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Identification and in vitro effectiveness test of four isolates of mercury-resistant bacteria as bioaccumulation agents of mercury

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Abstract

Bioaccumulation of mercury is one of the reclamation methods in ex-gold mining areas. Mercury-resistant bacteria can be used as a mercury bioaccumulation agent. The previous study in Mandor District West Kalimantan has collected four mercury-resistant bacteria from 62 samples with isolates code: HgTA1, HgTL2, HgRL and HgRA. The purpose of this study is to identify and verify the in vitro effectiveness ofthese four mercury-resistant bacteria. Identification of the bacteria is performed using16SrRNAsequencinganalysis, while the in vitro effectiveness test of the bacteria is done by using Canstein's selective media. The results show that the identification of bacterial isolates finds that HgTA1 and HgTL2 are *Bacillus subtilis*, HgRL is *Burkholderia cepacia*, and HgRA is *Burkholderia cenosepacia*. These three species of bacteria belong to the class of bacteria that are resistant to extreme conditions. Thus, these bacteria have anability toaccumulatemercury. Meanwhile, based on the test results of colony growth, detoxification abilities, mercury accumulation and bioaccumulation levels, three out of the four bacterial isolates are proven to be effective and superior to accumulate mercury i.e. HgRA, HgTA1 and HgTL2 isolates. Furthermore, these three isolates can be used as bioacculation agents of mercury-contaminated soil.

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Keywords: mercury resistant bacteria;mercury bioaccumulator.

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1. Introduction

Actually, illegal gold mining in Mandor District West Kalimantan is done by using surface mining systems. This activityhas changed the landscape, removedthe topsoil and destroyed the flora and fauna. The waste materials of ex-gold mining is called tailings. Each different type of mining and mine sites has the different characteristics and composition of tailings. The tailings in an ex-gold mining area in Mandor District are composed by quartz sand. The tailings area needs to be reclaimed.

Revegetation as part of reclamation activities in the tailings areas has limitation, in which these areas not only have limited organic matter and nutrients, and low soil microbial activity, but also contains mercury[1,2]. Mercury bioaccumulation can be one of the recommended options to restore these conditions. Mercury-resistant bacteria have the ability to accumulate mercury and change it into a form that is less or not dangerous[3]. After the tailings areas are clean fromthe mercury pollutants, physical and biological improvements in the tailings areas can be maximized so that revegetation can also be maximal. As the first step, collecting the effective mercury-resistant bacteria to accumulate mercury is very important.

Exploration and invention of mercury-resistant bacteria in the gold mine tailings area in Mandor District have been done, and four isolates of mercury-resistant bacteria have been obtained with isolates code: HgTA1, HgTL2, HgRL and HgRA. However, the identification and effectiveness test both in vitro and in vivo have not been done. The effectiveness of mercury-resistant bacteria in mercury accumulation is very important to know, to ensure the success of its application in the biological reclamation program using bioaccumulation techniques in the tailings area of the ex-gold mining.

The purpose of this study is to identify and verify the in vitro effectiveness of four isolates of mercury-resistant bacteria with isolates code: HgTA1, HgTL2, HgRL and HgRA.

2. Materials and Methods

2.1. BacterialIdentification

The previous study has found four mercury-resistant bacteria (isolates code: HgTA1, HgTL2, HgRL and HgRA) collected from 62 samples in the tailings area of ex-gold mining in Mandor District. Species identification of four mercury-resistant bacteria was performed by analysis of 16S rRNA sequencing and DNA isolation is performed using the alkaline lisys method. DNA amplificationwas performed using Universal primers for bacteria(16SF &1387R). Sequencing was done by ABI-Prism 3100-Avant Genetic Analyzer.

2.2. The Effectiveness Test of Bacteria

The effectiveness test of bacteria with isolates code HgTA1, HgTL2, HgRL and HgRA of the ability of mercury accumulation is done in vitro using Canstein's selective media[4].Each test is done with five replications. Mercury is added to the Canstein's selective media, for both solid and liquid forms with concentrations of 0ppm, 10ppm and 100ppm. The effectiveness is measured through several tests.

2.2.1. The Growth of Bacterial Colonies Test

1ml of liquid inoculums of mercury-resistant bacteria is inoculated in Petri dishes containing Canstein's selective media in various concentrations of HgCl2 (0ppm, 1ppm, 10ppm and 100ppm) while it is still warm (before it solidifies)[5]. Furthermore, bacterial cultures are incubated at room temperature. Observation on the growth of bacterial colonies is done daily for 14 days using a colony counter.