Isolation and Characterization of a 1,3-Dichloro-2-Propanol-Degrading Bacterium

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Arthrobacter sp. strain PY1, a bacterium having the ability to degrade 1,3-dichlotopropanol (1,3-DCP), was isolated from a soil sample of a chemical plant. Strain PY1 degraded 1000 mg/l (7.75 mM) of 1,3-DCP completely within 7 days, and the ability was elevated by acclimatization up to 4000 mg/l/week. Addition of nutrients such as peptone, glucose or glycerol showed no or slight effect on the degrading activity. These results suggest that strain PY1 is a useful organism in a biological control system for 1,3-DCP pollution. The ability to degrade 1,3-DCP was induced by addition of 1,3-DCP to the culture of strain PY1. A 1,3-DCP-degrading enzyme (Deh-PY1) was purified from the cytoplasmic fraction of strain PY1 by fractionation with ammonium sulfate, hydrophobic chromatography and anion exchange chromatography. Purified Deh-PY1 is a tetramer of a homogeneous subunit having a molecular weight of 20 kDa. Analysis of the N-terminal amino acid sequence of Deh-PY1 showed that the 31 residues were quite similar to those of known 1,3-DCP-dehalogenases of other organisms, *Arthrobacter* sp. strain AD2 and *Corynebacterium* sp. strain N-1074, although some differences in composition or enzymatic characteristics were observed. The *Km* value and *Vmax* of Deh-PY1 were 2.67 mM and 7.81 μmol/min/mg, respectively, and the optimum reaction temperature and pH were 40–50°C and 9.5–10.5.

Key words —— 1,3-dichloro-2-propanol, biodegradation, *Arthrobacter* sp., dehalogenase

INTRODUCTION

Environmental pollution caused by chlorinated organic compounds is currently a serious problem because of resistance to biodegradation and long-lasting toxicity. Chemical, physical and biological methods have therefore been developed to treat chlorinated organic compounds. The most energy- and resource-saving processes are biological treatment methods, which ingeniously use the microbe's metabolic ability to assimilate or detoxicate these compounds. There are drawbacks, however, such as slow reaction rate or difficulty in controlling the reactor because of the use of biological matter. To overcome these problems, it is important to obtain organisms having excellent ability including high degrading activity for the target compound or stability as the organism for the bioreactor.

Aliphatic halo-alcohols are widely used in industries or experimental laboratories as organic solvents or starting materials for the synthesis of various chemical products including medicines or pesticides. 1,3-dichloro-2-propanol (1,3-DCP) is an important aliphatic halo-alcohol for industrial chemical uses such as in hard resin, celluloid or paint industries.[1–3] The acute or chronic toxicity of various halogenated organic substances is well known and has been documented in detail. 1,3-DCP is known to be associated with liver dysfunction or mucous membrane toxicity.[3,4] Epichlorohydrin, a metabolite of 1,3-DCP, is also reported to have adverse genetic impact.[5]

Halogenated organic compounds are utilizable as a carbon source for growth of microorganisms if they are dehalogenated. Therefore, various studies have been made to isolate organisms with the ability to use such compounds in order to develop a process of eliminating the halogenated pollutants. Microorganisms dechlorinating 1,3-DCP have also been

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isolated and their characteristics investigated.[6–9] However, information on 1,3-DCP-degtrading organisms available for use in a biological control system remains inadequate.

We attempted to isolate bacteria with a good capability to degrade 1,3-DCP and obtained strain PY1 as a possible candidate for use in treatment of wastewater containing this pollutant. This paper reports the bacterial characteristics of PY1 as a 1,3-DCP-degrading bacterium and purification of the dehalogenating enzyme.

MATERIALS AND METHODS

Media ——— Mineral salt medium (MS medium) was prepared by mixing 500 ml of the basal medium shown below, 5 ml of magnesium solution ($MgSO_4 \cdot 7H_2O$, 10 g/l), 5 ml of calcium solution ($CaCl_2 \cdot 2H_2O$, 0.2 g/l), and 490 ml of water which were autoclaved separately. For an enrichment culture to accumulate 1,3-DCP-degrading bacteria, 1,3-DCP was added to the medium as sole carbon source (final concentration of 1000 mg/l). MS agar medium was prepared by adding 12 g of agar to 1 l of the medium. The composition of basal medium was as follows (in grams per liter): $(NH_4)_2SO_4$, 2.0; K_2HPO_4 2.0; $Na_2HPO_4 \cdot 12H_2O$, 15; $NaH_2PO_4 \cdot 2H_2O$, 30; $FeSO_4 \cdot 7H_2O$, 0.002. The dehydrated nutrient broth (NB) and nutrient agar (NA) media were purchased from Nissui Seiyaku, Ltd. (Tokyo, Japan).

Isolation of 1,3-DCP-Degrading Bacteria ——— Bacteria were isolated from twenty-four soil samples obtained from a chemical industry which uses 1,3-DCP. Approximately 1 g of soil sample was suspended in 10 ml of sterilized distilled-water and the suspension was left for about 10 min at room temperature to sediment the soil particles. Five-tenths ml of the supernatant was inoculated into 10 ml of MS medium containing 1,3-DCP as sole carbon source in a screw-capped 30 ml-test tube. In a preliminary experiment with 200 to 2000 mg/l of 1,3-DCP, some soil samples decomposed 1000 mg/l of 1,3-DCP completely within 7 days, whereas more days were needed for decomposition of 2000 mg/l. Therefore, a concentration of 1000 mg/l of 1,3-DCP was used for further surveys. The tube was incubated at 30°C for 7 days by shaking in the dark, and the growth was monitored by measuring the turbidity. After 3 subcultures by transferring an aliquot of culture which showed obvious growth to a new medium, a portion of the culture was streaked on a NA plate. Colonies formed on the plate were subcultured in MS medium containing 1000 mg/l of 1,3-DCP, and the residual amount of 1,3-DCP was assayed (as shown later) to confirm the 1,3-DCP-degrading ability. The isolates cultured in the 1,3-DCP-MS medium were mixed with glycerol (final concentration of 20%) and stored at –80°C until use.

Characterization of Bacteria ——— Isolates were preliminarily characterized based on colony color, morphology, Gram stain, motility, catalase, cytochrome oxidase and indole tests. An isolate, strain PY1, having the highest 1,3-DCP-degrading activity was subjected to further identification by analysis of the 16S rRNA gene. The template DNA from strain PY1 was obtained by the method of Saito and Miura.[10] The 16S rRNA gene of strain PY1 was amplified by PCR using the primer set f27 (5′-AGTTTGATCCTGGCTCAG-3′) and r1490 (5′-GTTACCTTGTTACGACTTC-3′) under standard PCR conditions.[11] The amplified fragment was purified by a QIAquick spin PCR purification kit (QIAGEN GmbH, Hilden, Germany) and sequenced by a Dye terminator cycle sequencing FS Ready Reaction kit (Perkin-Elmer Co., Foster City, CA, U.S.A.) and ABI 373A automatic DNA sequencer (Perkin-Elmer Co.) using 16S-specific primers f27 (5′-AGTTTGATCCTGGCTCAG-3′), f356 (5′-TACGGGAGGCAGCAG-3′), f530 (5′-GTGCCAGCAGCCGCGG-3′), f926 (5′-ACTCAAAGGAATTGACGG-37), and f1111 (5′-GCAACGAGCGCAACCC-3′).[12] DNA sequence was compared with the EMBL, DDBJ and Genbank Nucleotide Sequence databases. The 16S rRNA gene was aligned and edited with the CLUSTAL W program (version 1.5).[13]

Analytical Procedures ——— Gas chromatography to detect 1,3-DCP was performed with a GC-6AM instrument (Shimadzu, Kyoto, Japan) equipped with a 3 m × 3 mm i.d. glass column packed with Silicon DC550 (60–80 mesh chromosorb WAW) and a flame ionization detector (FID). Gas chromatography operating conditions were as follows: column temperature, 150°C; injection and detection temperature, 200°C; carrier gas (N_2) flow rate, 30 ml/min; hydrogen flow rate, 50 ml/min; and air flow rate, 900 ml/min. Chloride ion concentration was determined by an ion electrode method.[14] Bacterial growth was determined by monitoring the optical density at 600 nm (OD_{600}). Colony-forming units (CFU) were determined by inoculating 0.1 ml of sample on an NA plate, incubating the plate at 30°C overnight and counting the colonies formed. Pro-

tein concentration was determined by the method of Lowry *et al*. with bovine serum albumin as a standard.[15)]

1,3-DCP Degradation Test by Living Organisms ——— Five-tenths ml of preculture of isolate was inoculated into 10 ml of MS medium containing an appropriate amount of 1,3-DCP in a screw-capped 30 ml-test tube. The tube was incubated at 30°C in the dark with shaking at 140 rpm and the residual amount of 1,3-DCP was measured by GC-FID periodically as shown above.

Assay of the Dechlorinating Enzyme Activity ——— A mixture (1.0 ml) containing an enzyme preparation, 2 mM 1,3-DCP, 25 mM Tris/H_2SO_4 buffer (TSB, pH 7.0) and 3 mM $MgCl_2$ was incubated at 30°C for 20 min with shaking. The 1,3-DCP dechlorinating activity was determined by measuring the residual amount of 1,3-DCP by GC-FID. One unit of the enzyme was defined as the amount necessary to decompose 1 μmol of 1,3-DCP per minute.

Preparation of Cell-Free Extracts and Purification of 1,3-DCP-Degrading Enzyme ——— Strain PY1 was cultivated in MS medium supplemented with 1000 mg/l of 1,3-DCP as a sole carbon source. All subsequent operations for preparation of the cell-free extract were carried out at 4°C. The cells in the late log phase were harvested by centrifugation at 7000 × *g* for 20 min, washed twice with 50 mM TSB (pH 7.0), then resuspended in the same buffer (0.5 g wet weight/ml) and stored at –80°C until use. The freeze-thawed cell suspension was disrupted with an ultrasonic generator (Nissei, Japan) by 4 min-treatment (repeating a 30 sec-disrupting and 30 sec-cooling cycle). The sonicated cell suspension was centrifuged at 9000 × *g* for 15 min to remove unbroken cells and cell debris and the supernatant was further centrifuged at 100000 × *g* for 30 min. The cell free extract obtained as a clear supernatant by the second centrifugation was used for purification of the dechlorinating enzyme.

Solid ammonium sulfate was added to the cell-free extract to 60% saturation with stirring, and the resulting precipitate was collected by centrifugation at 10000 × *g* for 20 min. The collected precipitate was dissolved in 10 mM TSB (pH 7.0) containing 1 M ammonium sulfate and dialyzed against the same buffer. The dialyzed enzyme solution was applied to a Phenyl-Sepharose column (1.6 × 10 cm, Amersham Pharmacia Biotec, New Jersey, U.S.A.) equilibrated with 10 mM TSB (pH 7.0) containing 1 M ammonium sulfate. The column was washed with the same buffer and then eluted by 10 mM TSB (pH 7.0) containing ammonium sulfate of which the concentration was decreased stepwise from 0.5 to 0 M. The 1,3-DCP dehalogenating activity of the elution was assayed and the active fraction eluted by the ammonium sulfate-free TSB was concentrated by Ultrafiltration Membrane YM 30. The concentrated solution was dialyzed against 10 mM TSB and applied to a Fractogel EMD TMAE-650 (S) column (1.0 × 2 cm, MERCK, Dermstadt, Germany) equilibrated with 10 mM TSB (pH 7.5). The column was washed with the same buffer. The enzyme was eluted with 0 to 0.3 M linear gradient of ammonium sulfate in 10 mM TSB (pH 7.5), and the active fractions observed around 0.13 M ammonium sulfate were collected as the purified enzyme preparation.

Relative Molecular Mass (M_r) Determination ——— The molecular mass (M_r) of the denatured protein was determined by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) performed with Phast Gel gradient 8–25 (Amersham Pharmacia Biotec) by the procedure of Laemmli.[16)] The protein bands were visualized by staining with Coomassie brilliant blue R-250. The M_r of the enzyme subunit was determined by comparing the mobility with that of standard proteins.

The M_r of the native enzyme was estimated by gel filtration on Sephacryl S-200HR column (1.0 × 60 cm, Pharmacia) at a flow rate of 2.0 ml/min with elution of 10 mM TSB (pH 7.0). β-amylase (M_r = 200000), alcohol dehydrogenase (M_r = 150000), ovalbumin (M_r = 45000) and carbonic anhydrase (M_r = 30000) were used for the standard proteins.

N-Terminal Amino Acid Sequence ——— The peptide band of the enzyme separated by SDS-PAGE was electroblotted onto a MiniProBlot™ membrane (Applied Biosystems Japan, Tokyo, Japan). The blotted band was cut from the membrane, and the N-terminal amino acid sequence was determined with a model 491 protein sequencer (Applied Biosystems).

RESULTS

Identification of Isolated 1,3-DCP-Degrading Bacterium

Of the numerous isolates having 1,3-DCP-degrading activity, strain PY1 which had the highest activity was selected for further experiments. When strain PY1 was cultured in MS medium with 7.75 mM 1,3-DCP as sole carbon source, 1,3-DCP was degraded completely within 7 days. Strain PY1

was a motile Gram-positive rod form bacterium which was positive in the catalase test and negative in the oxidase and indole tests. Since 16S rRNA gene analysis of strain PY1 showed 98% homology with those of *Arthrobacter aurescens* and *Arthrobacter ilicis*, respectively, strain PY1 is thought to fall in the classification of an *Arthrobacter* sp.

1,3-DCP-Degrading Ability of Strain PY1

Figure 1 shows the growth of strain PY1, degradation of 1,3-DCP and chloride ion concentration in the culture medium. On the 4th day of cultivation, strain PY1 reached maximum growth and about 90% of 1000 mg/l (7.75 mM) 1,3-DCP was degraded, indicating assimilation of 1,3-DCP by strain PY1. With the degradation of 1,3-DCP, chloride ion was accumulated in the culture medium, finally reaching about 15 mM. Two moles of chloride ion are theoretically liberated from 1 mol of 1,3-DCP by the complete dechlorination. In this experiment, the amount of chloride ions detected was almost the same as the theoretical value, suggesting degradation of 1,3-DCP by dechlorination.

Strain PY1 degraded 1000 mg/l of 1,3-DCP completely within 7 days and the level was elevated by

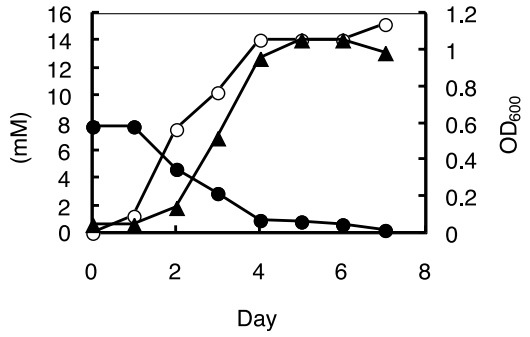

Fig. 1. Growth of Strain PY1 and Release of Chloride Ion Correlated with Degradation of 1,3-DCP

Strain PY1 was cultivated at 30°C for 7 days in MS medium containing 7.75 mM 1,3-DCP. (●), 1,3-DCP; (○), chloride ion; (▲), growth of strain PY1.

acclimatization. Namely, when strain PY1 was subcultured in medium containing 1,3-DCP of which the concentration was stepwise increased by 500 mg/l, the activity increased until 4000 mg/l of 1,3-DCP was degraded within 7 days (data not shown). Acclimatization to more than 4000 mg/l of 1,3-DCP was incomplete, although the bacterium was tolerant against a higher concentration. That is, when strain PY1 was cultivated in MS medium supplemented with 0.2% peptone containing 0–20000 mg/l of 1,3-DCP, the growth was not inhibited by 5000 mg/l of 1,3-DCP, whereas some delay of the growth was observed with the addition of 6000 mg/l and complete growth inhibition by 7000 mg/l.

Effect of the addition of carbon sources other than 1,3-DCP on the 1,3-DCP-degrading activity of strain PY1 is shown in Table 1. When 0.2 or 2% peptone was added to the culture medium, almost complete degradation of 1000 mg/l of 1,3-DCP within 7 days was observed similarly to cultivation without peptone. Carbon concentration of 2% peptone is 20 times higher than that of the 1,3-DCP in the medium, suggesting expression of the high ability of strain PY even in the presence of peptide nutrients which are easily utilized by the organism. Addition of a small amount of glucose (5 mM) or glycerol (10 mM) also did not affect the degrading activity, although some inhibitory effect was observed when a higher amount of glucose (50 mM) or glycerol (100 mM) was added; these amounts were equivalent to 10 times the carbon concentration of 1,3-DCP in the medium.

In Vitro 1,3-DCP-Degrading Activity

Cells of strain PY1 grown in MS medium containing 1,3-DCP were disrupted by ultrasonic treatment and the disrupted suspension was fractionated by differential centrifugation into 3 fractions, 9000 $\times g$ precipitate, 100000 $\times g$ precipitate and 100000 $\times g$ supernatant. As almost 1,3-DCP-degrading activity was observed in the 100000 $\times g$ supernatant (cell

Table 1. Effect of Nutrients on the 1,3-DCP-Degradation Ability of Strain PY1

	Addition of nutrient						
	None	Peptone		Glucose		Glycerol	
		0.2%	2%	5 mM	50 mM	10 mM	100 mM
Degradation (%)[a)]	98.4 ± 1.99	98.9 ± 0.29	97.7 ± 1.24	94.5 ± 1.75	82.9 ± 3.89	93.6 ± 1.14	83.9 ± 1.63

a) The nutrients were added to MS medium containing 1000 mg/l DCP. DCP degradation by strain PY1 was determined after 7 days cultivation in the medium and percent of degradation is indicated.

Table 2. Purification of Deh-PY1 from *Arthrobacter* sp. Strain PY1

	Total protein (mg)	Total activity (mU)	Specific activity (mU/mg-protein)	Yield (%)	Purification (fold)
Cell-free extract	392	4500	11.5	100	—
$(NH_4)_2SO_4$ (60% saturation)	80.4	1840	22.9	41	2.0
Phenyl Sepharose	28.8	1690	58.7	38	5.1
Fractgel EMD TEAE-650(s)	1.7	825	491	5	42.7

free extract), this fraction was used for further experiments. The cell free extract obtained from cells grown in 1000 mg/l 1,3-DCP-MS medium showed the degrading activity of 28.5 mU/mg-protein, whereas the extract from cells grown in nutrient broth had no activity. When peptone (0.2%), glucose (5 mM) or glycerol (10 mM) was added to the 1000 mg/l 1,3-DCP-MS medium, the cell free extracts from cells grown in this medium showed slightly reduced activity: 17.2 mU/mg-protein, 18.5 mU/mg-protein and 15.8 mU/mg-protein, respectively. These results suggest that the 1,3-DCP-degrading enzyme of strain PY1 is an enzyme induced by the existence of 1,3-DCP, but that the strain has the ability to produce the enzyme even in the presence of nutrients which are more utilizable than 1,3-DCP.

Purification of the 1,3-DCP-Degrading Enzyme

The 1,3-DCP-degrading enzyme was purified by ammonium sulfate fractionation, hydrophobic chromatography and anion exchange chromatography. A summary of the purification scheme for the enzyme is shown in Table 2. About 1.7 mg of protein was obtained from the culture of 12 l of MS medium containing 1000 mg/l of 1,3-DCP. The final preparation had specific activity of 491 mU/mg-protein. Figure 2 shows SDS-PAGE of the preparations of each purification step. The final preparation showed a single band of M_r of approximately 20 kDa, suggesting that the enzyme was purified to homogeneous preparation. The purified preparation was applied to a Sephacryl S-200 HR column and M_r of the native enzyme was estimated to be approximately 80 kDa, suggesting that the enzyme was composed of 4 identical subunits. The purified enzyme liberated 2 mol of chloride ion from 1 mol of 1,3-DCP in the assay system, suggesting that it was a dehalogenase (data not shown). Thus the enzyme was designated Deh-PY1.

The N-terminal amino acid sequence of the purified Deh-PY1 was analyzed and aligned with the

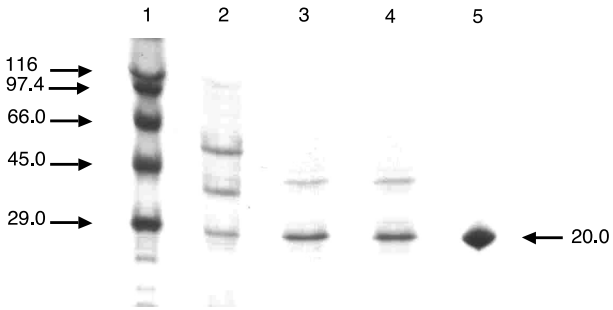

Fig. 2. SDS-PAGE of 1,3-DCP Dehalogenase of *Arthrobacter* sp. Strain PY1

Lanes; 1, molecular mass standards (shown in kDa); 2, Cell-free extract; 3, 60% saturated ammonium sulfate precipitate; 4, Phenyl-Sepharose elute; 5, Fractogel EMD TMAE 650 (S) elute.

DeH-PY1	MKIAL VTHAR HFAGP AAVEA LTRDG YTVVX H
AD2	MKIAL VTHAR HFAGP AAVEA LTQDG YTVVC H
Enzyme Ia	MKIAL VTHAR HFAGP AAVEA LTRDG YTVVC H
Enzyme Ib	MANGR KRE - - MANGR LAGKR VLLTN A

Fig. 3. Comparison of N-Terminal Amino Acid Sequence of Deh-PY1 from Strain PY1, AD2 from *Arthrobacter* sp. Strain AD2,[21] Enzyme Ia and Enzyme Ib from *Corynebacterium* sp. Strain N-1074[16]

X, unidentified residue.

sequences of already-known halogenases, 1,3-DCP-dehalogenase AD2 from *Arthrobacter* sp. strain AD2[17] and Enzyme Ia and Enzyme Ib from *Corynebacterium* sp. strain N-1074[18] (Fig. 3). Thirty-one N-terminal amino acids of Deh-PY1, AD2 and enzyme Ia were the same with some exceptions at the 23rd of AD2 and 30th of Deh-PY1 (unidentified), although they were different from those of enzyme Ib, another halo-alcohol-dehalogenase.

Enzymatic Characteristics of Deh-PY1

The optimum temperature and pH of Deh-PY1 were around 50°C and pH 10.5, respectively (data not shown). *Km* and *Vmax* values for degrading 1,3-

Table 3. Comparison of Haloalcohol Dehalogenases from Various Organisms

Enzyme	Organism	Holoenzeme Molecular mass (kDa)	Subunit Molecular mass (kDa)	Number of bands in native PAGE	*Km* (mM)
Deh-PY1	*Arthrobacter* sp. PY1	80	20	1	2.67
AD2	*Arthrobacter* sp. AD2	65	29	1	8.5
Enzyme Ia	*Corynebacterium* sp.	105	28	1	2.44
Enzyme Ib	N1074	118	32, 35	4	1.03
DehA	*Arthrobacter erithii* H10a	204	31.5, 34	5	0.105
Enzyme	Organism	*Vmax* (mmol/min/mg)	Optimum temperature (°C)	Optimum pH	Reference
Deh-PY1	*Arthrobacter* sp. PY1	7.81	40–50	9.5–10.5	This study
AD2	*Arthrobacter* sp. AD2	9	50	8.5	20
Enzyme Ia	*Corynebacterium* sp.	3.13	55	8.0–9.0	8, 18, 19
Enzyme Ib	N1074	148	45	8.0–9.0	
DehA	*Arthrobacter erithii* H10a	223	50	8.5–10.5	6

Table 4. Relative Activity of Haloalcohol Dehalogenases

Compound	Relative activity (%)				
	Deh-PY1	AD2	Enzyme Ia	Enzyme Ib	DehA
1,3-DCP	100	100	100	100	100
2,3-dichloro-1-propanol	10.0	0	0.3	0.089	0
1,3-dibromo-2-propanol	298	31100	12500	161	60
3-chloro-1,2-propandiol	27.0	10.0	37.5	0.922	0.1
1-chloro-2-propanol	118	11.0	22.7	18.5	27.0
1-bromo-2-propanol	534	3780	N.T.	N.T.	N.T.
2-chloroethanol	20.0	0	0.26	0.132	1.20
2-bromoethanol	83.0	102	57.4	7.91	28.0

Relative activity of Deh-PY1 obtained in this study was compared with values of various haloalcohol dehalogenases appearing in the references; AD2 of *Arthrobacter* sp. strain AD2,[20] Enzymes Ia and Ib of *Corynebacterium* sp. strain N1074,[8,18,19] DehA of *Arthrobacter erithii* H10[6]

DCP under the above optimum conditions are calculated to be 2.67 mM and 7.81 μmol/min/mg-protein, respectively (Table 3).

The relative activity of the Deh-PY1 on various halo-alcohols is shown in Table 4, together with the activity of other bacterial dehalogenases reported. The activity of the Deh-PY1 against 2,3-dichloro-1-propanol, an isomer of 1,3-DCP was only 10% of that against 1,3-DCP, whereas the activity against mono-chloro-propanol was slightly higher. The activity against bromo-substituted compounds was higher than that against corresponding chloro-compounds, although dehalogenases having more activity against bromo-compounds have been reported.

DISCUSSION

A bacterium with high ability to degrade 1,3-DCP was isolated from a soil specimen of a chemical industry which uses 1,3-DCP and was designated strain PY1. Strain PY1 was classified as *Arthrobacter* sp. by 16S rRNA gene analysis, and when it was cultivated in a mineral salt medium containing 1.3-DCP as a sole carbon source, the bacterium liberated a theoretical amount of chloride ion (2 mol from 1 mol of 1,3-DCP) and used the dechlorinated product as a nutrient of the growth. Metabolism of 1,3-DCP to glycerol through epichlorohydrin and 3-chloro-1,2-propanediol has been reported in some bacteria.[6,19,20] A similar pathway and assimilation of 1,3-DCP in strain PY1 is suggested.

The 1,3-DCP degrading activity of strain PY1 was raised to completely decompose 4000 mg/l of the substrate within 7 days by acclimatization. Al-

though other bacteria have been reported to have 1,3-DCP-degrading activity, information on their ability is insufficient. Van den Wijngaard *et al*. reported that *Agrobacterium radiobacter* strain AD1 could degrade 4 mM (about 516 mg/l) of 1,3-DCP completely in 6 days, but the ability of another bacterium, *Arthrobacter* sp. strain AD2, was lower than that of AD1.[17] The strain PY1 we isolated was able to degrade 4000 mg/l (31 mM) of 1,3-DCP, which was equivalent to a carbon concentration of 0.3% glucose. Although it is difficult to make an accurate comparison with the findings of other papers, strain PY1 is thought to be an organism having strong ability and may be a useful candidate for bioremediation of pollution caused by 1,3-DCP.

Wastewater or environmental water contains various organic substances which are effectively utilized by microorganisms as their nutrients. The presence of these substances may inhibit the biodegradation of organic pollutants. Fortunately, however, the inhibitory effect of the nutrients on the 1,3-DCP-degrading activity of strain PY1 was comparatively low. The addition of peptone, a protein digest, showed no inhibitory effect even at 20 times more carbon than 1,3-DCP, although glucose or glycerol showed a relatively weak inhibitory effect. This is an advantage for use of the organism in a biological control system. The 1,3-DCP-dechlorinating enzyme of strain PY1 designated as Deh-PY1 was observed in the cytoplasmic fraction, similar to various haloalcohol-dehalogenases reported in previous papers.[6,8,9,21] Purified Deh-PY1 having a molecular mass of 80 kDa was composed of 4 identical 20 kDa-subunits. The *Km* value and *Vmax* of Deh-PY1 were 2.67 mM and 7.81 μmol/min/mg, respectively, and the optimum reaction temperature and pH were 40–50°C and 9.5–10.5, respectively. Enzymatic characteristics and activity against various haloalcohols of Deh-PY1 are compared with enzymes reported by other authors in Tables 3 and 4. Deh-PY1 was obtained from a bacterium of the genus *Arthrobacter* similar to AD2[9,22] and DehA,[6] but their characteristics differ. Deh-PY1 and AD2 are homo tetramer and dimer, respectively, whereas DehA is a hetero polymer. Differences of a molecular mass of subunits and activity against haloalcohols are also observed in Deh-PY1 and AD2. N-terminal amino acid sequences, however, were quite similar between Deh-PY1 and AD2. Thus, Deh-PY1 of strain PY1 is thought to be a new 1,3-DCP dehalogenase, although very similar to the already-known enzymes.

A bacterium having high ability to degrade 1,3-DCP was isolated and a dehalogenating enzyme was purified from the isolate. Application of the strain and the enzyme to a biological control system of 1,3-DCP pollution are under investigation in our laboratory.

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