

Research Article

## The Genetic Organisation and Control of Putative Dehalogenase Gene Expression in *Bacillus megaterium* WSH-002

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**ABSTRACT**

Dehalogenase-producing bacterium has been extensively studied due to their ability to reduce pollution in the environment. A previous study reported that *Bacillus megaterium* WSH-002 contains a putative haloacid dehalogenase type II gene, therefore, the presence of other genes associated with the dehalogenase regulatory gene function and uptake of halogenated compounds are expected. In the *Bacillus megaterium* WSH-002 whole genome, type II dehalogenase (DehWSH002) and two other genes related to dehalogenase regulatory and haloacid uptake genes were identified suggesting its ability to control the expression of putative dehalogenase(s) and the uptake of halogenated compounds into the cells. The phylogenetic analysis showed that DehWSH002 shared common features with DehLBHS1 of *Bacillus megaterium* strain BHS1 and Had protein of *Bacillus megaterium* strain ATCC12872/QMB1551. The study concluded that the genome of *Bacillus megaterium* WSH-002 contains a dehalogenase gene designated as *dehWSH002* that is useful for biodegradation. In addition, further investigation of the adjacent genes suggested the presence of dehalogenase regulatory gene (*dehR*) and an uptake gene (*dehP*) in a single genetic organisation.

**Keywords:** *Bacillus megaterium* WSH-002, Dehalogenase gene (*dehWSH002*), Genetic organisation, Haloacid uptake gene (*dehP*), Regulatory gene (*dehR*)

### Introduction

Halogenated organic compound that has been widely used in industries as organic solvents, pharmaceuticals and agrochemicals have adversely affected the environment and health of other organisms. This is due to the toxicity and persistence of these chemicals [1, 2]. These organo-halogen compounds can be detoxified through the dehalogenation process by removing the halide ion that causes the compound to be toxic and recalcitrant [3]. Many dehalogenases-producing bacterium have benefited from their ability to utilize the organo-halogen compounds as their sole carbon source and thus reduce environmental pollution [4-11]. By far, *Rhizobium* sp. RC1 is the only bacterium that produces three dehalogenases, DehD, DehE, and DehL, each with different substrate specificities [5, 12]. Recent study by Wahhab et al. has elucidated haloacid dehalogenase type II gene sequence in *Bacillus megaterium*

BHS1 and their genome comparison analysis has demonstrated that it is closely related to *B. megaterium* WSH-002 [13]. Previous findings reported that, several putative haloacid dehalogenase related genes were found in *B. megaterium* WSH-002 through genes screening and annotation [14]. Interestingly, only one haloacid dehalogenase gene sequence was identified. To date, the genetic organisation of *B. megaterium* WSH-002 related to dehalogenase will be proposed from its full genome sequence, apart from investigating the phylogenetic relationship of haloacid dehalogenase type II of *B. megaterium* WSH-002 with the well establish dehalogenase producing bacteria.

### Material and Methods

#### Genome Sequence of Haloacid Dehalogenase

In this study, the gene locus of BMWSH\_4378 of *B. megaterium* WSH-002 was used for genetic

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locations of dehalogenase related genes.

**Genetic organisation of haloacid dehalogenase gene**

The location of the haloacid dehalogenase was referred to SEEDViewer databases to identify the intergenic genes and the length of gap between the regulatory gene and the uptake gene [15]. The genome mapping of haloacid dehalogenase was drawn to depict the location of the haloacid dehalogenase gene and the genes associated with dehalogenases.

**Phylogenetic analysis of haloacid dehalogenase gene**

The phylogenetic analysis was used to study the evolutionary relationship between organisms and identify the similarities and differences in terms of their gene or amino acid sequence [16]. The phylogenetic tree was constructed using MEGA X software based on dehalogenase amino acid sequences of each organism listed in Table 1 [17]. The parameter used was the Neighbour Joining Method with 1,000 bootstraps of replications [18, 19].

**Results and Discussion**

**The Gene location for dehalogenase, haloacid uptake, and regulatory**

The full genome sequence of *B. megaterium* was further analysed to identify the regulatory gene that governs the expression of dehalogenase. The genetic organisation of *B. megaterium* WSH-

002 related to dehalogenase has not yet been revealed from its genome sequence. In this study, the location of the regulatory gene and uptake permease gene were investigated. As shown in Table 2, the regulatory gene (BMWSH\_3724) that encodes for the transcriptional regulator was identified, and this gene was designated as ‘*dehR*’. Based on its location, it was hypothesised that this gene could control the expression of the dehalogenase gene (BMWSH\_4378) designated as ‘*dehWSH002*’. The presence of genes associated with the uptake of haloacid into the cells for biodegradation were identified as putative transport system permease protein (BMWSH\_3846) and an uncharacterised MFS-type transporter (BMWSH\_3847). Both were hypothesised as uptake genes and were closely located with a gap of 1556 bp. However, InterPro Scan analysis predicted the uncharacterized MFS-type transporter as an efflux system for lactose, glucose, cellobiose, maltose and other sugar compounds [32].

Meanwhile, the putative permease transport system was predicted as a Major Facilitator Superfamily (MFS) that target a wide range of substrates, including carbohydrates, amino acids, lipids, and other small molecules [33]. As studied by Jing [34], haloacid permease (DehrP) of *Rhizobium* sp. RC1 contains a member of the Major Facilitator Superfamily (MFS) transport protein which is a conserved domain consisting of a sugar signature and demonstrated that it is closely related to Metabolite: H<sup>+</sup> Symporter (MHS) family of haloacid transporters from *B. caribensis* MBA4

Table 1. List of dehalogenases found in various bacteria species for phylogenetic analysis

NCBI Accession Number	Gene Name	Organism	Length of amino acid	References
CAA46976	<i>hdl IVa</i>	<i>Burkholderia cepacia</i> MBA4	231	[20]
AAA27590	<i>dhlB</i>	<i>Xanthobacter autotrophicus</i> GJ10	253	[21]
CAA63861	<i>dhl VII</i>	<i>Pseudomonas fluorescens</i>	227	[22]
AAA25832	<i>hadL</i>	<i>Pseudomonas putida</i> AJ1	227	[23]
AAB32245	<i>L-DEX</i>	<i>Pseudomonas</i> sp. (strain YL)	232	[24]
BAA04474	<i>dehH109</i>	<i>Pseudomonas putida</i> strain 109	224	[25]
CAB38090	<i>dehII</i>	<i>Pseudomonas putida</i> PP3	227	[26]
CAA63794	<i>dehL</i>	<i>Rhizobium</i> sp. RC1	279	[27]
AAA63640	<i>dehCI</i>	<i>Pseudomonas</i> sp. CBS3	227	[28]
AMN16523	<i>bcfcd1</i>	<i>B. cereus</i> strain Indb1	283	[29]
WP_045293204	<i>dehLBHS1</i>	<i>B. megaterium</i> strain BHS1	219	[13]
-	<i>dehWH2</i>	<i>B. cereus</i> strain WH2	203	[11]
-	<i>dehGS1</i>	<i>B. megaterium</i> strain GS1	104	[30]
QOW03316	<i>dehH2</i>	<i>B. megaterium</i> strain H2	254	[10]
ADE68040	<i>had</i>	<i>B. megaterium</i> strain ATCC12872/QMB1551	219	[31]

Table 2. The location of BMWSH\_4378 of 2-haloalkanoic acid dehalogenase protein in the genome *Bacillus megaterium* strain WSH-002 (Accession Number: CP003017)

Gene Product	CDS Location	Gene Locus ID	Length (bp)	Length Gap Between Genes (bp)
Transcriptional regulator	3355158...3355739	BMWSH_3724 <i>dehR</i>	582	296
N1-spermidine/spermine acetyltransferase PaiA	3356559...3356035	BMWSH_3725	525	123,277
Hypothetical protein	3480207...3479836	BMWSH_3845	372	1643
Putative transport system permease protein	3480627...3481850	BMWSH_3846 <i>dehp</i>	1224	1556
Uncharacterized MFS-type transporter	3483397...3482183	BMWSH_3847	1215	496,295
Stress response protein CsbD	3979692...3979874	BMWSH_4377	183	32
2-haloalkanoic acid dehalogenase (EC 3.8.1.2)	3980565...3979906	BMWSH_4378 <i>dehWSH002</i>	660	129
Hypothetical protein	3981119...3980694	BMWSH_4379	426	-

\*\*The following information was retrieved from SEED Viewer database.

Table 3. The distance between transcriptional regulator, putative permease transport system and dehalogenase in genome of *Bacillus megaterium* WSH-002

Gene Product	CDS Location	Gene Locus ID	Length (bp)	Size of gap between genes(bp)
Transcriptional regulator	3355158...3355739	BMWSH_3724 <i>dehR</i>	582	124,888 ( <i>dehp</i> ) 624,167 ( <i>dehWSH002</i> )
Putative transport system permease protein	3480627...3481850	BMWSH_3846 <i>dehp</i>	1224	498,056
2-haloalkanoic acid dehalogenase (EC 3.8.1.2)	3980565...3979906	BMWSH_4378 <i>dehWSH002</i>	660	-

which is a subfamily of MFS [35-37]. Therefore, this study suggests that the putative transport system permease has a higher potential role as a haloacid uptake protein and designated as 'Dehp'. Table 2 and Table 3 show the position of putative *dehR*, *dehp* and *dehWSH002* genes. They were not closely related to each other. The gap between *dehR* and *dehp* was 124,888 bp whereas *dehR* and *dehWSH002* was 624,167 bp and the gap between *dehp* and *dehWSH002* was 498,056 bp. The location of *dehWSH002* was closed to the gene encoding stress response protein CsbD (BMWSH\_4377) and a hypothetical protein (BMWSH\_4379) with a gap of 32 bp and 129 bp, respectively. The transcriptional regulator gene (*dehR*) location was sited near spermidine/spermine acetyltransferase PaiA (BMWSH\_3725) with

a gap of 296 bp. In this study, only one haloacid dehalogenase gene was found in *B. megaterium* WSH-002, whereas in *Rhizobium* sp. RC1 revealed that more than one dehalogenase genes were present [12].

Figure 1, is the proposed location of the putative dehalogenase gene (*dehWSH002*) adjacent to the regulator gene (*dehR*) and an uptake gene (*dehp*) with an opposite direction of transcription. As studied by Huyop and Cooper [12], the dehalogenase E gene (*dehE*) was a neighbouring gene to *dehR* [12]. Wahhab et al. [13] demonstrated the haloacid dehalogenase gene (*dehLBHS1*) alongside its neighbouring gene, hypothetical protein, capsular biosynthesis protein and polyglutamate capsule biosynthesis protein CAPE in genome *B. megaterium* BHS1. The HTH-type transcriptional

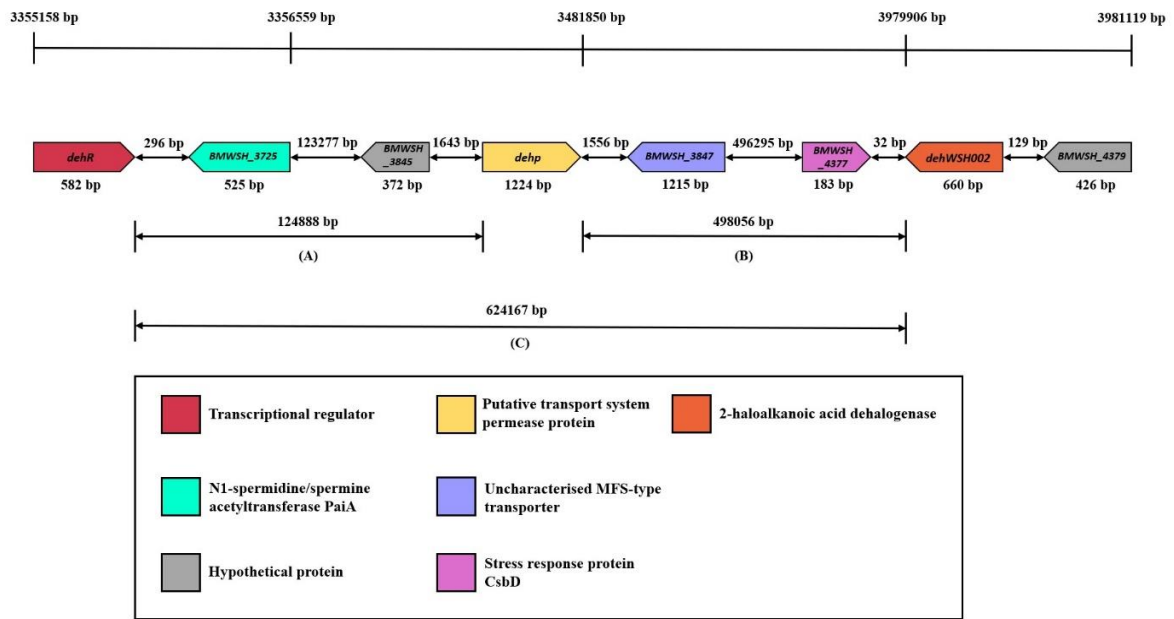


Figure 1. A propose genetic organisation of dehalogenase related genes (*dehR*, *dehp* and *dehWSH002*) based on the genome mapping of *Bacillus megaterium* WSH-002 (CDS region of 3355158 bp to 3981119 bp). The colours depict different type of protein encoding genes. The arrows with pentagon shaped showing gene locus and direction of transcription. The double arrows symbol indicates length of gap between genes in base pair (bp) unit. (A) The length of gap between transcriptional regulator and putative permease. (B) The length of gap between putative permease and haloacid dehalogenase (*DehWSH002*). (C) The length of gap between transcriptional regulator and haloacid dehalogenase.

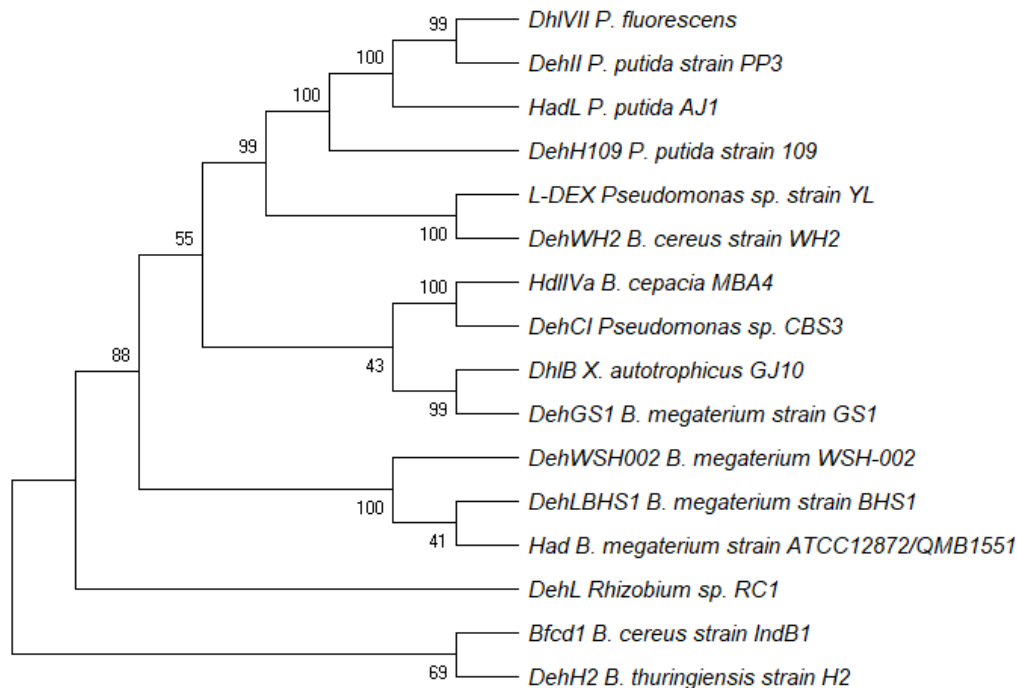


Figure 2. Phylogenetic tree was constructed using Neighbor-Joining method [18] with 1,000 bootstraps replication [19]. The dehalogenase of *Bacillus megaterium* WSH-002 was designated DehWSH002. The analysis involved 16 haloacid dehalogenases amino acid sequence from different species. The analysis was conducted using MEGA X [17].

regulator gene designated as *dehRBHS1* was reportedly found upstream of the gene sequence in genome *B. megaterium* BHS1 and also demonstrated the genetic location of the HTH-type transcriptional regulator gene (*dehRBHS1*) and L-2-haloacid dehalogenase gene (*dehLBHS1*) with the length of gap of 2,712 bp [13]. The gap between regulatory gene and haloacid dehalogenase gene in *B. megaterium* WSH-002 was considered relatively longer compared to *B. megaterium* BHS1 which has been previously reported 624,167 bp. However, the size of *dehWSH002* gene of *B. megaterium* WSH-002 (660 bp) was considered similar to *DehLBHS1* of *B. megaterium* BHS1 (659 bp). Therefore, this study proposed that the expression *dehWSH002* gene was initiated by the product of the adjacent regulator gene (*dehR*) found in *B. megaterium* WSH-002. Further investigation is required to enhance the insight into how these genes are being expressed and regulated in *B. megaterium* WSH-002.

#### Phylogenetic analysis of haloacid dehalogenase (*DehWSH002*)

To ascertain the relationship of *DehWSH002* with other selected dehalogenases, the phylogenetic tree was constructed based on the amino acid sequence. All dehalogenases that share common domain or families are shown in Figure 2. Besides that, it can be demonstrated that L-DEX, DhlVII, DehII, HadL and DehH109 were clustered together because they shared similar substrate specificity. Interestingly, the amino acid sequence of *DehWH2* has high identity to L-DEX. Previous study reported that Muslem *et al.* [11] has shown *DehWH2* of *B. cereus* strain WH2 has high identity of 79% to L-DEX of *Pseudomonas* sp. strain YL. The amino acid profiles showed several important amino acid residues shared between *DehWH2* and L-DEX. These residues were believed involved in the catalytic reaction that include aspartic acid, threonine, serine, lysine, tyrosine and asparagine [11]. Furthermore, *DehGS1* was found closely related to DhlB. Mashitah *et al.* [30] demonstrated that *DehGS1* of *B. megaterium* strain GS1 were 69% identity to DhlB of *X. autotrophicus* strain GJ10. Besides that, it can be demonstrated that *Bfcd1* is closely related to *DehH2* probably due to similar motifs or families shared between these two dehalogenases. In contrast, *DehL* from *Rhizobium* sp. RC1 were distantly related with other L-specific dehalogenase

probably due to evolutionary occurrence causing this gene to alter its sequence either insertion or deletion of the gene. Therefore, it was considered as an outgroup in phylogenetic tree.

#### Conclusion

A complete genetic organisation or an operon for dehalogenase gene expression was proposed that consist of dehalogenase type II gene (*dehWSH002*) adjacent to the regulator (*dehR*) and uptake (*dehp*) genes. The phylogenetic analysis strongly suggests that *DehWSH002* shared common features with *DehLBHS1* of *B. megaterium* strain BHS1 and *Had* of *B. megaterium* strain ATCC12872/QMB1551 since they can act on L-specific haloalkanoic acid. This study proposes genome of *B. megaterium* WSH-002 contains a haloacid dehalogenase gene that is useful for the degradation of halogenated compounds which can bring benefits to environmental remediations. However, the protein function of *DehWSH002* needs further studies such as protein modelling of the structure and functions to provide details regarding its conserved amino acid residues that are related to the dehalogenation mechanism. Besides that, the regulator and uptake gene functions also need to be investigated further using gene cloning method to prove its ability to control production of dehalogenase.

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