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A novel approach for rapidly and cost-effectively assessing toxicity of toxic metals in acidic water using an acidophilic iron-oxidizing biosensor

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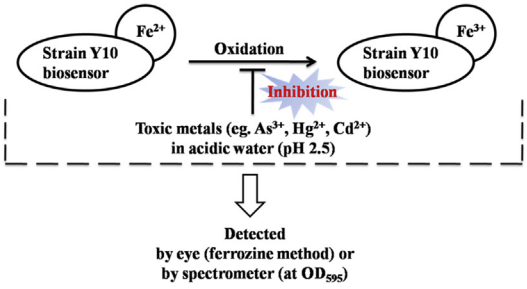
HIGHLIGHTS

- A novel approach for rapidly and cost-effectively detecting toxicity of toxic metals in acidic water was developed.
- An acidophilic iron-oxidizing bacterium Strain Y10 was isolated and characterized.
- The acidophilic IOB biosensor was used to detect toxicity of toxic metals in acidic water at pH 2.5.
- The colorimetric acidophilic IOB biosensor was developed.

GRAPHICAL ABSTRACT



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ABSTRACT

Contamination by heavy metals and metalloids is a serious environmental and health concern. Acidic wastewaters are often associated with toxic metals which may enter and spread into agricultural soils. Several biological assays have been developed to detect toxic metals; however, most of them can only detect toxic metals in a neutral pH, not in an acidic environment. In this study, an acidophilic iron-oxidizing bacterium (IOB) Strain Y10 was isolated, characterized, and used to detect toxic metals toxicity in acidic water at pH 2.5. The colorimetric acidophilic IOB biosensor was based on the inhibition of the iron oxidizing ability of Strain Y10, an acidophilic iron-oxidizing bacterium, by metals toxicity. Our results showed that Strain Y10 is acidophilic iron-oxidizing bacterium. *Thiobacillus caldus* medium (TCM) (pH 2.5) supplied with both $S_4O_6^{2-}$ and glucose was the optimum growth medium for Strain Y10. The optimum temperature and pH for the growth of Strain Y10 was 45 °C and pH 2.5, respectively. Our study demonstrates that the color-based acidophilic IOB biosensor can be semi-quantitatively observed by eye or quantitatively measured by spectrometer to detect toxicity from multiple toxic metals at pH 2.5 within 45 min. Our study shows that monitoring toxic metals in acidic water is possible by using the acidophilic IOB biosensor. Our study thus provides a novel approach for rapid and cost-effective detection of toxic metals in acidic conditions that can otherwise compromise current methods of chemical analysis. This method also allows for increased efficiency when screening large numbers of environmental samples.

1. Introduction

Contamination by toxic elements such as heavy metals and metalloids is a serious environmental and health concern. Toxic

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metals such as arsenic, cadmium, lead, and mercury can become highly accumulated in living organisms and threaten human health and the environment in many countries (Jarup, 2003; Sharma et al., 2008).

Toxic metals may enter and spread into agricultural soils through the processes of discharging waste and effluent directly or indirectly to soil, irrigation with contaminated water, application of inorganic fertilizer or pesticide containing such elements, and rock weathering (GimenoGarcia et al., 1996; Kashem and Singh, 1999; Mandal and Suzuki, 2002; Sharma et al., 2007). Toxic metals might subsequently transfer from agricultural soils into the human diet by plant uptake or soil ingestion by grazing livestock, which may increase the risk of human exposure and cause a variety of human diseases (Nicholson et al., 2003; Thornton and Abrahams, 1983). Therefore, a method for the efficient analysis of toxic metals in the environment is needed.

Chemical analyses such as atomic absorption spectroscopy, inductively coupled plasma-mass spectrometry or inductively coupled plasma-atomic emission spectrometry have been commonly used to determine the presence of toxic metals in water. Although these analytical methods are quantitative and sensitive, they are also time consuming and laborious, making it difficult to screen large numbers of environmental samples. In addition, chemical analysis alone for detecting toxic metals does not provide enough information to assess the environmental risk as it does not provide information about the mobility, bioavailability, and toxicity of the toxic metals present (González et al., 2011; Shetty et al., 2003; Woutersen et al., 2011). Hence, there is a growing need to develop an environmental monitoring method which can quickly and inexpensively detect toxic metals as well as provide biological and toxicological information in large numbers of environmental samples.

In recent years, several biological methods have been developed to detect metals' toxicity by observing the inhibition of biological or enzymatic activity (Durrieu and Tran-Minh, 2002; Hassan et al., 2010; Ishaque et al., 2006). Bacterial biosensors, which combine the promoter/operator elements of a stress response gene with different reporter genes, have also been established in several studies (Huang et al., 2015a, 2015b; Lee and Gu, 2003; Li et al., 2008; Liao and Ou, 2005,; Liao et al., 2006; Min et al., 1999). Recently, a sulfur-oxidizing bacterial (SOB) biosensor has been used for detecting toxic metals and other toxic chemicals in water (Hassan et al., 2012; Van Ginkel et al., 2010; Van Ginkel et al., 2011). However, these bacterial biosensors have some drawbacks or limitations. Few bacterial biosensors were constructed based on a color-based signal, which offers a simply colorimetric method for the detection of metals by the naked eye (Huang et al., 2015a). In addition, many toxic metals are present in extremely acidic industrial effluents (Sheoran et al., 2012) and this limits the application of most biological assays, which can only be used to detect toxic metals at near-neutral pH values. Furthermore, concerns have been raised about genetically modified organism-based biosensors that may have detrimental effects if released into the environment (Snow et al., 2005).

In this study we have developed a rapid and cost-effective color-based approach to detecting toxic metals and assessing their toxicity in acidic water by using an acidophilic iron-oxidizing biosensor. The acidophilic iron-oxidizing bacterium (IOB) Y10 was isolated and characterized from a geothermal soil. The acidophilic iron-oxidizing biosensor was used to detect the toxic metals arsenic, cadmium, mercury, and lead in acidic water (pH 2.5), based on the inhibition of the toxic metals on the iron-oxidizing ability of this bacterium.

2. Materials and methods

2.1. Isolation and cultivation of acidophilic bacteria from geothermal soil

A geothermal soil sample was taken from Yangminshan National Park, Taiwan. The region is known for its highly acidic soil due to the effects of chemical weathering from volcanic rocks and precipitation. The soil sample was enriched with liquid *Thiobacillus caldus* medium (Hallberg and Lindstrom, 1994) (TCM) (pH 2.5) (Supporting Information, STable 1) at 37 °C. After two weeks, the culture was carried with serial dilutions and spread onto solid TCM medium plates. The plates were incubated under the same condition until colonies were formed. Each single colony was carefully picked up and spread onto solid TCM medium plates at least five times.

2.2. Identification of acidophilic bacteria

Bacteria were identified based on the 16S rDNA method. A single colony was used as the DNA template for PCR. The universal primers VLIAO-16S-F:5′-GGAGCAAACAGGATTAGATACC-3′(forward) and VLIAO-16S-R:5′-TGCCAACTCTATGGTGTGTGACG-3′(reverse) were used for amplifying the 16S rDNA. The 25 μL reaction mixture contained the single bacterial colony, 2.5 μL reaction buffer (1×), 2.5 μL dNTPs (200 μM), 1 μL of each primer (0.5 μM), 0.25 μL Taq DNA polymerase (2.5 U), and 17.75 μL ddH_2O. The PCR was conducted with the initial denaturation at 94 °C for 5 min, 30 cycles of 94 °C for 0.5 min, 58 °C for 0.5 min, 72 °C for 0.5 min, and the final elongation of 72 °C for 7 min. The 16S rDNA in PCR product was purified using a BioMan kit (Taipei, Taiwan) and sequenced. The 16S rDNA sequence was compared with the Genbank database using the BLAST program. Phylogenetic analysis was conducted using the MEGA 6.06 program. Sequences obtained were deposited in the GenBank database with the accession number: KU183497.1 for bacterial Strain Y10.

2.3. Characterization of bacterial strain Y10

The growth curve of the bacterial Strain Y10 was evaluated by inoculating the bacterium into liquid TCM medium at pH 2.5 (Supporting Information, STable 1). The culture was incubated at 37 °C, 225 rpm. The optical density at 600 nm (OD_{600}) was measured at different time points over one week's incubation (168 h).

To determine the optimal pH and temperature for the Strain Y10, the bacterium was inoculated in TCM liquid medium. For pH assays, the incubated condition was carried out at 37 °C with different initial pH values of 1.5, 2, 2.5, 3, or 3.5. For temperature assays, the incubated condition was carried out at pH 2.5 at different temperatures of 30, 37, 45, or 50 °C. OD_{600} was measured at each sampling time point and generation time was calculated as described in Hallberg and Lindstrom (1994).

Different carbon and sulfur sources were used to determine whether the bacteria could grow under different nutrient conditions. Liquid TCM without solution C and D (pH 2.5) (Supporting Information, STable 1) was used as the growth medium. The carbon sources, including glucose, glycerol, citric acid, glutamic acid, and yeast extract; and sulfur sources, such as sulfur, thiosulfate, and tetrathionate were added both together or individually into the medium. The culture was incubated at 37 °C, 225 rpm. After several days' incubation, OD_{600} was measured to determine the bacterial growth.