UNIVERSITI PUTRA MALAYSIA

PHENOL DEGRADATION AND MOLECULAR ANALYSIS OF PHENOL HYDROXYLASE FROM *Pseudomonas cedrina*

TENGKU NUR SALEHA TENGKU KAMRUL

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TENGKU NUR SALEHA BINTI TENGKU KAMRUL

162297

DEPARTMENT OF BIOCHEMISTRY

FACULTY OF BIOTECHNOLOGY AND BIOMOLECULAR SCIENCES

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PENGESAHAN

Dengan ini adalah disahkan bahawa tesis projek yang bertajuk "Phenol degradation and molecular analysis of phenol hydroxylase from *Pseudomonas cedrina*" telah disiapkan serta dikemukakan kepada Jabatan Biokimia oleh Tengku Nur Saleha Binti Tengku Kamrul (162297) sebagai syarat untuk kursus BCH 4999 Projek.

Disahkan oleh,

..... Tarikh : .....

(Dr. Nur Adeela Binti Yasid)
Penyelia projek
Jabatan Biokimia
Fakulti Bioteknologi dan Sains Biomolekul
Universiti Putra Malaysia

..... Tarikh : .....

(Prof. Dato' Dr. Abu Bakar Salleh)
Ketua Jabatan Biokimia
Fakulti Bioteknologi dan Sains Biomolekul
Universiti Putra Malaysia

ABSTRACT

Phenols are toxic aromatic compounds that act as serious environmental pollutants especially in industrial wastewater. These phenolic compounds are harmful towards living organisms and can lead to serious health problems if the sources of phenol pollution are not being treated immediately. Thus, bioremediation process using microorganisms is a preferable method to remove phenol since the process is cheaper, effective and forms non-toxic end products. In this study, an aerobic fluorescent bacterium, *Pseudomonas cedrina* was successfully shown its potential ability to degrade phenol and utilise it as its sole source of carbon since it can degrade phenol concentration ranging from 0.1 to 1.0 g/L. Furthermore, the highest phenol removal rate of *P. cedrina* was at 0.3 g/L. Phenol hydroxylase, an enzyme that helps to degrade phenol into catechol, was amplified using specific primers at optimum temperature of 50.7°C. Besides that, DNA sequencing result has revealed that the size of DNA sequence of putative phenol hydroxylase is 1166 bp. However, BLAST algorithm results have shown negative results that phenol hydroxylase gene was not identified in *P. cedrina*. Therefore, several recommendation involving molecular genetic studies are emphasised for future work research.

ABSTRAK

Fenol merupakan bahan toksik yang bertindak sebagai pencemar alam terutamanya di dalam pembuangan air sisa di kawasan perindustrian. Bahan-bahan yang mengandungi fenol terbukti amat berbahaya kepada seluruh hidupan bumi dan berpotensi untuk mendatangkan masalah kesihatan yang teruk sekiranya sumber-sumber yang mengakibatkan pencemaran fenol ini tidak dipulihkan dengan segera. Oleh itu, process bioremediasi dengan menggunakan mikroorganisma merupakan kaedah yang sewajarnya dilakukan untuk penguraian fenol memandangkan proses ini amat menjimatkan kos, berkesan dan menghasilkan produk akhir yang tidak berbahaya. Di dalam kajian ini, *Pseudomonas cedrina* telah berjaya membuktikan keupayaannya untuk mengurai fenol dan menggunakan fenol tersebut sebagai sumber karbon dan tenaga setelah bakteria ini berjaya mengurai kepekatan fenol dari 0.1 sehingga 1.0 g/L. Tambahan pula, *P. cedrina* telah mencatatkan kadar penguraian fenol yang tertinggi pada kepekatan 0.3 g/L. Fenol hidroksilase, iaitu enzim yang membantu mengurai fenol menjadi katekol, telah diamplifikasikan dengan menggunakan primer tertentu pada suhu optimum iaitu 50.7°C. Selain itu, keputusan penjujukan DNA telah menunjukkan saiz urutan DNA yang dianggap fenol hidroksilase ialah 1166 bp. Walau bagaimanapun, keputusan kaedah BLAST telah memaparkan keputusan yang negatif di mana fenol hidroksilase tidak berjaya dikenalpasti di dalam *P. cedrina*. Justeru, beberapa cadangan yang melibatkan kajian genetik molekular telah dikemukan sebagai rujukan untuk kajian yang akan datang.

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LIST OF ABBREVIATIONS

%	Percent
4-AAP	4-aminoantipyrine
bp	Base pair
ºC	Degree celsius
CoA	Coenzyme A
DNA	Deoxyribonucleic acid
dNTP	Deoxyribose nucleotide triphosphate
et al.,	And friends
EtBr	Ethidium bromide
FAD	Flavin adenine dinucleotide
g	Gram
H_2O_2	Hydrogen peroxide
$K_2Fe(CN)_6$	Potassium ferric cyanide
K_2HPO_4	Dipotassium hydrogen phosphate
kb	Kilobase
KH_2PO_4	Potassium dihydrogen phosphate
L	Litre
M	Molar
mg	Milligram
$MgCl_2$	Magnesium chloride
min	Minute
ml	Millilitre
mM	Millimolar
MSM	Mineral salt medium
NaCl	Sodium chloride
NADH	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
nm	Nanometer
OD	Optical density
ppm	Parts per million
PCR	Polymerase chain reaction

RNA	Ribonucleic acid
rpm	Revolution per minute
sec	Second
TAE	Tris-acetate EDTA
TCA	Tricarboxylic acid
UV-VIS	Ultraviolet visible
µg	Microgram
µl	Microlitre
V	Voltage
w/v	Weight/volume

CHAPTER 1

INTRODUCTION

Petroleum hydrocarbons such as alkanes, aromatic and polyaromatic hydrocarbons are well known for their most widespread contaminants in the environment (Margesin *et al.*, 2013). Phenol and phenolic compounds are several examples of toxic aromatic compounds that act as ubiquitous pollutants, especially in industrial wastewater (Gad and Saad, 2008; Margesin *et al.,* 2013, Bonfa *et al.,* 2013). They are toxic towards microorganisms, aquatic flora and fauna, at even low concentrations (Margesin *et al.,* 2013; Bonfa *et al.,* 2013). Besides, they are also harmful to humans and animals if they are exposed to phenol compounds through inhalation, oral and dermal (Ohio Environmental Protection Agency, 2002; Michalowicz and Duda, 2007; Basha *et al.*, 2010). These phenolic compounds can induce genotoxic, carcinogenic, immunotoxic and haematological effects as well as having a high bioaccumulation rate (Gad and Saad, 2008). Therefore, phenol degradation is important for maintaining the environmental health.

Conventional remediation processes for phenol removal involve physico chemical processes such as solvent extraction, chemical oxidation and adsorption (Pradeep *et al.*, 2011; Chandana *et al.*, 2011). These treatment technologies are, however, expensive and tend to produce hazardous end products (Basha *et al.*, 2010; Pradeep *et al.*, 2011). Bioremediation is a preferable method to degrade phenol since the process is cheaper, effective and form non-toxic end products (Pessione *et al.*, 1999; Basha *et al.*, 2010; Pradeep *et al.*, 2011). A pure culture from *Pseudomonas* species is an example of soil microorganisms that acts as good phenol biodegrader (Shingler *et al.*, 1989; Basha *et al.*, 2010; Pradeep *et al.*, 2011; Mahiudddin *et al.,* 2012). *Pseudomonas cedrina,* which is under family of *Pseudomonas fluorescens* that produces phenol hydroxylase, plays a crucial role to degrade phenol and utilise it as its sole source of carbon and energy (Lin *et al.*,

2008; Behrendt *et al*., 2009). Hence, a comprehensive understanding is needed on the roles of phenol hydroxylase in *P. cedrina*.

Phenol can be degraded by both aerobic and anaerobic microorganisms (Basha *et al.*, 2010). Since *P. cedrina* is an aerobic microorganism, the enzyme phenol hydroxylase uses the molecular oxygen to hydroxylase phenol into catechol (Lin *et al.*, 2008; Behrendt *et al.*, 2009; Basha *et al.*, 2010; Mahiudddin *et al.*, 2012). The catechol ring can be cleaved by either *ortho*- or *meta* cleavage pathway (Basha *et al.*, 2010; Mahiudddin *et al.*, 2012). Different bacterial strains will metabolise phenol with different kind of cleavage pathways (Basha *et al*., 2010). However, *Pseudomonas fluorescens* and other *Pseudomonas* species work best in *meta*-cleavage pathway (Lin *et al.*, 2008; Basha *et al.*, 2010; Mahiudddin *et al.*, 2012). At the end of this process, both pathways will produce intermediates tricarboxylic acid cycle as their final products (Basha *et al*., 2010; Mahiudddin *et al*., 2012).

The researches on isolation and degradation of phenol by phenol degrading bacteria have been grown continuously over time. However, the comprehensive knowledge and understanding on the potential of *P. cedrina* in phenol biodegradation and its molecular genetic studies of phenol hydroxylase gene are lacking.

By considering the potential of *P. cedrina* in the biological treatment field and importance of molecular genetic studies of phenol hydroxylase for the subsequent enzyme purification (Shingler *et al.*, 1989), a research investigation is needed to be conducted.

Therefore, the present study was carried out with the following objectives:

1. To determine the highest phenol concentration that can be degraded by *P. cedrina*.
2. To isolate and purify phenol hydroxylase gene from *P. cedrina.*
3. To identify and analyse the amino acid and DNA sequences of phenol hydroxylase encoding region in *P. cedrina*.

1.1 Hypothesis

In this study, the expected results that need to be obtained at the end of this experiment are:

1. *P. cedrina* has the ability to degrade up phenol until at highest phenol concentration which is 1.0 g/L.
2. Phenol hydroxylase gene could be isolated from *P. cedrina*.
3. The DNA and amino acid sequences of phenol hydroxylase could be identified and analysed from *P. cedrina* using BLAST search program.

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