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PHENOL DEGRADATION AND MOLECULAR VALIDATION OF PHENOL HYDROXYLASE GENE OF Alcaligenes faecalis

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PENGESAHAN

Dengan ini adalah disahkan bahawa tesis projek yang bertajuk "Phenol degradation and molecular validation of phenol hydroxylase gene of *Alcaligenes faecalis*" telah disiapkan serta dikemukakan kepada Jabatan Biokimia oleh Nur Muhamad Syahir Bin Abdul Habib (161060) sebagai syarat untuk kursus BCH4999 Projek.

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ABSTRACT

Currently, phenol pollution has caused some environmental concerns as it causes severe toxicity towards human health and environmental conditions. Intensive efforts to reduce the contamination of pollutants have been done especially in bioremediation techniques. Many microbial species have been introduced to be utilised for contamination clean-up and one of them is *Alcaligenes faecalis*. A study on the phenol degrading ability and molecular analysis of *A. faecalis* was conducted. To study the phenol degrading ability of *A. faecalis*, the bacteria was incubated in six different concentrations of phenol – 0.1, 0.4, 0.5, 0.9, 1.0, and 1.5 g/L. The growth of bacteria and phenol degradation in each phenol concentration were monitored. Among all the concentrations studied, phenol concentration of 0.9 g/L showed the highest degradation rate. Meanwhile, the molecular analysis of the bacteria was carried out by isolating the phenol hydroxylase gene which is responsible for degrading phenol by using the designated primer. The gene was amplified by using PCR technique with an annealing temperature of 56.4°C. The expected size of the gene was between 300 - 400 bp. After DNA sequencing, molecular analysis was done and the DNA fragment obtained had a length of 337 bp. Next, BLAST search was used to confirm the sequence obtained was phenol hydroxylase gene isolated from *A. faecalis*. The BLAST result showed that the phenol hydroxylase gene was successfully amplified from the bacteria. These studies showed the hypothetical use of this bacteria to treat phenol-contaminated environment.

ABSTRAK

Kini, pencemaran fenol telah meningkatkan keprihatinan manusia terhadap alam sekitar kerana fenol telah menyebabkan kesan toksik yang teruk terhadap kesihatan manusia dan alam sekitar. Langkah-langkah intensif telah dilakukan untuk mengurangkan pencemaran terutamanya melalui teknik bioremediasi. Pelbagai spesis mikroorganisma telah diketengahkan untuk pembersihan bahan cemar tersebut dan salah satu daripada mikroorganisma tersebut adalah bakteria *Alcaligenes faecalis.* Satu kajian mengenai keupayaan menguraikan fenol dan analisis molekular oleh *A. faecalis* telah dijalankan. Bagi mengkaji keupayaan menguraikan fenol, bakteria ini telah diletakkan di dalam kepekatan fenol yang berbeza - 0.1, 0.4, 0.5, 0.9, 1.0, dan 1.5 g/L. Pertumbuhan bakteria dan tahap penguraian fenol oleh setiap kepekatan fenol telah dicatat. Berdasarkan hasil kajian, kepekatan fenol 0.9 g/L menunjukkan kadar penguraian yang tertinggi. Sementara itu, untuk menganalisis bakteria ini dari segi molekular, gen fenol hidroksilase yang bertanggungjawab menguraikan fenol telah diasingkan menggunakan primer gen yang telah direka. Seterusnya, gen itu diamplifikasikan menggunakan kaedah PCR bersama dengan suhu perlekatan iaitu 56.4°C. Saiz anggaran gen adalah diantara 300 - 400 bp. Selepas penjujukan DNA, analisis molekular telah dijalankan dan fragmen DNA yang diperolehi adalah 337 bp. Seterusnya, carian BLAST telah dibuat bagi mengesahkan jujukan DNA adalah gen fenol hidroksilase daripada *A. faecalis*. Hasil BLAST menunjukkan gen fenol hidroksilase berjaya diasingkan daripada bakteria ini. Kajian ini menunjukkan kebolehan penggunaan bakteria ini untuk merawat alam sekitar yang tercemar dengan fenol.

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% (w/v)	Percent concentration weight / volume
°C	Degree celsius
$(NH_4)_2SO_4$	Ammonium sulphate
µl	Microlitre
4-AAP	4-aminoantipyrene
bp	Base pair
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
g	Gram
kb	Kilobase
$K_2Fe(CN)_6$	Potassium ferric cyanide
K_2HPO_4	Di-potassium hydrogen phosphate
KH_2PO_4	Potassium dihydrogen phosphate
L	Litre
mg	Milligram
$MgCl_2$	Magnesium chloride
$MgSO_4$	Magnesium sulphate
min	Minutes
MSM	Mineral salt media
ml	Millilitre
mM	Millimolar
NaCl	Sodium chloride
nm	Nanometer
PCR	Polymerase chain reaction
rpm	Revolution per minute
TAE	Tris-acetate-EDTA
UV	Ultraviolet
V	Voltage

CHAPTER 1

INTRODUCTION

Phenol and phenolic compounds are being widely distributed within the environment partly because of natural processes and non-natural processes, due to human and industrial activities. These aromatic pollutants originated mainly from the industrial processes such as resin manufacturing, oil refineries, petrochemicals, steel, pharmaceuticals, dyes, textiles, plastic, as well as the pulp and paper industries (Annadurai *et al*., 2002; Duan, 2011; Ahmad *et al*., 2012; Mahiudddin *et al*., 2012).

Phenol is classified as a highly hazardous chemical for being a persistent compound. This is due to its toxicity, carcinogenic and mutagenic characteristics toward human being, fish, and environment as well as to several biochemical functions (Duan, 2011; Ahmad *et al*., 2012). The pollution effects of phenolic effluent have been reported worldwide and removing them has become a major environmental concern (Mahiudddin *et al*., 2012).

The removal of contaminants from the polluted area has been widely applied using different physical, chemical and biological methods. The biological technique for phenol reduction has been chosen as the technique that poses relatively low cost and offers complete mineralisation (Basha *et al*., 2010; Nawawi *et al*., 2010).

Microorganisms are heavily involved in the bioremediation process (Krastanov *et al*., 2013). In recent years, the number of research on microbial degradation has increased due to its sustainability ways of cleaning up

contaminated areas. Most microbes could quickly adapt and grow at extreme condition using those hazardous compounds as both their carbon and energy sources (Mahiudddin *et al*., 2012). Vast variety of microbes is known to be capable of degrading phenol under both aerobic and/or anaerobic conditions that are regulated by the action of enzymes and the microbial metabolism itself (Mahiudddin *et al*., 2012; Sridevi *et al*., 2012; Nawawi *et al*., 2014).

One of the phenol degrading bacteria that has been studied is the *Alcaligenes faecalis* (Rehfuss and Urban, 2005). Many present studies have shown that *A. faecalis* has the property as a resourceful phenol-degrading bacterium. The effectiveness of a certain catabolic pathway usually depends on the properties of the involved key enzyme (Jiang *et al*., 2007).

Phenol hydroxylase is the key enzyme that responsible for the conversion of phenol to catechol, which is the initial and rate-limiting step in phenol degradation pathways. Both single component and multi-component types of this enzyme have been identified with the latter recognised as the prevalent one in natural environments (Zhang *et al*., 2004).

Recent studies have shown that *A. faecalis* has the ability to degrade phenol using phenol hydroxylase enzyme. However, further research and studies are needed in obtaining a more specific information on the degrading mechanism of the enzyme.

Thus, a study was carried out with the following objectives:

1. To evaluate the ability of *A. faecalis* to degrade phenol.
2. To determine the phenol degradation rate by *A. faecalis* using different concentration of phenol.
3. To isolate and analyse the phenol hydroxylase gene from *A. faecalis*.

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