UNIVERSITI PUTRA MALAYSIA

ISOLATION, IDENTIFICATION AND CHARACTERIZATION OF PENTACHOLOROPHENOL DEGRADING BACTERIUM

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ISOLATION, IDENTIFICATION AND CHARACTERIZATION OF PENTACHOLOROPHENOL DEGRADING BACTERIUM

By

GAN BEE KOON

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia in Fulfillment of the Requirement for the Degree of Master of Science

July 2013

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Dedicated to my parents, family and friends.

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirements for the degree of Master of Science

ISOLATION, IDENTIFICATION AND CHARACTERIZATION OF PENTACHLOROPHENOL DEGRADING BACTERIUM

By

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July 2013

Chairman : Associate Professor Mohd Yunus Abd Shukor, PhD

Faculty : Biotechnology and Biomolecular Sciences

Pentachlorophenol (PCP) is a wide spectrum biocide applied in agriculture, industry and public health. PCP is lethal to all forms of life due to its nature to inhibit oxidative phosphorylation. Moreover, it is recalcitrant to biodegradation due to the stable aromatic ring and high chlorine content. Thus, the extensive use of PCP has led this chlorophenolic compound becoming a common environmental contaminant. The degradation of PCP is very important since it is highly toxic and carcinogenic. This study was conducted to isolate and characterize a potential PCP-degrading bacterium. The study also included the effectiveness of cell immobilization in enhancing PCP degradation by the bacterial cells as well as protecting the cells from the toxic effect of heavy metals which is the common co-contaminants at the sites of contamination. A potent PCP degrading bacterium has been isolated in this work. The bacterium was identified as *Klebsiella* sp. and assigned as *Klebsiella* sp. strain GBK1 based on 16S rDNA phylogenetic analysis. The 16S rDNA sequence has been submitted to genbank under the accession number FJ958193. The optimal PCP degradation conditions occurred at 30°C, pH 7 with 0.3 to 0.4 gL^{-1} ammonium chloride and 0.6% (w/v)

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glucose as supplemental carbon source. *Klebsiella* sp. strain GBK1 was able to degrade PCP with elevated concentration as high as 800 mgL^{-1}. The efficiency of PCP degradation by *Klebsiella* sp. strain GBK1 was enhanced by immobilization with gellan gum as compared to freely suspended cells. Immobilized *Klebsiella* sp. strain GBK 1 best degraded PCP with gellan gum concentration of 0.75% (w/v), bead size of 5 mm diameter (estimated surface area of 78.54 mm^2) and bead number of 500/100 ml medium. Degradation of PCP by both immobilized and free cells exhibited similar profile characteristics at lower concentrations of PCP. At higher concentration of PCP, the use of immobilized *Klebsiella* sp. strain GBK 1 resulted in rapid and extensive PCP degradation (degraded PCP up to 1750 mg/L) compared to free cells system whose PCP degrading ability is inhibited at concentration of 900 mg/L. Generally, the rate of PCP degradation was higher in immobilized *Klebsiella* sp. strain GBK1 and it is more tolerant to high concentrations of PCP (as high as 1750 mg/L). Also, the immobilized cells are more resistant towards heavy metals including As, Zn, Cr, Cd, Cu, Ni, Ag, Pb and Hg (as high as 20ppm) as compared to the free cells system whose activity were affected by 1ppm of heavy metals. In conclusion, *Klebsiella* sp. strain GBK1 is a potent candidate for bioremediation of PCP and its derivatives at chlorophenols contaminated sites and cell immobilization system was shown to be a more powerful system for biodegradation as compared to freely suspended cells system.

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Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia untuk memenuhi keperluan untuk Ijazah Master Sains

PEMENCILAN, PENGENALPASTIAN DAN PENCIRIAN BAKTERIA PENGURAI PENTAKLOROFENOL

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Pentaklorofenol (PCP) merupakan biosid yang luas spektrumnya dan digunakan dalam pertanian, industri dan kesihatan awam. PCP membawa maut kepada semua hidupan dengan sifatnya yang menghalang pengoksidaan fosforilasi. Selain itu, ia juga tahan daripada biodegradasi disebabkan gelang aromatiknya yang stabil dan kandungan klorinnya yang tinggi. Justeru itu, pengunaan PCP yang meluas telah menyebabkan kompaun klorofenolik ini menjadi satu bahan pencemar alam sekitar yang biasa. Biodegradasi PCP adalah sangat penting memandangkan ianya sangat toksik dan karsinogenik. Kajian ini dilakukan untuk memencilkan, mengenalpasti dan mencirikan bakteria pengurai yang berpotensi. Kajian ini juga merangkumi keberkesanan penyekat-gerakan sel untuk meningkatkan penguraian PCP serta melindungi sel bakteria daripada kesan toksik logam berat yang merupakan bahan pencemar bersama yang biasa terdapat pada tempat tercemar. Satu bakteria pengurai PCP yang berpotensi telah dipencilkan dalam kajian ini. Bakteria tersebut dikenal pasti sebagai *Klebsiella* sp. dan secara khususnya dikenal pasti sebagai *Klebsiella* sp. strain GBK1 berdasarkan analisa filogenetik 16S rDNA. Jujukan 16S rDNA bakteria

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tersebut telah dihantar ke bank gen dengan nombor perolehan FJ958193. Kondisi optimum penguraian PCP berlaku pada 30°C, pH 7 dengan 0.3 hingga 0.4 gL^{-1} ammonium klorida dan 0.6% (w/v) glukosa sebagai sumber karbon tambahan. *Klebsiella* sp. strain GBK1 berupaya untuk mengurai PCP dengan kepekatan setinggi 800 mgL^{-1}. Keberkesanan penguraian PCP oleh *Klebsiella* sp. strain GBK1 telah ditingkatkan dengan penyakat-gerakan sel menggunakan gam gellan berbanding dengan sel bebas. *Klebsiella* sp. strain GBK 1 yang disekat-gerakan terbaik menguraikan PCP pada kepekatan gam gellan 0.75% (w/v), 5 mm diameter saiz manik (keluasan permukaan anggaran of 78.54 mm^2) dan bilangan manik 500/100 ml media. Penguraian PCP oleh kedua-dua sel sekat-gerakan dan sel bebas menunjukkan ciri profil yang bersamaan pada kepekatan PCP yang rendah. Pada kepekatan PCP yang lebih tinggi, penggunaan *Klebsiella* sp. strain GBK 1 yang disekat-gerakan menghasilkan penguraian PCP yang laju dan meluas (mengurai PCP sepekat 1750 mg/L) berbanding dengan sistem sel bebas di mana keupayaan penguraiannya disekat pada kepekatan 900 mg/L. Umumnya, kadar penguraian PCP lebih tinggi untuk *Klebsiella* sp. strain GBK1 disekat-gerakan dan ia lebih toleran terhadap kepekatan PCP yang tinggi (sepekat 1750 mg/L). Juga, sel disekat-gerakan lebih tahan terhadap logam berat termasuk As, Zn, Cr, Cd, Cu, Ni, Ag, Pb and Hg (sepekat 20 ppm) berbanding dengan sistem sel bebas di mana aktivitinya dipengaruhi oleh 1 ppm logam berat. Kesimpulannya, *Klebsiella* sp. strain GBK1 merupakan calon yang berpotensi untuk bioremediasi PCP dan derivatifnya pada tempat pencemaran klorofenol dan sistem penyekat-gerakan telah dibuktikan sebagai satu sistem yang berkuasa untuk biodegradasi berbanding dengan sistem sel bebas.

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I certify that a Thesis Examination Committee has met on 11 July 2013 to conduct the final examination of GAN BEE KOON on her thesis entitled "Isolation, Identification and Characterization of Pentachlorophenol Degrading Bacterium" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citation which have been duly acknowledged. I also declare that it has not been previously or currently submitted for any other degree at Universiti Putra Malaysia or other institutions.

_____

GAN BEE KOON

Date: 11 July 2013

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