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Research Article

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 organohalogens 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]. Haloalkanoic 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

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cofactor-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 trichloroacetate) [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) (Table 2). 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

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%

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

Gene Locus ID	Protein Family		Molecular Function	
	Accession Number	Classification	Accession Number	Description
BMWSH_1544	IPR041492	Haloacid dehalogenase-like hydrolase	-	-
BMWSH_2800	IPR044266	Phosphoserine phosphatase YsaA	GO:0016787	hydrolase activity
			GO:0004647	phosphoserine phosphatase activity
BMWSH_3713	IPR041492	Haloacid dehalogenase-like hydrolase	-	-
BMWSH_4074	IPR000150	Cof family	GO:0016787	hydrolase activity
BMWSH_4378	IPR006328	L-2-Haloacid dehalogenase	GO:0016787	hydrolase activity
			GO:0019120	hydrolase activity, acting on acid halide bonds, in C-halide compounds
BMWSH_4521	IPR006439	HAD hydrolase, subfamily IA	GO:0016787	hydrolase activity

**The following information was retrieved from InterPro databases

ases [51, 52].

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 DhlB 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 L-2-haloacid dehalogenase and has demonstrated several features including active sites, homodimers, and HAD signature motifs. The conserved amino acid residue that acts as an active site nucleophile was aspartate residue (Asp8) found in DhlB and L-DEX from *X. autotrophicus* and *Pseudomonas* sp. YL respectively [63, 64]. Upon the attack of the substrate, this residue forms an ester intermediate and is subsequently hydrolysed by a water molecule [65]. The members of the L-DEX family have been reported

Table 4. The domain of 2-haloalkanoic acid dehalogenase protein gene BMWSH_4378 of *B. megaterium* WSH-002

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, Phospha-tase 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.

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

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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