ISOLATION AND CHARACTERIZATION OF HEAVY METAL RESISTANT PSYCHROTROPHS FROM ANTARCTICA

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A special dedication to my beloved family and friends
who showered me abundantly with their love and continuous support

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ABSTRACT

The present study reported the isolation and characterisation of heavy metal resistant bacteria from Antarctic water. Ten isolates were successfully isolated from seawater and ice lower glacier and two isolates named SW5 and ILG1 were selected for further study based on their high resistance towards heavy metals particularly, arsenite and arsenate. Both isolates have optimum growth at 20 ℃ to 30 ℃. Isolates SW5 and ILG1 were tested for their resistance to metal ions such as Cd(II), Cr(VI), As(III) and As(V) at different range of concentration namely, Cd(II) 3-30 ppm; Cr(VI) 15-135 ppm; As(III) 150-1500 ppm; and As(V) 150-7500 ppm. The minimal inhibitory concentration (MIC) of four heavy metals was determined in Low Phosphate Mediumfor each bacterial strain. The results of the test revealed that MIC for isolate SW5 was at 15 ppm, 30 ppm, 900 ppm and 7500 ppm for Cr(VI), Cd(II), As (III) and As(V) respectively while for ILG1, MIC was at 12 ppm for Cr(VI), 9 ppm for Cd(II), 750 ppm for As(III) and 3000 ppm for As(V). Overall, the order of toxicity of heavy metals to SW5 was in order of Cr(VI)> Cd(II)> As(III)> As(V) and for ILG1 was in order of Cd(II)> Cr(VI)> As(III)> As(V). Molecular characterization, 16S rRNA analysis revealed that isolate SW5 shares a 99.65% identity match to *Pseudomonas azotoformans* strain IAM 1603 while isolate ILG1 shares a 99.51% identity match to *Pseudomonas cedrina subsp cedrina* strain CFML 96-198. These bacteria could be used as a model microbial strain to study the mechanism of heavy metal resistance with potential application for bioremediation of heavy metals.

ABSTRAK

Kajian ini melaporkan pemencilan dan pencirian kerintangan bakteria terhadap logam berat yang dipencil dari air Antartika. Sepuluh bakteria telah berjaya dipencil dari air laut dan ais glasier. Dua bakteria yang dinamakan sebagai bakteria SW5 dan ILG1 telah dipilih untuk kajian lanjutan kerana mempunyai kerintangan yang tinggi terhadap logam berat terutamanya As(III) dan As(V). Kedua-dua bakteria mempunyai perbiakan yang optimum pada suhu 20℃ dan 30℃. Kerintangan bakteria SW5 dan ILG1 terhadap logam seperti Cd(II) , Cr(VI), As(III) dan As(V) telah diuji pada julat kepekatan yang berbeza iaitu Cd(II) 3-30 ppm, Cr(VI) 150-135 ppm, As(III) 150-1500 ppm dan As(V) 150-7500 ppm. Kepekatan perencat minimum (MIC) daripada empat logam telah ditentukan dengan menggunakan medium fosfat rendah untuk setiap bakteria. Keputusan ujian menunjukkan bahawa MIC untuk bakteria SW5 adalah pada 15 ppm untuk Cr(VI), 30 ppm untuk Cd(II), 900 ppm untuk As(III) dan 7500 ppm untuk As(V). Bagi ILG1, MIC telah ditentukan pada 12 ppm untuk Cr(VI), 9 ppm untuk Cd(II), 750 ppm untuk As(III) dan 3000 ppm untuk As(V). Keseluruhannya, urutan kerintanganan bakteria terhadap ion logam bagi SW5 adalah Cr(VI)> Cd(II)> As(III)> As(V). Manakala bagi ILG1, urutan kerintangan adalah Cd(II)> Cr(VI)> As(III)> As(V). Pencirian molekular 16S rRNA menunjukkan bahawa bakteria SW5 mempunyai 99.65% persamaan terhadap *Pseudomonas azotoformans* strain IAM 1603 manakala bakteria ILG1 mempunyai 99.51% persamaan terhadap *Pseudomonas cedrina subsp cedrina* strain CFML 96-198. Spesis-spesis tersebut boleh dijadikan sebagai model mikrob dalam kajian mekanisma kerintangan logam berat dan berpotensi dijadikan sebagai agen dalam biopemulihan logam.

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LIST OF ABBREVIATION AND SYMBOLS

ADMI	-	American Dye Manufacturers Institute
As(III)	-	Arsenite
As(V)	-	Arsenate
BOD		Biochemical Oxygen Demand
$CaCl_2$	-	Calcium chloride
Cd(II)	-	Cadmium
$Cd(NO_3)_2 \cdot 4H_2O$	-	Cadmium nitrate tetrahydrate
Cr(VI)	-	Chromium
HCl	-	Hydrochloric acid
ICP-OES	-	Inductively Coupled Plasma- Optical Emission Spectrometry
$K_2Cr_2O_7$	-	Potassium dichromate
KCl	-	Potassium chloride
LB		Luria Bertani
LPM	-	Low Phosphate Medium
MIC	-	Minimal Inhibitory Concentration
Na_2SO_4	-	Sodium sulphate
NaCl	-	Sodium chloride
NH_4Cl	-	Ammonium chloride

LIST OF SYMBOLS

g/L^{-1}	-	gram per litre
kPa	-	kilopascal
L	-	Litre
M	-	Molar
min	-	minute
mg/L	-	milligram per litre
mL	-	millilitre
μL	-	microlitre
ppm	-	parts per million
v/v	-	volume per volume
w/v	-	Weight per volume

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CHAPTER 1

INTRODUCTION

1.1 Research Background

Heavy metals are elements having atomic weights between 63.5 and 200.6, and have a specific gravity greater than 5.0. Some heavy metals are essential to living organisms including cobalt, copper, iron, manganese, molybedum, vanadium, strontium and zinc. However, excessive levels of essential metals can be harmful to organisms. Cadmium, chromium, mercury, lead, arsenic and antimony are non-essential heavy metals that are toxic to human. These heavy metals are relatively abundance in the Earth's crust and are frequently used in industrial process and agriculture (Srivastava and Majumder, 2008).

Heavy metals are persistent environmental contamination. The contamination is known as a critical environmental problem because the metals toxic effect and their accumulation throughout the food chain cause severe problems to the ecology and detrimental to human health (Malik, 2004). They can reside in the environment for hundreds of years once they are emitted to the environment. Besides that, biogeochemical cycles and the balance of some heavy metals in the environment have been drastically changed by human activities and it causes the accumulation of heavy metal in the soils, seawater, freshwater and sediment.

Growing interests among the researches towards heavy metal bioremediation applications are possibly due to its potential applications in industry and scientific novelty of the microorganism (Singh *et al.*, 2013). However, the success of bioremediation methods depends on having the right environmental factors, right microbes and right place for degradation to occur (Boopathy, 2000). Thus, there is an interest to discover the potential microorganism especially psychrotroph isolated from Antarctica which has the potential to perform bioremediation in cold environment. Human exploitation of heavy metals have been found in ice cores, sea water in Antarctica and Greenland (Bielicka *et al.*, 2005). Previous research shows that heavy metal resistant bacteria, *Aeromonas* sp. and *Pseudomonas* sp. have been detected in Antarctic seawater which showed tolerance to heavy metal such as mercury and chromium (De Souza *et al.*, 2006).

1.2 Problem Statement

Heavy metal contamination is one of the major environmental problems in the world. The study of heavy metal pollutions have become primary concern followed by the rapid development in urbanization and industrialization around the world. Although contamination and heavy metals exposure are not a recent phenomenon, the exposure of heavy metals to the populations remains the major issue despite effort at remediation (Monachese *et al.*, 2012). Many researches have been conducted to solve the problem of heavy metal contamination in water. According to Bestawy *et al.* (2013), environmental microorganisms that have been identified with the potential in bioremediation included *Enterobacter* sp., *Stenotrophomonas* sp., *Providencia* sp., *Chryseobacterium* sp., *Comamonas* sp., *Ochrobactrum* sp., and *Delftia* sp. which have the potential to degrade heavy metal such as copper, cadmium, chromium and cobalt.

The unfavourable conditions of environment have become the obstacle in bioremediation (Dash *et al.*, 2013). Most of the environment is characterized by the elevated or low temperature, or high salt concentration. Thus, bacteria from Antarctica which have been exposed to the unfavourable or extreme environmental conditions have the potential to be used for bioremediation of polluted extreme habitats. However, the extremophiles from Antarctica as the agents of bioremediation has yet to be fully discovered, most of which remain biochemically and metabolically unexplored. Currently, there is limited published literature in isolation of potential heavy metal resistant bacteria from Antarctica for the application of bioremediation. Hence, this research was undertaken to investigate the diverse properties of the adapted microbial strain and screen for potential application for bioremediation of heavy metals in cold environments.

1.3 Research Objectives

There are three objectives for this study:

i. To isolate psychrotrophic bacteria from Antarctic samples
ii. To screen the isolates for heavy metal resistance towards heavy metals.
iii. To identify the heavy metal resistance bacteria by using 16S rRNA gene sequence analysis.

1.4 Significance of the Study

Through this research, isolates of heavy metal resistant bacteria can be characterized and serve as an important step to further study the heavy metal resistance among the bacteria species from Antarctica. The metal resistant psychrotrophs could be used in bioremediation of heavy metal contaminated environment at lower temperature. Then, the detection of trends that emerged in heavy metal resistant of the bacterial strains can facilitates the implementation of effective control measure for potential heavy metal bioremediation applications.

1.5 Scope of the Study

This work studied heavy metal resistance characteristic of psychrotroph isolated from Antarctic water samples. Minimal inhibitory concentration of each isolates towards heavy metals was examined. Then, the heavy metal resistant bacteria were characterized by cellular and colony morphology. Molecular characterization was done by using 16S rRNA analysis to determine the identity of the bacteria.

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