FUNCTIONAL GENOMIC ANALYSIS AND MOLECULAR MODELING OF A NOVEL ALKALOTOLERANT DEHALOGENASE ENZYME FROM *Bacillus megaterium* DehLBHS1

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ABSTRACT

Extreme environments, such as alkaline lakes, are at risk of contamination by halogenated compounds. These halogenated products are recalcitrant toxicants posing hazards to human health and the environment, thus urgently need to be studied. In this research, an alkalotolerant bacterium was successfully isolated from the Turkish Blue Lake. The Biolog GEN III system and 16S rRNA analysis identified the bacterium as *Bacillus megaterium* strain BHS1. The BHS1 was able to use 2,2-dichloropropionic acid (2,2-DCP) as its sole carbon source and was found to grow well in alkaline conditions (pH 7.0−14.0) when supplemented with 2,2-DCP from 20 to 60 mM. This bacterium was also characterized at the genomic level using the HiSeq platform by *de novo* assembly. Genomic data were analyzed to demarcate DNA regions containing protein-coding genes and their functions. The present study showed the de novo assembly of the BHS1 genomic sequence unveiled a genome size of ~ 5.37 Mb with the average G + C content of 38% successfully. The predicted nuclear genome harbors 5,509 protein-coding genes, 1,353 tRNA genes, 67 rRNA genes and 6 non-coding (mRNA) genes. Genomic analysis suggested that BHS1 encodes a DehLBHS1 dehalogenase enzyme. Deduced amino acid sequence showed that it belongs to Group II dehalogenase with sequence identity (38.4%) to the previously described DehL-DEX YL. Homology modeling using I-TASSER was used to recreate the structure of the enzyme. Compared to other non-alkalophilic dehalogenases with a pI of 6.0, DehLBHS1, which had a theoretical pI of 6.66 in this study, showed a greater tendency to originate from a natural ecosystem rather than from a polluted environment. Homology-based structural modeling revealed that the surface charge of DehLBHS1 was negative, signifying that *Bacillus megaterium* has evolutionarily adapted to an alkaline environment. Findings from this study construed that bioprospecting for an effective halogen-degrading alkalotolerant bacteria in highly alkaline environments could be a safer and more stable means of bioremediation of polluted areas.

ABSTRAK

Persekitaran yang melampau, seperti tasik beralkali mempunyai risiko dicemari oleh sebatian halogen. Produk berhalogen adalah bahan toksik rekalsitran yang boleh mendatangkan bahaya kepada kesihatan manusia dan alam sekitar, justeru perlu dikaji dengan segera. Dalam penyelidikan ini, bakteria alkalotoleran telah berjaya dipencilkan dari Tasik Biru, Turki. Sistem "Biolog GEN III" dan analisa rRNA 16S telah mengenal pasti bakteria tersebut sebagai *Bacillus megaterium* strain BHS1. Strain BHS1 mampu menggunakan asid 2,2-dikloropropionik (2,2-DCP) sebagai sumber tunggal karbon dan didapati tumbuh dengan baik dalam keadaan beralkali (pH 7.0−14.0) pada kepekatan 2,2-DCP dari 20 hingga 60 mM. Bakteria ini juga dicirikan pada tahap genomik menggunakan platform Illumina HiSeq mlalui pemasangan *de novo*. Data genomik dianalisa untuk menyempadankan kawasan DNA yang mengkodkan protein dan fungsinya. Kajian menunjukkan pemasangan *de novo* mendapati saiz genom BHS1 adalah ~ 5.37 Mb dengan purata kandungan G + C sebanyak 38%. Genom nuklear diramalkan mempunyai 5,509 gen pengekodan protein, 1,353 gen tRNA, 67 gen rRNA dan 6 gen bukan pengekodan (mRNA). Data genomik BHS1 telah menemukan enzim dehalogenase DehLBHS1 dan jujukan asid amino menunjukkan ia tergolong dalam dehalogenase Kumpulan II dengan identiti jujukan (38.4%) kepada DehL-DEX YL yang dicirikan sebelumnya. Pemodelan homologi menggunakan I-TASSER telah berjaya membina struktur enzim tersebut. Berbanding dengan dehalogenase bukan alkalofilik lain dengan pI 6.0, DehLBHS1, yang mempunyai pI teoritikal 6.66 dalam kajian ini, menunjukkan kecenderungan yang lebih besar untuk berasal daripada ekosistem semula jadi dan bukannya daripada persekitaran yang tercemar. Pemodelan struktur enzim berasaskan homologi mendedahkan bahawa permukaan DehLBHS1 adalah bercas negatif menandakan bahawa *Bacillus megaterium* telah menyesuaikan diri secara evolusi kepada persekitaran alkali. Penemuan kajian ini boleh ditafsirkan bahawa bioprospek bakteria alkalotoleran yang berupaya merendahkan halogen secara berkesan dalam persekitaran yang sangat beralkali boleh menjadi kaedah bioremediasi yang lebih selamat dan stabil bagi kawasan tercemar.

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LIST OF ABBREVIATIONS

2,2-DCP	-	2,2-dichloropropionic acid
2CP	-	2-chloropropionic acid
3CP	-	3- chloropropionic acid
Å	-	Distance
ATP	-	Adenosine triphosphate
BCECF	-	2′,7′-bis-(2-carboxyethyl)-5(and-6)-carboxyfluorescein
Bh	-	Bacillus halodurans C-125
BLAST	-	Basic local alignment search tool
Bp	-	Bacillus pseudofirmus OF4
bp	-	Base pairs
CFAs	-	cyclopropane fatty acids
CL-	-	Concentration of Chloride Ion
CPA	-	Cation proton antiporters
COG	-	Clusters of Orthologous Groups
DEL	-	Deletion
DNA	-	Deoxyribonucleic acid
DNA	-	Deoxyribonucleic acid
Ec	-	Escherichia coli
Fq	-	FASTQ
GO	-	Gene ontology
H	-	Hour
HCHs	-	Hexachlorocyclohexanes
HIA	-	Halide ion assay
HLD	-	Haloalkane
HTH	-	helix-turn-helix-type
INS	-	Insertion
INV		Inversion
KEGG	-	Kyoto Encyclopedia of Genes and Genomes
LB	-	Luria-Bertani

M	-	Molar
MD		Molecular Dynamics
MCA	-	monoactate
MFCs	-	Microbial Fuel Cells
min	-	Minutes
ml	-	Mililiter
NCBI	-	National Center for Biotechnology Information
NIH	-	National Institutes of Health
ns	-	Nanoseconds
OD	-	Optical Density
OMPs	-	outer membrane porins
PCR	-	Polymerase chain reaction
PMF	-	Proton-motive force, a transmembrane electrochemical gradient of protons composed of the ΔpH (alkaline inside) and ΔΨ (negative inside)
PMF	-	Proton motive force
RDP	-	Ribosomal Database Project
Rg	-	Gyration
RMSD	-	Root mean square deviation
RMSF	-	Root mean square fluctuation
RNA	-	Ribonucleic acid
rRNA	-	Ribosomal RNA
RSO	-	right-side-out
SCWP	-	Secondary cell wall polymers
SCWPs	-	secondary cell wall polymers
SlpA	-	S-layer polymer
SMF	-	Sodium-motive force
SNP	-	Single nucleotide polymorphism
SV	-	Structural variation
TM-score	-	Template modeling score
TMS	-	Trans membrane segment
tRNA	-	Transfer RNA
ΔpH	-	Transmembrane pH gradient

ΔΨ	-	Transmembrane electrical gradient
A or Ala	-	Alanine
C or Cys	-	Cysteine
D or Asp	-	Aspartic acid
E or Glu	-	Glutamic acid
F or Phe	-	Phenylalanine
G or Gly	-	Glycine
H or His	-	Histidine
I or Ile	-	Isoleucine
K or Lys	-	Lysine
L or Leu	-	Leucine
M or Met	-	Methionine
N or Asn	-	Asparagine
P or Pro	-	Proline
Q or Gln	-	Glutamine
R or Arg	-	Arginine
S or Ser	-	Serine
T or Thr	-	Threonine
V or Val	-	Valine
W or Trp	-	Tryptophan
Y or Tyr	-	Asparagine
N	-	North
E	-	East

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CHAPTER 1

INTRODUCTION

1.1 Overview

The soda lake, Mavi Gölü (Blue Lake), in Turkey is a famous tourist destination known for its uniquely beautiful turquoise water that is retained in the lakebed from June to December. It is formed in the Göksu Creek, the only carbonated water that flows into the Black Sea. Given the lake's unique and rugged landscape, the identification/ characterization of the lake's culturable microbial communities is essential for expanding the repertoire of alkaliphilic microbes in fortifying available databases for alkaliphilic microbes. The data are particularly pertinent for basic research that elucidates various applications of alkaliphiles, such as in food industries, bioremediation, and medicine (Bagherbaigi *et al.*, 2013; Batumalaie *et al.*, 2018; Kevbrin, 2019; Neelam *et al.*, 2019). Despite being unpolluted, Mavi Gölü may contain organobromines and organohalogens, which are produced naturally in the lake. The organobromine and organohalogens are naturally produced by an array of the lake's biological and chemical processes. 2,2-dichloropropionic acid belongs to organohalogens, is significant environmental pollutants. Organohalogens were formerly thought to be entirely anthropogenic, however, over 5000 of these chemicals are now known to be generated naturally (Atashgahi *et al.*, 2018). It might trigger certain bacteria to produce dehalogenases (Gribble, 2000). Hence, we investigated the properties of dehalogenases produced by bacteria in natural ecosystems that produce environmentally halogenated compounds.

Many bacteria utilize halogenated compounds as an energy source with the aid of dehalogenases (Abel *et al.*, 2012b; Abdulgader Edbeib *et al.*, 2016; Adamu *et al.*, 2016; Edbeib *et al.*, 2017; Akcay and Kaya, 2019; Heidarrezaei *et al.*, 2020; Oyewusi *et al.*, 2020). These bacteria generate dehalogenases that catalyze the

cleavage of carbon-halogen bonds, which generally yields less toxic intermediates (Kurihara and Esaki, 2008). In particular, *Rhizobium* sp. RC1 remains to be the only bacterium known to produce three different dehalogenases, namely DehD, DehE, and DehL, each of which has unique substrate specificities (Hamid *et al.*, 2011; Huyop and Cooper, 2011; Huyop and Sudi, 2012; Sudi *et al.*, 2014; Adamu *et al.*, 2016). Because of the necessity to discover eco-friendly solutions for the bioremediation of halogenated chemicals in alkaline settings, alkaliphilic and alkali stable enzymes are preferred over none extremophilic enzymes. This is due to alkaliphilic bacteria' particular cellular enzymatic mechanisms, which allow them to flourish in very alkaline conditions. Conventional non-alkaliphilic bacteria are incapable of successfully removing organic contaminants at high pH levels. Alkaliphilic microorganisms are characterized by unique metabolisms which enables them to be adapted to extreme alkalinity. As a result, these bacteria are extremely important for the bioremediation of contaminated alkaline environments such as soda lakes.

Bacillus megaterium BHS1 can utilize 2,2-dichloropropionic acid (2,2DCP) as a sole carbon source and in agreement with the previous literature that *Bacillus megaterium* produces proteins of unknown function (Korneli *et al.*, 2013) that could potentially be used for bioremediation. Thus, to consider its appropriate applications and understand its adaptability to alkali-laden environments such as soda lakes, the acquisition of a full genomic sequence for *Bacillus megaterium* BHS1 is of interest. The genome data are processed to identify specific DNA regions containing protein-coding genes and functions reveals the general genome features and functional categories, as well as identifies the amino acid sequence for the dehalogenase enzyme and its type group. Having identified a specific dehalogenase, is to determine its function based on 3D structure and the active site as well as the interaction of substrate binding protein. The current work is the first study of the sequencing and structural-function characterization, as well as protein-ligand interactions, of a dehalogenase enzyme isolated from alkalotolerant bacteria. A combination of homological modeling, molecular docking, and simulation of molecular dynamics can assist to understand the mode of action of DehLBHS1 enzymes and their catalytic mechanisms.

1.2 Problem statement

Most pollutants that are chemically synthesized contain halogen atoms that are toxic and give a threat to the environment. Lately, some studies have pointed out the importance of alkaliphiles bacteria in the production of dehalogenase enzymes that have a significant role in pollutants degradation. This has opened the way to research being carried out into dehalogenase enzymes with the aim of exploiting the potential of this bacterial product. It is worth mentioning that there are many reports around the presence of different types of enzyme dehalogenase in the mesophilic environment (soil, water and sediments). Nevertheless, there is very little information about the existence of dehalogenase enzymes in high alkalinity environments. It is important to highlight here that there are no studies uncovered so far that involve aerobic alkaliphiles capable of degrading halogenated compounds 2,2-DCP.

The regulation of dehalogenase production in bacteria is not fully understood. It is uncertain whether it involves more than one regulatory gene. In some bacteria for example Rhizobium sp. RC1, three kinds of dehalogenases are produced, and it has been hypothesized that the production of these dehalogenases is controlled by a single regulator gene. Furthermore, the properties of dehalogenases in an alkaline environment have never been cited elsewhere and the presence of various contaminants in this environment is probable and must be handled.

Ultimately, the results of this study will aid in the exploitation and re-engineering of alkaliphiles as an effective bioremediation system to treat land and water bodies contaminated with halogenated compounds. Improvement of the quality of agricultural settings and decreased pollution will be amongst the advantages gained.

Therefore, this study focuses on investigating the genes responsible for degrading 2,2-DCP and those genes involved in the microbe adaptation to the alkaline environment. The current study will also investigate the full genomic profile of one organism known as *Bacillus megaterium* strain BHS1 to check the possible

operons present that involve in the regulatory aspect of dehalogenases production, that have not been reported earlier.

1.3 Objectives of research

1. To characterize and identify the genus and species of the isolated bacteria from Turkish alkaline lake water using the standard molecular techniques.
2. To carry out a genomic study of the bacteria and characterize its properties to degrade 2,2-dichloropropionic acid in an alkaliphilic environment based on growth on 2,2-dichloropropionic acid as a carbon source.
3. To predict and determine three-dimensional (3D) structure of the prospective alkotolerant dehalogenase and its structural characteristics that led to the alkalophilic adaptation of the enzyme as compared to their non-alkalotolerant homologues.

1.4 Significance of the study

Analysis of the complete genome of the identified bacteria may uncover the identity of dehalogenase operon genes expression and their adaptation to an alkaline environment. The Alkaline lakes are seldom studied searching for alkaliphile bacteria that are involved in the degradation of xenobiotics and a wide variety of hazardous industrial pollutants. The current study may provide one of the better alternative bioremediation to reduce pollution in the alkalinity environment. By identifying the bacterial operon genes involved in the degradation of the contaminant 2,2-dichloropropionic acid and their adaptation to a high alkalinity environment. Due to the limited studies about alkalinity microorganisms degrading halogenated compounds. As a result, in the future these microorganisms may represent a significant viable biocatalytic source of dehalogenases and intrinsically may serve as a cost-effective means to clean up. Identify the protein sequence and other cation/proton antiporter subunits that may contribute to the enzymes' alkaloadaptation. In turn, this may reveal important structural characteristics of

organisms and facilitates simulation as well as prediction of their structure and mechanism of adaption.

The current work contributes to improving substrate specificity and providing information on protein-ligand interactions between DehLBHS1 enzyme and their ligands to give insight into potential catalytic processes. This will be crucial in the future for protein engineering.

1.5 Scope of the study

The work involves the characterisation of the isolated bacteria from the alkaline water and growth establishment on 2,2-dichloropropionic acid as a carbon sole source. The analysis of the properties of the microbes to degrade 2,2-dichloropropionic acid and its potentials for tolerating of various concentrations of 2,2-dichloropropionic acid at different pH conditions were examined. The morphological and molecular approach used the 16S rRNA and Biolog^{TM} Gen III for its biochemical feats. The full genomic DNA to be analyzed by the Hi seq platform. Genome data will mark out the function of DNA containing protein-coding genes. Homology modeling for the structural enzyme and verification of model refinement is acceptable and corresponded to a good quality structure. The interaction of substrate binding protein will be investigated utilizing molecular docking. GROMACS was used to simulate the dynamic signature and conformational behavior of the protein-ligand complex. The findings of this work will provide significant insight into the enzyme for extending its substrate selectivity and catalytic activity.

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