UNIVERSITI PUTRA MALAYSIA

CHARACTERIZATION OF HEAVY-METAL-REMOVAL BACTERIA FROM THE PERSIAN GULF

HOSSEIN ZOLGHARNEIN

FPV 2005 2

CHARACTERIZATION OF HEAVY-METAL-REMOVAL BACTERIA FROM THE PERSIAN GULF

By

HOSSEIN ZOLGHARNEIN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Doctor of Philosophy

October 2005

DEDICATION

****

To

Memory of my parents whom their spirits will always be a part of mine

****

My wife Saya for years of love and dedication, and my sons Sina and Soheil

****

Thanks to Allah

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

CHARACTERIZATION OF HEAVY-METAL-REMOVAL BACTERIA FROM THE PERSIAN GULF

By

HOSSEIN ZOLGHARNEIN

October 2005

Supervisor: Professor Mohd Azmi Mohd Lila, PhD

Faculty: Veterinary Medicine

The study was carried out to isolate and screen high heavy metals resistant bacteria from Persian Gulf and enclosed industrial areas within 241,000 km^2. A total of 35 heavy metal resistant bacteria strains were identified from sediment and water samples collected. The resistance and biological capacity of the isolated bacteria were tested in a new formulated media, minimal salt solution (M.S.S), that mimics seawater. Isolated bacteria responded to media supplemented in range 0.5 to 2 mM of Zn, Cd, Cu and Pb by showing a prolonged lag phase and by decreasing growth rate.

Bacteria isolates, in the form of free or immobilized cells, are able to remove lead, copper, zinc and cadmium from solution. Removal of lead and cadmium from solution by some bacteria was very efficient, about 120 mg/g dry weight as high as 90%. Isolates tested presented distinct uptake capacities and the best results were obtained for *Delftia tsuruhatensis* and *Pseudomonas AU3411* respectively.

The diversity of isolated bacteria was examined by the phylogenetic analysis of 16S rRNA gene sequences. The phylogenetic analyses of the sequences revealed seven main taxonomic lineages. The phylogenetic tree illustrated discrimination between isolated bacteria from wastewater, industrials areas and marine environment. Some *Pseudomonas* strains isolated from marine environment were well differentiated from those of industrial wastewater. Members of the genera *Delftia* and *Bacterium* formed a monophyletic group within the subdivision of the class. There was a clear differentiation between two groups of *Pseudomonas* and other groups of bacteria in the phylogenetic tree.

The isolated bacteria were tested for the occurrence of plasmid using the modified alkaline lysate method. The study revealed that the frequency of the occurrence of plasmid in the heavy metals resistance bacteria was more than in the common bacteria. Multiple forms of plasmids were observed in 66% of the plasmid-carrying strains. Isolates bacteria from industrials wastewater showed the highest plasmid incidence (84.6%). In the marine environment there was a slightly higher incidence of plasmid in bacteria isolated from sediments (55.5%) compared to the water sample of the same origin (53.8%).

Scanning Electron Microscope (SEM) analyses showed *Pseudomonas* sp. accumulated heavy metals in the cell wall and along the external cell surfaces. This suggested that heavy metals uptake involves both surface phenomena and diffusion. Energy Dispersive X-ray (EDX) analysis confirmed heavy metals on the bacteria cell surface which was reported by SEM.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENCIRIAN BAKTERIA YANG DIPEROLEHI DARIPADA TELUK PARSI YANG BERUPAYA MENYINGKIR LOGAM BERAT

Oleh

HOSSEIN ZOLGHARNEIN

Oktober 2005

Pengerusi: Profesor Mohd Azmi Mohd Lila, PhD

Fakulti: Perubatan Veterinar

Beberapa jenis bakteria yang berintang logam berat telah diasingkan daripada sampel keladak dan air, yang diperolehi daripada Teluk Parsi dan kawasan industri disekitarnya dalam lingkungan 241,000 km^2. Sejumlah 35 jenis bakteria yang berintang logam berat telah dikenalpasti. Kesemua bakteria tersebut telah dicirikan berdasarkan keupayaan bakteria tersebut untuk tumbuh dan mengikatkan logam berat penting seperti zink, kadmium, kuprum dan plumbum pada kepekatan yang tinggi. Rintangan dan kemampuan biologi bakteria diuji dengan menggunakan media baru yang dirumuskan, Minimal Salt Solution (MMS), yang menyerupai air laut.

Aplikasi kadmium, plumbum, kuprum dan zink telah diselidik dengan menggunakan bakteria yang berbentuk tertambat dan bebas. Ujian dilakukan menggunakan dengan samada satu atau campuran beberapa logam berat. Terdapat banyak jenis bakteria yang mampu menyingkirkan kuprum, zink dan kadmium, samada sel bakteria dalam bentuk bebas atau tertambat. Satu atau lebih

campuran logam berat mempunyai afiniti yang berbeza terhadap pengikatan logam-logam berat. Kecekapan pengikatan untuk kadmium dan plumbum mencapai 90% peratusan atau lebih, berdasarkan berat kering sel bakteria dalam masa satu jam pendedahan. Bio-pengikatan kuprum dan zink mencapai 50%. Keupayaan pengikatan yang tertinggi ialah untuk Pb diikuti oleh Cd, Zn dan Cu. Walau bagaimanapun, penyingkiran logam berat adalah maksimum apabila bakteria didedahkan kepada sejenis logam berat sahaja pada satu masa.

Diversiti bakteria telah diuji dengan analisis filogenetik jujukan gen 16S rRNA . Analisis filogenetik pada jujukan tersebut mendedahkan tujuh jalinan taksonomi utama. Cabang filogenetik menunjukkan perbezaan antara bakteria yang diasingkan daripada kumbahan persekitaran industri dan air laut. Keputusan kajian menunjukkan perbezaan genetik yang baru, dan hubungan di antara bakteria yang diperolehi dari laut dan industri. Sebahagian bakteria pseudomonas yang diasingkan daripada persekitaran laut telah dicirikan/dibezakan dengan jelas daripada bakteria yang diasingkan daripada kawasan industri. Ahli genera *Delftia* dan *Bacterium* membentuk kumpulan monofiletik dalam sub-bahagian kelas tersebut. Terdapat perbezaan jelas di antara dua kumpulan pseudomonas dan kumpulan bakteria yang lain di dalam cabang filogenetik.

Bakteria tersebut telah diuji untuk menentukan kewujudan plasmid di dalam bakteria, dengan kaedah pemecahan beralkali. Kaedah ini adalah berkesan untuk pencirian dan pengenlpastian plasmid yang berlainan saiz, tanpa menggunakan bahan kimia toksik. Kajian mendedahkan kekerapan kewujudan plasmid dalam bakteria berintang logam berat adalah lebih tinggi berbanding dengan bakteria biasa. Kajian menunjukkan bahawa 66% daripada bakteria yang diasingkan

mempunyai plasmid besar atau kecil. Bakteria yang diasingkan daripada sisa air industri mempunyai peratusan kewujudan plasmid yang paling tinggi (84.6%). Di persekitaran laut pula, bakteria yang diasingkan daripada keladak mempunyai peratus kewujudan plasmid yang lebih tinggi (55.5%) berbanding dengan bakteria yang diasingkan daripada air laut (53.8%). Penemuan ini mencadangkan bahawa plasmid boleh terdapat pada kebanyakan jenis bakteria tetapi lebih cenderung kepada bakteria berintang logam berat.

Analisis Scanning Electron Microscope (SEM) menunjukkan bahawa spesies pseudomonas mengumpul logam berat dalam dinding sel dan sepanjang permukaan luar sel. Ini menunjukkan pengambilan logam-logam berat melibatkan fenomena permukaan dan penyebaran. Analisis Energy Dispersive X-ray (EDX) adalah kurang sensitif dan kurang dipercayai.berbanding dengan analisis penjerapan atom, walaupun EDX adalah satu cara yang cepat dan mudah untuk mengesan kehadiran logam berat ke dalam bakteria.

ACKNOWLEDGEMENTS

I would like to extend my gratitude to the members of my supervisory committee; Prof. Dr. Mohd. Azmi. Lila. Mohd, for his advice, kind and constant support, encouragement and help in all my difficulties.

I wish to express my deepest thankfulness to my co-supervisors; Prof. Dr Mohd Zamri. Saad, Assoc. Prof. Dr Abd. Rahim Mutalib, Assoc. Prof. Dr Che Abd. Rahim Mohamed, for their constructive guidance through out my study period.

Furthermore, my special thanks to Mr Kamarudin, and also John .Shia, Mrs. Sandy, Hayati, Dr Phong and Suria of the virology laboratory.

I am also grateful to the Department of Environment of Iran for providing me the facilities to collect sediments and water samples from Persian Gulf on the research ship Ghods, and also help in heavy metals analysis and isolation of bacteria and other laboratory work.

I would like to thank my wife and my sons, who patiently support me which away from home.

Last, I would like to thank many others, who help me during my study.

TABLE OF CONTENTS

LIST OF TABLE

LIST OF FIGURES

LIST OF ABREVIATIONS

$(NH_4)\ SO_4$	=	Ammonium sulfate
AAS	=	Atomic Absorption Spectrophotometer
ANOVA	=	Analysis of Variance
bp	=	base pairs
$CaCl_2$	=	Calcium chloride
$Cd(NO_3)_2$	=	Cadmium nitrate
$CsCl_2$	=	Cesium chloride
$CuSO_4$	=	Copper sulfate
dNTP	=	DNA deoxyribonucleic acid
ds	=	double stranded
EDTA	=	Ethylene diamine tetra acetic acid
EDX	=	Energy Dispersive X-ray
		Environment
$FeSO_4$	=	Iron sulfate
g	=	Gravity
Gm	=	growth media
K_2HPO_4	=	Di-potassium hydrogen orthophosphate
KH_2PO_4	=	Potassium di-hydrogen orthophosphate
Ls	=	Lab-scale
M.S.S	=	Minimal salt solution
MES	=	Morpholino ethanesulfonic acid
$MgSO_4$	=	Magnesium sulfate
$MnSO_4$	=	Manganese sulfate

NaCl	=	Sodium chloride
OD	=	Optical density
P	=	Probability
$PbNO_3$	=	Lead nitrate
PBS	=	Phosphate buffered saline
PCR	=	Polymerase chain reaction
pH	=	Negative logarithm of hydrogen ion
ppt	=	Part per thousand
ROPME	=	Regional Organization for the Protection of the Marine
rpm	=	rotation per minutes
SEM	=	Electron Microscope
TAE	=	Tris acetic acid
TE	=	Tris EDTA
TEMED	=	Tetra methyl ethylenediamine
UV	=	Ultra violet
$ZnSO_4$	=	Zinc sulfate