Molecular analysis of L-haloacid dehalogenase from *Arthrobacter ureafaciens* sp *S1*

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To my beloved family and husband

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ABSTRACT

Using enrichment technique a bacterium was isolated from soil showing the ability to grow on 20 mM 2, 2-dichloropropionate (2, 2-DCP) whether in solid or in liquid minimal media. Further characterization was carried out using 16S rRNA analysis. The partial 16S rRNA sequence (1450bp) was BLASTn in the NCBI database resulted in 95% identity to *Arthrobacter ureafaciens* (Accession number. FN433020.1). Therefore, this isolate was designated as *Arthrobacter ureafaciens* S1. In this study, we focused on the dehalogenase gene identification by PCR amplification using genomic DNA from *Arthrobacter* strain S1. Two kinds of primers DehA (reverse&forward) and DehB (reverse&forward) were used for dehalogenase gene amplification. The PCR results showed 1300 bp and 700 bp using DehA (reverse&forward) and DehB (reverse&forward), respectively. Moreover, the partial gene sequence was assembled, analyzed and compared with Gen Bank database in NCBI (National Center for Biotechnology Information) and converted into amino acids sequence. Using BLASTp tools, analysis showed distance relationship with L-2-haloacid sequence amino sequence that belonged to group II of α-halocarboxylic acid (αHA) dehalogenase. In conclusion, current investigations suggested that there were more than one dehalogenases were amplified and these dehalogenases maybe a novel based on the size of the amplified band patterns compared to the previously reported. However, further investigations need to be carried out.

ABSTRAK

Menggunakan teknik pengayaan bakteria telah diasingkan daripada tanah yang menunjukkan keupayaan untuk berkembang pada 20 mM 2, 2-dichloropropionate (2, 2-DCP) sama ada pepejal atau cecair media minimum. Pencirian selanjutnya telah dijalankan menggunakan 16S rRNA analisis. Jujukan rRNA 16S sebahagian (1450bp) adalah BLASTn dalam pangkalan data NCBI menyebabkan pengenalan 95% Arthrobacter ureafaciens (Accession nombor. FN433020.1). Oleh itu, ini mengasingkan telah ditetapkan sebagai Arthrobacter ureafaciens S1. Dalam kajian ini, kami memberi tumpuan kepada pengenalpastian gen dehalogenase oleh amplifikasi PCR menggunakan DNA genom dari S1 ketegangan Arthrobacter. Dua jenis primer DehA (berbalik & ke hadapan) dan DehB (berbalik & ke hadapan) telah digunakan untuk penguatan gen dehalogenase. Keputusan PCR menunjukkan 1300 bp dan 700 bp menggunakan DehA (berbalik & ke hadapan) dan DehB (berbalik & ke hadapan), masing-masing. Selain itu, jujukan gen separa telah dipasang, dianalisis dan dibandingkan dengan Jen Bank pangkalan data dalam NCBI (Pusat Maklumat Bioteknologi Nasional) dan ditukar ke dalam jujukan asid amino. Menggunakan alat-alat BLASTp, analisis menunjukkan hubungan jarak dengan L-2-haloacid urutan urutan amino yang dipunyai oleh kumpulan II asid α-halocarboxylic (αHA) dehalogenase. Kesimpulannya, siasatan semasa mencadangkan bahawa terdapat lebih daripada satu dehalogenases telah dikuatkan dan ini dehalogenases mungkin novel yang berdasarkan saiz corak jalur yang dikuatkan berbanding kepada yang dilaporkan sebelum ini. Walau bagaimanapun, siasatan lanjut perlu dijalankan.

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LIST OF ABBREVIATIONS

X	Xenophors
TCA	Trichloroethane
PCR	Polymerase chain reaction
CoA	CoEnzyme A
°C	Degree celcius
dATP	Deoxyadenosine triphosphate
dCTP	Deoxycytidine triphosphate
dTTP	Deoxythymidine triphosphate
dGTP	Deoxyguanosine monophosphate
Ta	Annealing temperature
Tm	Melting temperature
Bp	Base pair
QIAGen	QIA quick gel extraction kit
μL	Micro litre
mM	mili mole
NCBI	National center for biotechnology information
OD	Optical density
BLAST	Basic local alignment search tool
EC	Enzyme code
ATP	Adnosin Triphosphate
RNA	Ribonucleic acid
tRNA	Transfer RNA
Deh	Dehalogenase
3D	Three Dimensional
2,2-DCP	2,2-Dichloropropionate
A600nm / A460nm	Absorbance at 600 Nanometre / 460 Nanometre
BS	Basal Salt Solution
DNA	Deoxyribonucleic Acid
psi	Pound Force per Square Inch

rpm	Round per Minute
RNAse / rRNA	Ribonucleic Acidase / Ribosomal Ribonucleic Acid
TM	Trace Metal Solution
UV	Ultra Violet Ray
V	Voltage
vs	Versus
w/v	Weight per Volume Percentage

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CHAPTER 1

INTRODUCTION

1.1 Background

Xenobiotic is a chemical component and not be naturally produced by organic organisms. Xenobiotic compounds are termed in Greek root xeo and biotic life. One of the largest numbers of environmental pollutions has been caused by human activities in agriculture and industry areas that are named halogenated organic compounds. These compounds are very toxin and making health problems for human.

Halogenated compounds are largely used as herbicides, pharmaceuticals, fungicides, insecticides, and intermediate in organic synthesis. More than 130 chlorinated compounds have been isolated from bacteria, fungi, marine organism, plant and ferns. In recent fifty years ,many studies on biochemistry and genetics of Dehalogenase microorganisms have been done, in order to realize and evaluate the potential of Xenobiotic degradation in bacteria and natural microcosms due to deal with environmental problems. Biodegradation via microorganism have been investigated by several important aspects to understand the microbial physiology biochemistry genetics and molecular biology of Dehalogenation system. The carbon-halogen (fluorine (F), chlorine (Cl), bromine (Br), iodine (I)) bond has been cleaved

in metabolism and co-metabolisms pathways and some degradation of these compounds are an intermediate reaction (Fetzner and Lingens, 1994).

A huge number of bacteria and fungi have the capability to degrade organic pollutants .Furthermore, these bacteria and fungi that capable of using those compounds as their carbon and energy source (Wolfgang and Martin, 1997).

Some aerobic and anaerobic bacterium are capable the cleavage of halogen-carbon bond in the halogenated compounds by four processes in which oxygen or nitrate, sulfur as electron acceptors. The first process would be oxdisable substrate with either oxygen or nitrate as an electron acceptor; fermentative metabolisms which halogenated intermediate is as an electron acceptors; the third electron acceptor is liberation halide, and co-metabolic transformation that can be linked to release other metabolic (Janssen, Oppentocht and Poelarends, 2001)

1.2 Problem Statement

Halogenated organic compounds are the huge and main groups of Xenobiotic pollutions in the environment which are resulted from pesticide and herbicide, insecticide, fungicides, plasticizers and intermediates due to chemical syntheses, although these components are high risky and toxicity. The chlorinated compounds are as inhibitors in key reaction of central metabolisms. Meanwhile the high cost of the treatment contaminations and the economic removal of polluting halogenated have been estimated by microorganisms (Slater *et al.*, 1996).

Consequently, the aim of this study was revealed a better understanding of these mechanisms and pathways. The current study focused on isolation and identification of L-2.Haloacid gene sequence from *Arthrobacter* sp in order to understand the mechanisms biodegradation of Dehalogenase.

1.3 Objectives

The objectives of this study were:

1. To identify and characterize the respective L-2-haloacid gene by using PCR amplification and bioinformatics tools due to analysis of L-Haloacid gene.

1.4 Scope of Study

This research was conducted by the isolating of *Arthrobacter sp* from contaminated soil. The microorganisms were grown on 2, 2-dichloropropionate as a growth substrate. *Arthrobacter sp* was tested by the gram staining, the growth curve. PCR amplification was carried out to isolate L-halo acid gene. The genes were sequenced and bioinformatics analyses that proposed a great insight on the possible enzymes for biodegradation of halogenated compounds.

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