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Production of Chiral Chloropropanols using Stereospecifically Assimilating Bacteria

Abstract

Bacteria stereospecifically assimilating (S)- and (R)-2,3-dichloro-1-propanol or (S)- and (R)-3-chloro-1,2- propanediol were isolated from soils. These bacteria have dehalogenase activity for various halohydrins and epoxyhydrolase activity for aliphatic epoxides. Microbial resolution of (RS)-2,3-dichloro-1-propanol or (RS)-3-chloro-1,2-propanediol was performed and using these bacteria an industrial process was developed for the production of the respective highly optically active enantiomers.

Introduction

Large amounts of halogenated compounds are manufactured on an industrial scale and they have been utilized as solvents, agrochemicals, pharmaceuticals, and polymers. Recently, there have been many reports concerning the microbial degradation of low-molecular halogenated aliphatics (Vogel et al., 1987). The enzymatic dehalogenation of haloacids (Hardman and Slater 1981; Kawasaki et al., 1981a,b; Motosugi and Soda, 1983), haloalkanes (Janssen et al., 1987; Keuning et al., 1987; Scholtz et al., 1987) and dichloromethane (Brunner et al., 1980) have been reported. However, the microbial degradation of halogenated aldehydes, alcohols and esters has rarely been reported.

Stucki and Leisinger (1983) isolated a bacterium capable of degrading chloroethanol via chloroacetic acid, and with respect to the dehalogenation of a halogenated C3 alcohol by microorganisms, Castro and Bartnicki (1968) reported the degradation of 2,3-dibromo-1-propanol by *Flavobacterium*. Recently, Janssen and van den Wijngaard (1989) isolated three bacterial cultures capable of growing on 1,3-dichloro-2-propanol, epichlorohydrin (EP) and 3-chloro-1,2-propanediol (CPD) and purified haloalcohol dehalogenase from the strain *Arthrobacter* sp. AD2 (van den Wijngaard et al., 1991). This enzyme catalyzed the conversion of halogenated alcohols such as 1,3-dichloro-2-propanol, 1,3-

dibromo-2-propanol, CPD, 1-chloro-2-propanol and 1-bromo-2-propanol to the corresponding epoxides.

We have long studied the development of the microbial conversion of C3 halogenated aliphatic compounds to useful chemicals. Recently, we isolated novel bacteria capable of stereospecifically assimilating 2,3-dichloro-1-propanol (DCP) with the liberation of chloride ion, and developed the microbial resolution of optically active DCP and the synthesis of optically active epichlorohydrin (EP) (Kasai et al., 1992a, 1992b). Our microbial resolution was very effective and practical, so that the optically active epichlorohydrin obtained with this method was utilized for the syntheses of many useful compounds such as pharmaceuticals (Kawamura et al., 1990), pheromones (Imai and Nishida, 1990), and ferro-electric liquid crystals (Koden et al., 1990). More recently, we isolated novel bacteria stereospecifically assimilating (R)- and (S)-CPD from soil, and succeeded in the preparation of (S)- and (R)-CPD with highly optical purity using the bacterium by virtue of its stereospecific selectivity (Figure 1). These bacteria can produce optically active EP and CPD as useful chiral building blocks; they are not only useful for the degradation of halogenated compounds but also excellent biocatalysts for new chemistry. This report describes the characterization of the strains, the properties of the dehalogenating activity in the cell-free extracts of the bacteria used in the production of optically active EP, and CPD.
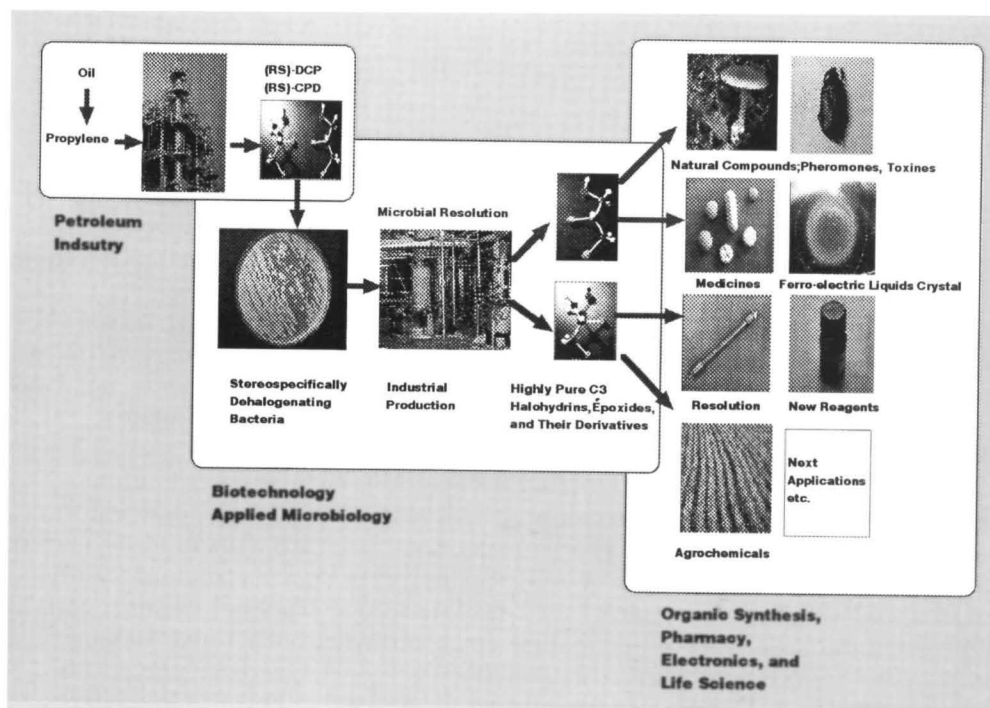

Fig. 1.   Microbial production of optically active C_3 building blocks and their use.

(R)- and (S)-DCP assimilating bacteria

Isolation of an (R)-DCP assimilating strain

From 300 soil samples, a bacterial strain, OS-K-29, which assimilated DCP as a sole carbon source, was isolated. Morphological and physiological characteristics of OS-K-29 showed that it belonged to the genus *Pseudomonas*.

The strain OS-K-29 could grow on DCP, which was gradually degraded, and CPD was formed with liberation of chloride ion. CPD accumulated in the logarithmic phase and disappeared completely in the stationary phase. The growth of the cells ceased and the final degree of degradation was estimated to be 50% regardless of the concentration of 2,3-dichloro-1-propanol (DCP) used. Therefore, the conversion of DCP was considered to be stereospecific. When the strain was cultured on DCP or 2,3-dibromo-1-propanol(DBP), the specific rotations of residual DCP, formed CPD, and residual DBP gave $[\alpha]_D^{25} - 10.50$ (c = 1.3, in CH_2Cl_2), $[\alpha]_D^{25} + 6.90$ (c = 1.2, in H_2O), and $[\alpha]_D^{25} - 12.00$ (c = 1.3, in CH_2Cl_2), respectively.

A possible degradation route by OS-K-29 of DCP was from CPD to glycidol to glycerol with EP as a tentative intermediate. The dehalogenation or epoxidation of halohydrins was slower than hydrolysis of epoxides, so accumulation of epoxide was hardly observed. However, alcohols formed from halohydrins were easily detected. The degradation activity of OS-K-29 seems to be similar to that of the previously reported *Flavobacterium* sp. which degrades 2,3-dibromo-1-propanol (Castro and Bartnick, 1968). The latter strain assimilated 2,3-dibromo-1-propanol via epibromohydrin, 3-bromo-1,2-propanediol, glycidol, and glycerol, and accumulated more epoxides than dehalogenated alcohols because of strong bromohydrin epoxidase and weak epoxyhydrolase activities. However, some distinct features between two strains were noted by us; first, in the conversion of DCP, EP was not detected in OS-K-29, and CPD accumulated instead. This might be due to a fast epoxy-opening reaction by strong epoxyhydrolase activity. Second, the conversion of DCP was stereospecific in OS-K-29, whereas *Flavobacterium* sp. did not show stereospecificity. The enzyme of OS-K-29 that causes stereospecific conversion of DCP seemed to be novel. This could be used for preparation of chiral compounds. Synthesis of optically active EP has been tried by various methods (Golding, 1988).

Isolation of an (S)-DCP assimilating strain

From about 1000 samples of soils collected at petrochemical plants, six (RS)-DCP assimilating bacteria and six (R)-DCP assimilating bacteria were found, while only one bacterial strain, DS-K-S38, which preferentially assimilated (S)-DCP as a sole source of carbon was isolated. This strain was found to belong to the genus *Alcaligenes* according to its morphological and physiological characteristics.

When strain DS-K-S38 was cultivated on (RS)-DCP, (S)-DCP was degraded with liberation of chloride ions. The cell growth ceased when the final degradation was estimated to be 52%. A trace amount of CPD (Kasai et al., 1990) and glycerol was detected in the logarithmic phase. These results were similar to those obtained with the (R)-DCP-assimilating strain OS-K-29 (Kasai et al., 1992 a). The specific rotation of residual (R)-DCP gave $[\alpha] = +10.7$ ($c = 1.25$, in CH_2Cl_2) and the value was in good accord with that of (S)-DCP ($[\alpha] = -10.5$, $c = 1.36$, in CH_2Cl_2) (Kasai et al., 1992 a).

Bacteria preferentially assimilating (R)-DCP ((R)-type) or (S)-DCP((S)-type) were isolated from soils. During the cultivation, in which (RS)-DCP was the single source of carbon, each bacterium degraded and assimilated half of the DCP; (R)-DCP or (S)-DCP.

Degradation of halogenated compounds

Table 1 shows the degradation activity of the (R)-DCP-assimilating strain OS-K-29 and the (S)-DCP assimilating strain DS-K-S38. Various low-molecular mass chlorinated aliphatic hydrocarbons and related epoxides (mainly C3 chlorinated compounds) were tested as to whether or not they were assimilated. Both strains could degrade chlorohydrins such as CPD, DCP, and glycidol, but

Table 1.   Degradation of various halogenated compounds by strain OS-K-29 and DS-K-S-38

	OS-K-29 Growth (at 660 nm)	Degradation (%)	DS-K-S38 Growth (at 660 nm)	Degradation (%)
Halohydrins				
3-Chloro-1,2-propanediol	0.52	63.4	0.85	56.8
1,3-Dichloro-2-propanol	0.14	73	0.04	0
2,3-Dichloro-1-propanol	0.2	42.6	0.17	36.7
2,3-Dibromo-1-propanol	0	47.6	0	0
Ethylenechlorohydrin	0.02	16.8	0.08	3.1
Propylenechlorohydrin	0.11	58.2	0.07	20.7
Butylenechlorohydrin	0.04	44.3	0.07	24.2
Epoxides				
Epichlorohydrin	0.48	>99	0	0
Epibromohydrin	0.01	75.1	0	0
Glycidol	0.75	>99	0.02	16.2
Propylene oxide	0.01	17.2	0	0
Haloacids and others				
Chloroacetone	0	4.5	0	0
n-Propylchloride	0	1.2	0	0
2-Chloropropionic acid	0	0	0	0
3-Chloropropionic acid	0	0	0	0

Five ml of synthetic medium containing various haloginated compounds (0.2%, v/v) in screw capped test tubes was inoculated with 0.1 ml of a preculture, and incubated at 30 °C for 24 h and shaken.

not chloroacetone and chloropropionic acid. CPD was a good carbon source for growth, but the other compounds were poor carbon sources. Comparing the degradation activity between strain DS-K-S38 ((S)-type) and strain OS-K-29 ((R)-type), it was found that the range of assimilable and degradable compounds was smaller for the (S)-type organism than for the (R)-type. 1,3-Dichloro-2-propanol and EP were good carbon sources for growth of the (R)-type, however, the (S)-type could not degrade and assimilate them, regardless of the concentration.

Enzymatic conversion

Figure 2 shows the activity of the cell-free extracts of the strains OS-K-29 and DS-K-S38. C3 halohydrins were dehalogenated, yielding dehalogenated alcohols. Epoxides, except for propylene oxide, were hydrolyzed and converted to the diols. Comparing the relative activity of cell-free extracts between the (S)-type and the (R)-type, some notable differences were found. In the case of the (R)-type, the conversion activity was high and broad; especially, high epoxyhydrolase activity was found. On the other hand, in the case of the (S)-type, the pattern of the relative activity for halohydrins was similar to that of the (R)-type, however, the activity was weak. The epoxyhydrolase activity of the (S)-type was very weak except for EP. Although EP was not assimilated, the epoxyhydrolase activity for EP was the highest.

The (S)-type and (R)-type were isolated as stereospecific DCP assimilating bacteria from soil samples collected from the same petrochemical plant site. CPD and glycerol were detected during degradation of DCP, and the pattern of relative activity
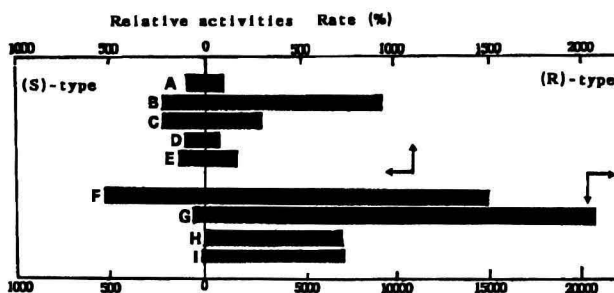

Fig. 2. Comparison of relative activities of cell-free extracts for halohydrins and epoxides between (S)-type and (R)-type. The conversion activities are expressed as percentages of the rate found with DCP(S)-type, 1.5 mU/mg protein; (R)-type, 1.1 mU/mg protein). Substrates A, DCP; B, 1,3-dichloro-2-propanol; C, CPD; D, propylene chlorohydrin; E, DBP, F, EP; G, epibromohydrin; H, propylene oxides; I, glycidol.

for halohydrins in the cell-free extracts resembled each other. Therefore, we considered that the degradation patterns were similar except the stereospecificity for DCP, which was dehalogenated and degraded to glycerol via CPD. Bacterial degradation of (RS)-DBP, in which the Cl of DCP is substituted for Br, was reported by Castro and Bartnicki (1968). DBP was converted by a halohydrin epoxidase to epibromohydrin, resulting in its accumulation. It was not determined whether or not dechlorination of (R)- and (S)-DCP occurred via EP, because epoxyhydrolase activity for EP was high compared to the dechlorinating activity for DCP which caused that EP did not accumulate. The enzyme which caused the dehalogenation of (R)- and (S)-DCP in the bacteria seemed to be novel because of its high stereospecificity. Stereospecific dehalogenation for DCP had not yet been described.

Production of (R)- and (S)-DCP and their conversion to (S)- and (R)-EP

Microbial resolution of DCP was carried out in simple synthetic medium using the stereospecific assimilating bacteria. The strain OS-K-29 or DS-K-S38 was precultured and inoculated into a synthetic medium containing (RS)-DCP as the single source of carbon. After the cultivation, the residual (R)- or (S)-DCP was purified using a charcoal column treatment, elution with acetone, and distillation *in vacuo*. The chiral purity of the each optically active DCP was 100% ee. (R)- and (S)-epichlorohydrin was obtained from DCP via treatment with aqueous NaOH with a yield of 74%. The chiral purity was determined as 99.3-99.4% ee through an examination of complexation gas chromatography (Kasai *et al.*, 1992a, 1992b).

Production of D-lactate from L-2-chloropropionate by a stereospecific dehalogenase of L-2-chloro-propionate-assimilating *Pseudomonas putida* has been reported. This system is similar to ours in the sense that the substrates are C3 compounds having the same chlorinated chiral center at the C2 position and the enzymes are stereospecific dehalogenases. Our substrate, however, has a hydroxyl group instead of a carboxyl group in the Cl position. Further characterization of the enzyme of these bacteria will be needed.

Generally, the resolution of liquid substances is very difficult since crystallization can not be carried out. Asymmetric syntheses of chiral EP has been developed, but these methods are not simple. For the resolution of DCP, some biochemical methods also have been reported. Iriuchijima and Kojima (1982) reported asymmetric hydrolysis of 1-acetoxy-2,3-dichloropropane with pancreatic or *Mucor* sp. lipases but the desired optical purity was not obtained.

This microbial resolution reported here was effective and useful for preparing (R)-and (S)-DCP and (S)- and (R)-EP. Residual (R)- and (S)-DCP were found to be optically pure, and highly pure (R)-and (S)-EP were prepared from these products. (RS)-DCP is produced economically by the petroleum industry and therefore, this method is considered applicable in practice. Habets-Crutzen *et al.*(1985) reported the formation of (S)-EP from allylchloride by microbial stereospecific epoxidation yielding product with an optical purity of 80-98% ee.

However, we consider that our method has advantages such as the higher solubility in water, its lower toxicity, and its higher boiling point than allylchloride. Since 1994, the microbial resolution is used on an industrial scale in Matsuyama, Japan. Currently, the (R)-and (S)-EP are being used in the synthesis of chiral drugs or natural products. It should also be possible to use these optically active compounds for easily obtaining C3 chiral building blocks.

(R)- and (S)-CPD assimilating bacteria

Isolation and characterization of strains assimilating (R)- and (S)-CPD

From 1000 soil samples, two bacterial strains, named DS-K-2D1 and DS-S-7G, were isolated that preferentially assimilated (S)-CPD and (R)-CPD as sole source of carbon, respectively. Strain DS-K-2D1 was identified as a member of the genus *Pseudomonas*, and DS-S-7G was identified as *Alcaligenes*.

Utilization of halogenated compounds

Table 2 shows the utilization and degradation of various halogenated and related compounds by strains DS-K-2D1 and DS-S-7G. The strains grew on racemic CPD, (R)-CPD or (S)-CPD and 3-bromo-1,2-propanediol but not on the other halohydrins, epoxides, haloacids and chloroacetone. Although (R)-CPD and 1,3-dichloro-2-propanol or (S)-CPD and DCP were slightly degraded by DS-K-2D1 and DS-S-7G, respectively, they did not support growth of the strains. Growth of the strain on racemic CPD medium was inhibited by the presence of 1,3-dichloro-2-propanol or 2,3-dibromo-1-propanol.

Degradation of halogenated and related compounds by cell-free extracts

The activities toward various halohydrins and related epoxides by cell-free extracts of strains DS-K-2D1 and DS-S-7G are summarized in Table 2.
In case of DS-K-2D1, halohydrins such as CPD, 3-bromo-1,2-propanediol, 1,3-dichloro-2-propanol, DCP, and propylene chlorohydrin were converted to the corresponding epoxides. The cell-free extracts had higher activity for bromohydrins than for chlorohydrins. The respective conversions were accompanied by the release of halide ions. The cell-free extracts also converted epichlorohydrin, epibromohydrin, GLD, and propylene oxide to the corresponding diols. The epoxide-opening activity for halogenated epoxides by cell-free extracts was high compared to that for non-halogenated epoxides. The degradation activity for (R)-CPD was 2.3 times higher than that for (S)-CPD. In case of DS-S-7G, the cell-free extracts had activities towards several halohydrins and epoxides which were converted to the corresponding epoxides and alcohols, respectively. Various halohydrins such as racemic CPD, 3-bromo-

Table 2. Comparison of degradation activities of cells and relative activities of the cell free extracts between strain DS-K-2D1 and DS-S-7G

| | DS-K-2D1 | | | DS-S-7G | | |
| | Growth | Degradation | Relative activity | Growth | Degradation | Relative activity |
	(at 660 nm)	(%)	by cell free extracts	(at 660 nm)	(%)	by cell free extracts
Halohydrins						
3-Chloro-1,2-propanediol	1.07	56.5	100	0.75	52.7	100
(R)-CPD	0.06	10	145	0.88	66.1	116
(S)-CPD	0.93	65.1	62.7	0.05	<5	47.5
3-Bromo-1,2-propanediol	0.35	67.6	894	0.44	49.5	72.7
1,3-Dichloro-2-propanol	0	13	136	0	<5	0
2,3-Dichloro-1-propanol	0	<5	23.3	0	11.5	36.4
Propylenechlorohydrin	0	<5	23.3	0	8.2	12.1
Epoxides						
Epichlorohydrin	0	<5	283	0	<5	30.3
Epibromohydrin	0	<5	300	0	<5	0
Glycidol	0	<5	34.2	0	<5	39.4
Propylene oxide	0	<5	39.1	0	13	0
Haloacids and others						
Chloroacetone	0	8	0	0	<5	0
n-Propylchloride	0	<5	0	0	<5	0
2-Chloropropionic acid	0	<5	0	0	<5	0

Method for degradation activities were the same as Table 1.
Relative activities were expressed as percentages of the rate found with CPD.

1,2-propanediol, DCP, and propylenechlorohydrin were mainly converted to the corresponding epoxides. These conversions were accompanied by the release of halide ions. Epichlorohydrin and glycidol were converted to the corresponding diols. Further enzymatic study of stereospecificity for (R)-CPD was done. We also found that (R)-CPD was converted to hydroxyacetone, and that the enzyme responsible was new, namely, a halohydrin dehydro-dehalogenase (HDDase) (Suzuki and Kasai, 1994).

Preparation of (R)- and (S)-CPD

A bacterial culture was inoculated into a synthetic medium containing (RS)-CPD as a single carbon source. (S)- or (R)-CPD was assimilated preferentially with the release of chloride ions, and (R)- or (S)-CPD was not converted. After cultivation, each CPD was purified by condensation and distillation from the culture filtrate. The optical purity of the purified (R)- and (S)-CPD was estimated to be greater than 99.5% ee by HPLC analysis of the tosylated derivatives (3-tosyloxy-1-chloro-2-propanol), respectively. Conversion to GLD was carried out according to the standard procedures. The optical purities of the formed (R)- and (S)-GLD were estimated to be greater than 99.3%ee and 99.4%ee, respectively, by gas chromatography (Suzuki and Kasai, 1991).

Chemical syntheses of (S)-CPD from methyl-6-chloro-6-deoxy-α-D-glucopyranoside and D-mannitol (Porter and Jones, 1982) are known, and also enzymatic and microbial preparations of (R)-CPD from racemic 1,2-diacetoxy-3-chloro-propane (Iriuchijima and Kojima, 1982b) and 1,3-dichloro-2-propanol, have been reported. Klunder *et al.* have reported a method for the preparation of optically active glycidol on the basis of the asymmetric oxidation of allyl alcohol (Klunder *et al.*, 1986., Gao *et al.*, 1987). However, these methods for generation of (R)-CPD and (S)-glycidol result in low optical purity or are not practical. Our microbial preparation of (R)-and (S)-CPD and the synthesis of (R)-and (S)-glycidol seems to be simpler, more effective and more practical than the other preparation processes described above. It is likely that these compounds can be produced on an industrial scale and it is possible using our procedure to produce highly optically active (R)- and (S)-CPD as well as optically active DCP and EP on a multiton scale.

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