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Isolation and Characterization of Pb Resistant Bacteria from Cilalay Lake, Indonesia

Kesi Kurnia[1,2], Nina Hermayani Sadi[2], Syafitri Jumianto[1]
[1]Universitas Al Azhar Indonesia, Jl Sisingamangaraja Kebayoran Baru, 12110 South Jakarta, Indonesia; [2]Indonesian Institute of Science, Jl Raya Jakarta-Bogor Km 46, Cibinong Bogor 16911, Indonesia. Corresponding author email: kesi.kurnia@gmail.com

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Abstract – Pollution of water environment with heavy metals is becoming one of the most severe environmental and human health hazards. Lead (Pb) is a major pollutant and highly toxic to human, animals, plants, and microbes. Toxic metals are difficult to remove from the environment, since they cannot be chemically or biologically degraded and are ultimately indestructible. Biological approaches based on metal-resistant microorganisms have received a great deal of attention as alternative remediation processes. This study aim to isolate and characterize Pb resistant of heterotrophic bacteria in Cilalay Lake, West Java, Indonesia. The water samples were collected along three points around Cilalay Lake. Water physical and chemical determination was performed using the Water Quality Checker. The bacterial isolates were screened on Triptone Glucose Yeast (TGY) agar plates. Afterwards selected isolates were grown on Nutrient Agar media 50% with supplemented Pb 100 ppm by the standard disk. Population of resistant bacteria was counted. The result from metal resistant bacteria indicated that all isolates were resistant. The most abundant type of resistant bacteria to lead was Gram negative more. Identified have metal resistant bacteria could be useful for the bioremediation of heavy metal contaminated sewage and waste water.
Keywords: Cilalay Lake, heavy metal, Pb resistant

Introduction

Heavy metal pollution of wastewater is a major environmental threat due to their toxicity on aquatic ecosystem and public health (Raja *et al.,* 2009; Naik *et al.,* 2012). They cannot be chemically or biologically degraded therefore they are difficult to remove from the environment. Moreover, accumulation of heavy metals in vegetation due to irrigation with waste water could have serious effects on human health (Joonu and Averal, 2012; N Gupta and Kumar, 2012). They can cause of damaging or reducing central nervous function, lower energy levels and damage to blood composition, kidneys, liver, lungs and other vital organs (Gupta and Kumar, 2012). Also, they are difficult to remove from the environment and to be powerful inhibitors of biodegradation activities (Selvi *et al.,* 2012). The five highest of metal toxicity for biological life are Hg^{2+}> Cd^{2+}> Ag^{2+}> Ni^{2+} > Pb^{2+} (Darmono, 2006).

Lead (Pb) is biologically non-essential and toxic heavy metals that is found in soil, water and air. It is a hazardous waste and highly toxic to human, animals, plants, and microbes (Raja *et al.,* 2009; Murthy *et al.,* 2014). It was a persistent environmental pollutant with half life of approximately 5000 years and biomagnifies through the trophic levels (Naik *et al.,* 2012). Lead found as by products of processes such as mining and ore processing, as well as burning of fossil fuels. Lead-resistant bacteria have been isolated (Murthy *et al.,* 2014). Some bacteria that resist to heavy metal will survive and grow in environment which accumulate of heavy metal. They have transport system in their cell membran to deny or decrease metal that enter to their sitoplasm (Arrizal *et al.,* 2013). Removal of heavy metal ions from wastewaters is essential due to their extreme toxicity towards aquatic life and humans. Joonu and Averal (2012) reported that the bioremediation of heavy metals using microorganisms has potential application in industry. Microbes may provide efficient and economical alternative to current methods. Also they play massive role in the bio-geochemical cycling of toxic heavy metals and also in cleaning up or remediating metal-contaminated environments (Selvi *et al.,* 2012). Different microbes have been proposed to be efficient and economical alternative in removal of heavy metals from water (Raja *et al.,* 2009).

The Cilalay Lake is situated at Indonesian Institute of Science-Cibinong in West Java, Indonesia. This is a natural aquatic ecosystem that has large amount 12.190 m^2. It does not receive input of various kinds of wastes and is pond with the original water source. The objective of current study is find out information about existence of various types of heterotrophic bacteria and isolate lead-resistant bacteria which can be used to clean-up industrial wastewater and sewage.

Materials and Methods

Sampling sites

The water samples were collected directly at the three sites in Cilalay Lake. The three sites selected from the lake were site a) inflow of water, site b) central part of the pond and site c) outflow of water (Figure 1). It is located in Indonesian Institute of Science area, Cibinong, West Java, Indonesia. The sampling network was designed to cover a wide range of Cilalay Lake, which reasonably represent the water quality of the lake system. The samples were stored in insulated cooler containing ice and transport to laboratory for bacteriological analysis. The determination of pH, conductivity, dissolved oxygen, and temperature was performed using the Water Quality Checker (HANNA Instruments). The experiments were performed in triplicate.

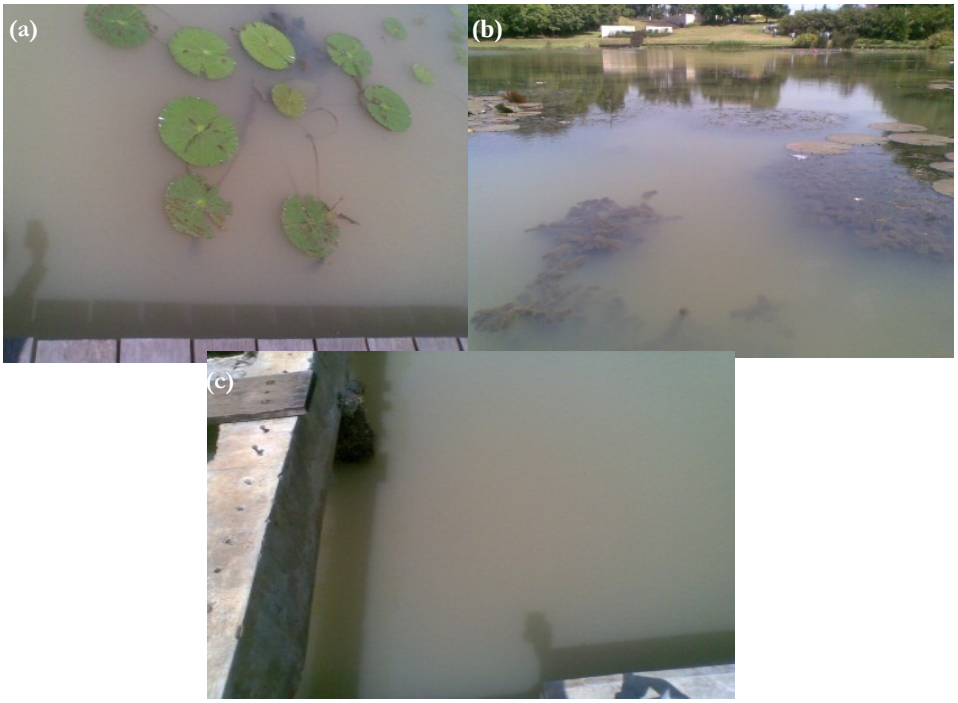

Figure 1. Location of sampling point in (a) inflow (b) central part (c) outflow of Cilalay Lake, West Java, Indonesia

Isolation of bacteria

Serial dilution techniques were used for isolation bacteria. Water sample was serially diluted in sterile distilled water from 10^{-1} to 10^{-4}. The bacterial isolates were screened on Triptone Glucose Yeast (TGY) agar plates by the standard spread plate. Afterwards plates were incubated for 5 days and the results of bacteriological analyses are presented in colony-forming units (CFU) of heterotrophic bacteria per 1 ml of water. Colonies differing in morphological characteristics were selected and used for further studies.

Characterization of bacteria

Gram Test. The potassium hydroxide (KOH) string test was used to differentiate bacterial isolates. A loop of growth from a bacterial colony was mixing in a suspension of 3% aqueous KOH on a glass slide. The test was considered positive if such a suspension gels or becomes viscous and strings out when the loop is lifted, the isolate is gram negative. Gram-positive cells do not form a viscous gel or string out.

Pb Resistance. Selected isolates were grown on Nutrient Agar (NA) 50% supplemented Pb 100 ppm. Isolates were incubated at 30°C for 3 days. Resistance of the selected isolates against Pb on NA agar plates was evaluated until the strains unable to grow colonies on the agar plates. Plates without any metal concentration were treated as reference plates.

Results and Discussion

Physical and chemical determination

The results showed fluctuations range of each parameter was not significant among the sites (Table 1). The pH range was suitable for aquatic biota. Nugroho (2002) reported pH of the water was influenced by several factors such as temperature, oxygen content, and the presence of cations. Increased respiration by all components of the ecosystem can improve the amount of carbon dioxide, therefore the pH of water decreased. The conductivity of water depends on the concentration of ions and water temperature, therefore more dissolved solids will affect the increase of DHL (Patil *et al.,* 2012). Low concentration of dissolved oxygen in Situ Cilalay can be caused by organic reshuffle not balance with the supply of oxygen (Light *et al.,* 2005). The reform process of organic matter requires oxygen therefore the oxygen concentration in the water will decrease.

Table 1. The main parameters of water quality of Cilalay Lake, West Java, Indonesia during the study

Site	pH	Conductivity (µmhos/cm)	Dissolved oxygen (mg/L)	Temperature (°C)
1	6.07	48.98	5.85	29.55
2	6.36	50.50	6.07	29.60
3	6.27	51.48	4.43	28.90
Average	6.23	50.32	5.45	29.35

Bacterial Isolation

Bacterial strains were characterized from three sites in Cilalay Lake (Figure 3). The plate viable count of bacteria isolates from water samples was in the range of 1.3 x 10^3 CFU/ml. Heterotrophic bacteria was known as decomposer and mineralizer organic chemical in their environment. They can obtain the food in the form of organic substances from the environment then remineralized and decomposed to nutrients. In the aquatic ecosystem, the decomposer organisms such as heterotrophic bacteria utilize the remaining organisms for decomposed into elements that are returned to the soil and the atmosphere as a nutrient can be reused by plants (Purnamaningtyasa and Syama, 2012).

Results show that 80% were Gram negative and the remaining 20% were Gram positive (Figure 3a). KOH string test are rapid and can differentiate bacteria well into Gram positive and Gram negative. Gram positive bacterial cell wall consists of a layer peptidoglycan (90%), whereas Gram-negative bacteria have a high content of lipids on his cell wall in the form of liposakarida and lipoprotein (Aminulloh, 2011). In Gram negative bacteria, the cell wall is only 1-3 layers thick. In the presence of potassium hydroxide, Gram negative cell walls are easily disrupted, releasing viscid chromosomal material, which causes the bacterial suspension to become thick and stringy. The cell walls of gram-positive bacteria are not affected by 3% KOH (Madigan *et al.,* 2012).

The growth of bacterial isolates in the presence of heavy metals is showed on Figure 3b. All isolates were found to be highly resistant to heavy metals where bacteria have seen growing around the disk (Figure 2). This study showed a high incidence of metal resistance for the bacterial isolates. Microorganisms have developed the mechanisms to cope with a variety of toxic metals for their survival in the environment enriched with such metals (Gupta and Kumar, 2012). The microbial resistance to heavy metal is attributed to a variety of detoxifying mechanism developed by resistant microorganisms such as complexation by xopolysaccharides, binding with bacterial cell envelopes, metal reduction, metal efflux etc. These mechanisms are sometime encoded in plasmid genes facilitating the transfer of toxic metal resistance from one cell to another (Powers, 1995). Such mechanisms of resistance may be based on metal retention on the surface of a cell, on intracellular transformation into less toxic forms, on the release of metal from a cell with the help of polymers, and lowering the permeability of a cell membrane. Many bacterial resistant systems for toxic metals are encoded by plasmids. The presence of the organisms that possess specific mechanisms of resistance to heavy metals increases destruction or transformation of toxic substances in the natural environment (Singh *et al.,* 2010).

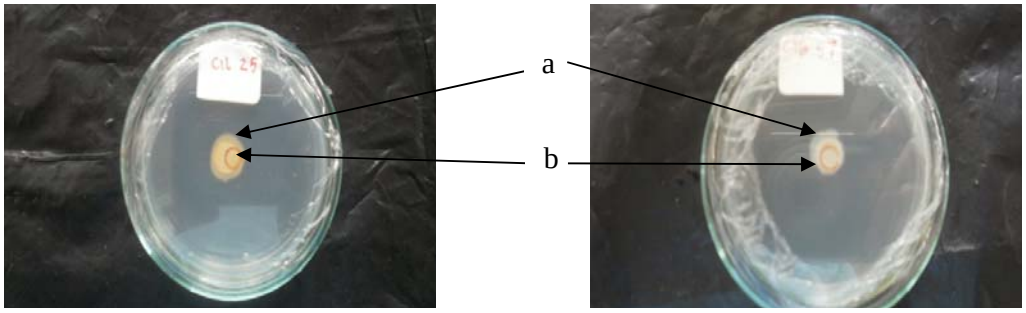

Figure 2. Lead-resistant bacteria by the standard disk method with supplemented Pb 100 ppm (a) growth bacteria (b) standard disk.

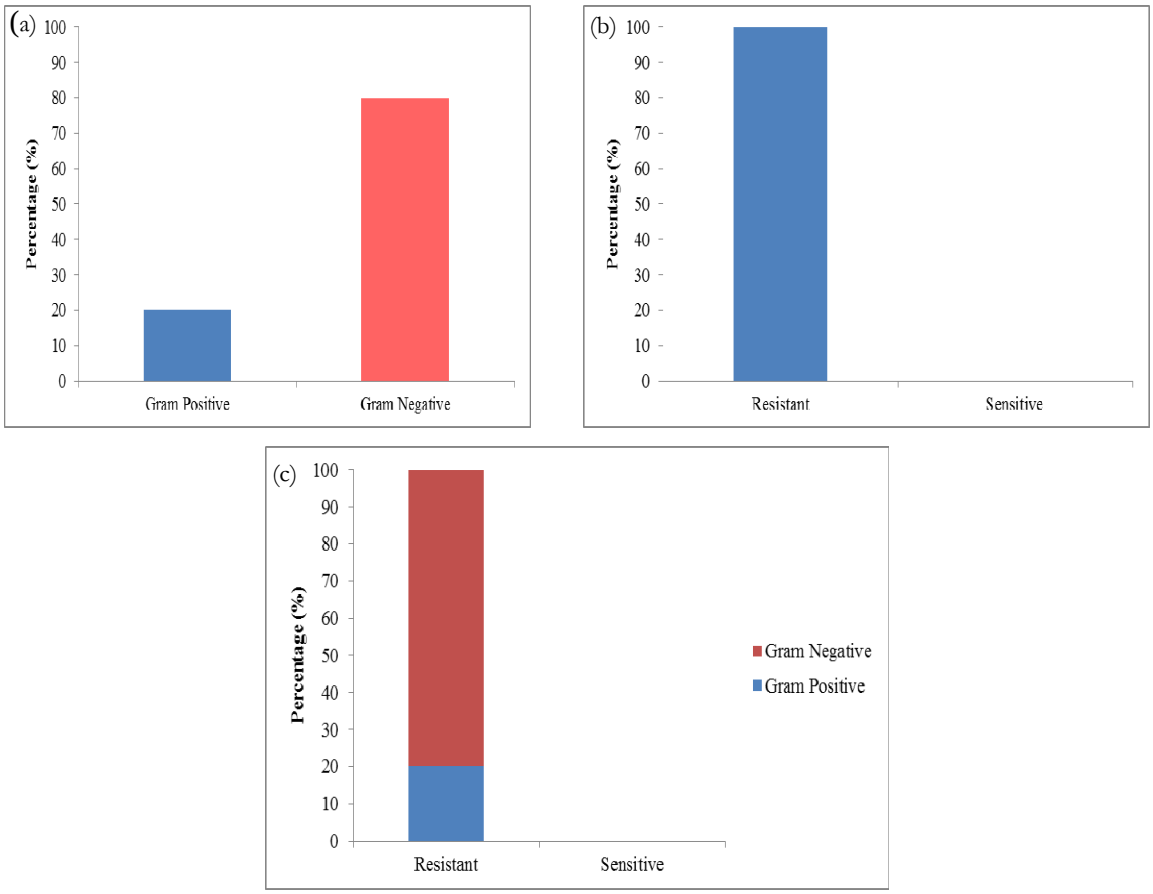

Figure 3. Characterization of bacterial isolates from Cilalay Lake, West Java, Indonesia (a) Gram characteristic (b) Lead-resistant characteristic (c) Relation between Gram and lead-resistant of bacterial isolates

About 80% Gram negative bacteria were resistant to lead, and 20% Gram positive bacteria were sensitive to lead (Figure 3c). In the present research, the abundance percentage of Gram negative bacteria was higher than percentage of Gram positive bacteria. Ahmad *et al.,* (2005) reported the Gram negative bacteria more resistance because their complex cells wall which bond and mobilize more the Pb^{2+} ion (Ahmad *et al.,* 2005). The mechanism of resistance to lead is due to an efflux by P-type ATPases and intracellular compounds complexation (Jankowska *et al.,* 2006). Several bacterial species utilize intra and extracellular binding mechanisms to avoid toxicity to Pb^{2+}. Other factors such as Plasmids, stress proteins, and membrane ion pumps, may also be responsible for this resistance (Hookoom and Puchooa, 2013).

Conclusions

The research has proved that heterotrophic bacteria isolated from the water of Cilalay Lake has resistant to lead (Pb). The most abundant type of lead-resistant bacteria was Gram negative. The identification of resistance bacteria against heavy metals may provide a useful tool for the simultaneous monitoring of several toxic pollutants in the environment. Bioremediation and recovery of heavy metals including lead from contaminated industrial effluents is a major challenge. The highly lead-resistant bacterial isolates bioaccumulating significant amount of lead may be employed for bioremediation of lead in contaminated environmental sites.

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