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The Consortium of Microbial Decomposers on Heavy Metal Resistant Waste to Improve Environmental Health

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Abstract. **Domestic wastewater is a source of pollutant microbes that cause various diseases and has the potential to be a source of transmission of pathogenic diseases carried through the water. Heavy metal contamination in waters, including domestic wastewater, affects water quality and biological components, including bacteria. This study aimed to find a consortium of tolerant strains bacteria originating from domestic wastewater in the form of a consortium to improve environmental health. The method used is to test heavy metal resistance using the streak plate method so that the slant contains $HgCl_2$ and $CuCl_2$. The resistance test is for bacterial strain consortium against various types and concentrations of heavy metals. The results showed a resistance of a consortium of strains of detergent-tolerant bacteria originating from domestic wastewater to various heavy metals up to a concentration of 40 mg/ L for Cu metals and 30 mg/ L for Hg metals. The composition of these strains is *Bacillus cereus strain BQAR-01d, Bacillus thuringiensis strain MSS-2, Bacillus cereus strain JDA-1*, and *Bacillus sp. B31* (2008). The four consortiums of heterotrophic bacterial strains can be developed into decomposers of domestic wastewater to improve environmental health.**

Keywords: resistance, bacterial strain consortium, wastewater, heavy metals, environmental health

INTRODUCTION

Domestic wastewater is a source of pollutant microbes that cause various diseases and has the potential to be a source of transmission of pathogenic diseases carried through the water. The domestic wastewater must be controlled and treated in advance with methods and management techniques that are environmentally sound to avoid negative impacts on public health and the environment. One of the wastewater treatments is by using waste decomposer tolerant of heavy metals. Heavy metals come not only from industrial waste but also from domestic wastewater. Heavy metal is a chemical pollutant that is very dangerous for human health. Cases of mercury pollution happened in the Minamata Bay of Japan in 1950-1963, which caused Minamata disease [1]- [3]. Thus, mercury pollution is highly dangerous and needs special attention from all parties. The amount of inorganic material produced depends on the source of wastewater it originates from. Usually, the inorganic material content of waste contains chlorides, sulfur, nitrogen, methane, toxic substances (such as copper, lead, silver, chromium, arsenic, boron, and cyanide) [4]- [9].

One of the efforts to handle domestic liquid waste in microbiology is by utilizing original microbial (indigen) isolates that can decompose the waste. Naturally, potential microbes, as decomposers, are obtained by isolating the waste and then cultured in vitro in the laboratory. The inoculants used originate from native microbes in the form of single compounds or groups of various species (consortia). The microbes that have the most potential in decomposing waste and killing pathogens are propagated in the laboratory to be used as starter decomposition of waste [5], [10]- [13]. Potential microbial strains in the decomposition of waste have been identified molecularly [11], [14], [15]. It can be introduced into domestic wastewater and will affect other microbes. Superior microbes must be tolerant of various types and concentrations of detergents. Microbes in the waste community itself interact with each other. The interaction varies, from beneficial interactions to mutually harmful interactions. However, in an environmentally friendly waste decomposition technology, effective and optimum conditions are sought, which are mutually beneficial so that the influence of interactions between microbes can reduce pollutants in wastewater [16- [18]. The problems of this research are 1) How is the resistance of a consortium of heterotrophic bacteria tolerant to detergents from domestic liquid waste against various heavy metals?

METHOD

The consortium of heterotrophic bacterial strains that grow is isolated and resistant to heavy metals Hg and Cu. Selected bacterial strains are resistant to heavy metals Hg and Cu. The heterotrophic bacterial strains are *Bacillus cereus strain BQAR-01d* (A), *Bacillus thuringiensis strain MSS-2* (B), *Bacillus cereus strain JDA-1* (C), and *Bacillus sp. B31* (2008) (D). The strain consortium consists of the ABC, ABD, BCD, and ABCD consortia [11].

Bacterial consortium strains derived from the PCR reaction were purified by the ethanol/EDTA precipitation method. A total of 20 microL suspensions of PCR results were put into a microcentrifuge tube and then added 5 microL of 125 mM EDTA and 60 microL of absolute ethanol, incubated at room temperature for 15 minutes and synthesized at a speed of 4,000-5,000 rpm (30 minutes). Supernatant discarded, pellet in a tube plus 60 microL of 70% ethanol, centrifuged 4,000 rpm at 40C (15 minutes). The supernatant is discarded, pellet sequences 16S rRNA are dried with speed vac at 40C (10-15 minutes), plus 20 microL Hi_in formamides, divortex briefly. Furthermore, the suspension is heated at a temperature of 950C (2-5 minutes) and immediately moved to cold temperatures, dipipiet 13 microL is inserted into the sample tube, and the sample is ready to tray and running—sequencing DNA nucleotides encoding 16S rRNA. The pure 16S rRNA coding DNA of each detergent tolerant isolate was sequenced using Bigdye V.3.1. The pure 16S rRNA sequencing process for each bacterial isolate was carried out according to the ABI PRIMS 310 genetic analyzer using a sequencer engine. The results of sequencing are files consisting of electropherograms and textfile DNA nucleotide sequences [16], [19]- [21].

Heavy metal resistance test used the streak plate method so that the slant contained $HgCl_2$ and $CuCl_2$. The concentrations of $HgCl_2$ tested were 1, 2, 3, 4, 5 mg/L, and continue until the highest mercury resistance was obtained. The concentrations of $CuCl_2$ tested were 5, 10, 15, 20, and 25 mg/L. The concentration was raised until the bacterial strain did not grow. The next concentrations used were 30, 35, 40, 45, 50, 55, 60, 65, and 70 mg/L. The study design with factorial CRD with the treatment was repeated 3 times. Heterotrophic bacterial culture was then incubated at 370C for 24 hours.

RESULT & DISUCSSION

The consortium resistance of heterotrophic bacterial strains to various heavy metals can be seen in Table 1 and Table 2.

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Table 1. The resistance of the Heterotrophic Bacteria Strain Consumption from Domestic Liquid Waste to $HgCl_2$ Replications 1, 2, and 3

No	Bacterial Consortium	Concentration of HgCl2 (mg / L) in NA-HgCl2 media																
		1	2	3	4	5	10	15	20	25	30	35	40	45	50	55	60	65	70
1	ABC	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
2	ABD	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
3	BCD	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
4	ABCD	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-

Information:
+: grows at an incubation temperature of 37OC 24 hours
- : do not grow at an incubation temperature of 37OC 24 hours
A: *Bacillus cereus strain BQAR-01d*
B: *Bacillus thuringiensis strain MSS-2*
C: *Bacillus cereus strain JDA-1*
D: *Bacillus sp. B31* (2008)

Table 2. The resistance of the Heterotrophic Bacteria Strain Consumption from Domestic Liquid Waste to CuCl2 Replications 1, 2, and 3

No	Bacterial Consortium	Concentration of CuCl2 (mg/L) in NA- CuCl2 media																
		1	2	3	4	5	10	15	20	25	30	35	40	45	50	55	60	65	70
1	ABC	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
2	ABD	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
3	BCD	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
4	ABCD	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-

Mercury contamination in water can affect water quality and biological components, including bacteria. Some bacteria are resistant to mercury, gram-negative and gram-positive bacteria, mesophyll, halophiles, and extremophile groups [14]. Mercury resistant bacteria is an example of physiological and genetic adaptation to mercury-contaminated environments of 1-10 mg / L [19], [22] and some are even mercury resistant at concentrations of 25 mg / L [19], [23].

Household waste, one of which is liquid, is a source of water pollution. Liquid waste can be in the form of water and other waste materials that are mixed (suspended) or dissolved in water. Household liquid waste includes soap, detergent, oil, pesticides, urine, used washing water, bathing

water, and many more. Household liquid waste can be found in various organic materials (e.g., vegetable, fish, rice, oil, human wastewater) carried by water. If not treated well, this liquid waste will have an impact on environmental pollution [24]-[26]. Lead (Pb) can enter the environment and the human body from various sources such as gasoline (petrol), recycled materials, battery disposal, toys, paints, pipes, soil, several types of cosmetics, foodstuffs, traditional medicines, and various other sources. Pb metal pollution in waste produced by households is pollution from products or materials used in households containing Pb metal. Cosmetics or beauty products are widely used in households. Cosmetics contain heavy metals such as lead (Pb), which is used as stabilizers and texturizers. Lead metal (Pb) in cosmetics is often found in lipstick, eye shadow, and eyeliner. The highest levels of lead (Pb) are found in easy red lipstick [19], [22], [27], [28].

Other types of food that contain high levels of Pb contaminants are vegetables. The average vegetable contains 28.78 ppm, far above the safe limit permitted by the Directorate General of Medicine and Food, equal to 2 ppm. Heavy metals in food do not occur naturally, but can also be the result of migration from the packaging material. Packaging using old newspapers is undoubtedly not appropriate because it allows the migration of heavy metals (especially Pb) from ink in the newspaper to food [21], [22], [29].

Metal in the industry is used to make household appliances such as spoons, forks, knives, and various other household appliances. Lead (Pb) is also used as a constituent of solder and as a formulation of pipe joints, which results in water for households having many possibilities of contact with Pb [21] ,[24]. It makes the waste generated by households to be contaminated by metals. The presence of heavy metal that lines the water pipe allows contact between household water and heavy metals. The United States found lead levels in drinking water to reach 50 µg / l caused by tendons and lead pipes coated with lead [3].

Bacteria possess the ability of resistance to metals, including mercury. Some of the mechanisms of bacterial resistance are a) Reduction of Hg^{2+} to Hg enzymatically or volatilization. This mechanism occurs in the cytoplasm [3]. Enzymatic reactions involve the merA gene that codes for mercury reductase and requires $NADH_2$ as an electron donor. Furthermore, volatile Hg is released from bacterial cells into the environment, b). Formation of mercury sulfide (Hg-S). The mechanism of Hg-S formation is carried out by anaerobic strains of *Clostridium cochlearium* species and Klebsiella aerogenes NCTC418 aerobically, c). It reduced membrane permeability to mercury $HgCl_2$. The decrease in membrane permeability is caused by the expression of two plasmids that encode the protein. These proteins can reduce the cytoplasmic membrane permeability to mercury and d) The mechanism of exopolysaccharide bioabsorption (EPS) on the outer walls of bacterial cells. EPS on the bacterial outer wall has two forms: CPS (capsular polysaccharides), a polymer on the surface of cells with covalent bonds. The second form, polysaccharide, is a polymer lender on the cell surface in the form of a matrix that is easily separated [18].

Mercury contamination in water can affect water quality and biological components, including bacteria. Some bacteria are resistant to mercury, both gram-negative and gram-positive bacteria, members of the mesophyll, halophil, and extremophile groups [14]. Mercury resistant bacteria is an example of physiological and genetic adaptation to mercury-contaminated environments of 1-10 mg / L [9], and some are even mercury resistant at concentrations of 25 mg / L [23].

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The bioreduction mechanism of Hg^{2+} to Hg enzymatically and bioabsorption mechanism in cell walls of mercury-resistant bacteria becomes a potential alternative to exploring and empowering mercury-resistant bacteria as candidates for bioremediation agents for mercury-polluted environments. Therefore, heterotrophic strains of detergent-tolerant bacteria originating from Malang domestic wastewater aside from being amylolytic, proteolytic, lipolytic, biopesticide have proven resistance to heavy metals.

CONCLUSION

The results showed a resistance of a consortium of heterotrophic strains of detergent-tolerant bacteria originating from domestic wastewater to various heavy metals up to a concentration of 40 mg / L for Cu metals and 30 mg / L for Hg metals. The composition of these strains is *Bacillus cereus* strain BQAR-01d, Bacillus thuringiensis strain MSS-2, Bacillus cereus strain JDA-1, and *Bacillus* sp. B31 (2008). The four consortiums of heterotrophic bacterial strains have the potential to be developed into decomposing domestic wastewater containing heavy metals (heavy metal bioremediator or decomposer) to improve environmental health.

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