UNIVERSITI PUTRA MALAYSIA

PARTIAL PURIFICATION AND CHARACTERIZATION OF MOLYBDENUM REDUCING ENZYME FROM *KLEBSIELLA OXYTOCA* STRAIN HKEEM

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MASTER OF SCIENCE

UNIVERSITI PUTRA MALAYSIA

October 2011

Dedicated to my parents, family and friends

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

PARTIAL PURIFICATION AND CHARACTERIZATION OF MOLYBDENUM REDUCING ENZYME FROM *KLEBSIELLA OXYTOCA* STRAIN HKEEM

By

LIM HUI KEEM

October 2011

Chairman: Associate Prof. Mohd. Yunus Abdul Shukor, PhD

Faculty: Biotechnology and Biomolecular Sciences

As a result of widespread application in numerous industrial processes, heavy metals have become a contaminant of many environmental systems. Molybdenum's ubiquitous application in many industrial products makes it a silent pollutant with levels as high as several hundreds of ppm. Thus in this study, the isolate molybdenum reducing bacterium, which showed the highest molybdenum reducing activity, were isolated from a soil sample collected near a steel factory in Selangor. This isolate was later identified as *Klebsiella oxytoca* strain hkeem through molecular phylogenetic analysis of sequenced 16S rRNA gene sequence. *Klebsiella oxytoca* strain hkeem reduced the heavy metal molybdenum to molybdenum blue optimally in selective low phosphate medium agar, at pH 7.3 and 30ºC, after 24 hours of incubation. In specific, *Klebsiella oxytoca* strain hkeem could reduce molybdenum to molybdenum blue under aerobic conditions in the medium with fructose as electron donor, yeast extract, phosphate ion (4.5 mM) and molybdate ion (80 mM). Partial purification and characterization were conducted on the molybdenum reducing enzyme, with anion exchange on Q-sepharose and gel filtration on Zorbax GFX-250. Based on the SDS polyacrylamide-gel

electrophoresis (SDS-PAGE), two bands were observed on the gel filtration fraction at 90 and 38 kDa, respectively. Meanwhile, the enzyme showed an optimum activity at substrate pH 5.5 and 25ºC. The kinetics of electron donor (NADH) and electron acceptor (LPPM) for the enzyme were followed the classical Michaelis-Menten rectangular hyperbolic curve. K_m and V_{max} for the electron donor substrate, NADH was 2.83 mM and 12.23 nmole molybdenum blue produced/min/mg/protein, respectively. However the K_m and V_{max} for the electron acceptor substrate phosphomolybdate (50 mM) were 1.66 mM and 32.06 nmole molybdenum blue/min/mg/protein respectively. Although many molybdenum-reducing bacteria have been isolated, molybdenum-reducing activity of *Klebsiella oxytoca* strain hkeem up to 80 mM of molybdate was reported as the most potent molybdenum-reducing isolate to date. Based on the results obtained, *Klebsiella oxytoca* strain hkeem was proven as a more powerful molybdenum reducer to develop a cost-effective bioremediation work, especially since bacterial molybdenum reduction has been suggested as an important remediation tool for cleaning up molybdenum pollutant in the environment.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra sebagai memenuhi keperluan untuk ijazah Master Sains

PENULENAN SEPARA DAN PENCIRIAN ENZIM PENURUM MOLYBDENUM DARIPADA STRAIN *KLEBSIELLA OXYTOCA* HKEEM

Oleh

LIM HUI KEEM

Oktober 2011

Pengerusi: Mohd. Yunus Abdul Shukor, PhD

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Akibat daripada penggunaan yang berleluasa dalam pelbagai proses perindustrian, logam berat telah menjadi bahan pencemar alam sekitar yang utama. Penggunaan molybdenum yang meluas dalam pelbagai produk industri, menjadikan molybdenum bahan pencemaran alam sekitar. Oleh itu, dalam kajian ini bakteria penurun molybdenum yang menunjukkan aktiviti penurunan molybdenum yang tinggi telah dipencilkan daripada sampel tanah berdekatan dengan kilang besi di Selangor. Pencilan ini dikenalpasti sebagai strain hkeem *Klebsiella oxytoca* melalui filogenetik molekul menggunakan jujukan gen 16S rDNA. *Klebsiella oxytoca* strain hkeem menurunkan logam berat molybdenum ke molybdenum biru dengan optimum pada agar media terpilih yang rendah fosfat, pada pH 7.3 dan 30ºC, selepas pengeraman selama 24 jam. Secara khasnya, *Klebsiella oxytoca* strain hkeem boleh menurunkan molybdenum ke molybdenum biru dibawah keadaan aerobik dalam media yang mengandungi fruktosa sebagai penderma elektron, ektrak yis, cas fosfate (4.5 mM) dan cas molybdate (80 mM). Penulenan separa dan pencirian enzim penurun molybdenum telah dijalankan dengan menggunakan penukar anion pada Q-sepharose dan penapisan gel pada Zorbax GFX-250. Berdasarkan elektroforesis SDS polyacrylamide-gel (SDS-PAGE), 2 jalur

diperhatikan pada bahagian penapisan gel pada 90 dan 38 kDa. Sementara itu enzim ini menunjukkan aktiviti tertinggi substrat ialah pada pH 5.5 dan 25ºC. Penderma elektron (NADH) dan penerima elektron (LPPM) enzim tersebut adalah berdasarkan kepada lengkungan hiperbolik segiempat Michaelis-Menten klasik. K_m dan V_{max} untuk substrat penderma elektron iaitu, NADH ialah 2.83 mM dan 12.23 nmole molybdenum biru terhasil/min/mg/protein. Manakala K_m dan V_{max} penerima elektron fosfomolybdat (50 mM) ialah 1.66 mM dan 32.06 nmole molybdenum biru/min/mg/protein. Walaupun banyak bakteria penurun molybdenum telah dipencilkan sebelum ini, keupayaan *Klebsiella oxytoca* strain hkeem menurunkan molybdenum sehingga 80 mM molybdat merupakan bakteria penurun molybdenum yang paling poten dipencilkan setakat ini. Bio-pemulihan telah dicadangkan sebagai kaedah yang lebih kos efektif dan penting untuk membersihkan bahan pencemar alam sekitar. Berdasarkan keputusan yang diperolehi dalam kajian ini, *Klebsiella oxytoca* strain hkeem telah terbuktikan sebagai penurun molybdenum yang paling berpotensi untuk membangunkan teknologi bio-pemulihan ini.

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I certify that an Examination Committee has met on 14th October 2011 to conduct the final examination of Lim Hui Keem on her thesis entitled "Partial Purification and Characterization of Molybdenum-reducing enzyme from *Klebsiella oxytoca* strain hkeem" in accordance with 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 degree.

Members of the Examination Committee are as follows:

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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DECLARATION

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

LIM HUI KEEM
Date: 14 October 2011

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