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THE POTENCY OF COPPER-RESISTANT BACTERIA *Cupriavidus* sp. IrC4 ISOLATED FROM INDUSTRIAL WASTEWATER TREATMENT PLANT IN RUNGKUT-SURABAYA AS A BIOREMEDIATION AGENT FOR HEAVY METALS

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ABSTRACT

Cupriavidus sp. IrC4 is a copper-resistant bacteria isolated from activated sludge in an Industrial Wastewater Treatment Plant in Rungkut-Surabaya, Indonesia. The purpose of this research was to study the potency of *Cupriavidus* sp. IrC4 as a bioremediation agent for copper, lead, mercury, and cadmium. Resistance of *Cupriavidus* sp. IrC4 to heavy metals were determined by measuring the minimum inhibitory concentration (MIC). Accumulation of copper, cadmium, and lead were determined by Atomic Absorption Spectrophotometer. *Cupriavidus* sp. IrC4 showed multiple resistance to heavy metals. The MICs of *Cupriavidus* sp. IrC4 to copper, lead, mercury, and cadmium were 16 mM, 15 mM, 6 mM, and 5 mM, respectively. The growth of *Cupriavidus* sp. IrC4 was inhibited by the addition of $CuSO_4$ in the medium. The bacteria survived in the presence of high copper concentration as shown by the extension of the lag phase up to 36 hours. The analysis demonstrated that the copper resistance of the bacteria was facilitated through the accumulation of copper. *Cupriavidus* sp. IrC4 accumulated up to 367.78 and 260.01 mg/gram dry weight of cells of copper and lead, respectively. The bacteria demonstrated growth in the medium containing the mixture of 0.5 mM copper, lead, cadmium and accumulated those heavy metals up to 0.14, 24.74, and 12.49 mg/g dry weight of cells, respectively. The high resistance and capability of *Cupriavidus* sp. IrC4 to accumulate heavy metals can be exploited in bioremediation process for removing heavy metals from industrial sewage.

Keywords: Accumulation, copper, *Cupriavidus* sp. IrC4,heavy metals, resistance.

INTRODUCTION

Heavy metal pollution of soil and wastewater is a significant environmental problem (Cheng, 2003). According to the World Health Organization (WHO, 2010), the metals of most immediate concern include copper, cadmium, lead, mercury, chromium, cobalt, nickel, and zinc. The presence of such metals in aquatic environments cause severe damage to aquatic life and killing microorganisms during biological water purification process (Vinodhini & Narayan, 2008). Moreover, these metals have exacting consequences on humans such as brain damage, reproductive failures, nervous system failures and tumor formation (Mahvi, 2008).

Copper, one of the most widely used heavy metals, is mainly employed in electrical and electroplating industries and in larger amounts is extremely toxic to living organisms. The presence of copper (II) ions, cause serious toxicological concerns, it is usually known to

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deposit in brain, skin, liver, pancreas and myocardium (Davis *et al*., 2000). Cadmium is the most dangerous metal ion characterized by high stability and toxicity. It is not degradable in nature and will thus, once released to the environment, stay in circulation. Cadmium is known to bind with essential respiratory enzymes (Nies, 2003) causing oxidative stress and cancer (Banjerdkji *et al.,* 2005). Lead (Pb) a major pollutant that is found in soil, water and air is a hazardous waste and is highly toxic to human, animals, plants and microbes (Low *et al*., 2000). Cadmium and lead, though non-essential and non-beneficial, are considered highly toxic to plants, animals and microbes (Ajmal *et al*., 1998).

The bioremediation of heavy metals using microorganisms has received a great deal of attention in recent years, not only as a scientific novelty but also for its potential application in industry. Conventional methods as precipitation, oxidation/reduction, ion exchange, membrane filtration and evaporation, though capable of eliminating these toxic metals from the environment, are extremely expensive and inefficient for metal removal from dilute solutions ranging from 1 to 100 mg of dissolved metal per liter (Volesky, 1990). Microorganisms and microbial products have been reported to efficiently remove soluble and particulate forms of metals, especially from dilute solutions, through bioaccumulation and therefore microbe-based technologies provide an alternative to the conventional techniques of metal removal/ recovery (Ozdemir *et al*., 2004). Metal accumulating bacteria can be used to remove, concentrate and recover metals from industrial effluents (Chowdhury *et al.,* 2008). The aims of this research were to analize the resistance of *Cupriavidus* sp. IrC4 to heavy metals, to study the effect of heavy metals added to bacterial growth, and to study the potency of bacteria to accumulated heavy metals.

MATERIALS AND METHODS

Bacterial strain, media and growth

Cupriavidus sp. IrC4 is copper-resistant bacteria isolated from activated sludge in an industrial wastewater treatment plant in Rungkut, Surabaya, Indonesia, under the accession number of JX398287 (Irawati *et al*., 2012). Bacterial isolate was grown in Luria Bertani (LB) agar containing the following (per liter) : tryptone: 10 g, yeast extract 5 g, NaCl 10 g, glucose 0,1 g, and pure agar 0.15 g. Stock of 1 M $CuSO_4$; 1M $Pb(NO_3)_2$; 0.25 mM $HgCl_2$; 1 M $Cd(NO_3)_2$ were added to the autoclaved media.

Heavy metals resistance

Resistance of *Cupriavidus* sp. IrC4 to heavy metals were determined by measuring the Minimum Inhibitory Concentration (MIC). The MICs of the strain against increasing concentrations of heavy metals on LB agar was evaluated by streak plate method until the strain unable to give colonies on the agar plates. The growing colonies at a given concentration were subsequently transferred to the next higher concentration. The evaluation of Minimum Inhibitory Concentration (MIC) was determined after 48 hours of incubation at 37ºC (Raja *et al*. 2009).

The Effect of Heavy Metals on Bacterial Growth

Cells were grown in LB broth supplemented with various concentration of copper sulphate and in medium without copper sulphate. The cultures were incubated at 37°C on a shaker (200 rpm). Growth was monitored by measuring optical density at 600 nm.

Cellular Heavy Metals Accumulation

Cells were grown in SBS broth containing the various concentration of copper and lead, and the mixture of copper, lead, and cadmium. The cells were incubated at 37°C with shaking at 200 rpm. The Cells were collected by centrifugation at 5000xg for 20 min at 4°C and washed several times with copper free phosphate buffer. The cell pellets were digested with HNO_3 at 100°C. Heavy metals content were determined by an Atomic Absorption Spectrophotometer. Dry weight of the cells from the same culture were determined (Cha & Cooksey, 1991).

RESULTS AND DISCUSSION

Heavy metals resistance

Cupriavidus sp. IrC4 showed highly degree of resistance to copper, lead, mercury, and cadmium with MIC of 16 mM, 15 mM, 6 mM, and 5 mM, respectively (tab. 1).

Table 1. The MICs of *Cupriavidus* sp. IrC4 to heavy metals

Heavy metals	MIC (mM)
$CuSO_4$	16
$Pb(NO_3)_2$	15
$HgCl_2$	6
$Cd(NO_3)_2$	5

The resistance of *Cupriavidus* sp. IrC4 to some heavy metals might be due to the selective pressure by pollution of industrial wastewater at Rungkut, the location where the strain has been isolated. Microorganisms undergo selection pressures in the presence of toxic compounds and develop resistance (Hideomi *et al*., 1977). Rungkut is frequently polluted with high concentrations of heavy metals such as copper, lead, cadmium, and mercury due to the industrial activities (Daud 1996). The ability of microbial strains to grow in the presence of heavy metals would be helpful in the waste water treatment where microorganisms are directly involved in the decomposition of organic matter in biological processes for waste water treatment, because often the inhibitory effect of heavy metals is a common phenomenon that occurs in the biological treatment of waste water and sewage (Filali *et al*., 2000).

The effect of copper added on Bacterial Growth

The growth of *Cupriavidus* sp. IrC4 was inhibited by the addition of 4 mM, 7 mM, and 10 mM $CuSO_4$ in the medium (Fig.1). The presence of high copper concentration affected the strain by reducing the growth rate. *Cupriavidus* sp. IrC4 survived in the presence of high copper concentration as shown by the extension of the lag phase with the addition of $CuSO_4$. The strain showed a lag phase and resumed to normal growth after the lag phase, but its cell density did not reach as high level as its cell density on medium without $CuSO_4$. The duration of the lag phase depends on the concentration of copper added to the medium. In the medium containing 7 mM $CuSO_4$, *Cupriavidus* sp. IrC4 extended the lag phase in 6 hours, whereas, this strain needed 36 hours of the lag phase when 10 mM was added in the medium. Copper in its ionic form is a required trace element but become toxic when in surplus. This toxicity is caused mainly by the intrinsic properties of copper, as free copper ions undergo redox cycling reactions alternating between Cu(I) and Cu(II). This also results in the transfer of electrons to hydrogen peroxide and the concomitant generation of hydroxyl radicals that readily attack and damage cellular biomolecules (Macomber *et al*., 2007).

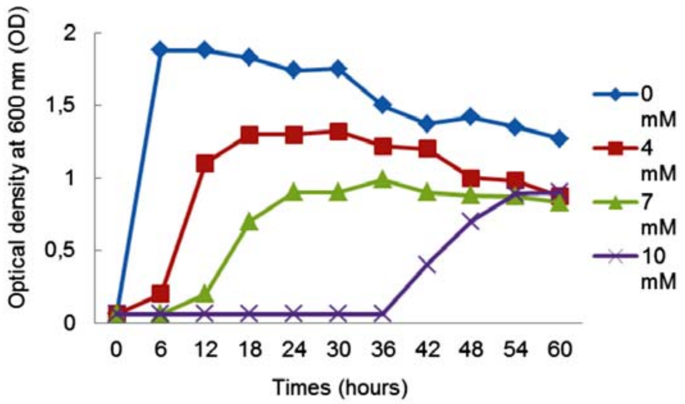

Figure 1. The growth of *Cupriavidus* sp. IrC4 in medium containing various concentration of $CuSO_4$

Heavy metals accumulation

It was shown that *Cupriavidus* sp. IrC4 has capability to accumulate copper and lead. The highest amount of copper and lead accumulated by the strain was 367.78 and 260.01 mg/gram dry weight of cells, respectively. The amount of cellular copper accumulation by *Cupriavidus* sp. IrC4 increased with increasing copper concentration up to 4 mM, 7 mM, and 10 mM $CuSO_{4,}$ respectively (fig.2). The previous study demontrated that the resistance mechanism of *Cupriavidus* sp. IrC4 to copper involved accumulation copper especially on the membrane fraction and restricted amount of copper inside the cytoplasm (Irawati *et al*., 2012). Copper ions are capable of catalyzing harmful redox reactions which result in the oxidation of lipid membranes and damage to nucleic acids. Some bacteria, developed detoxification systems to protect themselves from toxic concentrations of copper ions (Hoshino *et al*., 1999).

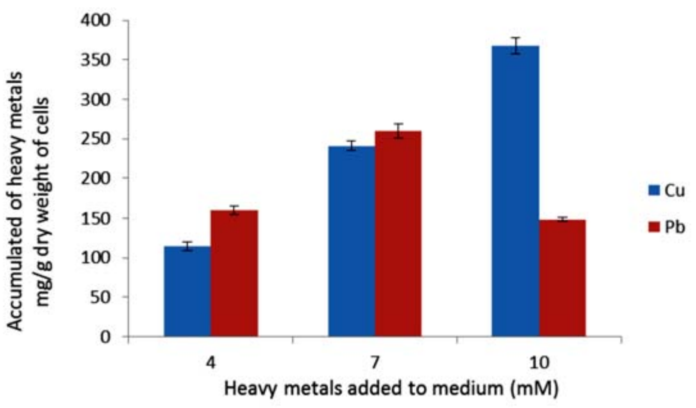

Figure 2. The potency of *Cupriavidus* sp. to accumulate copper and lead

Lead accumulation by this strain also increased with increasing lead concentration up to 7 mM but levelled off in medium containing 10 mM $Pb(NO_3)_2$ (fig. 2). It suggested that 10 mM $Pb(NO_3)_2$ was toxic for the strain. Nies (1999) reported that inside the cell, heavy-metal cations tend to bind to SH groups. By binding to SH groups, the metals may inhibit the activity of sensitive enzymes. Other heavy-metal cations may interact with physiological ions thereby inhibiting the function of the respective physiological cation. Many mechanisms of resistance are known in bacteria for maintaining intracellular homeostasis of metal ions (Nies, 1999). Two of which are the metabolically independent binding of these ions unto the cell surface (Binkley & Simpson, 2003) and metabolic production of binding proteins (Samuelson *et al.,* 2000).

Cupriavidus sp. IrC4 demonstrated growth in the medium containing the mixture of 0.5 mM copper, lead, cadmium and accumulated those heavy metals up to 0.14, 24.74, and 12.49 mg/g dry weight of cells, respectively (fig.3). *Cupriavidus* sp. IrC4 is a gram negative bacteria (Irawati *et al*., 2012). The outer membrane of gram negative bacteria, which consists of lipopolysaccharides, lipoproteins, and phospholipids carries a strong negative charge. Because the cell surface of bacteria carries a net negative charge due to the presence of carboxyl, amine, hydroxyl, phosphate and sulfhydrylgroups (Tortora *et al*., 2005), it can adsorb appreciable quantities of positively charged cationic metals (Scott & Palmer 1990). Capability of *Cupriavidus* sp. IrC4 to grow in a mixture of heavy metals and accumulate those heavy metals is very useful in biotechnology for the remediation of metal contaminated environments and can also be used in the construction of biomarkers for the detection of the presence of metals.

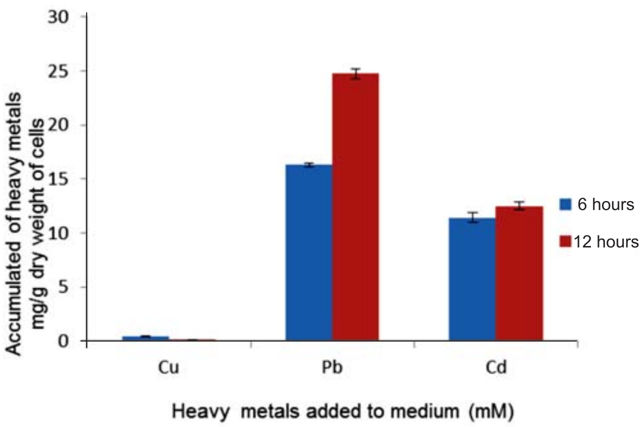

Figure 3. The potency of *Cupriavidus* sp. to accumulate copper, lead, and cadmium in medium containing a mixture of 0.5 mM $CuSO_4$, $Pb(NO_3)_2$, $Cd(NO_3)_2$

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REFERENCES

Ajmal, M., A. Mohammad, R. Yousuf, and A. Ahmad. 1998. Adsorption behaviour of cadmium, zinc, nickel & lead from aqueous solution Mangifera indica seed shell. *Indian Journal of Environmental Health*, 15–26.

Banjerdkji, P., P.Vattanaviboon, and S. Mongkolsuk. 2005. Exposure to cadmium elevates expression of genes in the oxy R and Ohr R regulons and induces cross-resistance to peroxide killing treatment in *Xanthomona scampestris*. *Appl. Environ. Microbiol*, vol.4:1843-1849.

Binkley, J, and J.A. Simpson. 2003. Heavy metals in wastewater treatment processes. *In: The Handbook of Water and Wastewater Microbiology*. Mara, D., and N. Horan (eds). London: Academic Press, 597-610.

Cha, J.S., and D.A. Cooksey. 1991. Copper resistance in *Pseudomonas syringae* mediated by periplasmic and outer membrane proteins. *Proc Nat Acad Sci*. USA, vol.10 : 8915-8919.

Cheng, S. 2003. Heavy metal pollution in China: origin, pattern and control. *Environmental Sciences and Pollution Research,* vol. 10, 192-198.

Chowdhury, S., M. Mishra, V.K. Adarsh, A. Mukherje, A.R. Thakur, and S.R. Chadhuri. 2008. Novel metal accumulator and protease secretor microbes from East Calcutta wetland. *American Journal of biochemistry and biotechnology*, vol.3:255-264.

Daud, A. 1996. *Analisis Kandungan Logam Berat Merkuri dan Kuprum pada Ikan dan Kerang serta Pengaruhnya terhadap Kesehatan Penduduk di Pesisir Pantai Kelurahan Sukolilo, Kecamatan Kenjeran, Kota Madya Surabaya*. Program Pascasarjana Universitas Airlangga. Surabaya.

Davis, J.A., B. Volesky, and R.H.S.F. Vierra. 2000. *Sargassum* seaweed as biosorbent forheavy meals. *Water Res*, vol.17: 4270-4278.

Filali, B.K., J. Taoufik, Y. Zeroual, F.Z. Dzairi, M. Talbi, and M. Blaghen. 2000. Waste water bacteria resistant to heavy metals and antibiotics. *Current Microbiology,* 151-156.

Hideomi, N., T. Ishikawa, S. Yasunaga, I. Kondo, and S. Mitsuhasi. 1977. Frequency of heavymetal resistance in bacteria from inpatients in Japan. *Nature,* 165-167.

Hoshino, N., T. Kimura, A. Yamaji, and T. Ando. 1999. Damage to the cytoplasmic membrane of *Escherichia coli* by catechin-copper complexes. *Free Rad. Biol. Med*, 1245-1250.

Irawati, W., T. Yuwono, H. Hartiko, and J. Soedarsono. 2012. Molecular and physiological characterization of copper-resistant bacteria isolated from activated sludge in an industrial wastewater treatment plant in Rungkut-Surabaya, Indonesia. *Microbiology Journal.* vol.3:107-116. DOI:10.5454/mi.6.3.3

Low, K.S., C.K. Lee, and S.C. Liew. 2000. Sorption of cadmium & lead from aqueous solution by spent grain. *Process Biochemistry*, 59–64.

Macomber, L., C. Rensing, and J.A. Imlay. 2007. Intracellular copper does not catalyze the formation of oxidative DNA damage in *Escherichia coli*. *J. Bacteriol*, vol.5:1616–1626.

Mahvi, A.H. 2008. Application of agricultural fibers in pollution removal from aqueous solution. *Int. J. Environ. Sci. Tech*, vol. 2: 275-285.

Nies, D.H. 1999. Microbial heavy metal resistance*. Applied Microbial Biotechnology.*, 51: 730- 750.

Nies, D.H. 2003. Efflux mediated heavy metal resistance in prokaryotes. *FEMS. Microbiol. Rev*. vol. 2-3:313-39

Ozdemir, G., N. Ceyhan, T. Ozturk, F. Akirmak, and T. Cosar. 2004. Biosorption of chromium (VI), cadmium(II) and copper (II) by Pentoea sp. TEM18. *Chemical Engineering Journal*, 249–253.

Raja, C.E., G.S. Selvam, and K. Omine. 2009. Isolation, identification and characterization of heavy metal resistant bacteria from sewage. *Int Joint Symp on Geodisaster Prevention and Geoenvironment in Asia*, 205-211.

Samuelson, P., H. Wernerus, M. Svedberg, and S. Stahl. 2000. Staphylococcal surface display of metal-binding polyhistidyl peptides. *Appl Environ Microbiol,* vol.3: 1243-1248.

Scott, J.A., S.J. Palmer. 1990. Sites of cadmium uptake in bacteria used for biosorption. *Appl Microbiol Biotechnol*, 221-225.

Tortora, G.J., D.R. Funke, and C.L. Case. 2005*. Microbiology: An Introduction*, 8th ed. San Fransisco, CA: Pearson Education Inc., publishing as Benjamin Cummings.

Vinodhini, R., and M. Narayanan. 2008. Bioaccumulation of heavy metals in organs of fresh water fish *Cyprinus carpio* (Common carp). *Int. J. Environ. Sci. Tech*, vol.2:179-182.

Volesky, B. 1994 Advances in biosorption of metals: Selection of biomass types. *FEMS Microbiology Reviews*, 291–302.

WHO, 2010. Guideline for drinking water quality recommendations, (Vol. 1), World Health Organization, Geneva.