CHARACTERIZATION OF BIOSURFACTANT PRODUCED BY *PSEUDOMONAS AERUGINOSA* EXPOSED TO CADMIUM AND COPPER

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ABSTRACT

Cadmium and copper are extensively used in different industrial sectors before being discharged as industrial effluent and causing pollution towards the environment and increases the health risk when being uptake by humans. The aims of this study were to evaluate the effect of Cd and Cu to induce production of biosurfactants and to characterize the biosurfactant produced using Fourier-Transform Infrared Spectroscope (FTIR). Results from the study showed that *P. aeruginosa* was able to tolerate Cu and Cd up to 2.0 mg/L and 0.1 mg/L respectively, which both concentration were 10x higher than the permissible limit allowed by the Environmental Quality Act (Industrial Effluent) Regulation, according to the Department of Environment, Malaysia. Recovery of biosurfactant from *P. aeruginosa* exposed to Cu and Cd were 0.051 g/g of bacterial cells and 0.125 g/g of bacterial cells, respectively. FTIR analysis obtained from control biosurfactant sample without metals induction, and biosurfactant samples induced with Cu and Cd, respectively showed major peaks at 3276 and 1351 cm^{-1} suggesting the presence of protein and lipid. This suggests that the type of biosurfactant produced by *P. aeruginosa* was lipoprotein in the presence of Cu or Cd, as in the normal condition without metal induction.

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LIST OF ABBREVIATION

Cd	Cadmium
$CdCl_2$	Cadmium chloride
CFU/mL	Colony-forming units per milliliter
cm^{-1}	Reciprocal wavelength
Cu	Copper
$CuCl_2$	Copper chloride
FTIR	Fourier Transform Infrared Spectroscopy
g	Gram
g/g	Gram biosurfactant/ gram bacteria
KBr	Potassium bromide
L	Litre
LB	Lysogeny Broth
mg	Milligram
mg/L	Milligram per litre
NaCl	Sodium chloride
OD	Optical density
rpm	Rounds per minute

CHAPTER 1

INTRODUCTION

Heavy metals are needed in diverse of fields like industrial, domestic, agriculture and even medical (Tchounwou, Yedjou, Patlolla & Sutton, 2012). Heavy metals are either being produced as a side product or used as a catalyst to accelerate the production process. Industrial effluent that contains heavy metal are being discharged without proper filtration or treatment (Buhari, & Ismail 2016), contributing to environmental heavy metal pollution. In order to arrest the situation, bioremediation techniques using biosurfactant are being given attention lately as compared to synthetic surfactants due to their excellent qualities, for instance, degradability, efficiency, lower toxicity and the environment compatibility (Moussa, Mohammed & Samak, 2014).

Numerous studies showed different bacteria biosynthesized different types of biosurfactants (Pacwa-Plociniczak, Plaza, Seget & Cameotra, 2011). According to Vijayakumar & Saravanan (2015), glycolipids like rhamnolipids are normally associated with *P. aeruginosa*, whereas trehalolipids are usually produced by *Mycobacterium*, *Arthrobacter* and *Nocardia* species, while sophorolipids are an example of biosurfactant produced by yeast. Despite that, it is unclear how different types of pollutants will affect the production of biosurfactants. For instance, Patowary, Patowary, Kalita & Deka (2017) reported on the induced production of rhamnolipids by *Pseudomonas aeruginosa*, exposed to crude oil. In comparison, Christova, Tuleva, Cohen, Ivanova, Stoev, Disheva & Stoineva (2011) demonstrated that *P. aeruginosa* cultured using different hydrocarbon source like glycerol, *n*-hexadecane and *n*-alkanes produced rhamnolipids with different ratio of rhamnolipids congeners that will decide the biosurfactant to be grouped mono-rhamnolipids or dirhamnolipids. However, to date, no studies have clearly demonstrated if different heavy metals will have induced different production of biosurfactant in *P. aeruginosa*.

Thus, in order to better understand the mechanism of bacteria regulating the toxicity of heavy metals via metal chelation by biosurfacatant, this study has two aims; (1) to evaluate the effect of heavy metals (Cadmium and Copper) on biosurfactants and (2) to characterize the biosurfactant using Fourier-transform Infrared

Spectroscopy which will help in the identification of the types of biosurfactants produced.

CHAPTER 2

LITERATURE REVIEW

2.1 HEAVY METALS

According to Jaishankar et al., (2014), heavy metals refer to groups of metal which has a specific density higher than 5 g/cm^3 such as cadmium, copper, mercury, lead and zinc. Heavy metals have extensive function in various industrial processes (Sharma, Singh & Siddiqi, 2014). Table 2.1 shows the industrial applications of different heavy metals. Due to the extensive use of heavy metals, they have caused pollution towards the environment. Amongst the various heavy metal, Cd and Cu were found to be most toxic towards the environment compared to others (Fargasova, 2004)

Table 2.1 Functions of different types of heavy metals in industry

Types of Heavy Metals	Function	Reference
Cadmium	- Used as electrode in batteries - Function as pigment and coating for plastic	Chunhuabundit, 2016
Copper	- Used in electrolytic refining process - Used as raw material for electronics	PubChem, 2018 (a)
Mercury	- Extensively used as industrial catalyst - Used in measuring equipment	Jan et al., 2015
Lead	- Manufacturing of automobiles - Act as catalyst in paints	Flora et al., 2012
Zinc	- As coating for iron - To create alloy	PubChem, 2018 (b)

2.1.1 Cadmium

Cadmium (Cd) is rarely present naturally in the environment, but is normally produced as a side product in industrial activities (Kim, Kim & Seo, 2015) i.e in paint production as color pigment, in alkaline batteries as electrode, in alloy production and amongst others (Jaishankar, Tseten, Anbalagan, Mathew & Beeregowda, 2014), which causes its released to the environment as industrial waste.

In Malaysia, there are several past studies that demonstrated level of Cd in different areas of Malaysia, which requires immediate attention to remediate them. Table 2.2 summarizes the concentration of Cd in different areas in Malaysia.

Table 2.2 Average concentration of Cd in different areas in Malaysia

Area	Average Concentration (mg/L)	Reference
Semenyih River, Selangor	0.98	Gasim et al., 2000
Bertam River, Pahang	0.83	Haron et al., 2016
Langat River, Selangor	0.04	Shazili et al., 2015

2.1.2 Copper

Copper is a naturally present heavy metal, which is used as a raw product for manufacturing copper coating, copper wire and are also incorporated with different metals to form brass. Cu compound are also used as preservatives for food, leather and wood (Pubchem, 2018b).

Past studies also showed that the concentration of Cu has reached a worrying rate as it could cause Cu poisoning if not being taken care of. Table 2.3 shows the summary of areas being polluted by Cu.

Table 2.3 Average Concentration of Cu in different areas in Malaysia

Area	Average Concentration (mg/L)	Reference
Laloh River, Johor	146.5	Maadin et al., 2016
Balok River, Pahang	24.2	Abdullah et al., 2015
Tunggak River, Pahang	0.4	Sujaul et al., 2013

2.2 Heavy Metals Regulations

Due to the toxicity of both Cd and Cu, different countries have set up different guidelines to regulate these metals as shown in Table 2.4.

Table 2.4 Summary of permissible limit of Cd and Cu in sewage effluent and drinking water.

Parameters	Cadmium	Copper	Reference
Permissible limit in sewage effluent (mg/L)	0.010	0.200	Department of Environment, 2009
Permissible limit in drinking water (mg/L)	0.003	1.000	Ministry of Health, 2000

2.3 REMEDIATION OF CADMIUM AND COPPER

Conventional methods to curb the heavy metals contamination can be divided into physical remediation and chemical remediation (Khalid et al., 2017). Although the remediation methods have shown promising efficiency and results, however the methods are not environmental friendly as many chemicals are used, such as chemical absorbent (Chen et al., 2017). Hence, biosurfactants has come into the spotlight due to its environmental friendly characteristic. Table 2.5 shows the comparison between different heavy metals remediation methods employed.