IDENTIFICATION OF DEHALOGENASE PRODUCING PSYCHROTROPHIC BACTERIA

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This dissertation is dedicated to my mother for her endless support and encouragement.

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ABSTRACT

2, 2 dichloropropionic acids (Dalapon) like most halogenated compounds are commonly used as herbicides and employed in agricultural areas and industries. Toxicity of these xenobiotic compounds causes serious environmental problems. Bacillus sp ih1 was isolated from top cliff soil collected from Antarctica. The bacteria was first grown on Antarctic bacterial medium and later transferred to a minimal medium containing 2, 2, dichloropropionic acid as carbon source. It grew slowly in the minimal media in different concentrations of 10mM, 20mM, 30mM and 40mM of 2, 2 DCP. The best growth was observed in 20mM of 2, 2-DCP with doubling time 32hours. To monitor the degradation activity of the bacteria, halide ion assay was carried out to check the release of chloride ion. The best release of chloride was 0.657 mmol/L in 20mM of 2, 2-DCP. The bacteria was identification using 16S rRNA, genomic DNA extraction method and PCR amplification of 16S rRNA was performed using universal primers 27F and 1492R. Nucleotide blast (BLASTn) showed 97% similarity with bacillus sp. Results from biochemical tests further confirm the bacteria as bacillus sp. Using phylogeny.fr, sequences from nucleotide blast result were used to build a phylogeny tree based on neighbour to neighbour joining.

ABSTRAK

Asid 2, 2 dichloropropionic (Dalapon) merupakan salah satu komponen sebatian halogen yang sering digunakan pakai dan dipraktikkan sebagai racun herba di dalam sektor pertanian dan industri. Kadar toksik di dalam sebatian xenobiotik ini menyebabkan berlakunya pencemaran alam sekitar yang serius. Bacillus sp ih1 telah diasingkan daripada sampel yag diperolehi dari tebing tinggi di Antartika. Pada mulanya bakteria ini telah dibiakkan di dalam media bakteria Antartika dan kemudiannya dipindahkkan ke dalam media minima yang mengandungi asid 2, 2, dichloropropionic sebagai sumber karbon. Bakteria ini telah menunjukkan kadar pertumbuhan yang perlahan di dalam media minima yang mempunyai kadar kepekatan 2,2 DCP yang berbeza iaitu 10mM, 20mM, 30mM and 40mM. Namun, kadar pertumbuhan yang terbaik bagi bakteria ini telah direkodkan di dalam media yang mengadungi 20mM 2,2- DCP dengan kadar pergandaan masa selama 32 jam. Bagi memerhati kebolehan degradasi bakteria ini, kaedah ion halide telah digunapakai bagi menguji kadar pelepasan ion klorin. Kadar pelepasan ion klorin yang terbaik yang telah direkodkan adalah 0.657 mmol/L di dalam 20mM 2,2-DCP. Kemudian, proses identifikasi bakteria ini telah dilakukkan dengan menggunakan kaedah 16s rRNA. Kedah pengektrakkan genomik DNA dan amplifikasi PCR telah dilakukkan dengan menggunakkan primer universal 27F dan 1492R. Kaedah BLASTn menunjukkan bahawa bakteria ini mempunyai 97% kadar persamaan identiti dengan bacillus sp. Tambahan lagi, keputusan analisa biokimia juga telah mengenalpasti bakteria ini tergolong dalam keluarga bacillus sp. Kemudian analisa pokok phylogeni telah dilakukkan dengan menggunakan susunan nucleotida yang diperolehi melalui kaedah BLAST berdasarkan sambungan jiran ke jiran.

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LIST OF ABBREVIATIONS

2, 2-DCP	-	2, 2 dichloropropianic acid
ABM	-	Antarctic Bacterial Medium
BLAST	-	Basic Local Alignment SearchTool
PCR	-	Polymerase Chain Reaction
NCBI	-	National Centre for Biotechnology Information
DNA	-	Deoxyribonucleic Acid
EDTA	-	Ethylene Diamine Tetraacetic Acid
MgSO4	-	Magnesium Sulphate
PUFAs	-	Polyunsaturated Fatty Acid
Rnase/rRNA	-	Ribonucleic Acidase/Ribosomal Ribonucleic Acid
bp	-	Base pairs Base pairs (Nucleotide)
kbp	-	Kilo base pairs
A600nm	-	Absorbance at 600 nanometer
A460nm	-	Absorbance at 460 nanometer
NaCl	-	Sodium chloride
ng	-	Nanogram
mM	-	Milimolar
µ	-	Specific growth rate
w/v	-	Weight per volume
µL	-	Micro litre
UV	-	Ultraviolet
v/v	-	Volume per volume
1000X	-	1000 Times Magnification

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

The chemically synthesized herbicide application in agricultural areas and industries has recorded remarkable successes by increasing products yield, but at the same time is of serious concern to the public. These recalcitrant pollutants can cause environmental pollution of underground water and rivers. Studies have also shown that these compounds can be carcinogenic to both human and animals (Zulkifly *et al.*, 2010). These halogenated xenobiotic compounds are converted to harmless form using the ubiquitous degradation ability of microorganism or by employing non biological method, but the former is more preferable because they are safer, economical, and environmental friendly (Haritash and Kaushik, 2009). Various microorganisms can degrade halogenated compounds and utilize its as carbon source. Therefore, perform a significant function in detoxification of halogenated compounds. These organisms achieved this by secreting an inducible enzyme which cleaved their carbon halogen bonds. These enzymes are called dehalogenases. These enzymes are found to be cluster in proteobacteria which have been characterized and found to be substrate specific. However, there are few literatures that reported about degradation of halogenated compounds by psychrotrophic microorganism. This study is very important because identification of bacteria that has the ability to degrade 2, 2, dicholoropropionic acid will be useful in future bioremediation of halogenated compounds in solving the environmental pollutions caused by these compounds.

1.2 Problem Statement

Environmental pollution by halogenated compounds and its adverse effects are among the most rising problems the world is facing today. Past century has witnessed a lot of advancement in industrial technology and intensification of agricultural practices, these xenobiotics functions as herbicide, insecticide, fungicides, pesticide solvents, and hydraulics (Fetzner and Lingens, 1994). Microbial degradation is one of the favoured ways in natural removal of these compounds from contaminated environments. However, more research need to be carried out about microbial degradation of halogenated compounds. Psychotropic bacteria grow at temperature range between 20°C to 40°C and are distributed widely in natural environment and adapt more to wide temperature ranges (Radjasa *et al.*,2001). A large proportion of earth surface is occupied by cold environment such as the arctic, Antarctic regions.

Despite an increasing number of microbial diversity assessments of polar regions in recent years, relatively little is known about their degradation ability especially halogenated compounds. Also, early studies of bacteria in Arctic environmental samples have focused much on abundance and diversity and to a certain extent; the influence of climatic conditions on these microbes with relatively few information about the potentials of the organisms recovered from such environment; in terms of biotechnological applications.

1.3 Objectives

The specific objectives of this research are:

i. To isolate psychotrophic bacteria from Antarctic environment using standard methods.
ii. To identify dehalogenase producing psychotrophic bacteria from Antarctica.

iii. To study the evolutionary relationship of isolated bacteria that degrade 2, 2 DCP.

1.4 Scope of the Study

This scope of this study include isolation of bacteria from Antarctica which will followed by testing its ability to degrade 2, 2 DCP. Identification of pure of isolated bacteria will be done using molecular approach. Determination of bacterial growth on 2, 2-dichloropropionic acid will be done by measuring absorbance at A_{680nm}. Evolutionary relationship of isolated bacteria will also be studied.

1.5 Significance of the Study

Considering the advantages biological methods have over non biological methods in decontamination of halogenated polluted sites (safer, cheaper, and environmental friendly), search for psychrotrophic organisms that has dehalogenase has increased. This is because psychrotrophic organisms have wide range temperature for growth (20°C up to 40°C) and still have good enzymatic activity. They can be supplied all year round because there are no seasonal fluctuations. Isolating psychrotrophic bacteria that produces dehalogenases will be useful in developing new bioremediation methods that can be use in different environment.This research will also contribute to the existing knowledge of psychrotrophic bacterial diversity in Antarctica.

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