METAL TOLERANCE AND ORGANIC COMPOUND UTILIZATION OF *BACILLUS* SP. ORIGINATED FROM WASTEWATER

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To my beloved mother and father

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ABSTRACT

Wastewater management has been one of the significant environmental concerns in the recent years. Organic material and heavy metal residues are among the contaminants of concern in wastewater and are spreading throughout the world along with the industrial progress. The use of microbes for the pollutant removal was a favourable alternative to conventional techniques. The aims of this study was (i) to identify bacterium JA based on 16S rRNA analysis, (ii) investigate the degree of bacterium JA tolerance towards various heavy metals (cobalt, copper, zinc, manganese and aluminium) and (iii) screen the ability of bacterium JA to utilize several selected compounds (glucose, glycerol, benzene, kerosene, sulfanilic acid and casamino acid) as the sole carbon and/or nitrogen source. Based on 16S rRNA analysis results, this bacterial strain was identified as *Bacillus* sp. In the heavy metals tolerance experiments, the maximum tolerance concentration of CuCl, $ZnSO_4$, $CoSO_4$ and $AlK(SO_4)_2$ in *Bacillus* sp. was 100mg/L, 100mg/L, 40mg/L and 500mg/L, respectively. In medium containing 100 mg/L of heavy metals (CuCl, $ZnSO_4$, $CoSO_4$, $MnCl_2$, and $AlK(SO_4)_2$), the growth rate (μ) of *Bacillus* sp. was 0.497 h^{-1}, 0.529h^{-1}, 0.093h^{-1}, 0.138 h^{-1}, and 0.303 h^{-1} respectively, which clearly showed that cobalt had the highest growth inhibitory effect to *Bacillus* sp. From the carbon utilization tests, glucose and glycerol were the preferred growth substrates, where glycerol was observed to give the highest absorbance value (OD_{600}=0.456) at 48 hours of incubation. No observable growth was seen when kerosene or benzene was supplied as the sole carbon source. Both sulfanilic acid (SA) and casamino acid (CA) were found independently to be used as sole nitrogen source, with CA was found to be a better source for the purpose of promoting the growth of *Bacillus* sp. No significant growth was observed when CA and SA were provided as carbon or carbon and nitrogen source.

ABSTRAK

Pengendalian air sisa buangan telah menjadi salah satu isu alam sekitar yang ketara dalam tahun-tahun kebelakangan ini. Bahan organik dan sisa logam berat adalah antara bahan pencemar pada air sisa yang membimbangkan dan tersebar di seluruh dunia seiring dengan perkembangan industri. Penggunaan mikrob untuk penyingkiran pencemar itu didapati menjadi alternatif yang baik berbanding teknik konvensional. Tujuan kajian ini adalah untuk (i) untuk mengenal pasti bakteria JA ini berdasarkan analisis 16S rRNA (ii) menyiasat tahap toleransi bakteria JA terhadap pelbagai logam berat (kobalt, tembaga, zink, mangan dan aluminium), dan (iii) menyaring keupayaan untuk menggunakan beberapa sebatian dipilih (glukosa, gliserol, benzena, minyak tanah, asid sulfanilic dan asid casamino) sebagai karbon tunggal dan/ atau sumber nitrogen. Berdasarkan kepada keputusan yang diperolehi daripada analisis 16S rRNA, strain bakteria ini telah dikenal pasti sebagai *Bacillus* sp. Dalam uji kaji toleransi logam berat, didapati kepekatan toleransi maksimum CuCl, $ZnSO_4$, $CoSO_4$ dan $AlK(SO_4)_2$ dalam *Bacillus* sp. adalah masing-masing 100mg/L, 100mg/L, 40mg/L dan 500mg/L. Dalam media yang mengandungi 100 mg/L logam berat (CuCl , $ZnSO_4$, $CoSO_4$, MnCl dan $AlK(SO_4)_2$), kadar pertumbuhan (μ) *Bacillus* sp. adalah 0.497 h^{-1}, 0.529 h^{-1}, 0.093 h^{-1}, 0.138 h^{-1}, dan 0.303 h^{-1} yang dengan jelas menunjukkan bahawa kobalt mempunyai keupayaan tertinggi untuk merencat pertumbuhan *Bacillus* sp. Daripada ujian penggunaan karbon, glukosa dan gliserol adalah substrat pilihan utama untuk pertumbuhan. Medium yang mengandungi gliserol diperhatikan telah memberi kadar pertumbuhan yang paling tinggi (OD_{600}=0.456) pada inkubasi 48 jam. Tiada pertumbuhan diperhatikan apabila minyak tanah atau benzena telah dibekalkan sebagai sumber karbon tunggal. Kedua-dua asid sulfanilic (SA) dan asid casamino (CA) didapati secara individu boleh digunakan sebagai sumber nitrogen tunggal, dengan CA didapati sumber yang lebih baik bagi tujuan menggalakkan pertumbuhan *Bacillus* sp. Tiada pertumbuhan ketara diperhatikan apabila CA dan SA disediakan sebagai sumber karbon atau karbon dan nitrogen.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

et al	And others
sp	Species
16S rRNA	16 small subunit of ribosomal ribonucleic acid
DNA	Deoxyribonucleic Acid
USA	United States of America
%	Percentage
µg	Microgram
L	Litre
RNA	Ribonucleic acid
G+C	Guanine-cytosine
mg	Milligram
ºC	Degree Celsius
rpm	Revolutions per minute
NA	Nutrient Agar
NB	Nutrient Broth
g	Gram
kPa	Kilo Pascal
mL	Millilitre
nm	Nanometre
PCR	Polymerase Chain Reaction
µL	Microliter
$MgCl_2$	Magnesium Chloride
dNTP	Deoxynucleotide Triphosphate
min	Minutes
EtBr	Ethidium bromide
V	Voltage
UV	Ultraviolet
h	Hour
OD	Optical Density
µ	Growth Rate
t_d	Generation time
~	Equivalence

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Background of Study

In the recent times, it is almost undeniable that industrialization intensifies environmental pollution that in return deteriorates several ecosystems by the accumulation of toxic metals. This form of contamination has definitely adversely affected many nations where industry practise is crucial to their economic structure. Among the sources of environmental heavy metal pollution are naturally available resources, agricultural activities, industrial activities, domestic effluent and atmospheric sources. Heavy metal pollution can exist either naturally or due to anthropogenic sources.

Treatment approaches can be classified into two; namely abiotic and biotic. Abiotic means include physico-chemical methods such as precipitation, ion exchange, electro-winning, electro coagulation, cementation, electro dialysis and adsorption. Biotic methods on the other hand involve the use of living organisms and products derived from them. Biological methods via bioaccumulation and biosorption have been demonstrated to possess good potentials to replace conventional methods for the removal of metals (Hussein *et al.,* 2004).

Factors that determine the extent of metal resistance in microorganisms can be derived as such; the type and mechanism of metal uptake, the role each metal plays in metabolism and the presence of genes located on plasmids and chromosomes. These were claimed to have been playing an essential role in forming resistance towards such toxic metals. The microorganisms have to be tolerant towards the metal in order to conduct further investigating for metal removal capability. Bioremediation method of bacterial use has been very beneficial in removal, recovering and transforming pollutants into a non-toxic compound. This is in due credit to their effective metabolic system in the elemental cycle that they have been known for long ago.

Among the numerous strains used in bioremediation, *Bacillus* sp. has been studied extensively for the ability to remove heavy metal from the environment (Krishna *et al.,*2003; Rajbhansi *et al.,*2008; Mahmood *et al.,*2013; Srabanti *et al.,*2013). *Bacillus* sp. are ubiquitous, hence they can be isolated from almost any part of the environment. A major part in the soil microflora comprises *Bacillus* sp. and they are also widespread in aquatic situates. It has also been reported, *Bacillus* sp. being present in various wastewater samples polluted with heavy metal residues (Rajbanshi., 2008).

The current study investigated the metal tolerance of a bacterium designated as strain JA, which was previous isolated from wastewater. The aims of this study included

i) to identify the strain based on complete sequence of 16S rRNA analysis ii) to investigate the tolerance of the strain towards selected heavy metals and the last aim of this study was to screen for the ability of the strain to utilize the selected organic compounds as sole carbon source.

1.2 Significance of Research

The ability of bacteria to treat and degrade a diversity of compounds is significant and has been used in waste processing and bioremediation. The heavy metal tolerant bacteria and organic pollutant-removing bacteria are significant in wastewater treatment since they could be utilized for bioremediation of heavy metal (Rajbhansi, 2008). Strain JA in this study was found to have ability to tolerate to some heavy metals and to remove organic compounds. These findings are important and the ability of strain JA to remove heavy metals from the wastewater would be further explored.

1.3 Research Objectives

Below are the objectives of this study.

i. To identify the strain using full sequence of 16S rRNA analysis
ii. To investigate the tolerance of the strain towards heavy metals
iii. To screen for the ability of the strain to use the selected organic compounds as carbon source.

1.4 Scope of Study

For identification purpose, genomic DNA strain JA was firstly extracted and 16S rRNA gene was amplified for sequencing. Then, the study emphasised on investigating the tolerance of strain JA towards heavy metals of varying concentration. In addition, strain JA was also screened for the ability to use selected organic compounds as carbon source for growth.

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