Further Analysis of *Burkholderia pseudomallei* MF2 and Identification of Putative Dehalogenase Gene by PCR

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Abstract: *Halogenated organic compounds are extensively and widely used as pesticides, herbicides, and antibiotics that contribute to the pollution. This research was aimed to further analyze and characterize a bacterium that has the ability to utilize 2,2-dichloropropionic acid (2,2-DCP) as a model to study dehalogenase enzyme production. Microscopic observation, biochemical tests and PCR technique were carried out in order to characterize the isolated bacterium. Strain MF2 showed its ability to grow on 10 mM 2,2-DCP liquid minimal medium with doubling time of 13 h with maximum chloride ion released of 19.8 µmolCl^-/mL. The 16S rDNA analysis suggested that strain MF2 belongs to the genus* Burkholderia. *This was supported by the microscopic observation and biochemical tests. Dehalogenase gene was observed when using only primers $dehI_{for1}$ and $dehI_{rev2}$ derived from group I deh PCR primer sequences, whereas no amplification using dhlB-314-forward and dhlB-637-reverse (group II dehalogenase) and haloacetate dehalogenase (H2-1157-forward and H2-1662-reverse) PCR primer sequences. The results suggested that, possibly, dehalogenase from MF2 was related to group I deh. In conclusion, strain MF2 showed the ability to utilize 2,2-DCP as sole source of carbon and energy. Further analysis revealed the MF2 strain consisted of dehalogenase gene that could be used for degradation of man-made halogenated compounds present in the environment. Using existing dehalogenase PCR primers, it was possible to amplify the dehalogenase genes sequence.*

Keywords: Burkholderia pseudomallei*; 2,2-dichloropropionate; biodegradation; dehalogenase gene; 16S rDNA gene*

■ INTRODUCTION

Environmental contamination from industrial chemicals, herbicide or pesticide from agricultural activities is an ongoing dilemma to the society. 2,2-dichloropropionate (abbreviation known as 2,2-DCP) or Dalapon® is a synthetic halogenated compound used in herbicides. The demand for herbicide in agriculture is increasing and its use is inevitable to improve crop yields in many developing countries. In some areas like in the Pineapple Plantation, Pekan Nenas Johor, Malaysia, it is believed that herbicide use will solve all weed problems. Xenobiotic compounds, mostly man-made chemicals when present in high concentrations in the environment can be very hazardous to the ecosystem and some resistant to degradation [1]. Among these xenobiotics, halogenated compounds are considered very toxic and

may cause enormous problems to human health and the environment. However, the answer to such predicament perhaps lie in the very ground we stand on. It is believed that microorganisms are capable of degrading xenobiotics [2-3].

Soil microorganisms such as *Rhizobium* sp., *Methylobacterium* sp. HJ1 and *Arthrobacter* sp. have been reported to be capable of utilizing such compounds as their sole carbon source for growth while liberating the organically bound chlorine as chloride ions at the same time [4-6]. The molecular structure of the 2,2-DCP consists of three carbons with the molecular formula $CH_3CCl_2CO_2H$ (Fig. 1). The first carbon is a carboxylic functional group (-COOH) and followed by two chloride substituents at C-2 of α carbon position. From chemistry point of view, the α-chlorination is particularly important within the halogenated propionic series because it results in herbicidal activity. Acids chlorinated in other positions do not possess phytotoxic properties unless they are also α-chlorinated. Similarly, increasing chain length reduces the herbicidal activity of the α-chloroacids, for instance 2,2-dichlorohexanoic acid being completely inactive. It has been reported that substitution of other halogens for chloride generally decreases herbicidal activity of these acids [7].

Biodegradation using microorganisms is one of the main and natural processes that help remove xenobiotic chemicals such as chloroaliphatic compounds from the environment [8-9]. Also, microorganisms can benefit from the use of the contaminant as an electron donor and carbon source to support growth. Hydrolytic dehalogenases represent the key position in the degradation of haloaliphatic compounds. These enzymes catalyze the cleavage of carbon-halogen bonds by nucleophilic substitution, replacing the halogen ion by a hydroxyl group derived from water [10]. Dehalogenase enzymes metabolize chlorinated substituents and the halogens are enzymatically removed to form non-halogenated compounds. In cases where biodegradation of halogenated compounds are lacking, it is often due to the inability of microorganisms to effectively metabolize such compounds with chemical structures to which these microbes have yet to be exposed during the course of evolution. In other words, recalcitrance

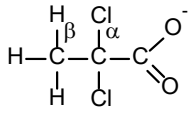

Fig 1. Basic structure of 2,2-DCP

of certain halogenated compounds generally is the result of a lack of efficient metabolic pathways. Nevertheless, there are reports of many microorganisms that have the ability to metabolize xenobiotic organohalogens [8]. This led to the idea that such microorganisms must have evolved their catabolic pathways during the past few decades and is thus suited to study the natural assembly of catabolic routes.

According to Slater et al. [11], dehalogenases can be classified based on substrate specificities. On the other hand, Hill et al. [12] classified dehalogenases based on genetic approach by investigating the diversity and molecular ecology of the dehalogenase genes. Using phylogenetic classification, dehalogenase is divided into group I and group II. These two families are evolutionarily unrelated and together represent almost all of α-halocarboxylic acid (αHA) *deh* genes as described. Group I is a non-stereospecific dehalogenase which act on C2-halogenated short chain aliphatic acids including D-isomer and L-isomer. For example, group I dehalogenases are able to act on D-2-chloropropionic acid (D-2-CP), L-2-chloropropionic acid (L-2-CP), D,L-2-chloropropionic acid (D,L-2-CP) and 2,2-DCP. Meanwhile, dehalogenases of group II is stereospecific that acts only on L-2-CP but not D-2-CP and 2,2-DCP [12]. Other kinds of dehalogenases, termed haloacetate dehalogenase (EC 3.8.1.3) that acts specifically on halogenated acetates to yield glycolate, are also in group II [13]. To understand how these dehalogenases function, *in silico* studies on enzyme-substrate binding need to be carried out provided that full dehalogenase sequence can be obtained in gene isolation by PCR [14-15].

Previous investigations have described that the molecular approach could be used to uncover genus/species and gene of interest like dehalogenase genes of the newly isolated microorganisms [16]. Therefore, the current study is focused on characterizing a bacterium isolated from mud soil from pineapple

plantation that can degrade 2,2-DCP and amplification of the putative dehalogenase gene using dehalogenase degenerate primers. In the current investigation, strain MF2 was further characterized and its putative dehalogenase classification was then determined.

■ EXPERIMENTAL SECTION

Isolation

The minimal growth media for growing bacteria was prepared as described by Hamid et al. [17]. Mud soil taken from a Pineapple agricultural area in Johor (10 g) was added into 250 mL conical flask containing 100 mL of minimal media with 10 mM of 2,2-DCP as the sole carbon and energy source. The bacterial culture was incubated in an incubator shaker for 3–7 days at 30 °C. For solid medium, Oxoid bacteriological agar No.1 (1.5% w/v) was added prior to sterilization. Bacterial culture was streaked onto the agar plate containing the same ingredients as the liquid minimal media. One pure colony was obtained after several subcultures by streaking on solid minimal media. The bacterial strain was characterized *via* morphological/biochemical tests as described in Bergey's Manual of Systematic Bacteriology [18].

Determination of Bacterial Growth

The extent of growth was determined by measuring the absorbance at A_{680nm} and the release of chloride ions was measured at A_{460nm} [19]. Measurement of the free halide released during the dehalogenation reaction was carried out by an adaptation of the method of Bergmann and Sanik [20]. The chloride ion released was defined 1 mM chloride is equivalent to 1 μmol Cl^-/mL.

PCR Amplification of 16S rDNA Gene

The chromosomal DNA was extracted using Qiagen DNA extraction kit as described by the manufacturer. PCR amplification was carried out using universal primers to amplify the 16S rDNA gene as described by Fulton and Cooper [21].

Partial Biochemical Tests

Different types of biochemical tests were carried to ascertain the identity of the bacteria by carrying out spore staining, catalase test, urease test, oxidase test, lactose utilization test, indole test, gelatin hydrolysis, motility test and nitrate reduction test.

PCR Amplification for Putative Dehalogenase Gene

The source of the primers were obtained from group I deh PCR primer sequences $dehI_{for1}$ and $dehI_{rev2}$ [12], *Xanthobacter autotrophicus* (dhlB314 and dhlB 637) belonging to group II *deh* PCR primer sequences and *Moraxella* sp. for primers dehH2-1157 and dehH2-1662 of the haloacetate dehalogenase [22]. The PCR primers and PCR cycles are summarized in Table 1 and 2, respectively.

Table 1. Dehalogenase oligonucleotide primers

Organisms	Genes	Primer sequences	Reference
	Group I Various sources	**$dehI_{for1}$ (Forward):** 5'-ACGCTGCGCGTGCCATGGGT-3' **$dehI_{rev2}$ (Reverse):** 5'-CGCAATCACATGGAAGTCACT-3'	Hill et al. [12]
Xanthobacter autotrophicus	Group II dehalogenase	**dhlB-314 (Forward):** 5'-TCTGGCGGCAGAAGCAGCTGG-3' **dhlB-637 (Reverse):** 5'-CGCGCTTGGCATCGACGCTGATG-3'	van Der Ploeg et al. [22]
Moroxella sp.	Haloacetate dehalogenase H-2	**dehH2-1157 (Forward):** 5'-CGGCACCCTCTACGATGTGCATTCGG-3' **dehH2-1662 (Reverse):** 5'-CATCCCATGGATTCGACGATACAAAGA-3'	Kawasaki et al. [13]

Table 2. PCR cycles for amplification of putative dehalogenase gene

Segments	Cycles	Temperature (°C)	Duration
1	1	94	2 min 30 sec
2	35	94	30 sec
		55	30 sec
		72	4 min
3	1	72	10 min
4	1	4	∞

DNA Sequencing and Molecular Analysis

The PCR products were purified using Promega Wizard® SV Gel and PCR Cleanup system. After purification, all PCR products were sent for sequencing at SOYGEN BIOTECHNOLOGY, Istanbul (Turkey).

Scanning Electron Microscopy (SEM)

Colonies of bacterial strain MF2 grown on solid media supplemented with 2,2-DCP for 24 h were excised as small agar blocks of 0.5 cm^3. The colonies were pre-fixed with 25% glutaraldehyde in 100 mM phosphate buffer (pH 7.2) for 2 h and then post fixed with 1% osmium tetroxide in the same buffer. The fixed cells were dehydrated in a series of increasing ethanol concentrations (30–95%) for 15 min and then in 100% ethanol for 20 min. The cells were substituted with absolute isoamyl acetate for 15 min and air-dried. The cells were coated with gold by using a gold sputter coater and examined with a Hitachi S.2500C (Hitachi Co., Japan) scanning electron microscopy.

■ RESULTS AND DISCUSSION

Analysis of Growth on 2,2-DCP Minimal Medium and Chloride Ion Released

The bacteria were screened on solid media containing 10 mM 2,2-DCP as a carbon source. After 7 days incubation, a pure colony was observed on 10 mM 2,2-DCP solid minimal medium. This colony was then grown in 10 mM 2,2-DCP liquid minimal medium. The bacterium showed the ability to utilize 2,2-DCP as sole source of carbon under aerobic conditions with doubling time of approximately 13 h (Fig. 2). Analysis of the chloride ions released was also determined with the maximum chloride released estimated at 19.8 µmol Cl^-/mL.

16S rDNA Gene Sequence Analysis

The 16S rDNA PCR product showed that approximately 1500 bp DNA band was amplified. All the 1544 bp 16S rDNA gene sequences were analyzed using BLASTn option (http:www.ncbi.nlm.nih.gov/BLAST/). The sequence matched with maximum identity of 99% to the bacterium *Burkholderia pseudomallei* (data not shown). Therefore, the current organism was designated as *Burkholderia pseudomallei* strain MF2.

Characterization based on biochemical properties and scanning electron microscopy (SEM) (Fig. 3) revealed the basic cellular morphology of the bacteria on

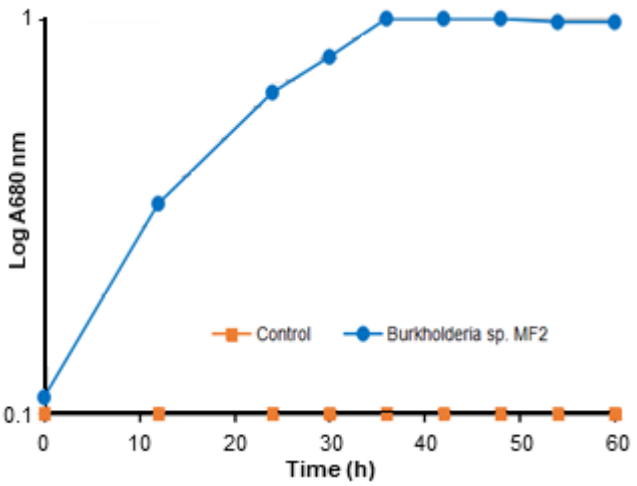

Fig 2. Maximum growth curve of *Burkholderia* sp. MF2 on 10 mM 2,2-DCP minimal medium. Control (*E. coli*) showing no growth in 10 mM 2,2-DCP minimal medium

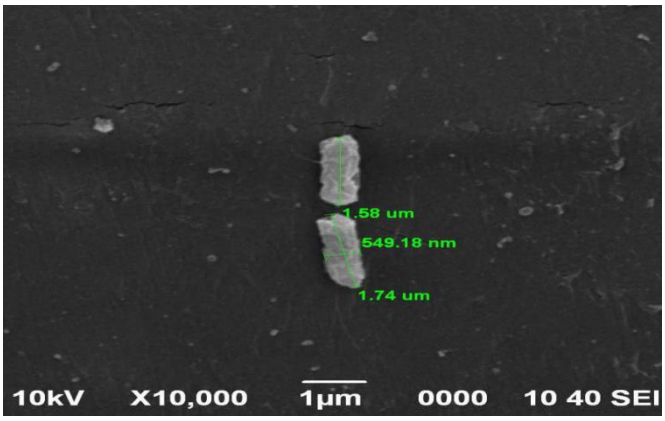

Fig 3. Electron microscopy analysis of strain MF2 appear as short rods with approximate length of 1.6–1.7 µm; width 0.55 µm (549 nm)

Table 3. Morphological and biochemical characterization of strain MF2

Properties	Details
Cell shape	rod
Size	length of 1.6~1.7 μm; width 0.55 μm
Colony morphology	Smooth, mucoid and somewhat elevated
Gram staining	Gram Negative
Spore staining	-
Oxygen requirement	Aerobic
Catalase	+
Oxidase	+
Urease	+
Gelatin hydrolysis	+
Citrate	+
Lactose utilization	-
Motility test	+
Nitrate reduction	-
Identity	*Burkholderia* sp.

+: positive, - : negative

solid minimal medium after 5 days incubation period at 30 °C. The bacteria were rod-shaped with colonies showing smooth, mucoid and somewhat elevated edges. Gram staining confirmed that strain MF2 was rod-shaped, Gram negative bacteria. Details of the physiological and biochemical properties of strain MF2 are depicted in Table 3.

Amplification of Putative Dehalogenase Gene

The PCR reaction was carried out as previously described and the PCR product was observed using gel electrophoresis. The result showed a single band of approximately 396 bp was amplified using primers related to group I dehalogenase. On the other hand, no amplification of DNA band was detected for the group II dehalogenase and haloacetate dehalogenase H-2.

Sequencing and Analysis of Putative Dehalogenase Gene

The partial sequence of putative dehalogenase was successfully sequenced and analyzed showing 360 bp DNA sequence (Fig. 4). The DNA Open Reading Frame (ORF) is a graphical analysis tool that can be used to find possible functional structural genes. The ORF Finder encodes for 120 amino acids only. The deduced sequence was short without start and stop codon. In order to check the identity of the current amino acids, multiple sequence analysis was carried out among both

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1    GCGATCGCGATCTTGGACGCGTTGAGGAGGATCCCGACAGCTTGCAAACATTTTGTATGG 60
1    AlaIleAlaIleLeuAspAlaLeuArgArgIleProThrAlaCysLysHisPheValTrp 20

61   GTTTTAACTGATCCAAAACTCCTTGCTGTCTCACTGAATTGGATTCAACCCGTCCTCAAG 120
21   ValLeuThrAspProLysLeuLeuAlaValSerLeuAsnTrpIleGlnProValLeuLys 40

121  AGCGATCTTTTTCTTGACTACGCCACCGGGCTCGTGCTTAAGTCTGACGGGGAAGATGAA 180
41   SerAspLeuPheLeuAspTyrAlaThrGlyLeuValLeuLysSerAspGlyGluAspGlu 60

181  ACCAGGAAAGTTTCGTCTCGCTTCAGATTTGCAGATGGTTTGACGCTGCTGGCACTCTGT 240
61   ThrArgLysValSerSerArgPheArgPheAlaAspGlyLeuThrLeuLeuAlaLeuCys 80

241  CTGGAACCAATCATTCGACCTGCTTCTTCTGCCGGAAGAATCCCTTGGGTTGCTTTATAC 300
81   LeuGluProIleIleArgProAlaSerSerAlaGlyArgIleProTrpValAlaLeuTyr 100

301  CCATTCGACGAAGTACCATCGGTGTTACGGGAGCTCCGAGGCCGGGGGCTCAAGCTAGCA 360
101  IleLeuSerAsnGlySerProHisSerIleAsnAlaValValSerAsnAlaGlyLeuArg 120
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Fig 4. Partial sequence of nucleotide and deduced amino acid of putative dehalogenase from strain MF2

groups I and II dehalogenases. The results showed there were no sequence identities with the known dehalogenases either group I or II, suggesting current isolate might be of a different kind of dehalogenase group. However, further analysis is needed to re-confirm this.

Burkholderia sp. can be found in the natural environment, such as soils, rhizospheres, water and from infected humans by melioidosis [23-26]. Very few halogenated aliphatic acids supported growth of the organisms. According to Watanabe et al. [27], there are differences between the bacteria isolated in the laboratory by enrichment and the bacteria performing the biodegradation *in situ*. The bacteria involved in biodegradation tend to be neglected more often when investigating pure culture isolates, since they are not always enriched in the process [28].

Burkholderia pseudomallei strain MF2 that was isolated from mud soil could grow on 2,2-DCP as sole source of carbon and energy. To allow growth on 2,2-DCP, there are some basic criteria to be fulfilled. First, the organism must either possess or synthesize dehalogenase which is capable of removing the substituent halogen(s) from halogenated compounds. Next, the dehalogenation product should be non-toxic and easily converted to an intermediate via the organism's central metabolic pathway. The halogenated compound should be able to enter cells either passively or by active transport in order to reach the site of dehalogenase activity. Last but not least, the halogenated compound should be non-toxic to the organism at normal intracellular concentrations [11].

This study sequenced the 1.5-kb 16S rDNA gene. The results of the sequence was analyzed by sequence comparisons to the sequence in the NCBI database. The phylogenetic analysis of 16S rDNA nucleotide sequence suggests the identity of the current isolate. Morphological and biochemical tests showed that MF2 is a rod-shaped Gram-negative bacteria. Based on the Bergey's manual of determinative bacteriology [18], the biochemical test supported the 16S rDNA indicating that strain MF2 belongs to the genus *Burkholderia* sp.

Cells doubling time of MF2 was calculated to be 13 h in 10 mM 2,2-DCP with concomitant release of chloride ions in the growth medium. The growth was relatively slower than that of *Serratia* sp [29], and more or less similar to the growth of *Rhizobium* sp. [30]. MF2 utilized 2,2-DCP as sole carbon source and it was believed to have eliminated the chlorine atom from the halogenated compound and produced pyruvate as a main product similar to what has been reported earlier by Slater et al. [11]. The maximum chloride ion released was 19.8 μmol Cl^-/mL, with almost all carbon source consumed after 42 h growth. It can be said that the MF2 strain exhibit a rather slow rate of 2,2-DCP utilization which could be attributed to a poor uptake system and/or low expression of the dehalogenase enzyme [31-35].

PCR amplification was used to detect partial dehalogenase genes. Group I *deh* genes, are specific towards both D and L forms of the substrate while group II *deh* genes are only active on the L form. Successful amplification of the putative dehalogenase gene was obtained using primers from group I suggesting the dehalogenase from MF2 might fall in the same category. In contrast, Bagherbaigi et al. [36] reported that strain S1 grew well on α/β–haloalkanoic acids (α/β-HA) and only group II primers [22] from *Xanthobacter autotrophicus* - dhlB314 and dhlB637 showed amplification. This observation was possibly due to L-2-haloacid dehalogenases being more common than group I dehalogenases. On the other hand, two possible reasons why using group I primers could not amplify the S1 gene: (1) the primers might not be universal for some bacteria, and (2) only a single dehalogenase gene may be present that acts on both αHA and βHA.

Rhizobium sp. RC1 was reported to contain more than one dehalogenase [34-35]. DehE or D,L-haloacid dehalogenase from *Rhizobium*sp. RC1 is unique because it can act on chiral carbons of both enantiomers. It is curious why *Rhizobium* sp. RC1 has more than one dehalogenases, when DehE alone can act on all of the substrates that DehD and DehL can also act on. Our current findings suggested that only a single dehalogenase may be present based on an *in silico* study by docking simulation [16,37-39]. Based on these findings, it can be inferred that some key catalytic residues of DehE is similar to that of dehalogenase of S1 that can grow on 3CP. However, more work involving

gene cloning and protein analysis are required for further characterization.

■ CONCLUSION

In conclusion, a molecular approach can be used to screen new genes of interest provided that the specific universal primers has no limitation. This study is the first study to investigate the presence of a putative dehalogenase gene in *Burkholderia pseudomallei* strain MF2 isolated from mud soil, associated with growth on 2,2-DCP or αHA. Using molecular tools, primers belonging to group I or II dehalogenases are possibly able to probe dehalogenase gene(s) present in the newly isolated microorganisms in the soil community.

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■ REFERENCES

[1] Rieger, P.G., Meier, H.M., Gerle, M., Vogt, U., Groth, T., and Knackmuss, H.J., 2002, Xenobiotics in the environment: Present and future strategies to obviate the problem of biological persistence, *J. Biotechnol.*, 94 (1), 101–123.

[2] van Pée, K.H., and Unversucht, S., 2003, Biological dehalogenation and halogenation reactions, *Chemosphere*, 52 (2), 299–312.

[3] Karpouzas, D.G., and Singh, B.K., 2006, Microbial degradation of organophosphorus xenobiotics: Metabolic pathways and molecular basis, *Adv. Microb. Physiol.*, 51, 119–225.

[4] Huyop, F., Yusn, T.Y., Ismail, M., Wahab, R.A., and Cooper, R.A., 2004, Overexpression and characterisation of non-stereospecific haloacid dehalogenase E (DehE) of *Rhizobium* sp, *Asia Pac. J. Mol. Biol. Biotechnol.*, 12 (1-2), 15–20.

[5] Huyop, F., Jing, N.H., and Cooper, R.A., 2008, Overexpression, purification and analysis of dehalogenase D of *Rhizobium* sp, *Can. J. Pure Appl. Sci.*, 2 (2), 389–392.

[6] Edbeib, M.F., Wahab, R.A., and Huyop, F., 2016, Characterization of an α-haloalkanoic acid–degrading *Pseudomonas aeruginosa* MX1 isolated from contaminated seawater, *Biorem. J.*, 20 (2), 89–97.

[7] Leasure, J.K., 1964, Metabolism of herbicides, halogenated aliphatic acids, *J. Agric. Food Chem.*, 12 (1), 40–43.

[8] Sinha, S., Chattopadhyay, P., Pan, I., Chatterjee, S., Chanda, P., Bandyopadhyay, D., Das, K., and Sen, S.K., 2009, Microbial transformation of xenobiotics for environmental bioremediation, *Afr. J. Biotechnol.*, 8 (22), 6016–6027.

[9] Edbeib, M.F., Wahab, R.A., Kaya, Y., and Huyop, F., 2017, In silico characterization of a novel dehalogenase (DehHX) from the halophile *Pseudomonas halophila* HX isolated from Tuz Gölü Lake, Turkey: Insights into a hypersaline-adapted dehalogenase, *Ann. Microbiol.*, 67 (5), 371–382.

[10] Schwarze, R., Brokamp, A., and Schmidt, R.J.F., 1997, Isolation and characterization of dehalogenases from 2,2-dichloropropionate-degrading soil bacteria, *Curr. Microbiol.*, 34 (2), 103–109.

[11] Slater, J.H., Bull, A.T., and Hardman, D.J., 1996, Microbial dehalogenation of halogenated alkanoic acids, alcohols and alkanes, *Adv. Microb. Physiol.*, 38, 133–176.

[12] Hill, K.E., Marchesi, J.R., and Weightman, A.J., 1999, Investigation of two evolutionarily unrelated halocarboxylic acid dehalogenase gene families, *J. Bacteriol.*, 181 (8), 2535–2547.

[13] Kawasaki, H., Tsuda, K., Matsushita, I., and Tonomura, K., 1992, Lack of homology between two haloacetate dehalogenase genes encoded on a plasmid from *Moraxella* sp. strain B, *J. Gen. Microbiol.*, 138 (7), 1317–1323.

[14] Adamu, A., Wahab, R.A., Shamsir, M.S., Aliyu, F., and Huyop, F., 2017, Deciphering the catalytic amino acid residues of l-2-haloacid dehalogenase (DehL) from *Rhizobium* sp. RC1: An *in silico* analysis, *Comput. Biol. Chem.*, 70, 125–132.

[15] Adamu, A., Shamsir, M.S., Wahab, R.A., Parvizpour, S., and Huyop, F., 2017, Multi-template homology-based structural model of L-2-haloacid dehalogenase (DehL) from *Rhizobium* sp. RC1, *J. Biomol. Struct. Dyn.*, 35 (15), 3285–3296.

[16] Nemati, M., Abdulghader, H.F., Gicana, R.G., Lamis, R.J.S., Ibrahim, N., Hamid, A.A.A., and Huyop, F.Z., 2013, Identification of putative Cof-like hydrolase associated with dehalogenase in *Enterobacter cloacae* MN1 isolated from the contaminated sea-side area of the Philippines, *Malays. J. Microbiol.*, 9 (3), 253–259.

[17] Hamid, A.A.A., Hamdan, S., Ariffin, S.H., and Huyop, F., 2010, Molecular prediction of dehalogenase producing microorganism using 16S rDNA analysis of 2,2-dichloropropionate (Dalapon) degrading bacterium isolated from volcanic soil, *J. Biol. Sci.*, 10 (3), 190–199.

[18] Bergey, D.H., and Holt, J.G., 1994, *Bergey's Manual of Determinative Bacteriology*, 9th Ed., Williams and Wilkins, Baltimore.

[19] Alomar, D., Hamid, A.A.A., Khosrowabadi, E., Gicana, R.G., Lamis, R.J., Huyop, F., and Tengku Abdul Hamid, T.H., 2014, Molecular characterization of monochloroacetate-degrading *Arthrobacter* sp. strain D2 isolated from Universiti Teknologi Malaysia agricultural area, *Biorem. J.*, 18 (1), 12–19.

[20] Bergmann, J., and Sanik, J.J., 1957, Determination of trace amounts of chlorine in naphtha, *Anal. Chem.*, 29 (2), 241–243.

[21] Fulton, C.K., and Cooper, R.A., 2005, Catabolism of sulfamate by *Mycobacterium* sp. CF1, *Environ. Microbiol.*, 7 (3), 378–381.

[22] van der Ploeg, J., van Hall, G., and Janssen, D.B., 1991, Characterization of the haloacid dehalogenase from *Xanthobacter autotrophicus* GJ10 and sequencing of the dhlB gene, *J. Bacteriol.*, 173 (24), 7925–7933.

[23] Fortin, N., Fulthorpe, R.R., Allen, D.G., and Greer, C.W., 1998, Molecular analysis of bacterial isolates and total community DNA from kraft pulp mill effluent treatment systems, *Can. J. Microbiol.*, 44 (6), 537–546.

[24] Dance, D.A.B., Limmathurotsakul, D., and Currie, B.J., 2017, *Burkholderia pseudomallei*: Challenges for the clinical microbiology laboratory–A response from the front line, *J. Clin. Microbiol.*, 55 (3), 980–982.

[25] Vial, L., Chapalain, A., Groleau, M.C., and Déziel, E., 2011, The various lifestyles of the *Burkholderia cepacia* complex species: A tribute to adaptation, *Environ. Microbiol.*, 13 (1), 1–12.

[26] Coenye, T., and Andamme, P., 2003, Diversity and significance of *Burkholderia* species occupying diverse ecological niches, *Environ. Microbiol.*, 5 (9), 719–729.

[27] Watanabe, K., Teramoto, M., Futamata, H., and Harayama, S., 1998, Molecular detection, isolation, and physiological characterization of functionally dominant phenol-degrading bacteria in activated sludge, *Appl. Environ. Microbiol.*, 64 (11), 4396–4402.

[28] Watanabe, K., and Baker, P.W., 2000, Environmentally relevant microorganisms, *J. Biosci. Bioeng.*, 89 (1), 1–11.

[29] Abel, E., Ibrahim, N., and Huyop, F., 2012, Identification of *Serratia marcescens* SE1 and determination of its herbicide 2,2-dichloropropionate (2,2-DCP) degradation potential, *Malays. J. Microbiol.*, 8 (4), 259–264.

[30] Allison, N., Skinner, A.J., and Cooper, R.A., 1983, The dehalogenases of a 2,2-dichloropropionate-degrading bacterium, *J. Gen. Microbiol.*, 129 (5), 1283–1293.

[31] Abel, E., Ibrahim, N., and Huyop, F., 2012, Identification of *Serratia marcescens* SE1 and determination of its herbicide 2,2-dichloropropionate (2,2-DCP) degradation potential, *Malays. J. Microbiol.*, 8 (4), 259–264.

[32] Abel, E., Pakingking Jr., R.V., Pagador, G., Wint, M.T., and Huyop, F., 2012, Characteristics of

dehalogenase from bacteria isolated from the gut of pond-reared rohu (*Labeo rohita*) Juveniles in Myanmar, *Adv. Biosci. Biotechnol.*, 3, 353–361.

[33] Jing, N.H., Wahab, R.A., Hamdan, S., and Huyop, F., 2010, Cloning and DNA sequence analysis of the alhoalkanoic permease uptake gene from *Rhizobium* sp. RC 1, *Biotechnology*, 9 (3), 319–325.

[34] Su, X., and Tsang, J.S.H., 2013, Existence of a robust haloacid transport system in a *Burkholderia* species bacterium, *Biochim. Biophys. Acta, Biomembr.*, 1828 (2), 187–192.

[35] Musa, M.A., Wahab, R.A., and Huyop, F., 2018, Homology modelling and *in silico* substrate-binding analysis of a *Rhizobium* sp. RC1 haloalkanoic acid permease, *Biotechnol. Biotechnol. Equip.*, 32 (2), 339–349.

[36] Bagherbaigi, S., Gicana, R.G., Lamis, R.J., Nemati, M., and Huyop, F., 2013, Characterisation of *Arthrobacter* sp. S1 that can degrade α and β-haloalkanoic acids isolated from contaminated soil, *Ann. Microbiol.*, 63 (4), 1363–1369.

[37] Cairns, S.S., Cornish, A., and Cooper, R.A., 1996, Cloning, sequencing and expression in *Escherichia coli* of two *Rhizobium* sp. genes encoding haloalkanoate dehalogenases of opposite stereospecificity, *Eur. J. Biochem.*, 235 (3), 744–749.

[38] Hamid, A.A.A., Wong, E.L., Joyce-Tan, K.H., Shamsir, M.S., Tengku Abdul Hamid, T.H., and Huyop, F., 2013, Molecular modelling and functional studies of the non-stereospecific α-haloalkanoic acid dehalogenase (DehE) from *Rhizobium* sp. RC1 and its association with 3-chloropropionic acid (β-chlorinated aliphatic acid), *Biotechnol. Biotechnol. Equip.*, 27 (2), 3725–3736.

[39] Batumalaie, K., Edbeib, M.F., Mahat, N.A., Huyop, F., and Wahab, R.A., 2018, In silico and empirical approaches toward understanding the structural adaptation of the alkaline-stable lipase KV1 from *Acinetobacter haemolyticus*, *J. Biomol. Struct. Dyn.*, 36 (12), 3077–3093.