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FROM MUD THAT ABLE TO DEGRADE
2, 2-DICHLOROPROPIONATE**

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Date : 22 JANUARY 2013

**ISOLATION AND CHARACTERIZATION OF PUTATIVE
BURKHOLDERIA sp. HY1 FROM MUD THAT ABLE TO
DEGRADE 2, 2-DICHLOROPROPIONATE**

HATEM MOHAMMED HADEED

A dissertation submitted in partial fulfillment of the
requirement for the award of the degree of
Master of Science (Biotechnology)

Faculty of Biosciences and Medical Engineering
UNIVERSITI TEKNOLOGI MALAYSIA

JANUARY 2013

I declare that this dissertation entitled “ISOLATION AND CHARACTERIZATION OF PUTATIVE *BURKHOLDERIA* sp. HY1 FROM MUD THAT ABLE TO DEGRADE 2, 2-DICHLOROPROPIONATE” is the result of my own research except as cited in the references. This dissertation has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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DEDICATION

To my beloved Father and Mother

To my beloved Brothers and Sisters

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HATEM M.HADEED, IRAQ

ABSTRACT

Halogenated organic compounds are extensively used as pesticides, herbicides, and antibiotics. However, using these chemicals in agriculture and industry in high concentration will make them extremely harmful to humans and animals. 2, 2-Dichloropropionate (2, 2-DCP) or Dalapon is one example of halogenated organic compounds that used in agriculture that can cause pollution. In this research, a bacterium strain HY1 was isolated from the mud taken from UTM agriculture area. HY1 showed its ability to degrade 2, 2-DCP by observing its growth on 2, 2-DCP liquid minimal media with doubling time of 42.15 hours. Result has shown that this bacterium grew best in 10mM 2, 2-DCP minimal medium. The activity of dehalogenation and growth pattern was directly proportional to the chloride ion released using colorimetric assay with maximum chloride ion released recorded at 0.748mmol/L from growth in 10mM of 2, 2-DCP. The 16S rRNA analysis and biochemical tests were carried out to identify the identity of the bacterium. From 16S rRNA analysis, the bacterium (HY1) had 95% identity to *Burkholderia* sp. HY1 is Gram negative bacterium and it had many similarities with *Burkholderia* sp. in terms of microscopic observation and biochemical tests. The PCR technique was carried out to determine dehalogenase gene of *Burkholderia* sp. whether related to group I or group II according to Hill *et al.*, (1999) classification system. The PCR amplification and sequencing results showed that the primers dhIB-314 and dhIB-637 showed PCR amplification and primers deh H2-1157 and deh H2-1662 (specific to degrade haloacetic acid) did not show any PCR amplification. This result suggests that a bacterium (HY1) only encode group I dehalogenase and its non-steroselectivity is in agreement with group I haloalkanoic acid such as (2,2DCP, D, L, 2-CP). BLASTp results showed that the partial gene had no significant sequence identity to the sequence in the database. It suggests may be it belongs to other group of dehalogenase.

ABSTRAK

Sebatian organik halogen digunakan secara meluas sebagai racun perosak, herbisid, dan antibiotik. Walau bagaimanapun, penggunaan bahan kimia dalam sektor pertanian dan industri dengan kepekatan yang tinggi akan membuatnya menjadi amat bahaya kepada manusia dan juga haiwan. 2, 2-dikloropropionik asid (2, 2-DKP) atau Dalapon ialah satu contoh sebatian organik berhalogen yang digunakan dalam pertanian dan boleh menyebabkan pencemaran. Dalam kajian ini, strain bakteria ST1 telah diisolasi dari lumpur yang telah diambil dari kawasan pertanian UTM. ST1 menunjukkan keupayaannya untuk mendegradasi 2,2-DKP melalui pemerhatian pertumbuhan dalam media minimum 2, 2-DKP dengan masa penggandaan sebanyak 42.15 jam. Keputusan kajian juga telah menunjukkan bahawa bakteria ini bertumbuh dengan baik dalam 10mM media minimum 2, 2-DKP. Aktiviti dehalogenasi dan corak pertumbuhan adalah berkadar terus dengan pengceraan ion klorida menggunakan kaedah ceraiian kolorimetrik. Pengceraan ion klorida maksimum direkodkan sebanyak 0.748mmol / L daripada pertumbuhan dalam 10mM 2,2-DKP. Analisis 16S rRNA dan ujian biokimia telah dijalankan untuk mengenal pasti identiti bakteria. Dari analisis 16S rRNA, bakteria (ST1) mempunyai identiti 95% dengan *Burkholderia* sp. ST1 adalah bakteria gram negatif dan ia mempunyai banyak persamaan dengan *Burkholderia* sp. melalui pemerhatian mikroskopik dan ujian biokimia. Teknik PCR telah dijalankan demi menentukan gen dehalogenase *Burkholderia* sp. Gen ini juga ditentukan sama ada ia berkait rapat dengan kumpulan I atau kumpulan II mengikut sistem klasifikasi Hill *et al.*, (1999). Amplifikasi PCR dan keputusan penjujukan menunjukkan bahawa primer-primer dh1B-314 dan dh1B-637 menunjukkan terdapat amplifikasi PCR dan primer-primer deh H2-1157 dan deh H2-1662 (degradasi spesifik haloasetik asid) tidak menunjukkan sebarang amplifikasi. Keputusan ini menunjukkan bahawa bakteria (ST1) hanya mengekod dehalogenase kumpulan I dan bukan steroselektif adalah persetujuan bagi kumpulan haloalkanoik asid seperti 2,2-DKP, D, L-2KP). Keputusan BLASTn menunjukkan bahawa separa gen tidak mempunyai jujukan identiti yang ketara kepada jujukan dalam pangkalan data. Dehalogenase ini mungkin menunjukkan bahawa ia tergolong dalam kumpulan yang lain.

TABLE OF CONTENTS

CHAPTER	TITLE	PAGE
	TITLE	I
	DECLARATION	Ii
	DEDICATION	Iii
	ACKNOWLEDGMENT	Iv
	ABSTRACT	V
	ABSTRAK	Vi
	TABLE OF CONTENTS	Vii
	LIST OF TABLES	Xi
	LIST OF FIGURES	Xiii
	LIST OF ABBREVIATIONS/ SYMBOLS	Xv
	LIST OF APPENDICES	Xvii
1	INTRODUCTION	1
	1.1 Background of study	1
	1.2 Halogenated Compound in Biosphere	3
	1.3 Problem Statement	4
	1.4 Objectives of Study	5
	1.5 Research Scope	5
	1.6 Significance of Study	6

2	LITERATURE REVIEW	7
2.1	The Properties of 2, 2-Dichloropropionate	7
2.2	Herbicidal Properties	9
2.3	Non Herbicidal Effects of 2,2-DCP	9
2.4	Molecular Fate of 2, 2-DCP	10
2.5	Biodegradation of Halogenated Aliphatic Acid	10
2.6	Bacterial Degradation of 2, 2-DCP	12
2.7	Basic Classification and Function of Dehalogenases	14
2.8	Microbial Degradation of Halogenated Compound	17
2.9	The Impact of Pesticides in The Environment and Human Concerns	20
2.10	The Chemistry of Halogenated Compound	23
2.11	The Mechanisms of Dehalogenation	25
2.12	Bacterial Identification by 16S rRNA Gene Analysis	28
2.13	Multiple Sequence Alignment	29
2.14	Basic Concept of Phylogenetic Tree	30
2.15	Phylogenetic Analysis Using Distance-Matrix approach	31
2.16	Neighbor-Joining Method	32
2.17	Mega 5.10 Software as Computational Tool	32
3	METHODOLOGY	34
3.1	Sample Processing and Purification	34
3.2	Preparation of Stock Solution	35
3.2.1	Composition of Growth Media (Liquid Media)	36
3.2.2	Composition of Growth Media (Solid Media)	37
3.3	Maintenance of Glycerol Stock Culture	38
3.4	Measurement of Microbial Growth	39

3.4.1	Growth Profile	39
3.4.2	Halide Ion Assay	39
3.4.2.1	Preparation of Working Reagent	40
3.4.2.2	Standard Curve and Sample Testing	41
3.5	Scanning Electron Microscopy (SEM)	42
3.6	Biochemical Test and Differential Staining	42
3.6.1	Gram Staining	42
3.6.2	Spore Staining (Schaeffer-Fulton Method)	43
3.6.3	Motility Test	44
3.7	Biochemical Test	44
3.7.1	Catalase Test	44
3.7.2	Citrate Test (Simmons Test)	45
3.7.3	Gelatin Liquefaction Test	46
3.7.4	Indole Test	46
3.7.5	MacConkey Agar (Lactose Utilization Test)	47
3.7.6	Nitrate Reduction Test	47
3.7.7	Oxidation Fermentation Glucose Test	48
3.7.8	Urease Test	49
3.7.9	Starch Test	49
3.7.10	Triple Sugar Iron Test	50
3.7.11	Casein Hydrolysis	50
3.8	Sterilization of Apparatus and Solutions	51
3.9	16S rRNA Genomic Analysis	51
3.9.1	DNA Extraction	52
3.9.2	Measuring of DNA Concentration	53
3.9.3	Polymerase Chain Reaction(PCR)	53
3.9.3.1	Identification of Dehalogenase Gene	54
3.9.3.2	Identification of Bacteria by 16S rRNA	56
3.9.4	PCR Product Purification	57

3.9.5	Agarose Gel Electrophoresis	57
3.9.6	Sequencing and Analysis of PCR Products	59
3.9.7	Phylogenetic Analysis of 16S rRNA Gene	59
4	EXPERIMENTAL RESULT	60
4.1	Isolation and of Bacteria from Agriculture Area	60
4.2	Microscopic Observation and Gram Staining	62
4.3	Scanning Electron Microscopy Result	64
4.4	Bacterial Growth Profile	65
4.5	Halide Ion Assay	67
4.6	Biochemical Tests Result	69
4.7	Molecular Analysis	71
4.7.1	DNA Extraction Result	71
4.7.2	Polymerase Chain Reaction of 16S rRNA Gene	72
4.7.3	Sequencing of 16S rRNA Gene	73
4.7.4	Phylogenic Tree Analysis	80
4.7.5	Pairwise Distance Analysis	81
4.7.6	Dehalogenase Gene Amplification Result	85
4.7.7	Sequencing and Analysis of Putative Dehalogenase Gene	86
5	DISCUSSION	90
6	CONCLUSION	93
7	FUTURE WORKS	95
	REFERENCES	96
	APPENDIX A~J	107~116

LIST OF TABLES

TABLE NO.	TITLE	PAGE
2.1	Examples of Microorganisms Involved in The Biodegradation of Chlorinated Aliphatic Hydrocarbons	12
2.2	Some Bacteria that Producing 2-haloalkanoic acid Hydrolytic Dehalogenase	15
2.3	The Function of Some Microbial Dehalogenases	16
2.4	Global Chemical Pesticides Market (1997)	21
2.5	Explanation of Major Groups of Chemical Pesticides	21
2.6	Electro Negativity in Pauling Unit	24
3.1	The Composition of Minimal Media of Stock Solution(10x Concentration)	35
3.2	Preparation of 100ml Culture of 10mM 2, 2-DCP Minimal Medium	37
3.3	Preparation of Solid Minimal Medium	38
3.4	Types of Halide Ions	40
3.5	Serial Dilution of NaCl (10mM) with Minimal Media	41
3.6	Oligonucleotide Primer Sequence	54
3.7	The Components in the PCR Reaction for Dehalogenase Gene Amplification	55
3.8	PCR Cycle for Putative Dehalogenase Gene Amplification	55
3.9	PCR Cycle for 16S rRNA Gene Amplification	56

4.1	Colony Characteristics of Bacteria HY1 on 10mM 2, 2-dichloropropionate	62
4.2	The Results of Morphological and Biochemical Tests	70
4.3	The Top 10 Entries Species in the Database	74
4.4	The Top 5 Entries in the Database that Show Highest Identity to HY1 Dehalogenase	89

LIST OF FIGURES

FIGURE NO.	TITLE	PAGE
2.1	Structure of 2, 2-Dichloropropionate	8
2.2	Degradability of Some Halogenated Aliphatic Compounds	11
2.3	Degradation Process of 2, 2- DCP	14
2.4	Calculation of Pk_a	24
2.5	Reaction Mechanisms of Dehalogenases Catalyzing the Hydrolytic Dehalogenation	26
3.1	Promega Marker Ladder (1 kb)	58
4.1	Pure Colonies after 7 Days Incubation in 10 mM of 2,2 DCP	61
4.2	Results of Gram Staining for Bacteria HY1 in Different Magnifying Powers	63
4.3	The Results of Scanning Electron Microscopy	64
4.4	The Turbidity of Bacterial Growth (Bacteria HY1) on 10mM 2,2-DCP After 4 Days Incubation	65
4.5	Growth Curve of Bacterium HY1 on 10 mM 2, 2-DCP	66
4.6	Standard Curve of Chloride Ion Released	67
4.7	Concentration of Chloride Ion (in mmol/L) Released in Minimal Medium Containing 10mM 2, 2-DCP	68
4.8	Correlation Between Growth and Chloride Ion Released in 10mM 2, 2-DCP of HY1	69
4.9	The Result of Gel Electrophoresis of Purified DNA	71

4.10	The Result of Gel Electrophoresis for PCR Product (16S rRNA Gene) for Bacterium HY1 Detected Under UV light	72
4.11	The Result of BLASTn Showed the Similarity Between Bacterium HY1 and Other Species	73
4.12	Strain HY1 16S rRNA Partial Sequence (1442 letters)	75
4.13	Multiple Sequence Before Alignment Using ClustalX2	76
4.14	Multiple Sequence After Alignment Using ClustalX2	76
4.15	The Nexus Format Results from ClustalX2 Before Editing	77
4.16	The Results of Nexus Format Using MEGA 5 Software After Editing	78
4.17	The Result of Alignment Between HY1 Sequence and The Top 10 Entries Sequences Using MEGA 5 Software	80
4.18	The Neighbour-Joining Phylogeny Tree of HY1 Using MEGA5 Software	81
4.19	The Result of Pairwise-Distance Analysis	82
4.20	Datasheet of Pairwise Distance Calculation Using MEGA5 Software	84
4.21	PCR Product for Deh-Gene (Gel Electrophoresis) for Bacterium HY1 Detected Under UV Light	86
4.22	Partial Sequence of Deh-Gene (dh1B-314) of Bacterium HY1	87
4.23	Nucleotide and Deduced Amino Acid Sequence of HY1	88

LIST OF SYMBOLS/ABBREVIATIONS

A	-	Absorbance
BLAST	-	Basic local alignment search tool
°C	-	Degree Centigrade Celsius
[Cl ⁻]	-	Concentration of Chloride Ion
2,2-DCP		2,2-Dichloropropionate
2-CP	-	2-chloropropionic acid
EDTA	-	Ethylenediaminetetraaceticacid, (HOOCCH ₂) ₂ N(CH ₂) ₂ N(CH ₂ COOH) ₂
EMBL	-	European Molecular Biology Laboratory
EtBr		Ethidium Bromide
kDA	-	Kilo Dalton
Hrs	-	Hours
Min	-	Minutes
Sec	-	Seconds
dNTPs	-	Deoxyribo Nucleotide TriPhosphates
PCR	-	Polymerase Chain Reaction
PCB		Polychlorinated Biphenyl
DNA	-	Deoxyribonucleic Acid
pKa		Acid dissociation constant
G	-	Gram
Mg	-	Milligram
µg	-	Microgram
Ng	-	Nanogram
gmol ⁻¹		Grams per mole

gcm ⁻¹		Grams per cubic centimeter
bp	-	Base pair
Kb		Kilo base
L	-	Liter
ml	-	Mililiter
μl	-	Microliter
mm	-	Micrometer
M	-	Molar
mM	-	Milimolar
μM	-	Micromolar
OD	-	Optical density
dH ₂ O	-	Deionized water
MgCl ₂	-	Magnesium chloride
CaCl ₂	-	Calcium chloride
LB	-	Luria Bertani medium
%	-	Percent
EDTA	-	Ethylene Diamine Tetra-Acetic acid
NaCl	-	Sodium chloride
w/v	-	Weight per volume
5'	-	5 prime-end
3'	-	3 prime-end
Rpm	-	Rotations per minute
V	-	Volts
TAE buffer	-	Tris-acetate –EDTA buffer
EDB		Ethylene Di-Bromide
A	-	Adenine
C	-	Cytosine
G	-	Guanine
T	-	Thymine
BLAST	-	Basic Local Alignment Search Tool
MEGA5	-	Molecular Evolutionary Genetics Analysis Software version 5
NCBI	-	National Centre for Biotechnology Information
M. W.	-	Molecular weight
HAA		Halogenated Alkanoic Acids

LIST OF APPENDICES

APPENDIX	TITLE	PAGE
A	The Calculation for Preparation of 2,2-DCP	107
B	Bacteria HY1 as Appeared Using Scanning Electron Microscopy	108
C	Growth Curve Calibration	109
D	Chloride Ion Assay Standard Calibration	110
E	Concentration of Chloride Ion (in mmol/L) Released in Minimal Medium Containing 10mM 2, 2-DCP	111
F	The Calibration of Relationship Between Growth and Chloride Ion Liberation of Bacteria HY1	112
G	Partial Sequence of Forward Primer of 16S rRNA from Bacterium HY1	113
H	Partial Sequence of Reverse Prime of 16S rRNA from Bacterium HY1	114
I	Protein BLAST Search of Dehalogenase Gene of Bacterium HY1	115
J	Viva Presentation Slides	116