Identification of Cadmium Resistant Bacteria Isolated from Silk Dyed Water. Case Study: Silk Weaving Small Enterprises Ban Chanplane Prokronchai District, Buriram Province

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Abstract

Bioremediation had been shown to be a powerful system for heavy metal pollution cleanup and prevention. Characterization ofthe cadmium (Cd)-resistant bacteria isolated from silk dyed water were performed in this research. I found that all 14 isolated bacteria have a toxicity tolerant of tolerate toxic $CdCl_2$ concentrations at 1,500 µM. Interestingly, the Cd binding capacity of all isolated bacteria was very high, ranging from 2.61 to 6.52 log [Cd(atom)]/cell when grown in the presence of 500 µM $CdCl_2$. Furthermore, the stability of Cd-bacteria complex of all isolated bacteria was effected by 1 mM EDTA. When grown in the presence of 500 µM $CdCl_2$, 3 isolates of Cd-resistant bacteria, including BRU03, BRU07,and BRU11 increasingly produced inorganic sulfide (1X to 3X). These results suggested that these Cd-resistant bacteria have potential ability to precipitate a toxic soluble $CdCl_2$ as non toxic insoluble CdS.

Keywords: Bioremediation, Cadmium resistant bacteria, Cadmium, Silk dyed water

1. Introduction

The textile industry is one of the largest polluters in the world. The World Bank estimates that almost 20% of global industrial water pollution comes from the treatment and dyeing of textiles. Some 72 toxic chemicals reach our water supply from textile dyeing. Many of these chemicals cannot be filtered or removed. The textile industry is second only to agriculture as the biggest polluter of clean water globally. Environmental pollution by dyed is a world wide problem causing vast areas of agricultural land to become non-arable and hazardous for animal, plant, and human populations (Eleni*et al*., 2007). Textile manufacturing dyes release aromatic amines (benzidine and toluidine), heavy metals, ammonia, alkalai salts, toxic solids and large amounts of pigments, chlorine, and a known carcinogen. One of the heavy metals whose concentration is increasing in environments is cadmium (Cd). Cadmiumwas used in different industrial processes as production of television tube phosphorus, alloy preparation, metal plating, nuclear reactor shields and rods, pigments, stabilizers, batteries, etc. (Hutton *et al*., 1987; Nriagu, Pacyna, 1988). Cd ion was a highly toxic metal and important element in food-chain contamination. Cadmium, in a variety of chemical forms, is unknown in biological functions (Pinto *et al*., 2004). Farmers in Asian countries, especially in Japan, confronted with Itai-Itai disease which was resulted from rice grains containing large amount of Cd (Akiko *et al*., 2006). The conventional physicochemical technologies such as electrochemical treatment, oxidation–reduction, ion exchange and membrane separation were inadequate and expensive to removing metals at lower concentrations (less than 100 mg/l). Bioremediation, which used microbes to detoxify and degrade environmental

contaminants, had received increasing attention in recent times to clean up a polluted environment (Farhadian*et al*., 2008; Radhika*et al*., 2006). Since microorganisms had developed survival strategies in heavy metal polluted habitats, their different microbial detoxifying mechanisms such as bioaccumulation, biotransformation, biomineralization and biosorption could be applied either *ex situ* or *in situ* to design economical bioremediation processes (Gadd, 2000; Lim *et al*., 2003).

Recently, there were reports about resistance, bioaccumulation and biotransformation capacity of Cd by isolated microorganisms under growing conditions such as *Bacillus* sp., *Pseudomonas* sp., *Aspergillus* sp., and *Trichoderma* sp. (Roane, Pepper, 2000; Haq*et al*.,1999)

2. Research Objectives

The objectives of this research were to isolate and identify as well as characterize cadmium-resistant bacteria for use in bioremediation from silk dyed at Silk Weaving Small Enterprises Ban Chanplane Prokronchai District, Buriram Province.

3. Research Methodology

3.1 Isolation of cadmium-resistant bacteria

Cd-resistant bacteria were isolated from silk dyed water. A portion 25 ml of silk dyed water was suspended in 25 ml nutrient broth (NB) and then incubated at 30 °C on orbital shaker at 150 rpm for 4 h. The 1 ml of suspension was serially diluted (in the range of 10^{-1} to 10^{-8}) and the each dilution solution was spread on nutrient agar (NA) in the absence and presence of $CdCl_2$ at 500 µM then incubated at 30 °C for 24 to 48 h.

3.2 Cell growth in response to Cd

11 isolates were grown in NB tube for starter bacteria. 1% starter were inoculated into the absence and presence of $CdCl_2$ at 500 µM and incubated at 30 °C, 150 rpm and take sample for measure at 600 nm every 3 h until 36 h.

3.3 Identification of cadmium-resistant bacteria

Cd-resistant isolates were identified by Gram-strain and biochemical conventional tests including conventional tube triple sugar iron agar, lysine iron agar, motility-indole-ornithine decarboxylase agar, phenylalanine-urea broth, ß-galactosidase (ONPG), citrate agar, the Voges-Proskauer test and deoxyribonuclease. In addition, the Cd-resistant bacteria were identified by API20NE system. All biochemical tests of all sample were performed in this study.

3.4 Cd binding capacity of Cd-resistant isolates

11 isolates were grown in NB medium containing 500 µM $CdCl_2$ incubated at 30 °C, 150 rpm for 24 h and harvested by centrifugation at 5000x g, 4 °C for 10 minutes. The pellets were then washed twice with sterile water, rewashed twice with EDTA solution (10 ml of 1 mM EDTA in 50 mM HEPES and 10 mMNaCl) and sterile water, respectively. The EDTA treated cells were resuspended with 10 ml sterile water and the Cd content was measured by atomic absorption spectrophotometer.

4. Research Results

Isolation of cadmium-resistant bacteria

During the selection of Cd-resistant bacteria, a total 24 isolates were able to tolerate to toxic Cd at 1500 µM. Only 11 isolates were randomly selected for further study.

Cell growth in response to Cd

To determine whether the Cd toxicity affected the growth of isolated bacteria, bacteria cells were grew in the absence and presence of toxic concentration of $CdCl_2$ at 500 µM. Cell growth was determined by measuring the optical density at 600 nm (Figure 1). In the absence of Cd, all isolated bacteria grew slightly better than in the presence of toxic concentration.

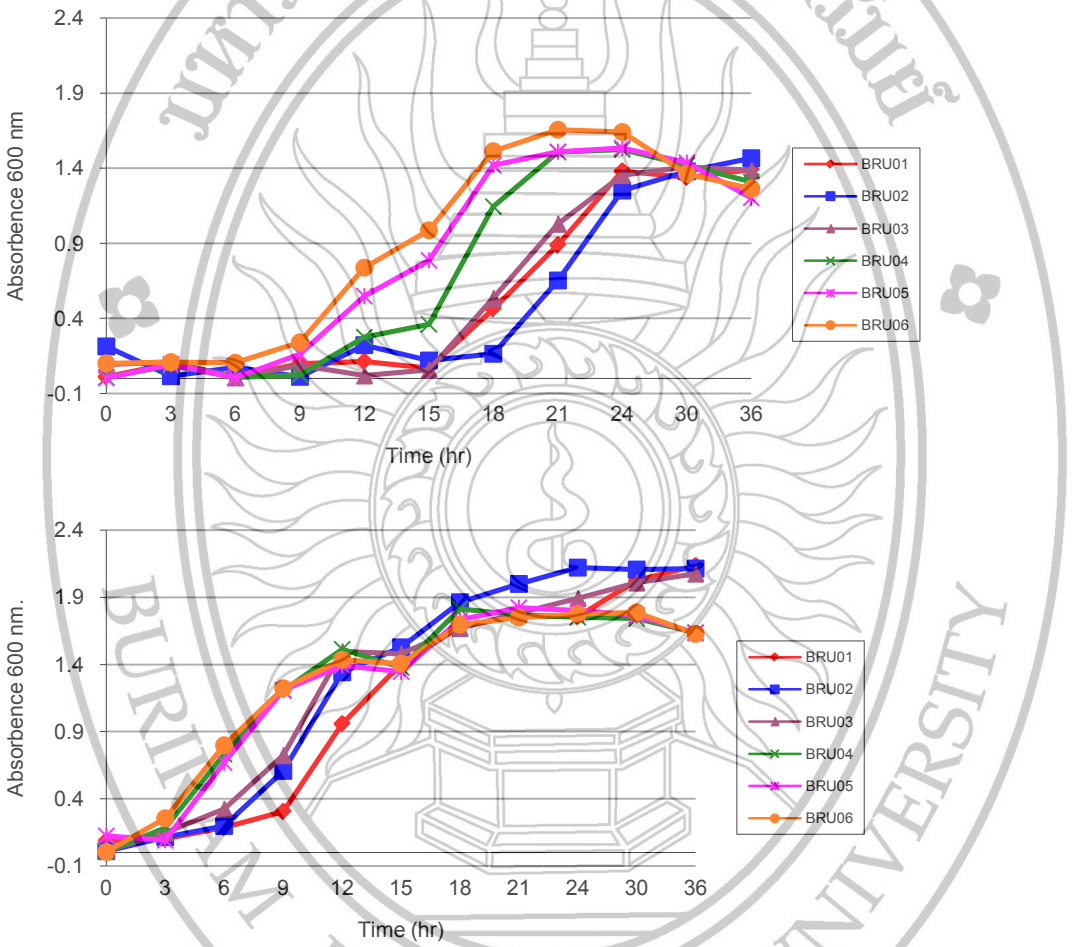

Figure 1. Growth curve of Cd-resistant bacteria in the presence $CdCl_2$ (A) and the absence of 500 µM $CdCl_2$ (B)

Identification of cadmium-resistant bacteria

11 Cd-resistant isolates were identified to gram-negative bacteria and then biochemical reactions of commercial identification system as follows; Biochemical tests and API20NE. Biochemical tests could one genuses. They are *Pseudomonas* sp. All *Pseudomonas*sp were identified their species by used API20NE system. All 11 isolates were *P. aeruginosa* which could be resistant of Cd up to 2500 µM. Structure of *P. aeruginosa* was shown in Table 1.

Table 1
Phenotypic characteristics of isolates BRU03, BRU07, and BRU11 determined by biochemical tests

Characteristics	BRU03	BRU07	BRU11
Gram reaction	-	-	-
Cell shape	Rod	Rod	Rod
Cell diameter, µm	0.8-1.4	0.8-1.4	0.8-1.4
Oxidase	+	+	+
Catalase	+	+	+
TSI medium	K/N/H_2S	K/N/H_2S	K/N/H_2S
Citrate	+	+	+
Motility	+	+	+
Mac	NFL	NFL	NFL
MR-VP	-/-	-/-	-/-
Growth at 4 °C	-	-	-
Lysine iron agar	-	-	-
ß-galactosidase	-	-	-
Urea	-	-	-
Starch	-	-	-
Gelatin	-	-	-
6% NaCl	-	-	-
Indole	-	-	-

Cd binding capacity of Cd-resistant isolates

In order to determine the ability of Cd-resistant bacteria to accumulate Cd from the environment, the Cd binding capacity of 24 isolates was measured. As show in Table 1, the Cd binding capacity of all isolated bacterial cells was 5.42 to 6.52 log[Cd(atom)/cell. I further studied the effect of EDTA on their Cd binding capacity

(Table 2). The results showed that the Cd binding capacity of all isolates were affect by1 mM EDTA.

Table 2

Cd binding capacity of Cd-resistant bacteria treated and untreated with EDTA

Isolate	Cd binding capacity Log Cd(atom)/Cell	
	[No EDTA]	[EDTA]
BRU01	6.58±0.18	5.20±0.14
BRU02	5.30±0.27	6.21±0.52
BRU03	4.38±0.13	6.52±0.32
BRU04	5.76±1.02	3.24±0.43
BRU05	5.39±0.86	3.73±0.61
BRU06	3.38±0.73	4.40±0.42
BRU07	4.90±0.54	5.52±0.84
BRU08	4.51±0.61	2.61±0.17
BRU09	4.96±0.72	4.83±0.95
BRU10	4.50±0.59	4.96±0.82
BRU11	4.66±0.77	5.20±0.84

5. Discussion

Agricultural soils were primarily contaminated with Cd^{2+} due to the excessive use of phosphate fertilizers, dispersal of sewage sludge and atmospheric deposition. Cd^{2+} was readily taken up by numerous crops including cereals, potatoes, rice and fruits (Ingwersen and Streck, 2005). Consumption of rice grown in paddy soils contaminated with Cd^{2+} may pose a serious risk to human health, because 22–24% of the total metal content in rice biomass was concentrated in the rice grains (Wang et al., 2003). Thus, contamination by Cd^{2+} is increasing in both human food and overall in the agricultural environment. Plants readily takes up Cd^{2+} from the soil. However, exposure to high levels of Cd^{2+} resulted in reduced rates of photosynthesis, chlorosis,

growth inhibition, browning of root tips, decreased water and nutrient uptake, and ultimately death (Marcano et al., 2002). Even metals exert their toxic effects on microorganisms through various mechanisms, and metal-tolerant bacteria could survive in these habitats and could be isolated and selected for their potential application in the bioremediation of contaminated sites (Piotrowska-Seget et al., 2005).

Many microorganisms in the soil are able to solubilize ‘‘unavailable’’ forms of heavy metal-bearing minerals by excreting organic acids. And many soil bacteria are tolerant to heavy metals and play important roles in mobilization of heavy metals (Gadd, 1990). In this study were found11 bacterial isolates which could be resistant to Cd. 11 isolates were *P. aeruginosa*. In this results indicated that the isolated BRU03 may be useful in preventing Cd diffusion in the soil environment. Further investigation of molecular characterization of Cd-resistant bacteria.

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