**JOURNAL OF TROPICAL LIFE SCIENCE**
2023, Vol. 13, No. 2, 369 – 376
http://dx.doi.org/10.11594/jtls.13.02.15

**Research Article**

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

Luqman Arif Zulkarnain*, Fahrul Huyop

Department of Biosciences, Faculty of Science, Universiti Teknologi Malaysia, 81310 UTM, Johor Bahru, Malaysia

*Article history*:
Submission August 2022
Revised December 2022
Accepted January 2023

**Corresponding author:*
E-mail: manarifz@yahoo.com

**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

*How to cite:*
Zulkarnain LA, Huyop F (2023) The Genetic Organisation and Control of Putative Dehalogenase Gene Expression in *Bacillus megaterium* WSH-002. Journal of Tropical Life Science 13 (2): 369 – 376. doi: 10.11594/jtls.13.02.15.

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: $H^+$ 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 | *hdl IVa* | *Burkholderia cepacia* MBA4 | 231 | [20] |
| AAA27590 | *dhlB* | *Xanthobacter autotrophicus* GJ10 | 253 | [21] |
| CAA63861 | *dhl VII* | *Pseudomonas fluorescens* | 227 | [22] |
| AAA25832 | *hadL* | *Pseudomonas putida* AJ1 | 227 | [23] |
| AAB32245 | *L-DEX* | *Pseudomonas sp.* (strain YL) | 232 | [24] |
| BAA04474 | *dehH109* | *Pseudomonas putida* strain 109 | 224 | [25] |
| CAB38090 | *dehII* | *Pseudomonas putida* PP3 | 227 | [26] |
| CAA63794 | *dehL* | *Rhizobium* sp. RC1 | 279 | [27] |
| AAA63640 | *dehCI* | *Pseudomonas* sp. CBS3 | 227 | [28] |
| AMN16523 | *bcfcd1* | *B. cereus* strain Indb1 | 283 | [29] |
| WP_045293204 | *dehLBHS1* | *B. megaterium* strain BHS1 | 219 | [13] |
| - | *dehWH2* | *B. cereus* strain WH2 | 203 | [11] |
| - | *dehGS1* | *B. megaterium* strain GS1 | 104 | [30] |
| QOW03316 | *dehH2* | *B. megaterium* strain H2 | 254 | [10] |
| ADE68040 | *had* | *B. megaterium* 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 *dehR* | 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 *dehp* | 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 *dehWSH002* | 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 *dehR* | 582 | 124,888 (*dehp*) 624,167 (*dehWSH002*) |
| Putative transport system permease protein | 3480627…3481850 | BMWSH_3846 *dehp* | 1224 | 498,056 |
| 2-haloalkanoic acid dehalogenase (EC 3.8.1.2) | 3980565...3979906 | BMWSH_4378 *dehWSH002* | 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

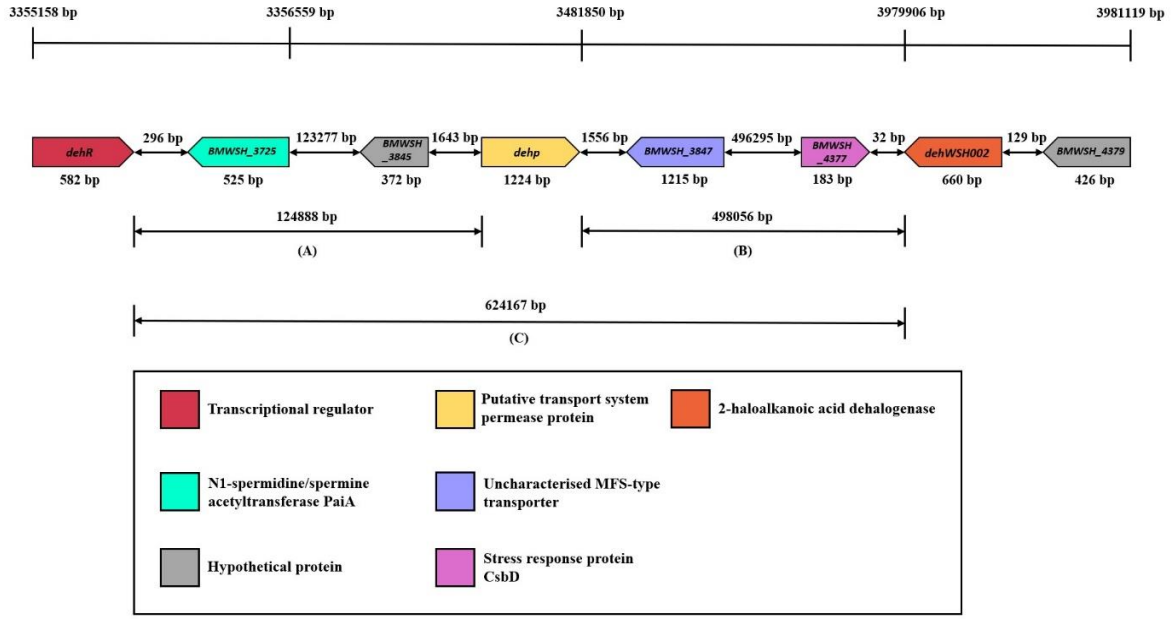


Figure 1. A propose genetic organisation of dehalogenase related genes (*dehR*, *dehp* and *dehWSH002*) based on the genome mapping of *Bacillus megaterium* WSH-002 (CDS region of 3355158 bp to 3981119 bp). The colours depict different type of protein encoding genes. The arrows with pentagon shaped showing 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.

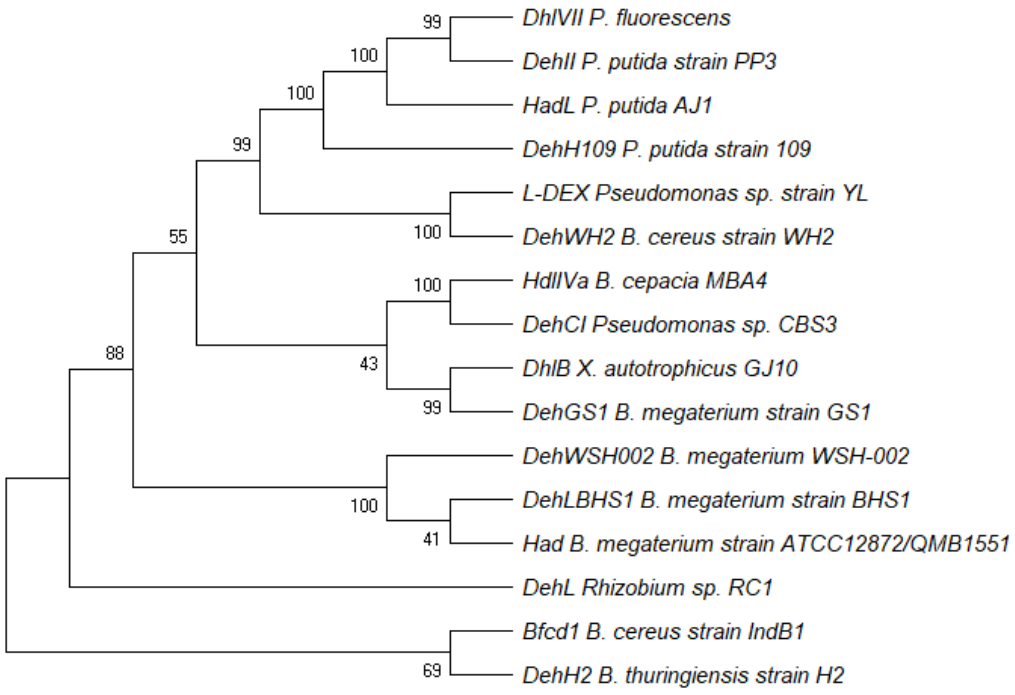


Figure 2. Phylogenetic tree was constructed using Neighbor-Joining method [18] with 1,000 bootstraps replication [19]. The dehalogenase of *Bacillus megaterium* WSH-002 was designated DehWSH002. The analysis involved 16 haloacid dehalogenases amino acid sequence from different species. The analysis was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

## Acknowledgment

The authors would like to express their appreciation and gratitude to FRGS (Ministry of Higher Education R.J130000.7854.5F189/ FRGS/1/2019/STG05/UTM/01/1) for financial assistance.

## References

1. Weissermel K, HJ Arpe (2008) Industrial organic chemistry. John Wiley & Sons.
2. Goldberg ED (1991) Halogenated hydrocarbons: past, present and near-future problems. Science of the total environment 100:17-28. doi:10.1016/0048-9697(91)90371-K
3. Janssen DB, Oppentocht JE, Poelarends GJ (2001) Microbial dehalogenation. Current Opinion in Biotechnology 12(3):254-258. doi:10.1016/S0958-1669(00)00208-1
4. Abel E, Pakingking Jr RV, Gregoria G, Wint MT, Huyop F (2012), Characteristics of dehalogenase from bacteria isolated from the gut of pond-reared rohu (*Labeo rohita*) juveniles in Myanmar. Advances in Biosciences and Biotechnology 3(4): 353–361. doi:10.4236/abb.2012.34051
5. Adamu A, Wahab RA, Huyop F (2016) L-2-Haloacid dehalogenase (DehL) from *Rhizobium* sp. RC1.

Springerplus 5 (1): 1-17. doi:10.1186/s40064-016-2328-9
6. Edbeib MFA, Wahab RA, Huyop F (2016) Characterization of an α-haloalkanoic acid–degrading *Pseudomonas aeruginosa* MX1 isolated from contaminated seawater. Bioremediation Journal 20(2): 89-97. doi:10.1080/10889868.2015.1114464
7. Edbeib MFA, Wahab RA, Kaya Y, 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. Annals of Microbiology 67(5): 371-382. doi:10.1007/s13213-017-1266-2
8. Akcay K, Kaya Y (2019) Isolation, characterization and molecular identification of a halotolerant *Bacillus megaterium* CTBmeg1 able to grow on halogenated compounds. Biotechnology & Biotechnological Equipment 33 (1): 945-953. doi:10.1080/13102818.2019.1631717
9. Heidarrezaei M, Shokravi H, Huyop F, Koloor SSR, Petrů M (2020) Isolation and characterization of a novel bacterium from the marine environment for trichloroacetic acid bioremediation. Applied Sciences 10 (13): 4593. doi:10.3390/app10134593
10. Oyewusi HA, Wahab RA, Huyop F (2020) Dehalogenase-producing halophiles and their potential role in bioremediation. Marine Pollution Bulletin 160: 111603. doi:10.1016/j.marpolbul.2020.111603
11. Muslem WH, Edbeib MFA, Aksoy HM, Kaya Y, Hamid AAA, Hood MHM, Wahab RA, Huyop F (2020) Biodegradation of 3-chloropropionic acid (3-CP) by *Bacillus cereus* WH2 and its *in silico* enzyme-substrate docking analysis. Journal of Biomolecular Structure and Dynamics 38 (11): 3432-3441. doi:10.1080/07391102.2019.1655482
12. Huyop F, Cooper RA (2011) Regulation of dehalogenase E (DehE) and expression of dehalogenase regulator gene (DehR) from *Rhizobium* sp. RC1 in *E. coli*. Biotechnology & Biotechnological Equipment 25 (1): 2237-2242. doi:10.5504/BBEQ.2011.0009
13. Wahhab BHA, Samsulrizal NH, Edbeib MFA, Wahab RA, Al-Nimer MS, Hamid AAA, Oyewusi HA, Kaya Y, Notarte KIR, Shariff AHM (2021), Genomic analysis of a functional haloacid-degrading gene of *Bacillus megaterium* strain BHS1 isolated from Blue Lake (Mavi Gölü, Turkey), Annals of Microbiology, 71 (1): 1-11. doi:10.1186/s13213-021-01625-9
14. Zulkarnain LA, Huyop FZ (2022) Genomic Analysis for Haloacid Dehalogenase in *Bacillus megaterium* WSH-002. Journal of Tropical Life Science, 12 (1) 73-82. doi:10.11594/jtls.12.01.07
15. Overbeek R, Olson R, Pusch GD, Olsen GJ, Davis JJ, Disz T, Edwards RA, Gerdes S, Parrello B, Shukla M (2014) The seed and the rapid annotation of microbial genomes using Subsystems Technology (RAST), Nucleic acids Research 42 (D1): D206-D214. doi:10.1093/nar/gkt1226
16. Patwardhan A, Ray S, Roy A (2014) Molecular markers in phylogenetic studies-A Review. Journal of Phylogenetics and Evolutionary Biology 2(2):1-9. doi:10.4172/2329-9002.1000131
17. Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. Molecular Biology and evolution, 35 (6): 1547. doi:10.1093/molbev/msy096
18. Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4 (4): 406-425. doi:10.1093/oxfordjournals.molbev.a040454
19. Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39(4): 783-791. doi:10.1111/j.15585646.1985.tb00420.
20. Murdiyatmo U, Asmara W, Tsang JS, Baines AJ, Bull AT, Hardman DJ (1992) Molecular biology of the 2-haloacid halidohydrolase IVa from *Pseudomonas cepacia* MBA4. Biochemical Journal 284(1): 87-93. doi:10.1042/bj2840087
21. van Der Ploeg JG, van Hall, Janssen DB (1991) Characterization of the haloacid dehalogenase from *Xanthobacter autotrophicus* GJ10 and sequencing of the *dhlB* gene. Journal of Bacteriology 173(24): 7925-7933. doi:10.1128/jb.173.24.7925-7933.1991
22. Brokamp A, Happe B, Schmidt FRJ (1996) Cloning and nucleotide sequence of a D,L-haloalkanoic acid dehalogenase encoding gene from *Alcaligenes xylosoxidans* ssp. *denitrificans* ABIV. Biodegradation 7 (5): 383-396. doi:10.1007/BF00056422
23. Barth, PT, Bolton L, Thomson JC (1992) Cloning and partial sequencing of an operon encoding two *Pseudomonas putida* haloalkanoate dehalogenases of opposite stereospecificity. Journal of Bacteriology 174 (8): 2612-2619. doi:10.1128/jb.174.8.2612-2619.1992
24. Nardi-Dei, V, Kurihara T, Okamura T, Liu JQ, Koshikawa, H, Ozaki H, Terashima Y, Esaki N, Soda K (1994) Comparative studies of genes encoding thermostable L-2-halo acid dehalogenase from *Pseudomonas* sp. strain YL, other dehalogenases, and two related hypothetical proteins from *Escherichia coli*. Applied and Environmental Microbiology 60(9): 3375-3380. doi:10.1128/aem.60.9.3375-3380.1994
25. Kawasaki H, Toyama T, Maeda T, Nishino H, Tonomura K (1994) Cloning and sequence analysis of a plasmid-encoded 2-haloacid dehalogenase gene from *Pseudomonas putida* no. 109. Bioscience Biotechnology and Biochemistry 58 (1): 160-163. doi:10.1271/bbb.58.160
26. Hill KE, Marchesi JR, Weightman AJ, (1999), Investigation of two evolutionarily unrelated halocarboxylic acid dehalogenase gene families. Journal of Bacteriology 181 (8): 2535-2547. doi:10.1128/JB.181.8.2535-2547.1999
27. Cairns SS, Cornish A, Cooper RA (1996) Cloning, sequencing and expression in *Escherichia coli* of two *Rhizobium* sp. genes encoding haloalkanoate dehalogenases of opposite stereospecificity. European Journal of Biochemistry 235(3): 744-749. doi:10.1111/j.1432-1033.1996.t01-1-00744.x
28. Schneider B, Müller R, Frank R, Lingens F (1991) Complete nucleotide sequences and comparison of the structural genes of two 2-haloalkanoic acid dehalogenases from *Pseudomonas* sp. strain CBS3. Journal of Bacteriology 173 (4): 1530-1535. doi:10.1128/jb.173.4.1530-1535.1991
29. Ratnaningsih E, Idris, I (2017) Cloning and expression of haloacid dehalogenase gene from *Bacillus cereus* IndB1. Indonesian Journal of Biotechnology 22 (2): 55-60.

30. Mashitah M, Roslan D, Huyop F, (2011) Molecular analysis of dehalogenase gene in genomic DNA of *Bacillus megaterium* strain GS1 isolated from volcanic area Gunung Sibayak. Journal of Biological Sciences 11 (5): 394-398.
31. Eppinger M, Bunk B, Johns MA, Edirisinghe JN, Kutumbaka KK, Koenig SSK, Creasy HH, Rosovitz MJ, Riley DR et al., (2011) Genome sequences of the biotechnologically important *Bacillus megaterium* strains QM B1551 and DSM319. Journal of Bacteriology 193 (16): 4199-4213. doi:10.1128/JB.00449-11
32. Lever J (1992) Cell and molecular biology of Na+/glucose symport: In Membrane Transport in Biology. Springer 56-72.
33. Madej MG, Kaback HR (2013) Evolutionary mix-and-match with MFS transporters II. Proceedings of the National Academy of Sciences, 110 (50): E4831-E4838. doi:10.1073/pnas.1319754110
34. Jing, NH, Wahab RA, Hamdan S, Huyop, F. (2010) Cloning and DNA Sequence Analysis of the Haloalkanoic Permease Uptake Gene from *Rhizobium* sp. RC 1. Biotechnology 9 (3): 319-325. doi: 10.3923/biotech.2010.319.325
35. Tse YM, Yu M, Tsang JS (2009) Topological analysis of a haloacid permease of a *Burkholderia* sp. bacterium with a PhoA-LacZ reporter. BMC Microbiology 9(1): 1-12. doi:10.1186/1471-2180-9-233
36. Saier Jr MH, Reddy VS, Tsu BV, Ahmed MS, Li C, Moreno-Hagelsieb G (2016) The transporter classification database (TCDB): recent advances. Nucleic Acids Research 44 (D1): D372-D379. doi: 10.1093/nar/gkv1103
37. Musa MA, Wahab RA, Huyop F (2018) Homology modelling and in silico substrate-binding analysis of a *Rhizobium* sp. RC1 haloalkanoic acid permease. Biotechnology and Biotechnological Equipment 32(2): 339-349. doi: ttps://doi.org/10.1080/13102818.2018.1432417.

This page is intentionally left blank.