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Epoxidation of alkenes by alkene-grown *Xanthobacter* spp.

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Summary. Newly isolated *Xanthobacter* spp. were able to grow on the gaseous alkenes like ethene, propene, 1-butene and 1,3-butadiene. Resting-cell suspensions of propene-, 1-butene- or 1,3-butadiene-grown *Xanthobacter* Py10 accumulated 1,2-epoxyethane from ethene. Ethene-grown *Xanthobacter* Py10 did not produce any 1,2-epoxyalkane from the alkenes tested. Furthermore, propene-grown *Xanthobacter* Py2 accumulated 2,3-epoxybutane from *trans*-butene and *cis*-butene but did not form epoxides from other substrates tested.

Introduction

The formation of 1,2-epoxyalkanes from alkenes has been studied in many micro-organisms. Most of these micro-organisms able to epoxidate alkenes are alkane-utilizing bacteria. The epoxidation of an alkene was first demonstrated by van der Linden (1963) who detected the formation of 1,2-epoxyoctane from octene by heptane-grown *Pseudomonas* cells. The epoxidation of alkenes by alkane-utilizing *Pseudomonas* cells was also reported by several other authors (Abbott and Hou 1973; de Smet et al. 1981; de Smet et al. 1983) and an octane-utilizing Corynebacterium also excreted epoxides in this way (Cardini and Jurtshuk 1970).

However, none of these bacteria were able to epoxidate gaseous alkenes. Hou et al. (1983) and Patel et al. (1983) reported that ethane-, propane- and butane-metabolizing bacteria excreted 1,2-epoxyalkanes from gaseous alkenes. Methanotrophic bacteria also catalysed the epoxidation of gaseous alkenes (Hou et al. 1979; Higgins et al. 1979; Higgins et al. 1980), and the methane-grown *Methylosinus trichosporium* was even found to be active on cycloalkanes as well as on aromatic compounds. The epoxidation of alkenes by these alkane-grown bacteria is due to the non specific alkane hydroxylase that is able to oxidize alkanes as well as alkenes.

Several micro-organisms isolated on alkenes have also been tested for their ability to epoxidate alkenes and for this purpose bacteria of the genera *Mycobacterium* (Habets-Crützen et al. 1984) and *Nocardia* (Furuhashi et al. 1981) grown on ethene or propene were used. The ethene- and propene-grown *Mycobacteria* were able to accumulate 1,2-epoxypropane and 1,2-epoxyethane, respectively (Habets-Crützen et al. 1984). Formation of epoxides by these bacteria is due to the substrate specificity of the 1,2-epoxyalkane degrading enzymes (Habets-Crützen et al. 1984). Furuhashi et al. (1981) reported that *Nocardia corallina* B276 accumulated 1,2-epoxypropane during growth on propene. Because the production rate of 1,2-epoxyalkanes by the *Mycobacteria* is rather low, we have tried to isolate some faster growing alkene-utilizing micro-organisms. Some fast growing *Xanthobacter* spp isolated on propene or 1-butene was the result of this attempt (van Ginkel and de Bont).

These organisms are especially interesting as potential epoxide producers because some of the epoxides were formed in high enantiomeric excess (Habets-Crützen et al. 1985). In this paper we present more detailed results on the accumulation of 1,2-epoxyalkanes from alkenes by ethene-, propene-, 1-butene- and 1,3-butadiene-grown *Xanthobacter*.

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Materials and methods

Chemicals. Gaseous alkenes and 1,2-epoxyethane were obtained from Hoek Loos, Amsterdam, The Netherlands. NADH was purchased from Boehringer, Mannheim, West-Germany. All other chemicals were obtained from Janssen Chimica, Beerse, Belgium.

Cultivation of the micro-organism. The micro-organisms were cultivated in mineral salts medium supplemented with the appropriate gaseous alkenes as described by Wiegant and de Bont (1980). Growth on different carbon sources was determined at 30° C in 100 cm^3 Erlenmeyer flasks containing 10 cm^3 mineral medium supplemented with the appropriate carbon source.

Analyses. Determination of gaseous alkenes and 1,2-epoxyalkanes has been described by de Bont et al. (1979). Protein concentration of washed cell suspensions was determined as described by Habets-Crützen et al. (1984).

Determination of the doubling times. The culture doubling times were measured as described by Habets-Crützen et al. (1984).

Oxidation of hydrocarbons and 1,2-epoxyalkanes. Preparation of washed cell suspensions has been described by de Bont and Harder (1978). The oxidation of hydrocarbons and the excretion of 1,2-epoxyalkanes by these washed cell suspensions were measured by incubating the cells in a 50 mM phosphate buffer (pH = 7.0) at 30° C in Hungate tubes. The appropriate gas (0.5 cm^3) or 0.1 cm^3 of a 100 mM 1,2-epoxyalkane solution in a 50 mM phosphate buffer (pH = 7.0) was injected in the Hungate tubes. Samples from the gas phase were withdrawn at regular intervals and analysed for hydrocarbons and 1,2-epoxyalkanes.

Results

Growth characteristics on hydrocarbons

The alkene-utilizing *Xanthobacter* spp. studied have been isolated on either propene (Py2, Py3, Py7, Py10, Py11, Py17) or 1-butene (By2) (van Ginkel and de Bont) and they were tested for growth on several other hydrocarbons. They did not grow on saturated hydrocarbons like methane, ethane, propane, butane, hexane, cyclohexane and hexadecane. Growth on unsaturated hydrocarbons like *trans*-butene, *cis*-butene, allene, ethyne and propyne did not occur either. Only ethene, propene, 1-butene and 1,3-butadiene supported growth. The culture doubling times of these organisms on propene and 1-butene ranged from 5 to 7 h, but growth on ethene and 1,3-butadiene was much slower. *Xanthobacter* Py10 was the fastest growing strain on ethene and 1,3-butadiene, and culture doubling times of this organism on ethene and 1,3-butadiene were 25 and 32 h, respectively.

Substrate specificity and excretion of 1,2-epoxyalkanes

Resting-cell suspension of propene-grown *Xanthobacter* were able to oxidize ethene, propene and 1-butene at rates varying from 38 to 83 nmoles of alkene oxidized per minute per mg protein (Table 1). The propene-grown *Xanthobacter* spp. oxidized propene and 1-butene without a transient extracellular production of the corresponding 1,2-epoxyalkanes but from ethene these whole cell suspensions excreted 1,2-epoxyethane. The production of 1,2-epoxyethane from ethene was not stoichiometric (Table 1). The behaviour of these micro-organisms towards alkenes was investigated in more detail by comparing washed cell suspensions of *Xanthobacter* Py10 grown on either ethene, propene, 1-butene and 1,3-butadiene. *Xanthobacter* Py10 was used because this strain grew fastest on ethene and 1,3-butadiene. Oxidation rates for ethene, propene, 1-butene and 1,3-butadiene were lower in ethene- and butadiene-grown cells than in propene and 1-butene-grown cells but in all cases the ratios of oxidation rates of ethene, propene, 1-butene and 1,3-butadiene of the ethene- and 1,3-butadiene-grown cells were comparable to the ratios of propene- and 1-butene-grown cells. A different pattern was observed for the oxidation of 1,2-epoxyalkanes by alkene-grown *Xanthobacter* Py10. 1,2-Epoxyethane was relatively poorly oxidized by cells grown on propene, 1-butene or 1,3-butadiene as compared with ethene-grown cells. The ethene-grown cells oxidized all 1,2-epoxyalkanes at rates that were comparable with the alkene oxidation rates. Consequently propene-, 1-butene- and 1,3-butadiene-grown cells excreted 1,2-epoxyethane

Table 1. Oxidation of alkenes and accumulation of 1,2-epoxyethane by washed cell suspensions of propene-grown *Xanthobacter* strains. None of the strains excreted 1,2-epoxypropane or 1,2-epoxybutane from propene or 1-butene

Strain	Substrate oxidation rate[a]			Product accumulation rate[a]
	Ethene	Propene	1-Butene	1,2-Epoxyethane
Py2	55	83	67	46
Py3	53	81	77	27
Py7	35	65	47	29
Py10	40	66	58	29
Py11	43	75	60	21
Py17	38	77	54	25
By2	43	79	46	20

[a] In nmol per minute per mg protein

but no other 1,2-epoxyalkanes, while ethene-grown cells did not accumulate epoxides at all (Table 2).

Substrate specificity towards other compounds

The substrate specificity towards other hydrocarbons was tested with propene-grown *Xanthobacter* Py2 cells. *Trans*-butene, *cis*-butene, 1-pentene, 1-hexene, and isoprene were oxidized, but epoxide accumulation was only detected during the oxidation of *trans*-butene or *cis*-butene (Table 3). The 2,3-epoxybutanes were not formed in stoichiometric amounts. Although *cis*-butene was oxidized faster than *trans*-butene, the 2,3-epoxybutane accumulation rate from *trans*-butene was more than twice as high as from *cis*-butene (Table 3). Other unsaturated hydrocarbons like allene, ethyne, propyne, limonene and myrcene were not oxidized. Furthermore, propene-grown *Xanthobacter* Py2 did not oxidize aromatic compounds like benzene, xylene, styrene and toluene or saturated hydrocarbons like methane, ethane propane, butane, pentane, hexane and cyclohexane. *Xanthobacter* Py2 did not oxidize CO either.

Table 2. Oxidation of alkenes and 1,2-epoxyalkanes and the accumulation of 1,2-epoxyalkanes by ethene-, propene-, 1-butene- and 1,3-butadiene-grown Xanthobacter Py10 cells

Growth substrate	Ethene	Propene	1-Butene	1,3-Butadiene
Oxidation rate[a]				
Ethene	20	40	39	11
Propene	30	66	65	20
1-Butene	29	64	63	21
1,3-Butadiene	11	17	16	9
1,2-Epoxyethane	15	10	13	3
1,2-Epoxypropane	25	60	58	20
1,2-Epoxybutane	25	62	60	18
3,4-Epoxy-1-butene	10	18	15	10
Accumulation rate[a]				
1,2-Epoxyethane	0	29	24	4
1,2-Epoxypropane	0	0	0	0
1,2-Epoxybutane	0	0	0	0
3,4-Epoxyl-butene	0	0	0	0

[a] In nmol per minute per mg protein

Table 3. Oxidation of unsaturated hydrocarbons and the formation of epoxyalkanes by washed cell suspensions of propene-grown *Xanthobacter* Py2

Substrate	Substrate oxidation rate[a]	Product detected	Product accumulation rate[a]
trans-Butene	60	2,3-Epoxybutane	41
cis-Butene	71	2,3-Epoxybutane	17
1,3-Butadiene	30	none	—
1-Pentene	18	none	—
Isoprene	4	none	—
1-Hexene	2	none	—

[a] In nmol per minute per mg protein

Discussion

Bacteria growing on either ethene, propene, 1-butene or 1,3-butadiene have been described by several authors (de Bont 1976; Heyer 1976; Cerniglia et al. 1976; Watkinson and Sommerville 1976) but until recently only bacteria belonging to the genera *Mycobacterium* (de Bont et al. 1980) and *Nocardia* (Watkinson and Sommerville 1976) had been isolated on these gaseous alkenes. Recent *Xanthobacter* isolates grew on both ethene, propene, 1-butene and 1,3-butadiene while the other alkene-utilizing micro-organisms grew only on a limited number of these gaseous alkenes (Habets-Crützen et al. 1984). A few alkene-utilizing *Mycobacterium* (de Bont et al. 1979) and *Nocardia* (Fujii et al. 1985) isolates were able to metabolize gaseous alkanes as well, but the alkene-utilizing *Xanthobacter* were not able to grow on saturated hydrocarbons, although other *Xanthobacter* have been isolated on cyclohexane (Trower et al. 1985) and butane (Coty 1967).

The alkene oxidation rates of *Xanthobacter* were higher than the oxidation rates of alkene-grown *Mycobacterium* (Habets-Crützen et al. 1984) which is probably a reflection of the higher growth rate of the *Xanthobacter*. These oxidation rates of the *Xanthobacter* are of the same order of magnitude as the activities of methane-, ethane-, propane- and butane-utilizing bacteria (Hou et al. 1979; Stirling and Dalton 1979; Hou et al. 1983; Patel et al. 1983). Oxidation rates of ethene, propene, 1-butene and 1,3-butadiene by cells of *Xanthobacter* Py10 grown on these four alkenes suggest that these alkenes were oxidized by the same enzyme. However, the further metabolism of epoxides in *Xanthobacter* Py10 is probably mediated by two different 1,2-epoxyalkane degrading enzymes because ethene-grown cells oxidized all 1,2-epoxyalkanes at the same rate whereas cells grown on propene or 1-butene oxidized 1,2-epoxypropane and 1,2-epoxybutane six times faster than 1,2-epoxyethane. Consequently, the *Xanthobacter* strains excreted 1,2-epoxyethane from ethene although not in stoichiometric amounts as did propene-grown *Mycobacteria* (de Bont et al.

1983; Habets Crützen et al. 1984). Unfortunately, neither 1,2-epoxypropane nor 1,2-epoxybutane accumulated from the respective alkenes. In *Xanthobacter* Py2 the oxidation of other unsaturated hydrocarbons was also tested and only from *trans*-butene and *cis*-butene an epoxide was detected. In both cases the formation of 2,3-epoxybutanes again was not in stoichiometric amounts. Higher 1-alkenes were also oxidized but at considerable lower rates. The substrate specificity of propene-grown *Xanthobacter* Py2 towards hydrocarbons is different from the substrate specificity of alkane utilizing bacteria but resembles the substrate specificity of *Mycobacteria*. Alkane-metabolizing micro-organisms are able to hydroxylate alkanes and epoxidate alkenes while alkene-utilizing *Xanthobacter* and *Mycobacteria* are only capable of epoxidating alkenes (Habets-Crützen et al. 1984). CO is not oxidized by propene-grown *Xanthobacter* Py2 whereas methanotrophic bacteria are able to oxidize this compound (Higgins et al. 1979).

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