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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)

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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 compound 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 dhlB-314 and dhlB-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 haloalkonoic 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 membuatkannya 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 pengceraian ion klorida menggunakan kaedah ceraian kolorimetrik. Pengceraian 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 dhlB-314 dan dhlB-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.

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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, $(HOOCCH_2)_2N(CH_2)_2N(CH_2COOH)_2$
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
Mg	-	Microgram
Ng	-	Nanogram
$gmol^{-1}$		Grams per mole

gcm^{-1}		Grams per cubic centimeter
bp	-	Base pair
Kb		Kilo base
L	-	Liter
Ml	-	Mililiter
Ml	-	Microliter
Mm	-	Micrometer
M	-	Molar
mM	-	Milimolar
µM	-	Micromolar
OD	-	Optical density
dH_20	-	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

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