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Degradation of dioxane, tetrahydrofuran and other cyclic ethers by an environmental *Rhodococcus* strain

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Summary. By enrichment and isolation techniques bacterial strains with the capacity to grow on aliphatic cyclic ethers (dioxane, tetrahydrofuran, 1,3-dioxolane) have been isolated. Six strains that degrade tetrahydrofuran were classified as belonging to the genus *Rhodococcus*. One of two strains that degrade dioxane instead of or in combination with tetrahydrofuran was further characterized and a hypothetical catabolic pathway comprising an initial 2-hydroxylation and several oxidation steps is postulated.

Introduction

While numerous publications deal with the microbial degradation of furan (Trudgill 1984), the information on biological degradability of the saturated cyclic ethers, tetrahydrofuran, dioxane, trioxane, 1,3-dioxolane and others, is scarce. Such structures may occur in natural compounds but are used in large quantities as solvents and chemical bulk products. Because of their high vapour pressure and water solubility, significant amounts will be released into the environment. Their fate is uncertain. In the assessment of biodegradability of fourteen chemicals tetrahydrofuran was classified as "not readily biodegradable" (Painter and King 1985), but there have been other publications on the degradation of dioxane (Orlowska et al. 1984) or tetrahydrofuran (Dimitrenko et al. 1987; Gvozdyak et al. 1988).

We report the isolation of strains with the ability to degrade unsubstituted and substituted saturated cyclic ethers from the environment and describe the growth characteristics and substrate specificities of a strain belonging to the genus *Rhodococcus*.

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Materials and methods

Organisms. All strains mentioned in Table 1 were isolated from enrichment cultures using liquid mineral medium A containing various carbon sources in concentrations from 0.02 to 0.05%. After several transfers in liquid medium a sample was transferred to nutrient broth agar. Pure isolates were conserved by lyophilisation.

Culture media and growth conditions. The media used were: mineral medium A (Trower et al. 1985); nutrient broth agar (Difco, Detroit, Mich., USA); seed medium (Thaler and Diekmann 1979). All incubations were at 30° C, in test tubes fixed at 45° inclination or in erlenmeyer flasks on a rotatory shaker at 175 rpm.

Analytical methods and techniques. Optical density (OD) was determined at 578 nm, and dry weight after collection of the sample on membrane filters, drying at 105° C for 1 h and weighing. Chemical oxygen demand (COD) was measured with the test kit LCK414 (Dr. Lange, Berlin, FRG). For the gas chromatographic determination of dioxane and tetrahydrofuran a Shimadzu (Kyoto, Japan) GC-9 AM equipped either with a Poropak Q (80/100) column or a Chromosorb W-HP column coated with 5% OV-17 100-200 mesh were used. The Gram stain was performed with the colour set of BioMerieux (Nürtingen, FRG), and the quinones were separated and identified according to Kroppenstedt (1982).

Chemicals. Dioxane and tetrahydrofuran were from Merck (Darmstadt, FRG). All other chemicals used for the degradation tests were purchased from Aldrich-Chemie (Steinheim, FRG) or were a gift from BASF (Ludwigshafen, FRG).

Results and discussion

Enrichment and isolation of strains

Mineral media containing dioxane, tetrahydrofuran or other cyclic ethers (see Table 1) as sole carbon sources in erlenmeyer flasks were inoculated with samples from forest soil or sludge from the settling basin of the aerobic waste-water purification plant of a chemical firm. When an increase in OD could be observed, cells were transferred to fresh media. After repeated transfer in liquid media and dilution streaks on solid media a number of pure strains were isolated (Table 1). Despite considerable efforts no strains were enriched or isolated

Table 1. Pure strains isolated from effluent samples and optical density (OD) values obtained under standard conditions

Substrates											
Dioxane-2,3-diol		Tetrahydrofuran		Tetrahydrofuran-3-ol		Tetrahydropyran		Tetrahydropyran-4-ol		Tetrahydropyran-4-one	
Strain	OD	Strain	OD	Strain	OD	Strain	OD	Strain	OD	Strain	OD
9/1	0.93	12	1.00	1	0.78	210	0.42	29	0.56	32/0	0.28
9/2	0.85	23	1.00	11	0.91	213	0.49				
18	0.96	113	1.00	12/0	0.68						
27/0	1.04	219	1.90	115	0.42						
103/1	1.0	220	1.60	116	0.32						
103/2	0.95	230	0.95	209	0.80						
103/3	0.83			211/1	0.95						
111	0.93			211/3	0.80						
				212/1	0.50						
				212/3	0.40						

Table 2. Changes in OD when tetrahydrofuran-degrading strains were grown on different substrates as the sole carbon source

Substrate	Strains					
	12	23	113	219	220	230
Tetrahydrofuran	0.84	0.23	0.55	0.89	0.37	0.13
Tetrahydrofuran-3-ol	0.12	0.00	0.04	0.04	0.03	0.00
Butan-1-ol	0.71	0.88	0.80	0.61	0.47	0.77
Butan-1,4-diol	0.04	0.00	0.05	0.00	0.05	0.00
Tetrahydropyran	0.10	0.00	0.25	0.10	0.00	0.10
Dioxane	0.00	0.00	0.31	0.34	0.00	0.00
Trioxane	0.00	0.00	0.00	0.00	0.00	0.00
Dioxane-2,3-diol	0.00	0.00	0.00	0.00	0.00	0.00
Cyclohexane	0.00	0.00	0.00	0.00	0.00	0.00
Cyclohexane-4-ol	0.00	0.00	0.00	0.00	0.00	0.00
Morpholine	0.03	0.00	0.00	0.05	0.00	0.00

Incubation at 30° C in silicone-stopper-covered test tubes containing 10 mM carbon source in 5 ml mineral medium

when dioxane or cyclohexane were the carbon substrates.

The enrichment, which was based on the method of Parkes (1982), led to a great number of strains capable of limited but significant growth on aliphatic oxygen heterocyclic substrates. Strains degrading dioxane-2,3-diol, tetrahydropyran, -4-ol and -4-one have not yet been studied in detail. Studies with strains degrading tetrahydrofuran-3-ol will be published elsewhere.

Characterization of tetrahydrofuran-degrading isolates

The six tetrahydrofuran-degrading strains showed identical morphological characteristics and were Gram-positive. Extraction and analysis of respiratory quinones revealed menaquinone-8 in all strains. From a comparison with *R. rhodnii* DSM 43336 and *R. terrae* DSM 43249 the isolates were preliminarily classified as belonging to the genus *Rhodococcus* (Lechevalier 1986). Their ability to grow on different substrates was compared (Table 2).

For further studies strain 219 was selected because of fast growth in minimal and complex media and its ability to degrade dioxane as well. The optimal temperature was 30° C and the optimal pH range was 6.5–7.0. At 7.5 mM tetrahydrofuran the maximal growth rate (μ) was 0.019 h^{-1} and the degradation rate was 28.5 mg tetrahydrofuran $\cdot h^{-1} \cdot g^{-1}$ dry weight at a yield ($Y_{X/C}$) up to 0.55 (dry weight/substrate carbon). Tetrahydrofuran- and dioxane-degrading ability was not lost during growth on complex media up to an OD_{578} of 75, therefore it seems unlikely that the initial enzyme is plasmid-encoded. After growth in complex media, a lag-phase of up to 24 h was observed, but there was no indication that the enzyme system for degradation was inducible.

Above a concentration of 10 mM (0.72 $g \cdot l^{-1}$) tetrahydrofuran the lag phase was prolonged, and the growth rate and yield reduced, but growth was not totally inhibited. Growth could be observed with as little as 0.22 mM (16 $mg \cdot l^{-1}$) tetrahydrofuran. Figure 1 shows the disappearance of tetrahydrofuran and dioxane in shake cultures. At the end of the logarithmic growth phase no tetrahydrofuran or dioxane could be detected by gas chromatography. The COD measured in the supernatant after centrifugation was zero, meaning that no intermediates had accumulated and the substances were totally mineralized.

The ability of *Rhodococcus* strain 219 to grow on different oygen- or nitrogen-containing compounds was tested with the 34 compounds listed in Table 3. In tests when combinations of tetrahydrofuran and dioxane or butan-1,4-diol were used as carbon sources it was shown that neither compound inhibited the degradation of the other.

The hypothetical catabolic pathway shown in Fig. 2 is in accordance with the list of compounds attacked by strain 219. Preferred substrates are cyclic ethers unsubstituted in the α-position to the ring oxygen. The presumed primary products of tetrahydrofuran or dioxane degradation, namely tetrahydrofuran-2-ol and dioxane-2-ol have not been identified yet and an initial dehydrogenation as hypothesised for the degradation of pyrrolidine (Jacoby and Fredericks 1959) and morpholine

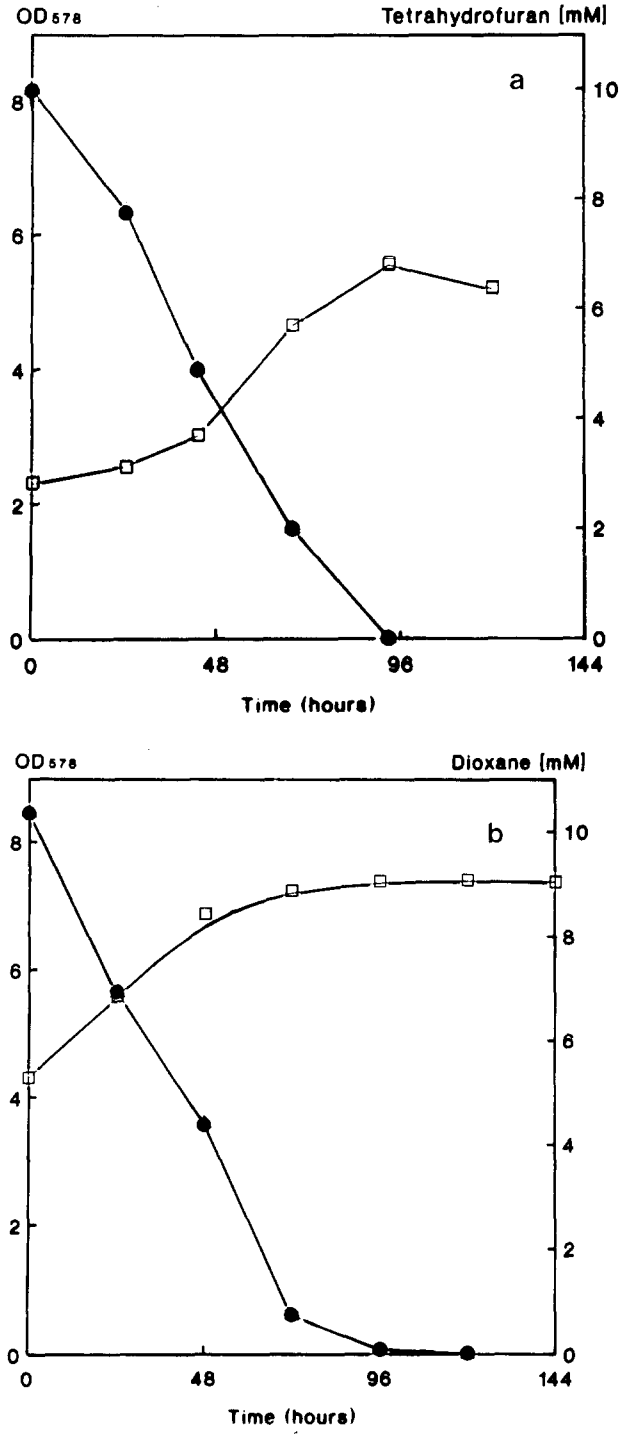

Fig. 1. Growth of *Rhodococcus* sp. strain 219 and degradation kinetics. Mineral medium A (100 ml) containing 10 mM carbon substrate in a 500-ml baffled erlenmeyer flask, covered with a silicone stopper, was inoculated with cells grown in seed medium. Incubation was at 30° C and 175 rpm on a rotatory shaker. Samples were analysed for optical density at 578 nm (OD_{578}) (□) and for carbon source (●) in a gas chromatograph. **a** Tetrahydrofuran (10 mM). **b** Dioxane (10 mM)

(Swain et al. 1991) cannot be excluded. The hypothesis is based:

1. On the fact that all the tetrahydrofuran-degraders (see Table 1) cannot grow on tetrahydrofuran-3-ol and all tetrahydrofuran-3-ol-degraders cannot grow on tetrahydrofuran (not shown).
2. Butyrolactone is a substrate metabolized as fast as tetrahydrofuran.
3. It was reported that a diureticum of the type 2-alkyl-2-methyl-tetrahydrofuran is metabolised in the rat via a hydroxylation at the C-atom adjacent to the ether oxygen (Meng and Kroneberg 1967).
4. Dioxane-2-one was identified as the major urinary metabolite of *p*-dioxane in rat (Woo et al. 1977).
5. By 2-hydroxylation and subsequent oxidation of the secondary alcohol to the ketone a lactone emerges that is a common intermediate in the degradation of cyclic hydrocarbons formed by Bayer-Villiger oxidation of the cyclic ketone, for instance cyclohexanone (Abril et al. 1989).

Table 3. Changes in OD when *Rhodococcus* strain 219 was incubated with aliphatic, ali- and heterocyclic hydrocarbons

Substance	OD	Substance	OD
Tetrahydrofuran	2.85	Succinic acid	1.46
Tetrahydrofuran-3-ol	0.00	Acetic acid	0.00
THF-2-CH_2OH	0.60	Propionic acid	1.00
2,5-Dimethoxi-THF	0.22	Piperazine	0.08
Butan-1,4-diol	3.00	1-Methyl-piperazine	0.00
Butyrolactone	1.65	1,4-Dimethyl-piperazine	0.00
Furan	0.37	Piperidine	0.08
Dioxolane	0.37	1-Ethyl-piperidine	0.15
Dioxolane-2-one	1.05	1-Methyl-piperidine	0.22
Propylencarbonate	1.50	Morpholine	0.00
Dioxane	2.32	*N*-Methyl-morpholine	0.00
Dioxane-2,3-diol	0.00	4-Ethyl-morpholine	0.00
Tetrahydropyran	0.95	*cis/trans* 2,6-Dimethyl-morpholine	0.00
Tetrahydropyran-4-ol	0.00	Methanol	0.00
Tetrahydropyran-4-one	0.00	Toluene sulphonic acid	0.00
n-Hexane	0.00	Nitrobenzenesulphonic acid	0.00
1-Octanol	0.15		
Fumaric acid	1.56		

Conditions were as in Table 2 but with substrates at 7.5 mM except for butan-1,4-diol (10 mM): THF, tetrahydrofuran

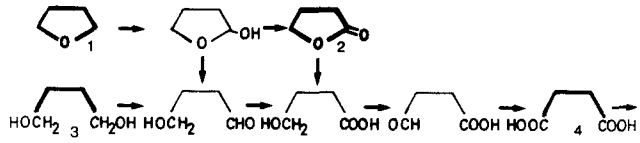

Fig. 2. Hypothetical catabolic pathway for C_4-substrates in *Rhodococcus* sp. strain 219. Substrates degraded (see Table 3) are shown in *bold* structural formula: *1*, tetrahydrofuran; *2*, butyrolactone; *3*, butan-1,4-diol; *4*, succinic acid

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References

Abril O, Ryerson CC, Walsh C, Whitesides GM (1989) Enzymatic Baeyer-Villiger type oxidations of ketones catalyzed by cyclohexanone oxygenase. Bioorg Chem 17:41–52

Dimitrenko GN, Gvozdyak PI, Udod VM (1987) Selection of destructor microorganisms for heterocyclic xenobiotics. Khim Tekhnol Vody 9:442–445

Gvozdyak PI, Udod VM, Dimitrenko GN (1988) *Xanthomonas* bacterial strain for removing tetrahydrofuran from wastewater. USSR patent no. SU 1375646. 23. 2. 1988 (CA 108:192259)

Jacoby WB, Fredericks J (1959) Pyrrolidine and putrescine metabolism γ-amino butyraldehyde dehydrogenase. J Biol Chem 234:2145–2150

Kroppenstedt RM (1982) Anwendung chromatographischer HP-Verfahren (HPTLC und HPLC) in der Bakterien-Taxonomie. GIT Labor-Medizin 5:266–275

Lechevalier HA (1986) Nocardioforms. In: Sneath PHA, et al. (eds) Bergey's manual of systematic bacteriology, vol 2. Williams and Wilkins, Baltimore, pp 1458–1579

Meng K, Kroneberg G (1967) Pharmakologie von *N*-(4′-Chlor-3′-sulfamoyl-benzolsulfonyl)-*N*-methyl-2-aminomethyl-2-methyl-tetrahydrofuran, einer neuen diuretisch wirkenden Verbindung. Arzneim-Forsch 17:659–671

Orlowska B, Jaroszynski T, Gomolka E (1984) Characteristics of activated sludge adapted to dioxane and *N*-methylpyrrolidone. Environ Prot Eng 10:47–57

Painter HA, King EF (1985) Ring test program 1983–84. Assessment of biodegradability of chemicals in water by manometric respirometry, Comm Eur Communities (Rep.) EUR, EUR 9962 (CA 103:183290)

Parkes RJ (1982) Methods for enriching, isolating, and analysing microbial communities in laboratory systems. In: Bull AT, Slater JH (eds) Microbial interaction and communities. Academic Press, London, pp 45–102

Swain A, Waterhouse KV, Venables WA, Callely AG, Lowe SE (1991) Biochemical studies of morpholine catabolism by an environmental mycobacterium. Appl Microbiol Biotechnol 35:110–114

Thaler M, Diekmann H (1979) The effect of manganese deficiency on lipid content and composition in *Brevibacterium ammoniagenes*. Eur J Appl Microbiol 6:379–387

Trower MK, Buckland RM, Higgins R, Griffin M (1985) Isolation and characterization of a cyclohexane-metabolizing *Xanthobacter* sp. Appl Environ Microbiol 49:1282–1289

Trudgill PW (1984) The microbial metabolism of furans. In: Gibson DT (ed) Microbial degradation of organic compounds. Dekker, New York, pp 295–308

Woo Y-T, Arcos JC, Argus MF, Griffin GW, Nishiyama K (1977) Structural identification of dioxane-2-one as the major urinary metabolite of *p*-dioxane. Naunyn-Schmiedeberg's Arch Pharmakol 299:283–287