GENOMIC CHARACTERIZATION AND IN SILICO ANALYSIS OF DEHALOGENASES FROM *MESORHIZOBIUM LOTI* STRAIN TONO

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DEDICATION

This dissertation is dedicated to my beloved family for their endless support and encouragement

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ABSTRACT

Halogenated compounds are extensively utilized in different industrial applications such as pesticides and herbicides and cause severe environmental problems because of their toxicity and persistence. Degradation of these compounds by the biological method is a significant method to reduce these recalcitrant. *Mesorhizobium loti* is important for nitrogen fixation in legume roots. Up to now, no report has indicated *Mesorhizobium loti* can produce dehalogenase enzymes. Thus, a total of twenty-five genomes of *Mesorhizobium loti* strains from the National Center for Biotechnology Information (NCBI) were analyzed. These strains notably carry dehalogenase genes and were further investigated. The relative ratio of haloalkane and haloacid dehalogenase type II or L-type from all twenty-five genomes was 26% and 74%, respectively, suggesting type II dehalogenase is common. Surprisingly, only *Mesorhizobium loti* strain TONO carries four dehalogenases and therefore it was further characterized. The chromosome of *Mesorhizobium loti* strain TONO contains four haloacid dehalogenase type II genes namely, *dehLt1* (MLTONO_2099), *dehLt2* (MLTONO_3660), *dehLt3* (MLTONO_4143), and *dehLt4* (MLTONO_6945), and their corresponding enzymes were designated as DehLt1, DehLt2, DehLt3, and DehLt4, respectively. The only haloalkane dehalogenase gene (MLTONO_4828) was located upstream of the *dehLt3* gene and its amino acid share 88% identity with DmlA of *Mesorhizobium japonicum* MAFF 303099. The putative haloacid permease gene designated as *dehrPt* (MLTONO_0284) was located downstream of the *dehLt1* and its amino acids show 69% identity with haloacid permease of *Rhizobium* sp. RC1. The gene encoding helix-turn-helix (HTH) motif family DNA-binding protein regulator and LysR family transcriptional regulator genes were also identified, possibly for regulatory functions. The type II dehalogenase, DehLt4 possess high sequence identity (48.18% and 42.73%) with the well-established DehIVa and L-DEX, respectively. Thus, in the current study, an in silico approach was used for homology modelling and docking assessment of newly identified DehLt4, type II dehalogenase to predict its ability to degrade selected haloalkanoic acids and haloacetate. The study aimed to establish the catalytic tendencies of the enzymes to optimally degrade the selected halogenated haloacids. The refined modelled structure of DehLt4 using GROMACS 5.1.2 software revealed satisfactory scores of ERRAT (94.73%), Verify3D (90.83%) and PROCHECK (99.05 %) assessments. Active site prediction by blind docking, and multiple sequence alignment indicated the catalytic triads for DehLt4 were Asp9-Lys149-Asn175. Both L-2-chloropropionic acid (L-2CP) and trichloroacetate (TCA) docked with DehLt4 exhibited binding energy of -3.9 kcal/mol. While the binding energy for D-2-chloropropionic acid (D-2CP) and monochloroacetate (MCA) was -3.8 kcal/mol and -3.1 kcal/mol, respectively. Thus, the findings of the study successfully identified the catalytic important residues of DehLt4 for possible pollutant degradation. The genomic studies as such, have good potential to be screened for new type of dehalogenases based on basic molecular structure and functions analysis.

ABSTRAK

Sebatian halogenasi digunakan secara meluas dalam aplikasi industri yang berbeza seperti racun perosak dan racun rumput-rumpai dan menyebabkan masalah persekitaran yang teruk kerana ketoksikan dan ketahanannya. Degradasi sebatian ini dengan kaedah biologi adalah kaedah yang signifikan untuk mengurangkan rekalsitran ini. *Mesorhizobium loti* penting untuk fiksasi nitrogen pada akar kekacang. Hingga kini, tidak ada laporan yang menunjukkan *Mesorhizobium loti* dapat menghasilkan enzim dehalogenase. Oleh itu, sejumlah dua puluh lima genom strain *Mesorhizobium loti* dari NCBI dianalisis. Strain ini terutama membawa gen dehalogenase dan disiasat lebih lanjut. Nisbah relatif haloalkane dan haloasid dehalogenase jenis II atau jenis-L dari kedua-dua puluh lima genom masing-masing adalah 26% dan 74%, menunjukkan dehalogenase jenis II adalah biasa. Yang mengejutkan, hanya strain *Mesorhizobium loti* strain TONO yang membawa empat dehalogenases dan oleh itu ia dicirikan lebih jauh. Kromosom *Mesorhizobium loti* strain TONO mengandungi empat gen haloasid dehalogenase jenis II iaitu, *dehLt1* (MLTONO_2099), *dehLt2* (MLTONO_3660), *dehLt3* (MLTONO_4143), dan *dehLt4* (MLTONO_6945), dan enzim ini masing-masing ditetapkan sebagai DehLt1, DehLt2, DehLt3, dan DehLt4. Satu-satunya gen haloalkane dehalogenase (MLTONO_4828) terletak di aliran atas gen *dehLt3* dan asid aminonya mempunyai identiti 88% dengan DmlA *Mesorhizobium japonicum* MAFF 303099. Putatif haloasid gen permease ditetapkan sebagai *dehrPt* (MLTONO_0284) terletak di aliran bawah *dehLt1* dan asid amino yang menunjukkan identiti 69% dengan haloasid permease daripada *Rhizobium* sp. RC1. Gen pengekodan helix-turn-helix (HTH) motif pengawal protein pengikat DNA keluarga dan gen pengatur transkrip keluarga LysR juga dikenal pasti, mungkin untuk fungsi pengawalseliaan. Dehalogenase jenis II, DehLt4 mempunyai identiti urutan tinggi (48.18% dan 42.73%) masing-masing dengan DehIVa dan L-DEX yang mapan. Oleh itu, dalam kajian semasa, pendekatan in silico digunakan untuk pemodelan homologi dan pengkajian dokumentasi DehLt4, dehalogenase jenis II yang baru dikenal pasti untuk meramalkan kemampuannya untuk menurunkan asid haloalkanoik dan haloasetat terpilih. Kajian ini bertujuan untuk menentukan kecenderungan mangkinan enzim untuk pengurangan secara optimum haloasid halogenasi yang terpilih. Struktur model DehLt4 yang dihalusi menggunakan perisian GROMACS 5.1.2 menunjukkan penilaian ERRAT yang memuaskan (94.73%), penilaian Verify3D (90.83%) dan PROCHECK (99.05%). Ramalan tapak aktif dengan pemasangan dok rambang, dan penjajaran urutan berganda menunjukkan triad pemangkin untuk DehLt4 adalah Asp9-Lys149-Asn175. Kedua-dua asid L-2-chloropropionic (L-2CP) dan trichloroacetate (TCA) dok dengan DehLt4 dipamerkan tenaga pengikatan -3.9 kcal / mol. Manakala tenaga pengikatan untuk D-2-chloropropionic acid (D-2CP) dan monochloroacetate (MCA) masing-masing adalah -3.8 kcal / mol dan -3.1 kcal / mol. Oleh itu, dapatan kajian berjaya mengenal pasti residu penting pemangkin DehLt4 untuk kemungkinan degradasi pencemaran. Kajian genomik seperti ini, berpotensi baik untuk disaring bagi jenis dehalogenases baru berdasarkan analisis struktur molekul dan fungsi asas.

TABLE OF CONTENTS

	TITLE	PAGE
	DECLARATION	**iii**
	DEDICATION	**iv**
	ACKNOWLEDGEMENT	**v**
	ABSTRACT	**vi**
	ABSTRAK	**vii**
	TABLE OF CONTENTS	**viii**
	LIST OF TABLES	**xi**
	LIST OF FIGURES	**xii**
	LIST OF ABBREVIATIONS	**xiv**
	LIST OF SYMBOLS	**xviii**
	LIST OF APPENDICES	**xix**
CHAPTER 1	**INTRODUCTION**	**1**
1.1	Background of the Study	1
1.2	Problem Statement	3
1.3	Research Objectives	4
1.4	Significance of the Study	4
1.5	Scope of the Study	5
CHAPTER 2	**LITERATURE REVIEW**	**7**
2.1	Halogenated Organic Compounds	7
2.2	Sources of Dehalogenases	9
2.3	Dehalogenase Enzyme Classification	11
2.4	Substrate Specificities and Mechanism of Bacterial Dehalogenase	11
2.5	Microbial Physiology: The Mechanism of Uptake System	14
2.6	DehIVa 3D Structure	15

2.7	Catalytically Important Residues in Haloacid Dehalogenase Group II	16
CHAPTER 3	**MATERIALS AND METHODS**	**19**
3.1	Experimental Design	19
3.2	Whole Genome Sequence Retrieval	19
3.3	Locus Structure prediction and Genetic Analysis of Dehalogenase Genes	21
3.4	Phylogenetic Analysis of Dehalogenase Amino Acid Sequence	21
3.5	Multiple Sequence Alignment and Conserved Amino Acid Prediction	21
3.6	DehLt4 Amino Acid Sequence Retrieval and Analysis	22
	3.6.1 Secondary and 3D structure prediction	22
	3.6.2 DehLt4 3D Structure Refinement and Validation	23
	3.6.3 Perpetration of Ligands 3D Structure for Molecular Docking	24
	3.6.4 Molecular Docking Calculation	24
CHAPTER 4	**RESULTS AND DISCUSSION**	**27**
4.1	Genomic Screening of Dehalogenase Gene(s)	27
4.2	Genetic Organization of Dehalogenase Genes of *Mesorhizobium loti* strain TONO	29
4.3	Phylogenetic Analysis of Dehalogenases from *Mesorhizobium loti* strain TONO	33
4.4	Multiple Sequence Alignment	34
4.5	Analysis of an Uptake Protein of DehrPt from *Mesorhizobium loti* Strain TONO	39
4.6	Primary Structure Analysis of DehLt4	40
4.7	Multiple Sequence Alignment of DehLt4 with Group II Dehalogenases	42
	4.7.1 Secondary Structure Analysis of DehLt4	43
	4.7.2 Homology Modelling of DehLt4	44
	4.7.3 DehLt4 model Refinement	46
	4.7.4 The Validation of DehLt4 Structure	48
	4.7.5 Active Site Residue Prediction	50

4.7.6 Molecular Docking Analysis 50

CHAPTER 5 CONCLUSIONS AND RECOMMENDATION 53

5.1 Conclusion 53

5.2 Recommendations for Future Work 54

REFERENCES 55

LIST OF PUBLICATIONS 71

LIST OF TABLES

TABLE NO.	TITLE	PAGE
Table 2.1	Types and uses of halogenated compound (Kemf, 2013)	9
Table 2.2	List of selected dehalogenases producing bacteria	10
Table 2.3	List of crystalized group II dehalogenases	18
Table 3.1	List of dehalogenase group II	22
Table 4.1	Complete genome features of *Mesorhizobium loti* from various strains. Type II and HKD (Haloalkane Dehalogenases) showing number of dehalogenase genes.	28
Table 4.2	Catalytically important residues in the crystallized haloacid dehalogenases and their conserved residues in *Mesorhizobium loti* strain TONO dehalogenases	37
Table 4.3	Physicochemical properties of DehLt4 enzyme	40
Table 4.4	Amino acid composition of DehLt4 from *Mesorhizobium loti* strain TONO	41

LIST OF FIGURES

FIGURE NO.	TITLE	PAGE
Figure 2.1	Hydrolytic dehalogenation of haloalkane dehalogenase (Adamu, 2018).	12
Figure 2.2	The reaction mechanism of L-2 haloacid dehalogenase.	14
Figure 2.3	The proposed mechanism of haloacid intake: H^+ Symport.	15
Figure 2.4	The ribbon representation of DehIVa 3D structure.	16
Figure 3.1	Experimental design of the research.	20
Figure 3.2	Structures of halogenated compounds used by this study.	24
Figure 4.1	Genetic organization of dehalogenase genes of *Mesorhizobium loti* strain TONO.	31
Figure 4.2	The Phylogenetic tree constructed by using the Maximum Likelihood method (Whelan and Goldman, 2001).	34
Figure 4.3	Multiple sequence alignment of four haloacid dehalogenases from *Mesorhizobium loti* strain TONO	35
Figure 4.4	Bar graph of comparing the percentage of each residue in haloacid dehalogenases	36
Figure 4.5	Multiple sequence alignment of four haloacid dehalogenase from strain TONO with group I dehalogenase	37
Figure 4.6	Multiple sequence alignment of haloalkane dehalogenase from *Mesorhizobium loti* strain TONO.	38
Figure 4.7	Pairwise sequence alignment of DehrPt from strain TONO with DehrP of *Rhizobium* sp. RC1	39
Figure 4.8	Amino acid sequence of DehLt4 from *Mesorhizobium loti* strain TONO.	41
Figure 4.9	Multiple sequence alignment of DehLt4 with DehIVa, and L-DEX.	42
Figure 4.10	The graph comparing the percentage of each amino acid in DehLt4 with DehIVa and L-DEX.	43
Figure 4.11	Secondary structure prediction of DehLt4 which contain strand (e), helix (h) and coil (c).	43

Figure 4.12	3D structure of DehIVa (template) and DehLt4 (model) after refinement.	44
Figure 4.13	RMSD plot of DehLt4 backbone atoms, during 50 ns MD simulation.	46
Figure 4.14	RMSF plot of DehLt4 backbone atoms fluctuations during 50 ns MD simulation.	47
Figure 4.15	The temperature stability during molecular simulation.	47
Figure 4.16	Verify3D plot of DehLt4 model.	48
Figure 4.17	Errat plot of DehLt4 structure.	49
Figure 4.18	Ramachandran plot of DehLt4 model.	49
Figure 4.19	LigPlot analysis for DehLt4-ligand	52

LIST OF ABBREVIATIONS

Arg	-	Arginine/R
Ala	-	Alanin
Asn	-	Asparagine/N
Asp	-	Aspartic acid/D
bp	-	Base pair
c	-	Coil (s)
$CFCl_3$	-	Trichlorofluoromethane
CCl_4	-	Tetrachloromethane
$CHCl_3$	-	Trichloromethane
C	-	Carbon
C-terminal	-	End of an amino acid chain; carboxyl-terminus
Cys	-	Cysteine/C
D-2CP	-	D-2-chloropropionic acid
Da	-	Dalton
DDBJ	-	DNA Data Bank of Japan
DDT	-	Dichlorodiphenyltrichloroethane
deh	-	Dehalogenase
DehCI	-	Haloacid dehalogenase type II from *Pseudomonas* sp. strain CBS3 Type I
DehH109	-	Haloacid dehalogenase type II from *Pseudomonas putida* strain 109
DehIVa	-	Haloacid dehalogenase type II from *Burkholderia cepacia* MBA4
DehL	-	L-specific dehalogenase from *Rhizobium* sp. RC1
dehLt1	-	Haloacid dehalogenase type II gene from *Mesorhizobium loti* strain TONO
dehLt2	-	Haloacid dehalogenase type II gene from *Mesorhizobium loti* strain TONO
dehLt3	-	Haloacid dehalogenase type II gene from *Mesorhizobium loti* strain TONO

dehLt4	-	Haloacid dehalogenase type II gene from *Mesorhizobium loti* strain TONO
dehrPt	-	An uptake gene from *Mesorhizobium loti* strain TONO
DhlB	-	Haloacid dehalogenase type II from *Xanthobacter autotrophicus* strain GJ10
DNA	-	Deoxyribonucleic acid
DOM	-	Dissolved organic matters
e	-	Strand (s)
EMBL	-	European Molecular Biology Laboratory
et al.	-	et alia (Latin); and others
ExPASy	-	EXpert Protein Analysis SYstem
fs	-	Femtosecond (s)
G+C%	-	Guanine+Cytosine content
Gln	-	Glutamine/Q
Glu	-	Glutamic acid/E
Gly	-	Glycine/G
GMQE	-	Global Model Quality Estimation
GOR	-	Garnier-Osguthorpe-Robson
GRAVY	-	Grand average of hydropathicity
GROMACS	-	GROningen MAchine for Chemical Simulations
h	-	Helix (s)
HAD	-	Haloacid dehalogenase
His	-	Histidine/H
HKD	-	haloalkane dehalogenase
II	-	Instability Index
K	-	Kelvin (s) (absolute temperature)
L-2CP	-	L-2-chloropropionic acid
L-DEX	-	L-2-haloacid dehalogenase from *Pseudomonas* sp. strain YL
Leu	-	Leucine/L
LINCS	-	Linear constraint solver
Lys	-	Lysine/K
MBR	-	Membrane bioreactor
MCA	-	Monochloroacetate

MD	-	Molecular dynamics
MEGA	-	Molecular Evolutionary Genetics Analysis
Met	-	Methionine/M
MultAlin	-	Multiple alignment
NCBI	-	National Centre for Biotechnology Information
NPSA	-	Network Protein Sequence Analysis
NPT	-	Amount of substance (N), pressure (P) and temperature (T)
ns	-	Nanosecond (s)
N-terminal	-	Start of a protein; amine-terminus
ORF	-	Open Reading Frame
PDB	-	Protein Data Bank
Phe	-	Phenylalanine/F
pI	-	Protein isoelectric point
Pro	-	Proline/P
RMSD	-	Root mean square deviation
RMSF	-	Root mean square fluctuation
SDM	-	Site-directed mutagenesis
Ser	-	Serine/S
S_N2	-	Bimolecular nucleophilic substitution
sp.	-	Species
SPC	-	simple point-charges
TCA	-	Trichloroacetate
Thr	-	Threonine/T
Trp	-	Tryptophan/W
typeII	-	dehalogenase type II
Tyr	-	Tyrosine/Y
UCSF	-	University of California, San Fransisco
Val	-	Valine/V
VCM	-	Vinyl chloride monomer
2,2-DCP	-	2,2 dichloropropionic acid
3-CP	-	3-chloropropionic acid

54a7 - Force field parameter sets which is for application to aqueous/a polar solutions of proteins, nucleotides, and sugars

LIST OF SYMBOLS

%	-	Percentage
Å	-	Angstrom (s)
Na^+	-	Sodium ion
P	-	Pressure
T	-	Temperature
ϕ	-	phi
ψ	-	psi

LIST OF APPENDICES

APPENDIX	TITLE	PAGE
Appendix A	Physiochemical properties of DehIVa from *Burkholderia cepacia* MBA4	67
Appendix B	Physiochemical properties of L-DEX from *Pseudomonas* sp. strain YL	69

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

A significant group of halogenated organic compounds such as chlorinated hydrocarbons was produced and extensively utilized in agriculture and various industrial applications. This resulted in severe environmental dilemmas due to their toxicity and persistence and effect the well-being of humans (Edbeib *et al.*, 2020). Fortunately, many bacterial species produce dehalogenases that could breakdown these toxic compounds and convert them into harmless products (Adamu *et al.*, 2020). Most of these bacteria species used organohalides as their sole source of carbon and energy (Heidarrezaei *et al.*, 2020). Dehalogenase catalyzes halogenated organic pollutants' by cleaving of carbon–halogen bonds. Many dehalogenases were identified, for example haloalkane dehalogenase (Pries *et al.*, 1994), fluoroacetate dehalogenase (Zhang *et al.*, 2004), haloalkanoic acid dehalogenase (Allison *et al.*, 1983; Slater *et al.*, 1996; Slater *et al.*, 1979), and 4-chlorobenzoyl-CoA dehalogenase (Yang *et al.*, 1994). Haloalkanoic acid dehalogenase catalyzes haloalkanoic acids and produces hydroxy-acid and a halide (Hill *et al.*, 1999).

The 2-haloacid dehalogenases were classified into three groups based on the substrate specificities (Slater *et al.*, 1995), whereas the genetic approach by Hill *et al* (1999) classified dehalogenases into groups I and II. Group II was stereoselective dehalogenases acting only on L-2-haloacids. While group I dehalogenases were non-stereospecific dehalogenases can react with both D and L form of substrates producing L- and D-hydroxy acids, respectively or can retain the chemical's isomeric pattern (Hill *et al.*, 1999). D-specific dehalogenase was categorised as group I as well. Slater *et al.* (1979) showed that a dehalogenase crude extract from *Pseudomonas putida* PP3 grown on 2-chloropropionate, dehalogenate only a small amount of 3-chloropropionate. Recently, these types of dehalogenases have received more attention

since not many dehalogenases remove chloride from the β-carbon of chloroalkanoic acids (Mesri *et al.*, 2009; Ng and Huyop, 2007).

The genetic organization of *Rhizobial* dehalogenases and their operon was proposed based on gene cloning analysis. The location of *dehE* and *dehD*/*dehL* were far from clear. However, it was strongly believed that a single regulator gene (*dehR*) controls all three dehalogenases (Adamu *et al.*, 2020; Adamu *et al.*, 2016). To understand how dehalogenases is regulated, soil bacteria like *Mesorhizobium loti* species is studied due to the fact that its full genome sequence is readily available in the GenBank (Shimoda *et al.*, 2016). Therefore, soil bacteria which is closely related to *Rhizobium* sp. RC1 like *Mesorhizobium loti* was further investigated. There were several other bacteria species with the ability to degrade pollutants had been reported (Oyewusi *et al*., 2020a). However, not many studies reported on the use of whole-genome sequencing to reveal their genetic sources of pollutant degradation potential. The current study screened several *Mesorhizobium loti* strains for the presence of specific dehalogenases. By nature, the *Mesorhizobium loti* isolated from soil, regarded as rhizosphere bacterial of agronomic significance due to their ability to form nitrogen-fixing interactions with leguminous plants (Shimoda *et al*., 2016; Yamaya-Ito *et al*., 2018). Analyzing dehalogenase genes from this bacterium will predict other possible functions aside from nitrogen-fixation.

Herein, we partially analyze the complete genome sequence of *Mesorhizobium loti* strains in view of predicting their pollutant's degradation ability just by focusing on the dehalogenase enzymes family. The metabolic novelty of *Mesorhizobium loti* strain TONO and its potential for use in bioremediation provided the motivation for complete genome sequence analysis. Here, we describe bioinformatics analysis that reveals diverse metabolic capabilities of *Mesorhizobium loti* strain TONO that may be useful for bioremediation of halocetate and haloacid pollutants (Shimoda *et al*., 2016). The dehalogenase genes arrangement including their upstream and downstream regions and their non-coding sequences for the possible regulator and promoter binding sites in the complete genome sequence were investigated. The newly identified dehalogenase genes were aligned using multiple sequence comparison and phylogenetic study was carried out. We also highlighted on the regulatory element that

might present and predicted to be potential in dehalogenase regulatory mechanism for *Mesorhizobium loti* strain TONO. In addition to that, haloacid uptake gene was also identified. This approach will provide an integrative knowledge to understand the complete bioremediation process. Besides that, the genome study of *Mesorhizobium loti* strain TONO revealed four haloacid dehalogenase type II. Among them dehalogenase type II namely DehLt4 possess high percentage similarity with will studied, DehIVa and L-DEX, was chosen for the current study. The 3D structure of DehLt4 from *Mesorhizobium loti* strain TONO and its catalytically important residues have not been characterized. Understanding the structure of DehLt4 in *Mesorhizobium loti* strain TONO is essential for understanding of its function and mechanism. Thus, we constructed a homology model of the DehLt4 using DehIVa from *Burkholderia cepacia* MBA4 as a template. The DehLt4 model provided insight of folding and also identification of the substrate-binding location, which helps in the recognition of the catalytic residues. Therefore, the catalytically important amino acids function in the catalytic mechanism of DehLt4 is essential to understand the dehalogenation process in *Mesorhizobium loti* strain TONO.

1.2 Problem Statement

It was curious that some bacteria like *Rhizobium* sp. RC1 has more than one dehalogenase while others have a single dehalogenase gene in their entire genome. To date, the genomes of *Mesorhizobium loti* strains in the perspective of dehalogenase(s) have not been searched and there is no report to show the genome of *Mesorhizobium loti* strain TONO contains dehalogenase genes. Therefore, dehalogenases, haloacid uptake gene, their locations including their upstream and downstream genes in the genome of *Mesorhizobium loti* strain TONO for possible regulators and promoters is further investigated in order to insight the regulation of dehalogenase genes. The three-dimensional structure of dehalogenase type II denoted as DehLt4 is not available, as well as its catalytic important residues from *Mesorhizobium loti* strain TONO.

1.3 Research Objectives

The objectives of this research were as follows:

1) To screen dehalogenase gene(s) in the genomes of *Mesorhizobium loti* different strains.

2) To investigate dehalogenase genes location and other functional genes related to the complete operon of these dehalogenases in the genome of *Mesorhizobium loti* strain TONO.

3) To determine the relationship of dehalogenases from *Mesorhizobium loti* strain TONO with other dehalogenases.

4) To predict 3D structure and catalytic important residues of DehLt4 from *Mesorhizobium loti* strain TONO.

1.4 Significance of the Study

This study investigates the genomes of *Mesorhizobium loti* various strains to find dehalogenase genes for possible pollutants degradation. The location of dehalogenase genes, and dehalogenase functional related genes are searched in the genome of *Mesorhizobium loti* strain TONO. The outcome of this research reveals dehalogenases in the genome of *Mesorhizobium loti* strains for clean-up of environmental pollutions. Additionally, the genome study related to dehalogenase gene in *Mesorhizobium loti* strain TONO shed a light in understanding how these genes are regulated. DehLt4 share a high similarity with other well studied dehalogenases. Furthermore, this study elucidated the molecular details of DehLt4 structure, and it identified the important catalytic amino acid residues of the enzyme. In the absence of crystallized structure, modelling in 3D structure of a homologous protein is the only reliable method to achieve the structural information. Understanding the structure of proteins is essential for basic understanding of the mechanisms and

interactions of proteins with substrates. In addition, it can be used to find out the specific function of these proteins.

1.5 Scope of the Study

The scope of the study covers the analysis of the full genomes sequence of *Mesorhizobium loti* strains using bioinformatics tools to identify dehalogenase(s) from the full genomes. The genomes sequences used in this research were retrieved from NCBI GenBank. At the same time, bioinformatic tools were used for detection of possible dehalogenase operons and dehalogenase accessory genes in the genome of *Mesorhizobium loti* strain TONO. The dehalogenase genes arrangement in the genome of *Mesorhizobium loti* strain TONO was extensively studied. Phylogenetic analysis was used for evolutionary studies of dehalogenases form *Mesorhizobium loti* strain TONO. In addition, this study employed the used of various computational methods to characterise DehLt4 at molecular level. The DehLt4 was further analysed by homology modelling. The modelled structure was refined by molecular dynamic simulation using GROMACS 5.1.2 software package. Furthermore, The DehLt4-substrate complex conformation was conducted by molecular docking calculations.

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