ISOLATION AND SCREENING OF CADMIUM-RESISTANT BACTERIA FROM CADMIUM CONTAMINATED SOIL

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Cadmium (Cd) contamination is an important environmental pollution problem due to its toxic effect. Cd contamination in soil causes adverse effects on many living organisms including soil microorganisms. The quantity and activities of soil microbes may be changed. Soil samples were collected from 5 sampling sites at a zinc mine located near Maetoa creek in the Maesod district, Tak province, Thailand. These soil samples were analyzed Cd concentration by atomic adsorption spectrometry. Cd concentration in soil samples ranged from 36.2 to 204.1 mg kg⁻¹ of soil. Soil sample from site 1 contained the highest Cd concentration. For isolation Cd-resistant bacteria, soil suspensions were plated on 0.1-strength TSA amended with 4 mM CdCl₂. High numbers of Cd-resistant bacteria (3.5x10⁴ CFU g⁻¹ of dry soil) were found in the soil sample collected from sampling site 1. Six isolates of Cd-resistant bacteria from culturable bacteria on CdCl₂-amended 0.1-strength TSA plates were chosen based on their colony morphology and their potential to grow on TSA plate supplemented with 4 mM CdCl₂. Six strains of isolated bacteria were screened high potential of Cd resistance levels by the growth inhibition zone assay. The results showed that inhibition zone against 3M CdCl₂ of isolate TAK1 isolated from soil sample at sampling site 1 was 14.17±1.28 mm. It was highly resistant to cadmium toxicity as compared to other isolates. Ribosomal 16S DNA sequencing identified the TAK1 isolate as Ralstonia sp. It could be concluded that Ralstonia sp. TAK1 isolated from Cd-contaminated soil showed the potential to resist against Cd toxicity. It had the ability to survive in an environment where the level of Cd is high. These finding might lead to the use of Ralstonia sp. TAK1 for microbial based remediation in Cd-contaminated soil.

Key words: Cadmium; Cadmium-resistant bacteria; Ralstonia sp.

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Introduction: The contamination of cadmium (Cd), an important heavy metal, in the environmental media results from both industrial and agricultural activities. Discharge of Cd into the environment causes direct and indirect effects on human health and other living organisms. Cd is well-known for being highly toxic to soil microorganisms even at very low concentrations (Nies, 1999). The exposure of microorganisms to excessive concentrations of Cd adversely affects their growth, morphology and biochemical activity (Roane and Kellogg, 1996). In heavy metal contaminated sites, soil bacteria are usually exposed to heavy metals resulting in the establishment of heavy metal-resistant bacterial populations (Piotrowska-Segat et al., 2005). Thus, this study focused on Cd concentration in soil and the quantity of Cd-resistant bacteria in Cd-contaminated soil at a zinc mine and in the vicinity of a paddy field. In addition, Cd-resistant bacteria were isolated from contaminated soil and screened for high potential of Cd-resistant bacteria.

Methods: Five composite soil samples were collected from the top 10 cm of soil surface horizon at zinc mine and in the vicinity paddy field located near Maetao Creek in Madsod district, Tak province, Thailand. Cd concentrations in soil samples were analyzed by atomic adsorption spectrometry. Appropriated dilutions of soil suspensions were plated on on 0.1-strength tryptic soy agar (TSA) amended with 4mM CdCl₂ for enumeration and isolation of Cd-resistant bacteria. Cd resistance levels in each isolated bacterium were determines by growth inhibition zone assay. The high potential Cd-resistant bacterium was selected for identification by 16S rDNA sequencing.
Results and Discussion: Quantity of Cd-resistant bacteria isolated from Cd-contaminated soils

Cd concentrations in five soil samples ranged from 36.2 to 204.1 mg.kg$^{-1}$ of soil (Fig.1). The highest Cd concentration in the soil sample was found at sampling site 1 and its concentration was exceed the permissible limits of soil quality standard for agricultural use in Thailand (Std. 37 mg.kg$^{-1}$ of soil) (National Environment Committee, 2005). High numbers of Cd-resistant bacteria were found in the soil sample collected from sampling site 1 (Fig.1). Culturable numbers of Cd-resistant bacteria in Cd contaminated soil were higher than that in uncontaminated soil (Roane and Pepper, 2000). The development of metal resistant populations and metal resistance levels are directly proportional to the concentrations of metal exposure (Roane and Kellogg, 1996). In addition, six isolates of Cd-resistant bacteria were chosen for further study based on their colony morphology and their potential to grow on TSA plate supplemented with 4 mM CdCl$_2$.

Screening and identification of potential Cd-resistant soil bacteria

The resistance levels against Cd toxicity were determined in the exponential-phase cultures of six bacterial isolates using a growth inhibition zone assay. Bacterial TAK1 isolated from soil sample at sampling site 1 was highly resistant to 3M CdCl$_2$ toxicity as compared to other isolates (Fig.2). This result showed that bacteria isolated from highly Cd-contaminated site exhibited higher resistance levels than bacteria isolated from uncontaminated soil. In addition, several investigators have reported that bacterial communities in highly metal-contaminated soil are more resistant than uncontaminated soil communities (Roane and Pepper, 2000; Piotrowska-Seget et al., 2005).

From morphological study, isolate TAK1 is Gram-negative and short-rod shape. Using 16S rDNA sequencing, the Cd-resistant bacterium, TAK1, showed 96% similarity to Ralstonia sp. from GenBank database accession number AB167178. Thus, isolate TAK1 could be identified as Ralstonia sp. Resistance to Cd is more prevalent in Gram-negative bacteria than Gram-positive bacteria (Piotrowska-Seget et al., 2005). The resistant mechanisms to Cd toxicity of Ralstonia sp. TAK1 are being investigated.

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References: