EFFECTS OF HEAVY METALS AND SELECTED CARBON COMPOUNDS ON THE GROWTH OF BACTERIUM CA

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Dedicated to my beloved parents,
and my siblings

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ABSTRACT

Nowadays environment has been suffering from the contamination by heavy metals and organic compounds. Modernization, urbanization and industrialization further increase the levels of contamination. In this work, the ability of bacterium CA previously isolated from textile waste water was investigated to tolerate to aluminium, copper, cobalt, zinc and manganese at different concentrations. The MIC were found to be 400, 200, 40, 60, 300 mg/L, respectively, the growth rate at MIC were 0.1732, 0.417, 0.0885, 0.2824 and 0.4046 h^{-1}, respectively, and the doubling time values at MIC were 4.005, 1.66187, 7.83, 2.4539 and 1.7128 h^{-1}, respectively. Different organic compounds were supplied as sole carbon sources at chemically defined media. The bacterium CA could grow on the CDM provided with casamino acids, casamino acids (as both carbon and nitrogen source), glucose and glycerol with growth rate of 0.0297, 0.0317, 0.04 and 0.0492 h^{-1}, respectively. Whereas the bacterium failed to grow when sulfanilic acids, acrylamide and benzene were only source of carbon. Bacterium CA was found to be sensitive to tetracycline hydrochloride and kanamycin sulfate with clear zones of 2.8 and 2.6 cm, respectively, while it was resistant to XY-12 and ampicillin trihydrate. Moreover, bacterium CA was found to be closest with *Bacillus thuringiensis* with accession number NR_102506 via 16S rRNA gene analysis. In conclusion, bacterium CA was positive to tolerate to heavy metals, results were significant in case of aluminium, manganese and copper. Bacterium CA was able to grow on different organic compounds that indicated the presence of different mechanisms of carbon source up take and metabolism in the bacterium system. The obtained results suggested the bacterium CA could be potentially used for the treatment of wastewater contamination containing heavy metals and organic compounds.

ABSTRAK

Pada masa kini persekitaran telah mengalami pencemaran logam berat dan sebatian organik. Pemodenan, perbandaran dan pengindustrian meningkatkan lagi aras pencemaran. Dalam kajian ini keupayaan bakteria CA yang sebelum ini diasingkan daripada air sisa tekstil untuk bertolak ansur-dengan aluminium, kuprum, kobalt, zink dan mangan pada kepekatan yang berbeza. disiasat dan kepekatan renjatan minimum (MIC) adalah 400 200, 40, 60, 300 mg/L, masing-masing. Kadar pertumbuhan bakteria pada MIC adelah 0.1732, 0.417, 0.0885, 0.2824 dan 0.4046 h-1, masing-masing, dan doubling masa nilai pada MIC adalah 4.005, 1.662, 7.83, 2.4539 dan 1.7128 h^{-1}, masing-masing. Sebatian organik yang berbeza dibekalkan sebagai sumber karbon yang tunggal di media CDM. Bakteria CA dapat bertumbuh di CDM yang dibekalkan dengan asid casamino, asid casamino (bertindak sebagai sumber karbon dan nitrogen), glukosa dan glycerol dengan kadar pertumbuhan sebanyak 0.0297, 0.04, 0.0317 dan 0.0492 h^{-1}, masing-masing. Manakala bakteria gagal tumbuh di media CDM apabila asid sulfanilic, acrylamide dan benzene dibekalkan sebagai sumber karbon. Bakteria CA telah didapati sensitif kepada tetracycline hydrochloride dan kanamycin sulfat dengan zon-zon yang jelas 2.8 cm dan 2.6 cm masing-masing. Bakteria CA didapati tidak sensitif terhadap XY-12 dan ampicillin trihydrate. Selain itu, bakteria CA telah dikenalpasti sebagai *Bacillus thuringiensis* (NR_102506) melalui kaedah analisis 16S rRNA gen. Kesimpulannya, bakteria CA positif untuk bertolak ansur untuk logam berat terutamanya, aluminium, mangan dan tembaga. Bakteria CA ini telah berjaya bertumbuh dengan menggunakan sebatian organik yang berbeza sebagai sumber karbon menunjukkan kehadiran mekanisma yang berbeza di dalam sistem bakteria. Keputusan yang diperolehi mencadangkan bakteria CA berpotensi untuk digunakan untuk rawatan pencemaran logam berat dan sebatian organik.

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LIST OF ABBREVIATIONS

AH	-	Aliphatic Hydrocarbon
Bacterium CA	-	An isolated bacterium from textile waste water
Cd	-	Cadmium
CDM	-	Chemically Defined Media
CO_2	-	Carbon dioxide
Cr	-	Chromium
Cu	-	Copper
DDTs	-	Dichloro Diphenyl Trichloroethanes
DNA	-	Deoxyribonucleic acid
EDTA	-	Ethylenediaminetetraacetic acid
EMP	-	Embden–Meyerhof–Parnas
EOC	-	Emerging Organic Contaminant
GAC	-	Granular Activated Carbon
HBC	-	Hexachlorobenzene
Hg	-	Mercury
HMP	-	Hexose monophosphate
k	-	Specific growth rate
MCL	-	Maximum Contaminant Level
MEGA	-	Molecular Evolutionary Genetics Analysis
MIC	-	Minimal inhibitory concentration
BLASTn	-	nucleotide Basic Local Alignment Search Tool
NCBI	-	National Center for Biotechnology Information
OD	-	Optical Density
PAC	-	Powder Activated Carbon
PAH	-	Polycyclic Aromatic Hydrocarbon
Pb	-	Lead

PCB	-	Polychlorinated Biphenyl
PCR	-	Polymerase Chain Reaction
rRNA	-	Ribosomal Ribonucleic acid
STP	-	Sewage Treatment Plant
TAE	-	Tris-acetate-EDTA
t_d	-	Doubling time (Generation time)
USEPA	-	United States Environmental Protection Agency
UV	-	Ultraviolet
WHO	-	World Health Organization
Zn	-	Zinc

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CHAPTER 1

INTRODUCTION

1.0 Background

Urbanisation and modernisation have given human beings a number of advantages. These include the improvement of the quantity and quality of food, clothes and entertainment products. Despite of their positive features, such developments pose negative impacts on ecosystem through releasing of effluents and they are reason behind the increase of proportion of contaminants like heavy metals, organic compounds and others in the environment (Nagajyoti *et al.*, 2010; Chopra *et al*., 2009; Pérez-López *et al*., 2003).

Contaminants like heavy metals and organic compounds are introduced into environment through industrial effluents and these compounds give negative impacts to the environments and living organisms. Hence the innovation of removal treatments such as physical, chemical and biological approaches is urgent. The examples of physical treatments are activated carbon adsorption and membrane filtration, Chemical treatments are chemical precipitation, ion exchange and chemical advanced oxidation, and Biological treatments are biosorption, phytoremediation and bioleaching. Biological means are preferred over physical and chemical means because biological treatments are eco-friendly, cost effective and promising (Vargas-Garcia *et al.*, 2012;

Sutton *et al.,* 2013; Szulc *et al.,* 2014; Umrania, 2006; Fosso-Kankeu and Mulaba-Bafubiandi, 2013; Colin *et al.*, 2012).

Bacteria have been applied in bioremediation processes such as in bioadsorption treatment process in layer by layer fattening strategy (*Pseudomonas sp.* as biosorbent*)* to remove heavy metals from industrial effluents (Luo *et al.,* 2014) and in membrane bioreactor treatment to remove micro contaminants including organic compounds (Luo *et al.,* 2014). Bacteria have numerous mechanisms, various kind of enzymes and have unique structure to tolerate and remove the contaminants of different kinds including heavy metals and organic compounds.

In this study, bacterium CA previously isolated from textile wastewater was further characterized through assessment of its ability to tolerate certain heavy metals at determined concentrations, then its capability to utilize different organic compounds as carbon source, in addition, the effects of antimicrobial agents on the growth of bacterium CA was studied. Eventually it was identified via 16S rRNA method.

1.1 Significance of the Work / Problem Statement

Bioremediation that uses microorganisms to clean up an environment is relatively cost-effective compared to conventional physical or chemical processes (Abdel-El-Haleem, 2003). The bacterium CA was able to tolerate some heavy metals even at high concentrations and was positive to grow on some organic compounds, hence such results postulate that bacterium CA could be a potential option to remove of heavy metals and organic compounds from the contaminated area.

1.2 Objectives

a) To investigate the tolerance of the bacterium CA isolated from textile wastewater towards heavy metals.

b) To screen for the ability of the bacterium CA to use the selected organic compounds as carbon source.

c) To test the effect of antimicrobial agents on the growth of bacterium CA.

d) To identify the bacterium CA based on 16S rRNA gene analysis.

1.3 Study Scope

Bacterium CA was firstly examined to tolerate (aluminium, cobalt, zinc, copper and manganese). Its ability was screened to utilize the organic compounds (casamino acids, sulfanilic acids, glucose, glycerol, acrylamide and benzene) as sole carbon source for growth. Effects of antibiotics on the growth bacterium CA were examined. Finally, the bacterium CA was identified based on the 16S rRNA gene analysis.

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