

Overexpression and characterisation of non-stereospecific haloacid Dehalogenase E (DehE) of *Rhizobium* sp.

Fahrul Z. Huyop^{1*}, Tan Yea Yusn¹, Marcella Ismail¹,
Roswanira Ab Wahab² and Ronald A. Cooper³

¹Biology Department, University Technology Malaysia, 81310 Skudai, Johor, Malaysia

²Chemistry Department, University Technology Malaysia, 81310 Skudai, Johor, Malaysia

³Biochemistry Department, University of Leicester, United Kingdom

Received 13 October 2003 / Accepted 12 February 2004

Abstract. The *Rhizobium* sp. dehalogenase enzyme (DehE) produced by heterologous expression of the cloned gene in *E. coli* was purified and characterised. The K_m , K_{cat} and the Specificity Constants were determined. The enzyme shows non-stereospecific and could act on D_L and L_L isomer of 2-chloropropionate. The derived amino acid sequence of DehE showed little identity to *Pseudomonas putida* 113 DL-DEX (39% homology) but there was significant identity to two other dehalogenases (DehI and DhlIV) – 72% homology, that act non-selectively on 2-chloropropionate. The amino acid sequence of *dehE* contains 4 cysteine residues but their involvement in the mechanism of catalysis is still unclear based on their end product configuration. The K_m , K_{cat} and Specificity Constants values for brominated compound suggested that this compound is a better substrate for growth and for the DehE enzyme.

Key words: dehalogenase, DehL, DehE, DehD, *Rhizobium* sp.

Abbreviations: D_L -2-CP: D_L -2-chloropropionate; D_L -2-BP: D_L -2-bromopropionate; 2,2-DCP: 2,2-dichloropropionate; D_L -2,3-DCP: D_L -2,3-dichloropropionate; DCA: dichloroacetate; MCA: monochloroacetate; TCA: trichloroacetate; MBA: monobromoacetate; DBA: dibromoacetate; TBA: tribromoacetate; 3-CP: 3-chloropropionate

INTRODUCTION

Halogenated compounds are widely used as herbicides and insecticides. They cause serious environmental pollution and human health problems as a result of their toxicity, persistence and transformation into hazardous metabolites. Haloalkanoate dehalogenases (Deh) are microbial enzymes that catalyse cleavage of the carbon-halogen bond by a hydrolytic mechanism. Various dehalogenases have been found and characterised (Hardman, 1991; Janssen *et al.*, 1994, Pang and Tsang, 2001; Tsang and Sze, 2002 and Weightman *et al.*, 2002). All these enzymes were classified based on their substrate specificity (Slater *et al.*, 1997).

There were more than one dehalogenases in *Rhizobium* sp.. DehL for example acts on L_L -isomer of D_L -2-CP and several other 2-haloacids and forms D_L -lactate whereas DehD acts exclusively on D_L -2-haloacids (e.g. D_L -2-chloropropionate) and forms the corresponding L_L -2-hydroxyacids (e.g. L_L -lactate). The third *Rhizobium* sp. dehalogenase, DehE, catalyse the dehalogenation of both isomers of 2-haloacids such as D_L -2-CP (Leigh, 1986). The reaction of DehE proceeds with inversion of configuration when chiral substrates were used and the enzyme was class 2I (Slater, *et al.*, 1997). Thus, dehalogenase that shows non-stereospecificity are useful not

only for the degradation of toxic halogenated compounds but also for the production of optically active 2-hydroxyalkanoic acids, which are important as starting materials in the chemical industry.

It was curious that *Rhizobium* sp. had DehD and DehL when DehE on its own could act on all the identified substrate and shows non-stereospecific. And only DehE could utilise 2,2-DCP on which the organism was isolated. A possible explanation of this phenomenon was that *dehE* gene had evolved from *dehD* and *dehL* genes and in so doing had gained the additional ability to act on 2,2-DCP. Since more than one dehalogenase has been found in some bacteria growing on haloalkanoates but the physiological significance and role of each enzyme is unclear (Schneider *et al.*, 1991; van der Ploeg *et al.*, 1991; Nardi Dei, *et al.*, 1994). Equally, little is known about the genetic regulation of dehalogenase synthesis. Here we describe expression and further characterisation of DehE using cloned *dehE* gene from *Rhizobium* sp. in *E. coli* system.

*Author for Correspondence.

Mailing address: Biology Department, University Technology Malaysia, 81310 Skudai, Johor, Malaysia. Tel: 07-55 34556 Fax: 07-556 6162; E-mail: fahrul@bio.fs.utm.my

MATERIALS AND METHODS

Bacterial strains, plasmids and growth conditions.

E. coli K-12 strain BL21(DE3) (Studier and Moffat, 1986) was used as a host for plasmid pT7-7 (Tabor and Richardson, 1985). Cells were grown aerobically at 30°C in Luria-Bertani medium (Miller, 1972). Ampicillin (100 µg/ml) and 0.01 mM isopropyl thio-β-D-galactoside (IPTG) were incorporated as appropriate. Growth was followed by measurement of the absorbance at $A_{680\text{nm}}$.

Construction of a clone overexpressing dehE. To enable large amount of dehalogenase E enzyme to be produced an *NdeI* site was incorporated before the initiation codon of the *dehE* coding sequence and an *EcoRI* site downstream of the termination codon were introduced via PCR, using appropriate primers based on the gene sequence. The 0.9 kb PCR product was digested with *NdeI* and *EcoRI* and then ligated into the corresponding sites of pT7-7 to give pJS771.

PCR procedures. Primers used for introducing *NdeI* and *EcoRI* sites for construction of a *dehE* expression plasmid were a) 5' GGAGCACACATATGTTAAACGCTGCG 3' b) 5' AGAAGAATTCTGGTATTCATAAGTAGTCC 3'. The introduced restriction sites are underlined. The amplification reactions contained, in 50 µl with 100 ng template DNA (*Rhizobium sp.* chromosomal DNA), 25 pmol each of primers (a) and (b), 250 pmol each dNTP, 20 mM Tris pH 8.8, 10 mM KCl, 10 mM (NH₄)₂SO₄, 4 mM MgSO₄ and 0.1% (mass/vol.) Triton X-100. After denaturation at 95°C for 5 min, followed by cooling 1U Vent polymerase was added. The PCR reaction was carried out in 30 cycles of the following parameters: denaturation, 95°C for 1 min; annealing, 52°C for 1 min; extension, 72°C for 2 min. The reaction mixture was electrophoresed on a 0.8% agarose gel and the 0.9 kb band extracted, digested with *NdeI* and *EcoRI*, then ligated into similarly digested pT7-7.

Expression of dehE in E. coli and preparation of cell-free extracts.

Extracts were prepared from recombinant cells in the mid-exponential to late-exponential phase of growth ($A_{680\text{nm}}$ 0.4-0.6). Bacteria were harvested by centrifugation at 10 000 g for 10 min at 4°C. The cell pellet was washed in 0.1 vol of 0.1 M Tris acetate (pH 7.6) buffer and suspended in 0.04 vol of the same buffer. The cell suspension was sonicated at 0°C for 30 s at an amplitude of 10 µm, using an MSE soniprep 150 ultrasonicator. Unbroken cells and cell wall debris were removed by centrifugation at 20,000 g for 15 min at 4°C.

For purification of DehE, *E. coli* K-12 strain BL21(DE3) transformed with pJS771 (*dehE*⁺) was grown aerobically at 20°C on Luria broth/ampicillin to an absorbance $A_{680\text{nm}}$ of 0.4 at which point 0.01 mM IPTG was introduced and the

incubation continued for a further 20 h. Extracts were prepared in 0.1 M Tris acetate buffer (pH 7.6). Protein was measured by the biuret method, using crystalline BSA as standard (Gornall *et al.*, 1949).

Purification of DehE. Purification was carried out using a Pharmacia system Fast Protein Liquid Chromatography (FPLC) procedure. Purifications were performed at 4°C in a cold room. Crude extract from BL21(DE3) [pJS771] (*dehE*⁺) prepared as described above were ultracentrifuged at 120,000 g for 90 min at 4°C. An approximately 6 mg protein (6 U enzyme with 2,2-DCP as substrate) was loaded onto a MonoQ HR 5/5 anion exchange column (Pharmacia-Biotech) equilibrated with 20 mM sodium phosphate, 1 mM EDTA, 1 mM dithiothreitol (DTT), 10 % (mass/vol) glycerol (pH 7.6) and eluted at a flow rate of 1 ml/min using a linear gradient from 20 mM to 200 mM sodium phosphate over 15 ml. DehE was eluted at approximately 80mM sodium phosphate.

Gel Filtration analysis. Gel filtration was carried out using two columns of Superose 12 (Pharmacia-Biotech) connected in series. Columns were equilibrated overnight using a buffer containing 20 mM Tris acetate, 0.1 M sodium acetate pH 7.6. The sodium acetate is used to increase the ionic strength and stop any interaction with the gel matrix. Samples (0.2ml) from the MonoQ step were applied to the gel filtration column at approximately 0.5 g protein per-run. The column was run at the flow rate 0.4 ml/min. For molecular weight determination the columns were calibrated using molecular weight standards from SIGMA (USA) and the relative molecular weights were: β-amylase (200 kDa), alcohol dehydrogenase (150 kDa), bovine serum albumin (66 kDa), carbonic anhydrase (29 kDa) and cytochrome C (12.4 kDa).

Assay of dehalogenase activity and kinetic analysis.

The enzyme reaction was carried out at 30°C in a mixture of 5 ml 0.09 M Tris acetate (pH 7.5), substrate and enzyme. Samples were removed at 5 min intervals, and the free halide was determined colorimetrically (Bergman and Sanik, 1957). The colour was allowed to develop for 10 min at room temperature and measured at $A_{460\text{nm}}$. Enzyme activity (1U) was defined as the amount of enzyme that catalyses the formation of 1 µmol halide ion/min. The assay was carried in duplicate.

SDS-PAGE analysis. SDS-PAGE mini-gels were prepared using the Mini-Protean II kit (Biorad). The composition formulation for resolving gel were prepared with a 12% Acrylamide/bisacrylamide; 0.1% Sodium dodecyl sulphate (SDS) in 375 mM Tris.Cl pH 8.8 and were polymerised by the addition of 0.1% ammonium persulphate and 0.05% Tetramethyl-ethylenediamine (TEMED). Stacking gel were prepared at 5% Acrylamide/bisacrylamide in 125 mM Tris.Cl pH 6.8 but were otherwise identical. Samples were prepared

by heating the protein for 2 min at boiling temperature with 0.3 vol. of sample buffer. The sample buffer consisted of 2% SDS; 0.1% bromophenol blue; 10% glycerol; 100 mM DTT in 50mM Tris.Cl pH 6.8. Gels were generally run for 45 minutes at a constant voltage of 200V in a buffer which was 25 mM Tris, 250 mM glycine (pH 8.3) with 0.1% SDS. The protein bands were stained with Coomassie blue R250 solution (comprising 0.5% (w/v) Coomassie blue R250 in 45% (v/v) methanol; 10% acetic acid) for 3 hours. Gels were destained in a solution of 7.5% (v/v) acetic acid; 5% methanol for 16 hours. Gels were calibrated using Mark12 Unstained Standard (Invitrogen).

Computer analysis. Sequence analysis was carried out using the DNA Strider programme. Sequence alignment using the Genetics Computer Group (GCG) package was used to identify regions of similarity between two sequences. Multiple sequence alignment were made using Multialign programme (Corpet, 1988). International databases were searched using the BLAST programme (Altschul *et al.*, 1990) (<http://www.ncbi.nlm.nih.gov>).

RESULTS

DehE enzyme activity. Cells were harvested and extract was prepared and checked for DehE activity. Growth condition and expression temperature other than stated showed lower enzyme activity against 2,2DCP. Using the optimised condition the enzyme activity against 2,2DCP was 1.20 $\mu\text{molCl}/\text{min}/\text{mg}$ protein.

Purification of DehE enzyme. The fraction had 2.7 U enzyme and a specific activity of 2.1 U/mg with 2,2DCP as substrate respectively, with a recovery of 93% in total. Analysis of the fraction by SDS-PAGE showed that at least 95% of the protein seen was accounted for by a 32 kDa band (Figure 1). Based on the protein gel analysis the protein from this one step purification was sufficiently pure for further characterisation.

Molecular weight and substrate structure. The native molecular weight of DehE was estimated to be about 64 kDa by gel filtration. The subunit structure studied by SDS-PAGE was 32 kDa for DehE as shown in Figure 1. From the subunit molecular weight of 32 kDa, this suggests that the protein is dimer.

Substrate specificity and enzyme kinetics. The substrates used were divided into two groups, substrate suitable for growth and substrate for the enzyme. It was found that DehE can act on all of the substrates and does not show any substrate specificity (Table 1).

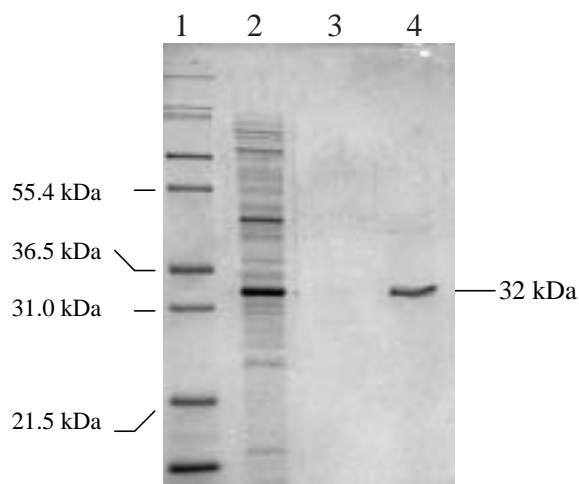


Figure 1. SDS-PAGE analysis of the purification of DehE. Lane 1: Protein markers (Invitrogen); Lane 2: Crude extract of DehE; Lane 3: MonoQ fraction 12; Lane 4: MonoQ fraction 13 (6 μg).

The K_m values for both chlorinated and brominated compounds were compared as in Table 1. The K_m results did not show any significant differences between chlorinated and brominated propionate. However, the K_m values for chloro- and bromoacetate decreased from monohalogenated acetate to trihalogenated acetate. In addition, the K_m for TBA was equivalent to that for TCA. The K_{cat} values for growth substrates varied with 2,2-DCP being the fastest substrate molecule converted to product whereas D-2-CP was the slowest.

The best way to compare the catalytic efficiencies for different substrate by DehE was to compare the ratio K_{cat}/K_m . This parameter is known as the Specificity Constant and was given in Table 1. The results showed all brominated compound has higher Specificity Constant value compared to the chlorinated substrate.

Database analysis of dehalogenases by amino acid sequence comparison. The amino acid sequence of DehE was compared to those from the database (NCBI) and showed high identity to that from *Alcaligenes xylosoxidans* ssp. DhIIIV (72% identity) (Brokamp *et al.*, 1997), DehI from *Pseudomonas putida* PP3 (72% identity) (Topping, 1992) and DL-DEX from *Pseudomonas* sp. strain 113 showed 39% identity (Nardi-Dei *et al.*, 1997) (Figure 2).

Since all these three enzymes were reported to be sensitive to sulphhydryl blocking reagents their protein sequences were compared in order to identify any consensus cysteine residue in the protein (Figure 2). Each of the amino acid sequences of DhIIIV and DehI contain two cysteine residues (at positions 42 and 288), D, L-DEX contains one cysteine residue (at position 178) and DehE contains 4 cysteine

Table 1. Kcat and Specificity Constants for DehE enzyme for all the substrates

Substrate for Growth:	Kcat(sec ⁻¹)	Km	Specificity Constant (M ⁻¹ sec ⁻¹)
D-2CP	08.16	5.20x10 ⁻⁴ M (0.52 mM)	1.56 x 10 ⁴
D-2BP	20.66	4.60 x10 ⁻⁴ M (0.46 mM)	4.40 x 10 ⁴
L-2CP	13.43	4.10x10 ⁻⁴ M (0.41 mM)	3.27 x 10 ⁴
L-2BP	13.43	2.90 x10 ⁻⁴ M (0.29 mM)	4.60 x 10 ⁴
D,L-2CP	10.03	3.50 x10 ⁻⁴ M (0.35 mM)	2.86 x 10 ⁴
D,L2BP	12.40	2.20 x10 ⁻⁴ M (0.22 mM)	5.64 x 10 ⁴
2,2-DCP	05.58	1.90x10 ⁻⁴ M (0.19 mM)	2.94x 10 ⁴
Substrate for Enzyme	Kcat(sec ⁻¹)	Km	Specificity Constant (M ⁻¹ sec ⁻¹)
D,L-2,3DCP	01.44	3.60 x10 ⁻⁴ M (0.36 mM)	0.40 x 10 ⁴
MCA	25.83	1.19 x10 ⁻³ M (1.19 mM)	2.17 x 10 ⁴
DCA	01.65	3.60 x10 ⁻⁴ M (0.36 mM)	0.46 x 10 ⁴
TCA	00.20	3.10 x10 ⁻⁴ M (0.31 mM)	0.65 x 10 ³
MBA	89.90	2.18 x10 ⁻³ M (2.18 mM)	4.12 x 10 ⁴
DBA	14.46	8.80 x10 ⁻⁴ M (0.88 mM)	1.64 x 10 ⁴
TBA	02.06	3.20 x10 ⁻⁴ M (0.32 mM)	0.64 x 10 ⁴

residues (at position 42, 128, 256 and 288). The results showed a conserved cysteine residue between DehE, DhIIV and DehI at position 42 and 288. However, these cysteines were not seen in DL-DEX enzyme.

Pseudomonas putida PP3 was also reported to have another dehalogenase, DehII (Topping, 1992). DehII was classified as type 2I according to Slater *et al.*, (1997) classification. Although DehII is in the same group as DehE from the *Rhizobium sp.*, (Leigh, 1986) however, it did not share any identity to DehE (sequence not shown).

DISCUSSION

DehE enzyme was obtained using cloned *dehE* gene in *E. coli* K-12 strain BL21(DE3) transformed with pJS771 (*dehE*⁺). Optimum condition for expression of the *dehE* gene was at 20°C on Luria broth/ampicillin with 0.01 mM and less enzyme activity detected at temperature above 20°C. The enzyme was purified and characterised. The present investigation showed that DehE enzyme could act on both enantiomers for _D- and _L-2-CP and all chloro- and bromoacetates. This confirmed the previous analysis that DehE purified from crude extract of *Rhizobium sp.* showed non-stereospecific whereas DehD and DehL showed their specificity for each D and L isomers of _{D,L}-2-CP (Leigh, 1986).

DehE was able to act on _{D,L}-2,3-DCP. Only chloride from one position either _D- or _L- was released, presumably from carbon 2 because dehalogenase from *Rhizobium sp.* did not react with 3CP (Allison, 1981). Based on this information presumably the chloride at carbon 3 was not attacked and the product of dehalogenation was proposed to be 2-hydroxy-3-chloropropionate.

DL-DEX of *Pseudomonas putida* strain YL (Lui, *et al.*, 1994), DL-DEX from *Pseudomonas putida* strain 113 (Nardi-Dei, *et al.*, 1997) and DhIIV from *Alcaligenes xyloxydans* ssp. *denitrificans* ABIV (Brokamp *et al.*, 1997) have similar features to those of DehE of *Rhizobium sp.*, in that both act on _D- and _L-2-CP by inversion of the optical configuration of substrate-product and are classed in Class 2I (Slater *et al.*, 1997). Their subunit molecular weights (YL: DL-DEX 36 kDa; 113: DL-DEX 35 kDa; ABIV: DhIIV 32 kDa; *Rhizobium sp.*: DehE 32 kDa) were similar but they were distinct in the subunit structure to that of *Pseudomonas putida* strain YL DL-DEX that has a monomer structure. However, *Rhizobium sp.* DehE and *Pseudomonas putida* strain 113 DL-DEX consist of two identical subunits whereas subunit structure for DhIIV not reported. In addition, the amino acid sequence comparison between these two enzymes (DehE and 113: DL-DEX) showed only 39% identity (Figure 2). The amino acid sequences of DehE and DhIIV showed 72% sequence identity whereas DehI from *Pseudomonas putida* PP3 also showed high percent identity to DehE (72%). DehI from *Pseudomonas putida* PP3 maintains the optical configuration of substrate-product and classified as Class 2R and not in the same class as DehE (Class 2I). Enzymes from Class 2R differ from Class 2I enzymes in their ability to dehalogenate both _D- and _L- isomers with retention of product configuration.

Dehalogenase (DehI) from *Pseudomonas putida* PP3 (Topping, 1992) was estimated to have a native molecular mass of 64 kDa which corresponded to SDS-PAGE analysis of 33 kDa, suggesting possibly a protein dimer in active state. The amino acid sequence of DehE from *Rhizobium sp.* could not be compared to the amino acid sequence of D,L-DEX from *Pseudomonas putida* strain YL DL-DEX because DL-DEX sequence is not available in the database (Lui *et al.*, 1994).

	1		50
DehE	...MLNAAYP	PQISQSDVGG	EMEATYENIR QTLRVPWVAF
DhlIV	...MTNPAYF	PQLSQLDVSQ	EMESTYEDIR LTLRVPWVAF
DehI	...MTNPAYF	PQLSQLDVSQ	EMESTYEDIR LTLRVPWVAF
D, LDEX	MSHRPILKNF	PQVDDHQASQ	KLGDLYNDIH DTLRVPWVAF
			GIRVMSQFPH

	51		100
DehE	YLPVANARTA	EAMSTRYAEQ	AADELRRSL LSTIEPKVDLK
DhlIV	YLPVANARTA	EAMSTRYAEQ	AADELRRSL LSTIEPKVDLK
DehI	YLPVANARTA	EAMSTRYAEQ	AADELRRSL LSTIEPKVDLK
D, LDEX	FVPAAEWALK	PQISTRVYAE	GADKVBREAII
			IPGSAPANPT
			PALLANGWSE

	101		150
DehE	AQIEEVRVRRV	NAPNYGNPKY	IMMITALCES FNLRPVGGG
DhlIV	GEIEKVRVRL	YAPNYGNPKY	LLLITALSES MQMRPVGGA
DehI	GEIEKVRVRL	YAPNYGNPKY	LLLITALSES MQMRPVGGA
D, LDEX	EETAKLKATL	DGLNYGNPKY	LILISANNEA
			WHGRDAGGGA
			GKRLDSVQSE

	151		200
DehE	SVPKGHPEGM	DPLLSLVNAN	EAPPEVQTLK
DhlIV	SIPKGHPEGM	DPLLSLVNAN	EAPPEVQTLK
DehI	SIPKGHPEGM	DPLLSLVNAN	EAPPEVQTLK
D, LDEX	RLPVGLEQV	EKP.HLIDPE	AADDVQCLL
			RDIRDAPLHH
			GPASDFVLA

	201		250
DehE	NWPEFLQIAT	DEALAPVVRT	ETFDLKAREL
DhlIV	NWPKVLQIVT	DEVLARVART	EQYDAKSREL
DehI	NWPKVLQIVT	DEVLARVART	EQYDAKSREL
D, LDEX	AWPDYLEIAP	RDTLKQVALT	TEFELTTSRI
			RKIAREHVRR
			FDGAGGVAMR

	251		300
DehE	ELMSTPTGPE	IAGLTGILFM	YQRFIPDITI
DhlIV	ELMSMLTPNE	IAGLTGILFM	YQRFIADITI
DehI	ELMSMLTPNE	IAGLTGILFM	YQRFIADITI
D, LDEX	DMADRMTPEE	IAGLTGILFM	YNRFTADITV
			AIIRLKQAFG
			SAEDATENKF

	301		
DehE	PVZ.....		
DhlIV	PIZ.....		
DehI	PIZ.....		
D, LDEX	RWVPTKGGZ		

Figure 2. Multiple sequence alignment of *Rhizobium* sp. DehE with *Alcaligenes xylosoxidans* ssp. *denitrificans* ABIV DhlIV [Brokamp *et al.*, 1997], *Pseudomonas putida* PP3 DehI [Topping, 1992] and *Pseudomonas* sp. strain 113 D,L-DEX [Nardi-Dei *et al.*, 1997] [* indicates sequence identity]

According to Weightman *et al.* (1982), there are two different catalytic mechanisms involved in these dehalogenation reactions, one is generalised catalysis, which inverts substrate product configuration, and the other mechanism comprises an active -SH group from a cysteine residue leading to retention of configuration. The first mechanism is thought not to be affected by thiol reagents while the second is vigorously inhibited. There are also enzymes in Class 2I more or less affected by thiol reagents including DehE. This effect should be handled with care in proposing a specific reaction since there are some dehalogenases inhibited, although no cysteine is involved in the dehalogenating reaction. The amino acid sequence of DehE has 4 cysteine residues that two of them clearly matched to DhlIV and DehI. But it is still unclear if they are involved in the mechanism of catalysis since they are grouped in 2I.

There is very little information in the current literature regarding dehalogenase K_m values. Some of the reported values were very high compared to the current investigation. The calculated K_m values of D,L-DEX from *Pseudomonas putida* strain 113 for L-2CP, D-2CP and MCA were 0.9, 4.2

and 4.8mM, respectively (Nardi-Dei *et al.*, 1997). These values were generally high compared to the K_{ms} of the pure DehE enzyme from the cloned *debE* gene from *Rhizobium* sp. with 0.56, 0.85 and 1.88 mM using the same substrates. However, there are no K_m values reported for other dehalogenases from the same group as DehE, DhlIV from *Alcaligenes xylosoxidans* ssp. *denitrificans* ABIV (Brokamp, *et al.*, 1997) and DehII (Topping, 1992).

CONCLUSIONS

The presence of more than one dehalogenase is not fully understood. *Rhizobium* sp. was the only reported organism to make all three forms of dehalogenase and the physiological role for each enzyme is unclear. In current investigation using cloned dehalogenase *debE* gene reconfirmed the previous analysis that dehalogenase enzyme isolated from *Rhizobium* sp. could act on all the identified substrate and shows non-stereospecificity. An investigation of the kinetic properties for example the K_{cat} , K_{ms} and the Specificity Constant values were varied according to the substrate. It has been suggested that brominated compound is always a better substrate compared to chlorinated compound. This agreed to the earlier investigation that the growth of *Rhizobium* sp. in D,L-2BP was faster (doubling time of 5 to 6 hours) whereas growth in 2,2DCP was slower (doubling time 12 hours) (Allison, 1981). Various values of kinetic properties for dehalogenases might be observed as indicated by the presence of more than one dehalogenases in some of the bacterial species. Currently, little is known about the genetic regulation of dehalogenase synthesis and isolation of the regulator gene may shed light on this question.

ACKNOWLEDGEMENTS

This work was supported by the Malaysian Government and Short Term Research Grant: 71874/71925. The organism was originally isolated from garden soil Nottingham University, United Kingdom and the author thanks the LATE Dr. Berry E.K.M. for the isolate.

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