zeyer and Kocher 1988). Although various authors described the utilization of 2,4-dinitrophenol and 4,6-dinitro-2-methylphenol (DNOC) (Simpson and Evans 1953; Gundersen and Jensen 1956; Germanier and Wuhrmann 1963; Tewfik and Evans 1966; Jensen and Lautrup-Larsen 1967; Schmidt and Gier 1989; Hess et al. 1990) little is known about the degradative pathway of di- and polynitrosubstituted phenols. Bruhn et al. (1987) successfully applied a new technique to enrich microorganisms which utilize different mono- and dinitrophenols as sole nitrogen source in the presence of a readily degradable carbon and energy source. One of the newly isolated strains, *Pseudomonas sp.* N 26-8, was able to mineralize 2,6-dinitrophenol (2,6-DNP). Degradation of 2,6-DNP was shown to be strictly O_2-dependent and in presence of ammonia resting cells liberated two mol of nitrite per mol of substrate.

When laboratory strains were screened for the ability to utilize nitrophenols as a nitrogen source *Alcaligenes eutrophus* JMP 134, originally isolated as a 2,4-dichlorophenoxyacetic acid (2,4-D) degrading bacterium (Pemberton et al. 1979), was shown to mineralize 2.6-DNP. In order to elucidate the catabolic sequence of 2,6-DNP in the present investigation pathway-deficient derivatives of JMP 134 were obtained by transposon mutagenesis. Instead of *Alcaligenes eutrophus* JMP 134 we used the cured derivative JMP 222 (Pemberton et al. 1979) as recipient for transposon mutagenesis, since it avoids preferential insertion of transposon Tn5 into plasmid pJP4 of strain JMP 134 (R. H. Don, personal communication).

Materials and methods

Organisms

The suicide plasmid pLG221::Tn5 in its host *Escherichia coli* LE 392, *hsd* R514 *sup* E44 *sup* F58 *lac* Y1 *gal* K2 *gal* T22 *met* B1 *trp*

Offprint requests to: H.-J. Knackmuss

Non-standard abbreviations. 2,4-D, 2,4-dichlorophenoxyacetic acid; 2,6-DNP, 2,6-dinitrophenol; HNMA, 2-hydroxy-5-nitromuconic acid; HNPA, 2-hydroxy-5-nitropenta-2,4-dienoic acid; NB, nutrient broth; NMR, nuclear magnetic resonance; NPG, 4-nitropyrogallol; O.D., optical density; t_R, retention time; UV/Vis, ultraviolet/visible

R55 (Murray et al. 1977) was used as transposon donor. Strain LE 392 is resistent to kanamycin (Kan^r, due to Tn5), but sensitive to streptomycin (Sm^s). *Alcaligenes eutrophus* JMP 134 ($2,4\text{-}D^+$, $2,6\text{-}DNP^+$) as recipient was replaced by his plasmid-free derivative JMP 222 ($2,4\text{-}D^-$, $2,6\text{-}DNP^+$, Sm^r) (Pemberton et al. 1979). All strains were cultivated at 30 °C. The organisms were incubated in liquid cultures on a rotary shaker (Typ RC 106, Infors AG, Basel, Switzerland) at 100 rpm or on agar plates containing 1.5% (w/v) oxoid agar No. 1 (Oxoid Ltd., London, UK).

Media

Nutrient broth 0.8% (w/v) (Difco, Detroit, USA), supplemented with 0.5% (w/v) NaCl, was used as complete medium.

Where antibiotics and amino acids were used they were incorporated into media at the following final concentrations: Streptomycin 0.1 $g\,l^{-1}$; kanamycin, arginine, histidine, valine, isoleucine, and leucine, all 0.05 $g\,l^{-1}$.

In order to select kanamycin resistent derivatives of strain JMP 222, the mineral medium described by Dorn et al. (1974) was used containing 10 mM succinate as carbon source and the antibiotics and amino acids cited above (medium A).

The following media (B, M_1, M_2, and M_3) were applied to select the pathway deficient mutants. The basic components as described previously (Bruhn et al. 1987) were supplemented with trace element solution (Pfennig and Lippert 1966), 1 $ml\,l^{-1}$, antibiotics and 0.5 mM 2,6-DNP. Additionally, amino acids (medium M_1), 10 mM succinate (medium M_2) or 1 $g\,l^{-1}$ $(NH_4)_2SO_4$ (medium M_3) was added to media M. Medium M_2 also was used for subcultivation of strains JMP 222-α1D and JMP 222-α1S.

Transposon mutagenesis

Mutagenesis of JMP 222 was performed by conjugal transfer. Donor and recipient strains were grown overnight in NB and 0.5 ml portions of each culture were mixed. Cells were collected on filter discs (Sartorius, Göttingen, FRG) and placed on NB agar plates. After incubation for 22 h at 30 °C biomass was resuspended in 0.9% (w/v) NaCl solution.

Selection of mutants

Selection for 2,6-DNP pathway-defective mutants was performed in two steps. First the cell suspension (0.1 ml) was plated on mineral medium agar plates (medium A). The composition of this medium allowed growth of streptomycin and kanamycin resistant cells only, even if they were auxotrophic for valine, leucine, isoleucine, arginine, or histidine. In the second step 12000 of these mutants were transferred to microtiter plates containing medium B. Since this medium contained only 2,6-DNP and antibiotics, 4 groups of defective mutants were excluded from growth: a) auxotrophic cells which could not synthesize at least one of the five amino acids, b) mutants defective in assimilation of nitrite, c) mutants defective in a 2,6-DNP transport system or in an enzyme of the initial sequence of the pathway, and d) mutants deficient in a lower part of the pathway capable to use 2,6-DNP as nitrogen but not as carbon and energy source. Growth could easily be judged by decolorization of the yellow medium. 188 mutants belonging to one of these four groups were differentiated by transferring them to media M_1, M_2 and M_3, respectively.

Resting cell experiments

The organisms were grown with 2 mM ammonia and 10 mM succinate in mineral medium B without antibiotics (0.5 mM 2,6-DNP served as inducer), harvested during late exponential growth, and resuspended in 25 mM phosphate buffer pH 7.4 (optical density at 546 nm = 5). The cell suspensions were incubated on a water bath shaker (New Brunswick, Edison, NJ, USA) at 30 °C. For resting cell experiments 0.5 mM 2,6-DNP was added to JMP 222-α1D, JMP 222-α1S or the parent strain JMP 222. For turnover of 4-nitropyrogallol (NPG) or 2-hydroxy-5-nitropenta-2,4-dienoic acid (HNPA) by resting cells of JMP 222 both these substrates were used at a final concentration of 0.5 mM. In each case 1 mM $(NH_4)_2SO_4$ was added to prevent assimilation of released nitrite.

Analysis of substrates and metabolites

2,6-DNP, nitrite, HNPA, and NPG were identified and quantified by reversed-phase high performance liquid chromatography (Liquid Chromatograph 655A-11, Merck, Darmstadt, FRG; Lichrospher column SC 125 RP 8, particle diameter 5 µm, Bischoff, Leonberg, FRG; Spectroflow detector 783, Kratos, Karlsruhe, FRG or L-3000 Multi Channel Photo Detector, Merck, Darmstadt, FRG). Separation was achieved with an isocratic solvent system of acetonitrile/water (3:7, v/v) containing 2 $ml\,l^{-1}$ 85% H_3PO_4. The compounds were detected by measuring UV-absorption at 210 nm. Additionally, nitrite was quantified by a colorimetric test (Montgomery and Dymock 1961).

Isolation and identification of 2-hydroxy-5-nitropenta-2,4-dienoic acid

To identify its structure, the metabolite was produced by 2,6-DNP oxidation by resting cells of strain JMP 222-α1D. The biomass was removed by centrifugation (4000 g; 20 min; 5 °C). The cellfree culture fluid was acidified to pH 2 with amidosulfonic acid which destroys the liberated nitrite. The fluid was extracted two times with equal volumes of ethylacetate. The combined organic phases were dried over $MgSO_4$ and evaporated. The yellow-brown partly crystalline product was identified as HNPA by means of its mass spectrum and ^1H- as well as ^{13}C-NMR spectra. A Varian-MAT 711 mass spectrometer (Varian, Palo Alto, Calif., USA) was used for recording the mass spectrum at a source temperature of 350 K and 70 eV. ^1H-NMR and ^{13}C-NMR spectra were obtained in $(CD_3)_2CO$ with tetramethylsilane as internal standard at 75.47 and 300.13 MHz, respectively, with a Bruker CXP 300 spectrometer (7.046 T, Bruker, Rheinstetten, FRG).

Preparation of cell-free extracts

The organisms were grown with 2 mM ammonia and 10 mM succinate in mineral medium B without antibiotics, harvested during late exponential growth, and resuspended in acetone/20 mM Tris/HCl-buffer pH 7.9 (1:9, v/v). Cell suspensions were disrupted by using a French press (Aminco, Silver Spring, Md., USA). Cell debris was removed by centrifugation at 14000 g for 20 min (ultracentrifuge Centrikon T-1055, Kontron, Zürich, Switzerland).

Experiments with cell-free extracts

In order to test for enzyme activities, 50 µl of the cell extract (about 10 mg ml^{-1} protein) were mixed with 900 µl 50 mM Tris/HCl-buffer pH 7.2 and 5 µl 10 mM NPG. Formation of the product was followed photometrically by recording overlay-spectra (200 to 400 nm; Uvikon photometer 810 P, Kontron, Zürich, Switzerland). Additionally, at different times of the enzymatic reaction samples were taken, the protein precipitated by ultrafiltration (Centricon 10, Amicon, Witten, FRG), and the filtrate analyzed by HPLC.

Chemicals

All chemicals were of the highest purity commercially available. 2,6-DNP was obtained from Fluka (Buchs, Switzerland), NPG was synthesized as described by Einhorn et al. (1904).

Results

Transposon mutagenesis

Because the majority of mutants obtained from transposon (Tn5) mutagenesis was unstable only 36 could clearly be identified as: auxothrophs (26), mutants defective in the assimilation of nitrite (7), mutants which were deficient in either a 2,6-DNP uptake system or in the initial step of 2,6-DNP degradation (2), and mutants which were blocked further downstream in the catabolic sequence (1).

The frequency of transfer was approximately 2×10^{-5}. Among ten mutants examined the frequency of revertants ranged from 10^{-6} to 10^{-9}. Reversions were also shown in presence of kanamycin. Only one mutant was able to grow on media M_1 and M_2 but not on medium B. This mutant, designated *Alcaligenes eutrophus* JMP 222-α1D, changed the colour of 2,6-DNP containing media from yellow to lemon. During growth with 0.5 mM 2,6-DNP and 10 mM succinate the doubling time was 11 h.

After plating JMP 222-α1D on 2,6-DNP/succinate agar a spontaneous mutant was obtained which generated a reddish brown instead of a lemon coloration. Obviously this mutant, designated *Alcaligenes eutrophus* JMP 222-α1S, accumulated another metabolite.

Both mutants, JMP 222-α1D and JMP 222-α1S, can use 2,6-DNP as sole source of nitrogen but not as carbon and energy source. The activity for 2,6-DNP degradation is induced even in the presence of ammonia.

Isolation and identification of metabolites

Resting cells of strain JMP 134 and strain JMP 222 turned over 2,6-DNP releasing stoichiometric amounts of nitrite (2 mol of nitrite per mol of 2,6-DNP; data not shown). In contrast, cells of the mutant strains JMP 222-α1D and JMP 222-α1S eliminated only 1 mol of nitrite per mol of 2,6-DNP. Turnover of 2,6-DNP by resting cells of strain JMP 222-α1D generated a single metabolite which was detected by HPLC analysis (t_R = 3.10 min, conditions described above) (Fig. 1). The metabolite could be extracted from the culture fluid with ethylacetate. The yellow-brown, partly crystalline product exhibited an absorption maximum at 376 nm (pH 7.4) and an acidic character in water. It was identified as HNPA by means of mass (Table 1) and NMR spectroscopy (Table 2). The mass spectrum (EI, 70 eV) shows, besides the correct molecular ion (m/z *159*, 7%), a prominent fragment for the combined loss of $H^{\cdot}$ and CO_2 (or $HO^{\cdot}$ and CO) at m/z *114*. The loss of $NO_2^{\cdot}$ (m/z *113*) is fairly weak as expected for an sp^2-bound NO_2 group. The ^{13}C-NMR shows five carbon resonances (sp^2 carbon atoms) in the 100–170 ppm range. Three of these, pertaining to olefinic –CH= carbon atoms, correspond directly to the three olefinic protons in the ^{1}H-NMR spectrum. These protons are mutually correlated by vicinal olefinic coupling constants (12.0, 13.2 Hz), i.e. constitute a –CH=CH–CH= substructure.

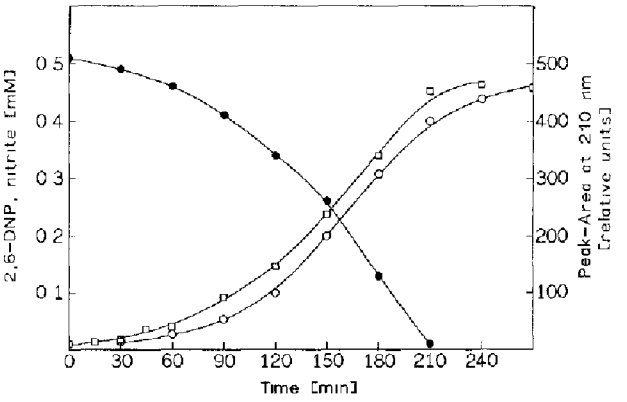

Fig. 1. Turnover of 2,6-DNP by resting cells of strain *Alcaligenes eutrophus* JMP 222-α1D. Cells of strain JMP 222-α1D, grown with 0.5 mM 2,6-DNP, 2 mM ammonia, and 10 mM succinate, were resuspended in phosphate buffer (25 mM, pH 7.4, $O.D._{546\,nm}$ 5). The cell suspension was incubated on a rotary shaker at 30 °C. Concentrations of 2,6-DNP (●) and HNPA (○) were determined by HPLC, nitrite (□) was quantified by a colorimetrical test

Table 1. Mass spectroscopic data of 2-hydroxy-5-nitropenta-2,4-dienoic acid (HNPA) (electron energy 70 eV, source temperature 350 K)

Mass m/z	Fragments	Relative intensities (% base peak)
159	$M^{+\cdot}$	7
114	$M-H^{\cdot}, -CO_2]^+$	39
113	$M-NO_2^{\cdot}]^+$	6
98	$M-HO^{\cdot}, -CO_2]^+$	3
97	$M-H_2O, -CO_2]^+$	5
86	$O_2N-CH-CH-CH_2]^+$	16
83	$ON-CH-CH-CH-CH_2]^{+\cdot}$	5
73	$O=C-COOH]^+$	5
69	$CH_3-CH-CH-CO]^+$	9
68	$CH_2-CH-CH-CO]^{+\cdot}$	43
60	$CH_2=NO_2]^{+\cdot}$	31
57	$O=CH-C=O]^+$	13
55	$CH_2=CH-C=O]^+$	8
46	$NO_2]^+$	5

Table 2. ^{1}H- and ^{13}C-NMR data of 2-hydroxy-5-nitropenta-2,4-dienoic acid (HNPA) (in $(CD_3)_2CO$, 300 K, nominal frequency 75.47 MHz and 300.13 MHz, respectively, tetramethylsilane as internal standard).

$\delta(^{13}C)$ [ppm]		$\delta(^1H)$ [ppm]		Coupling constants [Hz]	
C-1	164.49				
C-2	150.96				
C-3	103.56	3-H	6.427	^3J (3-H, 4-H)	12.0
C-4	132.71	4-H	8.025	^3J (4-H, 5-H)	13.2
C-5	140.10	5-H	7.582		

Table 3. Strain characteristics referring to substrates and metabolites

	Substrates	used[a] as source of		Metabolites released by resting cells (mol per mol of substrate)	
		carbon and energy	nitrogen	nitrite	others
JMP 134	2,6-DNP	+	+	2	
	NPG	+	+	1	
	HNPA	−	−	0	
JMP 222	2,6-DNP	+	+	2	
	NPG	+	+	1	
	HNPA	−	−	0	
JMP 222-α1D	2,6-DNP	−	+	1	1 HNPA
	NPG	−	−	n.d.	
	HNPA	−	−	n.d.	
JMP 222-α1S	2,6-DNP	−	+	1	1 NPG
	NPG	−	−	n.d.	
	HNPA	−	−	n.d.	

[a] + indicates utilization of substrate in liquid culture. In case of NPG as substrate turnover but no growth was observed. This is probably due to the chemical instability of NPG in mineral medium. − designates no utilization or turnover of substrate
n.d. not determined

The spontaneous mutant JMP 222-α1S accumulated a different metabolite in the culture fluid which distinguished itself by an absorption maximum at 380 nm (pH 7.4). This product was identified with authentic NPG by chromatographic properties (t_R = 2.90 min) (Einhorn et al. 1904). NPG is produced in stoichiometric amounts. The two metabolites, HNPA and NPG, were tested as substrates with resting cells of the parent strain JMP 222. NPG is completely metabolized at substrate concentrations ≤4 mM. During this reaction resting cells released 1 mol of nitrite per mol of substrate (Table 3). 2,6-DNP is shown to be an inducer of the enzymes involved in the metabolism of NPG. Thus, NPG is clearly identified as a key metabolite of 2,6-DNP degradation pathway. In contrast, turnover of HNPA (≤0.3 mM) by strain JMP 222 is extremely slow irrespective of the cells being induced by 2,6-DNP or not. Nitrite could not be detected as a metabolite. These results indicate that HNPA is an artifact or a spontaneous reaction product of a metabolite not yet identified.

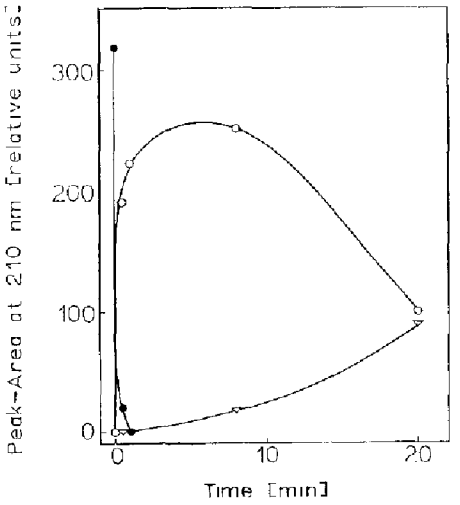

Fig. 2. Turnover of NPG by cell-free extract of strain *Alcaligenes eutrophus* JMP 222. Cells were grown in mineral medium with 0.5 mM 2,6 DNP, 2 mM ammonia, and 10 mM succinate. NPG (●), HNPA (▽), and the initial product of ring cleavage (unknown metabolite (○)) were analyzed by HPLC

Table 4. Comparison of UV/Vis-spectra of 2-hydroxy-5-nitropenta-2,4-dienoic acid (HNPA) and the putative 2-hydroxy-5-nitromuconic acid (HNMA) which appears during the turnover of 4-nitropyrogallol (NPG) by cell extracts of JMP 222 and JMP 134[a]

Metabolite	λ_{max1} [nm]	λ_{max2} [nm]	Relative intensities (I_{max2}/I_{max1})
HNPA	243 ± 5	374 ± 5	2.6 ± 0.2
HNMA	250 ± 5	384 ± 5	2.1 ± 0.1

[a] The spectra were recorded during a HPLC analysis in a solvent system of methanol/water (2.5:7.5, v/v) containing 0.005 mM Pic A reagent

Enzyme activities in JMP 222

Although 2,6-DNP is not oxidized by cell extracts of JMP 222, NPG is readily turned over at concentrations ≤0.1 mM. The disappearance of the substrate correlated with the formation of a metabolite (HPLC analysis: t_R = 1.70 min). This intermediate is further transformed to HNPA in the presence but also in the absence of enzymes (Fig. 2). It exhibits an UV/Vis-spectrum very similar to that of HNPA (Table 4). Due to its instability, particularly under acidic conditions (half-time ≤2 min at pH 2), the product could not yet be isolated and identified.

Discussion

A characteristic feature of the catabolism of nitrophenols is the elimination of nitrite (Simpson and Evans 1953;

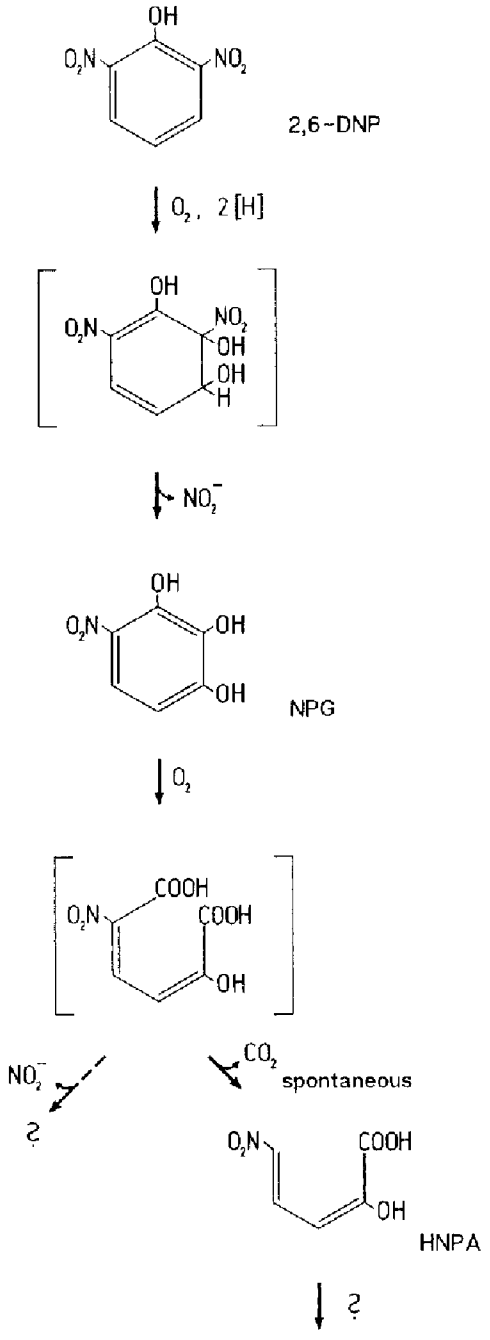

Fig. 3. Proposed initial steps of the catabolic pathway of 2,6-DNP in *Alcaligenes eutrophus* JMP 222 and JMP 134

Gundersen and Jensen 1956; Germanier and Wuhrmann 1963; Jensen and Lautrup-Larsen 1967; Schmidt and Gier 1989; Hess et al. 1990).

For 2-nitrophenol and 4-nitrophenol oxygenolytic mechanisms were discussed for the liberation of this anion (Spain et al. 1979; Zeyer and Kearney 1984; Zeyer et al. 1986; Zeyer and Kocher 1988). In both cases nitrophenol monooxygenases were identified converting 2-nitrophenol to catechol and 4-nitrophenol to hydroquinone. Earlier observations (Bruhn et al. 1987) and the present results indicate that the release of nitrite from 2,6-DNP is due to a dioxygenase.

NPG was identified as a key metabolite of 2,6-DNP degradation which suggests an initial catabolic step as shown in Fig. 3. Obviously, dioxygenation in position 2 and 3 of the aromatic ring generates an unstable dihydrodiol. This by spontaneous rearomatization and elimination of nitrite leads to NPG which is subject to ring cleavage in 2,3-position.

During turnover of NPG by cell-free extracts of JMP 134 and JMP 222 a metabolite appears which is readily converted into HNPA, both in the presence and in the absence of cell-free extract. The UV/Vis-spectrum of this metabolite is very similar to that of HNPA (Table 4). From these results we suggest 2-hydroxy-5-nitromuconic acid (HNMA) to be the initial ring cleavage product which by spontaneous decarboxylation generates HNPA (Fig. 3). Accumulation of HNPA may be due to a missing enzyme in the defective mutant JMP 222-α1D. Obviously, in the crude extract this activity is also absent, so that HNPA is the only detectable endproduct of ring cleavage of NPG. Significantly, in liquid cultures of strain JMP 222 degrading 2,6-DNP small amounts of HNPA could be detected by HPLC. This indicates that HNPA is a "dead end"-metabolite and not degraded further by the cells.

Interestingly, two different mechanisms must exist for the elimination of nitrite from 2,6-DNP. Obviously, the first nitrogroup is eliminated by a dioxygenase, whereas the liberation of the second nitrogroup as nitrite must take place after ring cleavage. Since the catabolic activity for 2,6-DNP is induced also in the presence of ammonia detoxification of the xenobiotic compound must be the primary function of this property. In addition JMP 134 can also utilize 2,6-DNP as sole source of nitrogen, carbon and energy, and thus must harbour a hitherto unknown catabolic sequence for 2,6-DNP.

Acknowledgement. We thank W. Rozdzinski, University of Stuttgart, for measuring the mass spectrum.

References

Bruhn C, Lenke H, Knackmuss H-J (1987) Nitrosubstituted aromatic compounds as nitrogen source for bacteria. Appl Environ Microbiol 53: 208–210

Dorn E, Hellwig M, Reineke W, Knackmuss H-J (1974) Isolation and characterization of a 3-chlorobenzoate degrading pseudomonad. Arch Microbiol 99: 61–70

Einhorn A, Cobliner J, Pfeiffer H (1904) Über das Pyrogallol. Chem Ber 37: 113–114

Germanier F, Wuhrmann K (1963) Über den aeroben mikrobiellen Abbau aromatischer Nitroverbindungen. Pathol Microbiol 26: 569–578

Grosjean D (1985) Reactions of o-cresol and nitrocresol with NO_x in sunlight and with ozone-nitrogen dioxide mixtures in the dark. Environ Sci Technol 19: 968–974

Gundersen K, Jensen HL (1956) A soil bacterium decomposing organic nitro-compounds. Acta Agric Scand 6: 100–114

Hallas LE, Alexander M (1983) Microbial transformation of nitroaromatic compounds in sewage effluent. Appl Environ Microbiol 45: 1234–1241

Hess TF, Schmidt SK, Silverstein J, Howe B (1990) Supplemental substrate enhancement of 2,4-dinitrophenol mineralization by a bacterial consortium. Appl Environ Microbiol 56: 1551–1558

Jensen HL, Lautrup-Larsen G (1967) Microorganisms that decompose nitroaromatic compounds with special reference to dinitro-ortho-cresol. Acta Agricol Scand 17: 115–126

Kearney PC, Kaufman DD (1975) Herbicides: chemistry, degradation and mode of action. Marcel Dekker, New York

Montgomery HAC, Dymock JF (1961) The determination of nitrite in water. Analyst 86: 414–416

Murray NE, Brammar WJ, Murray K (1977) Lamboid phages that simplify the recovery of in vitro recombinants. Mol Gen Genet 150: 53–61

Pemberton JM, Corney B, Don RH (1979) Evolution and spread of pesticide degrading ability among soil micro-organisms. In: Timmis KN, Pühler A (eds) Plasmids of medical, environmental and commerical importance. Elsevier/North Holland Biomedical Press, Amsterdam, pp 287–299

Pfennig N, Lippert KD (1966) Über das Vitamin B_{12}-Bedürfnis phototropher Schwefelbakterien. Arch Mikrobiol 55: 245–256

Schmidt SK, Gier MJ (1989) Dynamics of microbial populations in soil: Indigenous microorganisms degrading 2,4-dinitrophenol. Microbiol Ecol 18: 285–296

Simpson JR, Evans WC (1953) The metabolism of nitrophenols by certain bacteria. Biochem J 55: XXIV

Spain JC, Wyss O, Gibson DT (1979) Enzymatic oxidation of *p*-nitrophenol. Biochem Biophys Res Commun 88: 634–641

Tewfik MS, Evans WC (1966) DNOC-metabolism. Biochem J 99: 31P

Zeyer J, Kearney PC (1984) Degradation of o-nitrophenol and m-nitrophenol by a *Pseudomonas putida*. J Agric Food Chem 32: 238–242

Zeyer J, Kocher HP, Timmis KN (1986) Influence of parasubstituents on the oxidative metabolism of o-nitrophenols by *Pseudomonas putida* B2. Appl Environ Microbiol 52: 334–339

Zeyer J, Kocher HP (1988) Purification and characterization of a bacterial nitrophenol oxygenase which converts ortho-nitrophenol to catechol and nitrite. J Bacteriol 170: 1789–1794

Zoeteman BCJ, Harmsen K, Linders JBHJ, Morra CFH, Slooff W (1980) Persistent organic pollutants in river water and ground water of the Netherlands. Chemosphere 9: 231–249