Genomic Analysis for Haloacid Dehalogenase in *Bacillus megaterium* WSH-002

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**ABSTRACT**

Bacterial dehalogenation is one of the processes that can reduce environmental pollutions. The attributes of *B. megaterium* that can grow in a polluted environment suggested that its genome contains pollutant degrading genes. To date, there were no reports related to dehalogenase in *B. megaterium* WSH-002 and how it was regulated. Therefore, the presence of environmentally important genes that can detoxify organohalogenics in many microbial genomes, including *B. megaterium* WSH-002 will be investigated. The genome sequence of *B. megaterium* WSH-002 was retrieved from NCBI databases. It was then annotated through the RAST server to identify all the putative dehalogenase gene sequences. The selected gene sequence was converted into amino and went through BLASTp via UniProt database. The highest percentage identity of the amino acid sequence to any dehalogenases was subjected to further identification of specific dehalogenase domain using InterPro Scan server. The results from genome annotations have shown its potential for bioremediation due to the presence of putative dehalogenase protein. Only one type of haloacid dehalogenase was identified. It was classified as haloacid dehalogenase type II because its amino acid sequence is highly identical with HAD_type_II and HAD_L2-DEX. The study concluded that the genome of *B. megaterium* WSH-002 contains a haloacid dehalogenase gene that is useful for the biodegradation of halogenated compounds. In the future, further investigation on the expression of the dehalogenase gene as recombinant protein and to study its protein structure and functions will be considered.

**Keywords:** *Bacillus megaterium* WSH-002, Genomics, Haloacid dehalogenase

**Introduction**

Halogenated compounds are widely found in the environments such as lakes, soil, groundwater, and rivers [1]. These compounds can be naturally produced by microorganisms or being produced by chemically synthesised [2]. The synthetic halogenated compound is being produced as active ingredients for the production of herbicides, pesticides, and organic solvents [3-5]. Haloalkaneic acids are one of the compounds widely used in the agriculture industry. It has the properties of being toxic to the environment, which could cause harmful effects to human health [6]. Several human diseases were caused by these organo-halogen compounds, including digestive disorders, oral infections, organ damage, toxic reproduction, skin and respiratory irritations [7, 8]. Malaysia is one of the countries in the Asia Pacific that uses pesticides extensively in many agricultural activities. Approximately 1.5 million hectares of land were used for rubber tree cultivation and 0.6 hectares for oil palm trees [9]. As being studied by Awang and his colleagues [9], the extensive usage of pesticides in agriculture accounted for nearly 50% of the total number of 5,152 cases of human poisoning in Malaysia. Statistically, the number of these toxic compounds gradually increased from 50 naturally produced compounds in 1968 to more than 5,000 in 2015 and surprisingly still increasing [10].

Microbial dehalogenases have been widely studied and proven to degrade various halogenated compounds [11-13]. These dehalogenases have been grouped into hydrolytic, haloalcohol, and...
calfactor-dependent [14]. Haloacid dehalogenase is classified under hydrolytic dehalogenase which hydrolyses haloalkanoic acids and is converted into hydroxyl compound [15]. The process of dehalogenation is designated as the initial step in the degradative pathway and is the most crucial in degrading chlorinated aliphatics. The mechanism involves cleaving the halogen bonds by nucleophilic substitution, replacing the halogen ion with hydroxyl group derived from water. In addition, dehalogenation involves in degradation of chlorinated aliphatic acids such as α-chloro substituted of D- and L-haloalkanoates (2,2-dichloropropionate and/or D- and L-chloropropionate) and haloacetates (monochloroacetate, dichloroacetate, and tri-chloroacetate) [16, 17]. Bacillus megaterium is a Gram-positive bacterium, ubiquitous from soil to seawater, sediment, rice paddies, honey, and dried food [18]. This bacterium was considered an ideal organism for industrial application used for more than 50 years [18]. Plasmids in Bacillus sp. commonly carrying genes involved in the degradation of toxic compounds, biocontrol, antibiotic, and heavy metal resistance gene and are transferable among genus or species [19-22]. Interestingly, B. megaterium was able to degrade halogenated compounds as a carbon source through the production of dehalogenase enzymes and deserved further investigation [23-27].

According to World Health Organization (WHO), genomics is defined as studying genes and their functions [28]. Microbial genomes encompass all chromosomal and extrachromosomal genetic material. Microbial genomics in bacteria is considered very diverse [29]. Bacterial genomes usually, consists of a single circular chromosome but some species also contain more than one chromosome, such as in Deinococcus radiodurans, or linear chromosomes such as in Bacillus subtilis strain, or a combination of linear and circular chromosomes such as in Agrobacterium tumefaciens [30-32]. The study of microbial genomes helps us better understand the genetic composition that contributes to their tangible characteristics that can be further utilized for specific functions [33]. For instance, microbial dehalogenation can be seen as interesting potential outcomes for the bioremediation process and from a genomics and bioinformatics perspective. Recent genomic research has been used to identify gene and metabolic pathways, essential for the whole gene expression to investigate the co-expressed genes [34, 35].

The current study will ascertain partial genetic organisation related to dehalogenases and their operon to regulate dehalogenases. There are no reports on haloacid dehalogenase in B. megaterium strain WSH-002. However, many literatures reported B. megaterium were able to produce dehalogenase-like enzymes and limited studies explained the use of whole-genome sequencing to elucidate genetic sources of pollutant degradation potential [36, 37]. In the most recent report, B. megaterium strain BHS1 was found to contain haloacid dehalogenase type II gene (dehLBHS1) [38] and the strain BHS1 was reported closely related to B. megaterium WSH-002 [39]. Hence, it can be hypothesised that B. megaterium WSH-002 contains a dehalogenase gene which is potential for bioremediation. This study screens and partially annotates the whole genome for possible putative dehalogenase genes in B. megaterium strain WSH-002. This will shed light in the future on the genetic organisation and regulation of dehalogenases for haloacid degradation.

**Material and Methods**

**Genome retrieval and genome annotation**

The genome sequence of B. megaterium WSH-002 with the Accession Number of CP003017 was obtained from the National Center for Biotechnology Information (NCBI) databases (www.ncbi.nlm.nih.gov/) and downloaded as FASTA format. The genome sequence of B. megaterium WSH-002 was then uploaded in Rapid Annotation using Subsystem Technology (RAST) server (rast.nmpdr.org/) to perform genome annotation [40]. The setting parameter for genome annotation was set as default. After completion, the annotated genome sequence was downloaded as an Excel file (xls).

**Screening for putative haloacid dehalogenase gene**

The screening on the genome sequence was to search for the putative haloacid dehalogenase genes. The identified putative haloacid dehalogenase gene was translated into amino acid sequence followed by protein BLAST search using Uniprot databases [41, 42]. For further analyses, the highest amino acid sequence identity to any related dehalogenases will be selected.
Determining of Protein Domain and Families of Putative Haloacid Dehalogenase

The selected putative haloacid dehalogenase gene sequence from B. megaterium WSH-002 was further analysed to identify and predict the protein family domain using InterPro Scan online software (www.ebi.ac.uk/interpro) [43].

Results and Discussions
Screening for Putative Haloacid Dehalogenase Gene from B. megaterium WSH-002

The annotated genome was downloaded as an xls file and the process of screening the possible putative haloacid dehalogenase sequence was done manually. In total, at least 12 possible putative haloacid dehalogenase genes were found in the genome, as shown in Table 1. The longest sequence was gene locus BMWSH_2800 (789 bp) and the shortest BMWSH_2297 (270 bp).

All twelve genes were BLASTn using NCBI databases. It was found that only 6 gene sequences showed the highest identity to dehalogenase genes, BMWSH_1544 (97.6%), BMWSH_2800 (99.2%), BMWSH_3713 (95.1%), BMWSH_4074 (99.6%), BMWSH_4378 (98.2%) and BMWSH_4521 (99.5%) as shown in Table 2.

BMWSH_1544 (97.6%) showed that B. megaterium WSH-002 has highly similar to B. megaterium ATCC 12872/QMB1551 since most of the gene sequences found in B. megaterium WSH-002 was homologous to B. megaterium ATCC12872/QMB1551 [44].

Meanwhile, there were several gene loci of putative haloacid dehalogenase that have shown results of other than dehalogenases (BMWSH_0693, BMWSH_0746, BMWSH_1820, BMWSH_2297, BMWSH_4020). For example, gene locus BMWSH_0693 has a high similarity to HD domain-containing protein (98.4%). This domain was classified as a superfamily that contributes the major functionality of phosphohydrolase which catalyses both metal dependant and independent phosphomonoesterase and phosphodiesterase reactions for various ranges of substrates including CCA-adding enzymes, uridylyl transferases dGTPase, polyA polymerases, and stringent-response guanosine polyphosphate hydrolase [45, 46].

Table 1. List of all possible putative haloacid dehalogenase genes in the genome Bacillus megaterium WSH-002

| No. | Gene Product | CDS Location (bp) | Gene Locus ID | Strand (+/-) | Length (bp) |
|-----|--------------|-------------------|---------------|--------------|-------------|
| 1.  | Hydrolase (HAD superfamily), YqeK | 685729_686301 | BMWSH_0693 | +           | 573         |
| 2.  | HAD-superfamily hydrolase-like protein | 735987_735412 | BMWSH_0746 | -           | 576         |
| 3.  | Hydrolase, haloacid dehalogenase-like family | 1473045_1473788 | BMWSH_1544 | +           | 744         |
| 4.  | HAD-superfamily hydrolase, subfamily IIB | 1698317_1697544 | BMWSH_1820 | -           | 774         |
| 5.  | Hydrolase, HAD superfamily | 2061582_2061313 | BMWSH_2297 | -           | 270         |
| 6.  | 2-haloalkanoic acid dehalogenase (EC 3.8.1.2) | 2289639_2290310 | BMWSH_2542 | +           | 672         |
| 7.  | 2-haloalkanoic acid dehalogenase (EC 3.8.1.2) | 2520695_2521483 | BMWSH_2800 | +           | 789         |
| 8.  | Hydrolase, haloacid dehalogenase-like family protein BCZK2594 | 3349662_3348991 | BMWSH_3713 | -           | 672         |
| 9.  | Hydrolase (HAD superfamily) in the cluster with DUF1447 | 3641408_3640626 | BMWSH_4020 | -           | 783         |
| 10. | Hydrolase, haloacid dehalogenase-like family | 3693107_3693904 | BMWSH_4074 | +           | 798         |
| 11. | 2-haloalkanoic acid dehalogenase (EC 3.8.1.2) | 3980565_3979906 | BMWSH_4378 | -           | 660         |
| 12. | Hydrolase, haloacid dehalogenase-like family | 4127620_4126961 | BMWSH_4521 | -           | 660         |
Besides that, gene locus BMWSH_0746 also has a high similarity to nucleotidase (99.0%). This protein consists of a domain that is remotely related to HAD-like hydrolase domains and SCOP HAD-like hydrolase superfamily. This enzyme has a significant role in the digestion of nucleic acids since it catalyses the hydrolysis of a nucleotide into a nucleoside and a phosphate [47]. For instance, the conversion of adenosine monophosphate to adenosine. Most bacteria can utilize nucleotides as sources of purines or pyrimidines but these have to be dephosphorylated by extracellular nucleotidases before entering the cell [48].

According to Table 2, gene locus BMWSH_2297 is similar to cysteine-rich CPCC domain protein (97.8%). This protein family was identified as an uncharacterised functional protein although it can be found in bacteria, archaea, eukaryotes, and viruses. This type of domain constitutes six conserved cysteines and a conserved CPCC sequence motif. In contrast, the gene locus BMWSH_4020 is highly similar to Cof-like hydrolase [49]. Cof-like hydrolase associated with dehalogenase from Enterobacter allows this bacterium to grow on a medium containing halogenated compound as the sole carbon source [49]. However, the specialized function of this protein remains obscure. Interestingly, gene locus BMWSH_2542 has a similarity to L-2-haloalkanoic acid dehalogenase belongs to Bacillus sp. AFS018417 [50] although the percentage of protein identity is insignificant. L-2-haloalkanoic acid dehalogenase is common among many bacteria species but not D-2-haloalkanoic acid dehalogen

### Table 2. The results of BLASTp from UniProt Databases

| Gene Locus ID | Protein Name                                      | Species                                | Accession Number | Percentage Identity |
|---------------|--------------------------------------------------|----------------------------------------|------------------|---------------------|
| BMWSH_0693   | HD domain-containing protein                      | Bacillus megaterium (strain ATCC 12872 / QMB1551) | D5DSX3           | 98.4%               |
| BMWSH_0746   | Nucleotidase                                      | Bacillus megaterium (strain ATCC 12872 / QMB1551) | D5DSD5           | 99.0%               |
| BMWSH_1544   | Haloacid dehalogenase-like hydrolase             | Bacillus megaterium (strain ATCC 12872 / QMB1551) | D5E2W1           | 97.6%               |
| BMWSH_1820   | HAD-superfamily hydrolase, subfamily IIB         | Bacillus megaterium (strain ATCC 12872 / QMB1551) | D5DZR9           | 88.7%               |
| BMWSH_2297   | Cys_rich_CPCC domain-containing protein          | Bacillus megaterium (strain ATCC 12872 / QMB1551) | D5DW41           | 97.8%               |
| BMWSH_2542   | L-2-haloalkanoic acid dehalogenase               | Bacillus sp. AFS018417                 | A0A2A8S566       | 67.4%               |
| BMWSH_2800   | HAD-superfamily hydrolase, subfamily IA, variant 3 | Bacillus megaterium (strain ATCC 12872 / QMB1551) | D5DTA0           | 99.2%               |
| BMWSH_3713   | Haloacid dehalogenase-like hydrolase domain protein | Bacillus megaterium (strain ATCC 12872 / QMB1551) | D5E2P3           | 95.1%               |
| BMWSH_4020   | Cof-like hydrolase                               | Bacillus megaterium (strain ATCC 12872 / QMB1551) | D5E0B2           | 98.8%               |
| BMWSH_4074   | HAD-superfamily hydrolase, subfamily IIB         | Bacillus megaterium (strain ATCC 12872 / QMB1551) | D5E062           | 99.6%               |
| BMWSH_4378   | Haloacid dehalogenase, type II                   | Bacillus megaterium (strain ATCC 12872 / QMB1551) | D5E2F6           | 98.2%               |
| BMWSH_4521   | Haloacid dehalogenase-like hydrolase family protein | Bacillus megaterium (strain ATCC 12872 / QMB1551) | D5E193           | 99.5%               |
Further analysis of gene locus with putative dehalogenase genes

The six highest percentage of gene locus was further analysed and summarised shown in Table 3. The gene locus BMWSH_1544 and BMWSH_3713 were classified as haloacid dehalogenase-like hydrolase (IPR041492). This protein belongs to the large superfamily of diverse enzymes that catalyse carbon or phosphoryl group transfer reaction on a wide range of substrates using aspartate as an active site in nucleophilic catalysis [53]. However, their molecular function was unable to predict. Gene locus BMWSH_4074 and BMWSH_4521 were known as Cof family (IPR000150) and HAD hydrolase, subfamily IA (IPR006439). Both gene loci showed the same molecular function, which has hydrolase activity (GO:0016787). Catalyzing the hydrolysis of various bonds such as C-O, C-N, C-C, phosphoric anhydride bonds [54, 55] but did not predict any specialized functions of the proteins. Furthermore, gene locus BMWSH_2800 was classified as phosphoserine phosphatase YsaA (IPR044266) which has the function of hydrolase specifically acting on ester bonds in the phosphate-containing compound which catalyses the dephosphorylation reaction of phosphoserine yielding serine and phosphate (GO:0004647) [56, 57]. However, its functional properties were significantly different from haloacid dehalogenases. The gene locus BMWSH_4378 was classified as haloacid dehalogenase type II because of the presence of conserved domain HAD_L2-DEX (cd02588) and HAD_type_II (TIGR01428) found in putative haloacid dehalogenase protein sequence (Table 4). According to Marchler-Bauer et al. [58], the domain HAD_type_II in Conserved Domain Databases (CDD) was classified as 2-haloalkanoic acid dehalogenase type II which catalyses the hydrolytic dehalogenation of L-2-haloalkanoic acids to produce the corresponding D-2-hydroxyalkanoic acids [58, 59]. This domain belongs to HAD superfamily of aspartate-nucleophile hydrolase [60]. This domain was also conserved in DhIB and Hdl IVa from Xanthobacter autotrophicus GJ10 and Burkholderia cepacia MBA4 respectively [61, 62]. The HAD_L2-DEX domain in the CDD was classified as hydrolase activity, acting on acid halide bonds, in C-halide compounds. **The following information was retrieved from InterPro databases**

| Gene Locus ID | Accession Number | Classification | Accession Number | Description |
|--------------|------------------|----------------|------------------|-------------|
| BMWSH_1544  | IPR041492        | Haloacid dehalogenase-like hydrolase | -               | hydrolase activity |
| BMWSH_2800  | IPR044266        | Phosphoserine phosphatase YsaA | GO:0016787 | phosphoserine phosphatase activity |
| BMWSH_3713  | IPR041492        | Haloacid dehalogenase-like hydrolase | -               | hydrolase activity |
| BMWSH_4074  | IPR000150        | Cof family     | GO:0016787 | hydrolase activity |
| BMWSH_4521  | IPR006439        | HAD hydrolase, subfamily IA | GO:0016787 | hydrolase activity |

Table 3. The prediction of protein family classification and their molecular function

**ases [51, 52].**
to occur as homodimers including L-DEX from *Pseudomonas* sp. YL [66]. The dimer of DhlB is more tightly packed compared to L-DEX due to the absence of a small atrium subdomain that provides a major contribution to the dimer interface [63]. Most of the amino acid residues involved in dimerization are conserved in the family of L-2-haloacid dehalogenase [67]. Therefore, this study suggested that predicted domains found in gene locus BMWSH_4378 contribute to the functional properties of haloacid dehalogenase type II that exhibit the function of hydrolase specifically acting on acid halide bonds in halogenated compounds [68]. Due to this evidence, the gene locus BMWSH_4378 as putative haloacid dehalogenase was subjected to future studies for its structure and functions.

**Conclusion**

DNA annotation or genome annotation allows the identifying the locations of genes and all of the coding regions in a genome and determining what

| Accession Number | Classification of Protein Families | Short Name | Contributing Member Database Entries |
|------------------|-----------------------------------|------------|--------------------------------------|
| IPR006439        | HAD hydrolase, subfamily IA (superfamily) | HAD-SF_hydro_IA | InterPro |
| TIGR01493        | HAD hydrolase, family IA, variant 2 (subfamily) | HAD-SF-IA-v2 | TIGRFAMS |
| TIGR01549        | HAD hydrolase, family IA, variant 1 (subfamily) | HAD-SF-IA-v1 | TIGRFAMS |
| PR00413          | HAD family (family) | HADHALOGNASE | PRINTS |
| IPR006328        | L-2-Haloacid dehalogenase (superfamily) | 2-HAD | InterPro |
| cd02588          | L-2-haloacid dehalogenase (domain) | HAD_L2-DEX | CDD |
| TIGR01428        | haloacid dehalogenase, type II (family) | HAD_type_II | TIGRFAMS |
| IPR041492        | Haloacid dehalogenase-like hydrolase (superfamily) | HAD_2 | InterPro |
| PF13419          | Haloacid dehalogenase-like hydrolase (family) | HAD_2 | Pfam |
| IPR023214        | HAD superfamily (superfamily) | HAD_sf | InterPro |
| G3DSA:3.40.50.1000 | HAD superfamily/HAD-like (Homologous superfamily) | - | CATH-Gene3D |
| IPR023198        | Phosphoglycolate phosphatase-like, domain 2 (domain) | PGP-like_dom2 | InterPro |
| G3DSA:1.10.150.240 | Putative phosphatase; domain 2 (Homologous superfamily) | - | CATH-Gene3D |
| IPR036412        | HAD-like superfamily (superfamily) | HAD-like_sf | InterPro |
| SSF56784         | HAD-like (Homologous superfamily) | - | SUPERFAMILY |
| SFLDF00045       | 2-haloacid dehalogenase (family) | 2-haloacid_dehalogenase | SFLD |
| SFLDG01135       | C1.5.6: HAD, Beta-PGM, Phosphatase Like (family) | C1.5.6:_HAD_Beta-PGM_Phospha | SFLD |
| PTHR43316        | Hydrolase, Haloacid Dehalogenase-Related (family) | - | PANTHER |
| PTHR43316: SF3   | Haloacid Dehalogenase, Type II (AFU ORTHOLOGUE AFUA_2G07750)-Related (family) | - | PANTHER |

**The following information was retrieved from InterPro databases.**
those genes do. Here, putative dehalogenases were identified from the whole genome studies in *B. megaterium* WSH-002. It was curious why a single bacterium may have more than one dehalogenase with similar or different functions. However, the presence of dehalogenase regulatory and uptake genes was not detected. This study allows researchers to study not only the genes which code for the important proteins that keep the cell to survive in the highly contaminated area but also the regions of the DNA that have other important roles, such as the regulation of the specific gene(s) particularly dehalogenases need to be investigated.

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