CHARACTERIZATION AND SCREENING OF THERMOPHILIC STRAINS FOR PHENOL DEGRADATION

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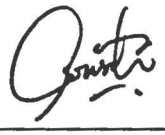

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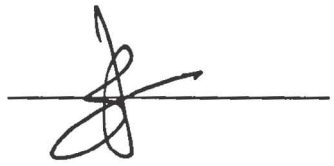

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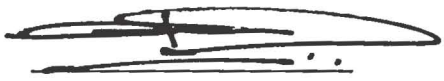

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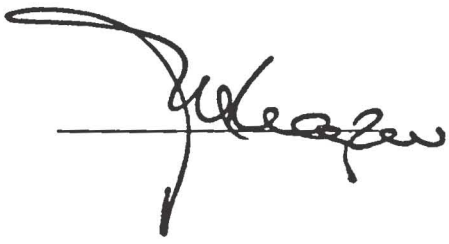

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PENCIRIAN DAN PENGSKRINAN TERMOFILIK STRAIN UNTUK DEGRADASI FENOL

ABSTRAK

Kajian ini adalah mengenai pencirian, pengskrinan, dan penilaian enzim bakteria penguraian fenol yang berpotensi. Objektif kajian ini adalah untuk mencirikan dan menskrinkan bakteria yang dapat mengurai fenol serta penilaian enzim ke atas bakteria tersebut menggunakan catechol 2,3 dioxygenase. Tiga termofilik bakteria daripada NAedEFBs-Cr_I, NAdEFBus-Cr_I, and RdEFBs-Cr_I dicirikan morfologi koloni dan morfologi selnya. Ketiga-tiga sel ini adalah cocci, aerobik bakteria, tidak mempunyai endospora, tidak berupaya mengurangkan sulfur, dan berwarna krim. NAedEFBs-Cr_I dan RdEFBs-Cr_I adalah bakteria Gram-positif dan tidak bergerak manakala NAdEFBus-Cr_I adalah bakteria Gram-negatif dan bergerak. Ketiga-tiga bakteria ini juga diuji lengkungan pertumbuhannya di dalam kaldu Ramsay goncangan 200 rpm selama 24 jam. Suhu optimal untuk ketiga-tiga bakteria ini adalah pada suhu 40 °C. Antara ketiga-tiga bakteria tersebut, NAdEFBus-Cr_I paling berpotensi dalam mendegrad fenol iaitu bertumbuh subur di dalam agar Ramsay dengan mengandungi 0.5 mM, 1.0 mM, dan 1.5 mM fenol. Bakteria ini juga bertumbuh dengan nilai paling tinggi di dalam kaldu Ramsay yang mengandungi fenol 0.5 mM, 1.0 mM, dan 1.5 mM diukur menggunakan CFU mL^{-1} dan Optical Density (OD) 600 nm. Keputusannya adalah 4.14×10^6 CFU mL^{-1}, 3.58×10^6 CFU mL^{-1}, dan 3.46×10^6 CFU mL^{-1} ; 0.843, 0.804, and 0.817. Catechol 2,3 dioxygenase digunakan untuk mengesan penguraian fenol dengan menggunakan aliran meta. Pembentukan warna kuning dengan menggunakan teknik sembur dan teknik tabung uji menunjukkan ketiga-tiga bakteria tersebut mengurai fenol dengan aliran meta.

ABSTRACT

This research is about characterization, screening, and enzymatic assay for potential phenol degrader. The objectives of this research are to characterize the thermophilic strains, to screen the thermophilic strains towards utilization of phenol at various concentration, and to detect the catechol 2,3 dioxygenase activities. Three thermophilic strains that isolated from Oil Palm Empty Fruit Bunch (OPEFB), namely NAedEFBs-Cr_I, NAdEFBus-Cr_I, and RdEFBs-Cr_I were characterized by their colony morphology and cellular morphology. All these three strains are cocci, aerobic bacteria, have no endospore, are not able to reduce sulphur, and cream in colour. For NAedEFBs-Cr_I and RdEFBs-Cr_I, both are Gram-positive and not motile bacterium whereas NAdEFBus-Cr_I is a Gram-negative and motile bacterium. All the three strains were tested for their growth curve at 40 °C, 50 °C, and 60 °C for 24 hours incubation at 200 rpm. The growth temperature is at 40 °C. Among the three strains, NAdEFBus-Cr_I is the most tolerance toward phenol where it can grow dense in phenol-containing Ramsay Agar at concentrations of 0.5 mM, 1.0 mM, and 1.5 mM. This strain also shows the highest growth of cells in phenol-containing Ramsay broth tested by CFU mL^{-1} and Optical Density (OD) 600 nm at different concentrations of phenol. The results are 4.14×10^6 CFU mL^{-1}, 3.58×10^6 CFU mL^{-1}, and 3.46×10^6 CFU mL^{-1} ; 0.843, 0.804, and 0.817 respectively. Catechol 2,3 dioxygenase was used to detect the catabolism of phenol by *meta*- pathway. Yellow formations in both spray plate method and test-tube method showed that NAedEFBs-Cr_I, NAdEFBus-Cr_I, and RdEFBs-Cr_I degrade phenol via *meta*-pathway.

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LIST OF SYMBOLS

°C	-	degrees Celsius
OD_{600}	-	optical density at 600
g	-	gram
$g\ L^{-1}$	-	gram per litre
h	-	hour (duration)
hr	-	hour (time)
L	-	litre
min	-	minutes
$mg\ L^{-1}$	-	milligram per litre
mM	-	millimolar
mL	-	mililitre
$\mu g\ L^{-1}$	-	microgram per litre
$\mu g\ kg^{-1}$	-	microgram per kilogram
µL	-	microlitre
nm	-	nanometer
%	-	percent
s	-	second
v/v	-	volume per volume

LIST OF ABBREVIATIONS

CFU	-	colony forming unit
EFB	-	empty fruit bunch
NA	-	nutrient agar
OPEFB	-	oil palm empty fruit bunch
pH	-	hydrogen ion concentration
ppm	-	parts per million
psi	-	pounds per sq. in
rDNA	-	ribosomal deoxyribonucleic acid
RETL-Cr1	-	Ramsay Effluent of Treatment Lagoon-Cream 1
RM	-	Ramsay medium
rpm	-	revolutions per minute
sp.	-	species
UV	-	ultraviolet

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CHAPTER 1

INTRODUCTION

1.1 Introduction

Environmental pollution, especially with the hazardous and recalcitrant toxic chemicals, is one of the major problems faced by the developing countries (Saravanan *et al.*, 2008). Phenol and its derivatives are widely used in manufacture of a variety of chemicals, such as antioxidants, biocides, and disinfectants which are the major constituents of wastes produced by many industrial processes such as coal carbonization, petroleum and coal tar distillation (Wu *et al.*, 2006). Phenol discharged into the environment by various industrial operations, which include petroleum refineries, textile, dyeing, phenolic resin manufacturing, glass fibre units, varnish industries and smelting related to metallurgical operations (Saravanan *et al.*, 2008).

Phenol has been listed as the priority pollutant in the list of United States Environmental Protection Agency. Minute quantities of phenol and its derivatives results in high levels of toxicity in the effluent stream and also gives a foul odour to the effluent water (Bapat *et al.*, 2008). In treating phenolic compounds, the biological

method has attracted more attention than physical and chemical methods because many different types of microorganisms are known to utilize phenol as their sole carbon and energy sources (Yang & Lee, 2007).

The use of bioremediation in the treatment of hazardous waste is a relatively new concept, yet it is a rapidly growing trend in environmental management. A significant factor in the development of bioremediation has been the enactment of environmental laws and regulations that favour waste treatment rather than waste disposal (Eweis *et al.,* 1998).

A common method for isolating microorganisms from nature is enrichment culture technique. After the isolating method, screening is the second step to get the phenol degrades bacteria. Screening mean any of a number of procedures that permits the sorting of organisms by phenotype or genotype by allowing growth of some type but not others (Madigan & Martinko, 2006).

Oil Palm production is a major agricultural industry in Malaysia. It contributes about US$ 7.3 billion in export earnings each year, mostly from the export of palm oil (Suhaimi & Ong, 2001). In the process of extraction of palm oil from oil palm fruit, a lignocellulosic material oil palm empty fruit bunch (OPEFB) is generated as a waste product. Approximately fifteen million tons of OPEFB biomass waste is generated annually throughout Malaysia by oil palm mills. In practice this biomass is burned in incinerators by palm oil mills which create environmental pollution problems in nearby localities (Nur Hazwani & Piakong, 2008).

Mulching currently accounts for only fraction of the EFB that are discarded; these are normally burnt in incinerators for the ash as fertilizer. Currently there is much interest in utilizing palm oil waste in general. Composting has been suggested as an alternative to incineration of the waste as the process converts the waste, which is essentially organic in nature, into humus that is suitable for crop production. In composting, the higher-plant material breaks down under the influence of aerobic thermophilic microorganisms present in the waste to a material rich in organic nutrients (Thambirajah *et al.,* 1995).

A thermophile is an organism which grows at a higher temperature than most other organisms. Generally, as a wide range of bacteria, fungi, and simple plants and animals can grow at temperatures up to 50 °C according to Bains (1993). Thermophilic and hyperthermophilic microorganisms are interesting for more than just basic biological reasons. These organisms offer some major advantages for industrial and biotechnological processes, many of which run more rapidly and efficiently at high temperatures. Enzymes from thermophiles are capable of catalyzing biochemical reactions at high temperatures and are typically more stable than enzymes from mesophiles, thus prolonged the shelf life of enzyme preparations (Madigan & Martinko, 2006).

The significant of this study is to characterize and screen the thermophilic phenol-degrading strains isolated from Oil Palm Empty fruit Bunch (OPEFB) at three different concentrations of phenol since phenol is the main source of pollutant in the environment.

1.2 Objectives of Study

The aim of this study is to investigate the ability of microorganisms isolated from Oil Palm Empty Fruit Bunch (OPEFB) to degrade phenol with the objectives listed below.

1. To characterize and identify thermophilic bacteria coded NAedEFBs-Cr_1, NAdEFBus-Cr_1, and RdEFBs-Cr_1 isolated from Oil Palm Empty Fruit Bunch (OPEFB).

2. To screen the thermophilic bacteria coded NAedEFBs-Cr_1, NAdEFBus-Cr_1, and RdEFBs-Cr_1 towards utilization of phenol at various concentration of 0.5mM, 1.0mM, and 1.5mM at 40°C.

3. To carry out enzymatic assays to detect 2,3 dioxygenase activity for *meta*-pathway.

CHAPTER 2

LITERATURE REVIEW

2.1 Phenol

Phenol is an aromatic compound that is used as raw material for the production of a variety of resins, including phenolic, epoxy, polycarbonate, and polyamide, for various applications (Fang *et al.*, 2006). The applications include phenolic resins as construction materials for automobiles and appliances, epoxy resins as adhesives, polycarbonate for soft-drink containers, and polyamide for various applications (Herbert *et al.*, 1995). Phenol is now one of the most common toxic environmental pollutants, which mainly originates from industrial processes (Wei *et al.*, 2007). The annual production of phenol is around 1.25×10^9 kg (Boopathy, 1995).

Phenol is a toxic and potentially carcinogenic chemical; the release of phenol into the environment is of great concern (Fang *et al.*, 2006). Due to their toxicity to microorganisms phenolic compounds may often cause the breakdown of wastewater treatment plants by inhibition of microbial growth (Margesin *et al.*, 2004). Despite causing considerably damage and threat to the environment, phenol is also

resistant to natural biodegradation and continues to persist in the environment for a longer time (Saravanan *et al.*, 2008). Phenolic compounds are toxic by ingestion, contact, or inhalation, even at low concentrations (Yang & Lee, 2007). Therefore, this compound needs to be disposed off the environment in a safer and easier pathway.

2.1.1 Physical and Chemical Properties of Phenol

Phenol, or C_6H_5OH is an aromatic compound with one or more hydroxyl groups attached to the benzene ring structure (Arutchelvan *et al.*, 2005) (Figure 2.1). Phenol is a white crystalline solid which melt at 43 °C and liquefies upon contact with water. It has a characteristic acrid odour and a sharp burning taste. It is a weak acid and very sensitive to electrophile substitution reactions and oxidations in its ionized form (WHO, 1994). The physical and chemical properties of phenol are summarized in Table 2.1.

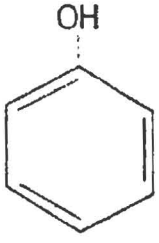

Figure 2.1 Chemical structure of phenol (Piakong, 2006)

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