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The Potential of Indigenous Bacteria for Removing Cadmium from Industrial Wastewater in Lawang, East Java

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ABSTRACT

Heavy metals have been used in various areas around the world especially in the industrial sector. Heavy metals contamination is very dangerous for ecosystem because of its toxicity for some organisms. Cadmium (Cd) is a dangerous metal pollutant that can cause remarkable diverse of toxic effects, in particular for humans and animals. The use of bacteria as bioremediation agents has been widely studied because more efficient, less cost, and environmentally friendly strategy. This present study aimed to isolate and identify Cd-resistant bacteria from the industrial disposal site. Wastewater samples were collected from disposal site of agar flour industry in Lawang Malang, East Java. The collected wastewater effluent was analyzed for physicochemical properties. Isolation of Cd-resistant bacteria was carried out using serial dilution. Bacterial isolates were observed and tested for their effects on the content of Cd. The content of Cd was tested daily using Atomic Absorption Spectroscopy (AAS) for seven consecutive days. Data was analyzed using one-way ANOVA ($p < 0.05$) and Tukey test. Characterization of potential bacterium was performed using bacterial identification kit. Four bacteria isolates have been successfully isolated from the wastewater sample. There was a statistically significant difference between groups as determined by one-way ANOVA ($F = 1229.62$, $p = 0.00$). A Tukey post hoc test revealed that all conditions are significantly different from each other. The content of Cd in wastewater sample was statistically significantly lower after taking the A isolate (3.39 mg/L, $p = 0.00$), B Isolate (1.47 mg/L, $p = 0.00$), C Isolate (1.15 mg/L, $p = 0.00$), and D isolate (1.95 mg/L, $p = 0.00$) compared to the control treatment (5.11 mg/L, $p = 0.00$). Two of the most potential isolates identified as *Pseudomonas flourescens* (C isolate) and *Enterobacter agglomerans* (B isolate).

Keywords: *Heavy metals, cadmium, wastewater, potential bacteria*

INTRODUCTION

The negative impact of the growing economy, especially in the industrial sector is the increasing environmental pollution when the industrial waste cannot be handled properly. The industrial waste is hazardous waste. There are different types of hazardous wastes that have the potential to pollute the environment such as heavy metals. Heavy metals have been used in many different areas around the world. Various industries use some heavy metals on pro-duction process which produce wastes containing a heavy metal such as leatherworking, mining, and smelting of metalliferous, electroosmotic, fertilizer and pesticide industry and application, electroplating, electrolysis, photography, etc. [1]. The indiscriminate release of heavy metals into the soil and waters is a major worldwide health concern, as they cannot be broken down become non-toxic forms and therefore have long-lasting effects on the ecosystem [2]. Heavy metal pollution has negative impacts on various organisms, especially humans and animals.

Many studies have reported the dangerousness of heavy metals for various organisms. The dangerous metals called 'toxic trio' are cadmium (Cd), lead (Pb), and mercury (Hg), which their biological function has not been found, yet [3]. Among those heavy metals, Cd is the dangerous one due to its toxicity, in particular for

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humans and animals. Cd can cause various of toxic effects such as teratogenicity, carcinogenicity, endocrine or reproductive damage, and mutagenicity [4]. Candidate critical effects of long-term cadmium exposure were those on bone and kidneys [5]. Human exposure to Cd occurs chiefly through inhalation or ingestion [6]. Therefore, the problem of Cd pollution should be resolved as early as possible.

Like most metals, Cd is not bio-transformed [4]. Conventional methods have been applicated for removing metal ions from an aqueous solution such as evaporation, ion exchange, precipitation, filtration, membrane technologies, electrochemical treatment, etc. [1]. However, most of the strategies are ineffective and extremely expensive [1,7].

Bioremediation is an innovative technology available for removing of heavy metals in various areas. Several studies have reported that bacteria are a potential agent to eliminate heavy metals from the environment as effective, less cost, and environmentally friendly strategy [8, 9, 10, 11]. Another advantage of using bacteria for removing heavy metal is that they are a biological material which can be subject to regeneration [12]. Even more, the bacteria can be obtained from pollution area. The effectiveness of metal removal greatly depends on the affinity between the microbial cell wall and the metal, and this can be achieved using indigenous bacteria isolated from mine areas [13]. Also, the successful of bioremediation can be supported by the bacterial design approach and by understanding the activity of bacteria in the contaminated sites, their mechanism to control the growth, their metabolic capabilities and their response to environmental changes [2].

The aim of the present study was to isolate and identify Cd-resistant bacteria from the industrial disposal site. The understanding of indigenous bacteria characteristics is expected to be used as the database for bioremediation technology.

MATERIALS AND METHODS

Source of wastewater

The wastewater samples were collected aseptically using sterilized flasks from the main reservoir pond of disposal site in an agar flour industry in Lawang Malang, East Java. Samples were treated and analyzed in Laboratory of Microbiology, State University of Malang. The collected wastewater effluent was analyzed for physicochemical properties, i.e., color, chemical oxygen demand (COD), biological oxygen demand (BOD), dissolved oxygen (DO), total suspended solids (TSS), pH, and content of Cd in wastewater.

Isolation of cadmium resistant bacteria

For propagation of Cd resistance bacteria, 50 mL of wastewater samples were mixed with Luria Bertani medium up to 500 mL and were shaken at 100 rpm for a week. $Cd(NO_3)_2$ was added to increase the contain of Cd in the medium. Thus, that medium contains Cd up to 10 ppm for selecting Cd-resistant bacteria. A serial dilution was made up to 10-12 from the wastewater treated sample. 0.1 mL of each dilution was spread onto nutrient plates containing Cd up to 10 ppm. After incubation at 37°C for 24 hours, the growth of the bacterial colonies was observed.

Removal of cadmium by isolated bacterium

Isolates of bacteria that have been found were tested for their effects on the content of Cd in wastewater. A total of 5 mL pure culture of each colony was added to 45 mL sterile wastewater containing Cd. As a control, distilled water was used as a substitute. These flasks of treatment were shaken at 100 rpm. The content of Cd was determined using the wet-ashing method. The measurement was carried out daily using Atomic Absorption Spectroscopy (AAS) for seven consecutive days. Data was analyzed by one-way ANOVA with p = 0.05 followed by Tukey test.

Characterization of potential bacterium

The potential bacterium for removal Cd was gram stained and characterized using various biochemical tests such us glucose fermentation, lysine decarboxylase, TSI test, indole, urease test, etc. The biochemical tests were performed using Oxoid Microbact™ Biochemical Identification Kits.

RESULTS AND DISCUSSION

Wastewater characteristics

The effluent of agar flour industry that used as wastewater sample had some characteristics. The characteristics of wastewater sample scores were very high compared to standards set by East Java Governor Regulation No. 72/2013 regarding Standard of Wastewater Industry and Other Business Activities (Table 1). The wastewater sample that analysed had a brownish colour. The sample had biological oxygen demand (BOD), chemical oxygen demand (COD), and Dissolved Oxygen (DO) value of 206.88 mg/L, 350.40 mg/L, and 1.32 mg/L, respectively. BOD is the amount of dissolved oxygen required for the biochemical decomposition of organic compounds by a microorganism and the oxidation of certain inorganic materials. In another hand, the amount of dissolved oxygen needed to cause chemical oxidation of the organic material in water is called COD. The higher BOD and COD levels indicate that water samples are highly polluted [14]. Moreover, the low levels of DO (less than 2 mg/L) would indicate not only poor water quality but also would have difficulty in sustaining much sensitive aquatic life [15].

The total suspended solids (TSS) score of the measurement results was 305.00 mg/L. Higher concentration of TSS in wastewater sample is an index that it is more polluted [14]. Suspended solids in wastewater can increase the turbidity of water so that it reduces the penetration of light and plant production [16].

The measurement results showed a pH value of 4.7. This result indicates that the wastewater needs further evaluation. Water with an abnormal range of pH may cause a nutritional imbalance or may contain a toxic ion [17]. The pH values of water less than 5 and greater than 10 indicate the presence of industrial wastes and non-compatibility with biological operations [18].

For heavy metal content, this sample contained Cd for 5.44 mg/L. The content of Cd in the water system can be harmful to aquatic life due to its toxicity and carcinogenicity. Moreover, Cd has the ability to concentrate along the food chain. Therefore, treatment must be needed, before discharge the wastewater in water bodies.

Isolation of cadmium resistant bacteria

The serial dilution method succeeded in isolating four isolates of Cd-resistant bacteria from the wastewater sample. Each of isolated colonies was picked up and were sub-cultured onto nutrient agar medium for further analyzed. Those four isolates were coded by A, B, C, and D. The morphological characteristic has been shown in Table 2. The similar research has reported that two Cd-resistant gram-negative bacteria have successfully been isolated from polluted Yamuna River in India [19]. In other studies, twelve cadmium-resistant bacteria (include both gram-negative [75%] and gram-positive [25%]) have been isolated from hospital wastewaters in Algeria [20].

Removal of cadmium by isolated bacterium

Statistical analysis showed that all of the isolate has a significant difference in reducing the content of Cd in wastewater samples (Figure 1). There was a statistically significant difference between groups as determined by one-way ANOVA ($F = 1229.62$, $p = 0.00$). During the seven days, the content of Cd reduced from 5.28 to 3.39 mg/L (isolate A treatment), 5.23 to 1.47 mg/L (isolate B treatment), 5.33 to 1.15 mg/L (isolate C treatment), 5.23 to 1.95 mg/L (isolate D treatment), while a decrease of 5.23 to 5.11 mg/L for control treatment.

Furthermore, a Tukey post-hoc test revealed that all conditions are significantly different from each other. The content of Cd in wastewater sample was statistically significantly lower after taking the A isolate (3.39 mg/L, $p = 0.00$), B Isolate (1.47 mg/L, $p = 0.00$), C Isolate (1.15 mg/L, $p = 0.00$), and D isolate (1.95 mg/L, $p = 0.00$) compared to the control treatment (5.11 mg/L, $p = 0.00$). The C isolate has shown the highest ability for decreasing the content of Cd on wastewater, followed by B isolate, D isolate, and A isolate. This result showed that all of the isolates have a potential for removing Cd. There are at least three types of microbial processes that can influence toxicity and transport of metals and radionuclides: biotransformation, biosorption and bioaccumulation, and degradation or synthesis of organic ligands that affect the solubility of the contaminants [21]. As yet, it is not known if Cd^{2+} can be reduced to Cd^{0} biologically [22]. On the other hand, many studies

Table 1. Physicochemical characteristics of the wastewater sample

Parameters	*Wastewater Sample*	*Governor Regulation No. 72/2013 Threshold*
Color	Brownish	Not Mentioned
BOD	206.88 mg/L	50 mg/L
COD	350.40 mg/L	100 mg/L
DO	1.32 mg/L	Not Mentioned
TSS	305.00 mg/L	200 mg/L
pH	4.7	6.0 up to 9.0
Cadmium	5.44 mg/L	0.05 mg/L

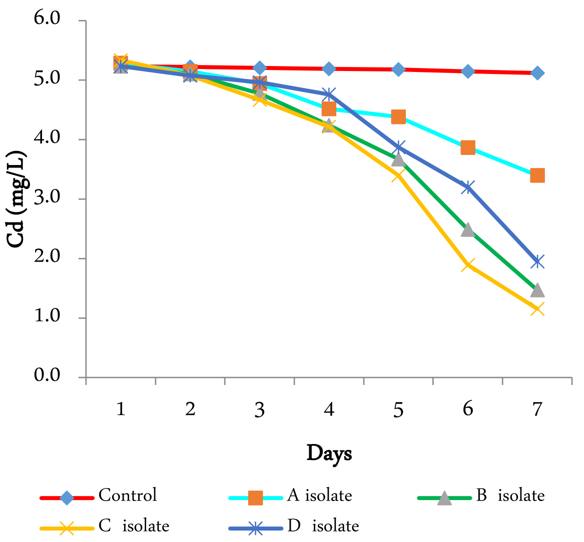

Figure 1. A decrease in the content of cadmium in wastewater samples

Table 2. Morphological characteristics of bacterial isolates

Morphological Characteristic	*Bacterial Isolates*			
	A	*B*	*C*	*D*
Size of colony	7 mm	5 mm	4 mm	8 mm
Shape of colony	Round	curled	round	irregular
Elevation colony	Raised	convex	raised	umbonate
Edge	Undulat	rhizoid	lobate	rhizoid
Colony color	Yellowish	yellowish	yellowish	yellowish
Gram reaction	Negative	negative	negative	negative
Texture	Mucoid	mucoid	mucoid	mucoid
Motility	Motil	motil	motil	motil

Tabel 3. Result of biochemical tests using gram negative bacteria kit

No	*Reaction*	*B isolate*	*C isolate*
1.	Oxidase	-	+
2.	Nitrate Reduction	+	-
3.	Lysine Decarboxylase	-	-
4.	Ornithine Decarboxyl	-	-
5.	H_2S Production	-	-
6.	Acid from Glucose	+	-
7.	Acid from Mannitol	+	-
8.	Acid from Xylose	-	+
9.	ONPG	+	-
10.	Indole	-	-
11.	Urea Hydrolysis	+	+
12.	Voges Proskauer	+	+
13.	Citrate Utilization	+	+
14.	Tryptophan Deaminase	+	-
15.	Gelatin Liquefaction	+	+
16.	Malonate Inhibition	-	+
17.	Acid from Inositol	-	-
18.	Acid from Sorbitol	-	-
19.	Acid from Rhamnose	+	-
20.	Acid from Sucrose	-	-
21.	Acid from Lactose	-	-
22.	Acid from Arabinose	+	+
23.	Acid from Adonitol	+	-
24.	Acid from Raffinose	-	-
25.	Acid from Salicin	-	-
26.	Arginin Dihydrolase	-	-
	Species	*Enterobacter agglomerans*	*Pseudomonas flourescens*
	Percent Probability	99.99%	99.09%

Note : (+) = Positive reaction, (-) = Negative reaction

have reported that some bacteria possess a potential for biosorption or bioaccumulation activity of Cd [8, 23, 24, 25]. Metal biosorption by biomass mainly depends on structure and components of the cell wall such as peptidoglycan, teichoic acids, and lipoteichoic acids that are all necessary chemical components of bacterial surface structures [1].

Characterization of potential bacterium

According to gram staining result, those four bacteria are negative gram bacteria (Table 2). Therefore, the further analysis carried out using *Microbact TM GNB 12A/B/E 24 Identification Kit*. Analysis of the two most potent bacteria showed that the species of bacteria are *Pseudomonas flourescens* and *Enterobacter agglomerans*, respectively (Table 3).

Several studies have reported the potential of metal-resistant bacteria in the treatment of heavy metals contamination. Researchers have indicated that immobilized cells of *P. fluorescens* SM1 strain were quite effective in bioremediation of major toxicants present in Indian water bodies, including heavy metal [26]. The functional groups such as hydroxyl, carboxyl, amide, and phosphate groups which identified on *P. fluorescens* surface by FTIR technique could possibly be involved in the metal binding of Zn, Co, Cd, Pb, Cu, Cr, and Fe [27].

E. agglomerans or *P. agglomerans* can be termed as heavy metal tolerant bacteria. A study has reported that *P. agglomerans* JCM1 and *Enterobacter asburiae* JCM 6051 may have the potential to remove heavy metals from contaminated effluents before discharging them into river waters [19]. *E. agglomerans* was also reported as flocculant-producing bacteria that can remove nickel and cadmium [28].

Bacteria have developed various mechanisms for tolerating heavy metals. The bacterial resistance mechanism to heavy metal such (1) produce the compounds that can bind and detoxify metals inside the cell; (2) excreted metals via efflux transport systems; (3) absorbing metal by utilizing the structure of the cell envelope; (4) release of metal chelators into extracellular; (5) accomplished by intra- and extracellular mechanisms [29]. In the case of Cd, the Cd-resistant bacteria could be divided into three groups; the largest group consisted of bacteria resistant to Cd by effluxion it from the cells, the other two groups were capable of by binding Cd or detoxifying it [22]. The microbial can interact with heavy metals via many mechanisms such as biosorption, bio-accumulation, bio-transformation, bio-leaching, bio-mineralization, etc. The interactions may be used as the basis of potential bioremediation strategies [21, 30].

CONCLUSION

Four Cd-resistant bacteria isolates have been successfully isolated from the industrial disposal site. Two of the most potential bacteria for removing Cd identified as *P. flourescens* and *E. agglomerans*. Those bacteria can be furthermore utilized to reduce heavy metal pollution.

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