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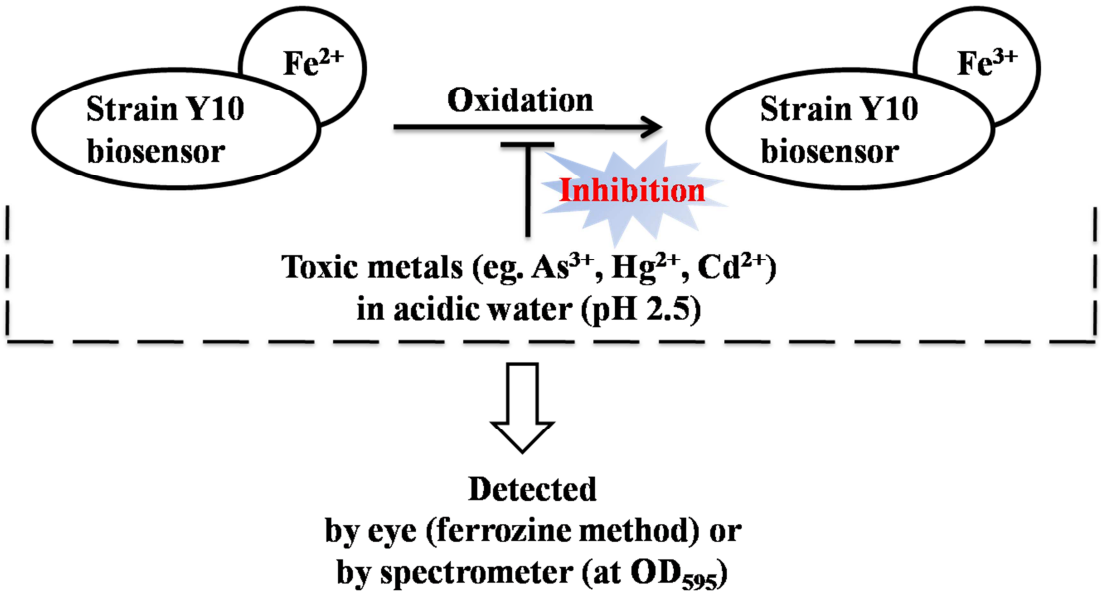
Graphic Abstract



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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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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.

Keywords: acidic water; toxic metals; iron-oxidizing bacteria; biosensor

1. Introduction

Contamination by toxic elements such as heavy metals and metalloids is a serious environmental and health concern. Toxic 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; Huang et al., 2015b; Lee and Gu, 2003; Li et al., 2008; Liao et al., 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

97 medium plates at least five times.

98

99 *2.2. Identification of acidophilic bacteria*

100 Bacteria were identified based on the 16S rDNA method. A single colony was used as
101 the DNA template for PCR. The universal primers
102 VLIAO-16S-F:5'-GGAGCAAACAGGATTAGATACC-3'(forward) and
103 VLIAO-16S-R:5'-TGCCAACTCTATGGTGTGTGACG-3'(reverse) were used for
104 amplifying the 16S rDNA. The 25 μL reaction mixture contained the single bacterial colony,
105 2.5 μL reaction buffer (1X), 2.5 μL dNTPs (200 μM), 1 μL of each primer (0.5 μM), 0.25 μL
106 Taq DNA polymerase (2.5 U), and 17.75 μL ddH_2O. The PCR was conducted with the initial
107 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
108 0.5 min, and the final elongation of 72 °C for 7 min. The 16S rDNA in PCR product was
109 purified using a BioMan kit (Taipei, Taiwan) and sequenced. The 16S rDNA sequence was
110 compared with the Genbank database using the BLAST program. Phylogenetic analysis was
111 conducted using the MEGA 6.06 program. Sequences obtained were deposited in the
112 GenBank database with the accession number: KU183497 for bacterial Strain Y10.

113

114 *2.3. Characterization of bacterial Strain Y10*

115 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 ^{o}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.

For ferrous oxidation assay, ferrozine assay is a widely used method for detection of iron (Stookey, 1970). This chemical forms a purple precipitate by binding the ferrous ion, but not the ferric ion (Stookey, 1970). The bacterial culture (OD_{600} = 0.3) was harvested and

centrifuged at 5,000 rpm. The cell pellet was resuspended in Solution A of TCM (Supporting Information, STable 1). 1 mM ferrous iron (Fe^{2+}) was added into the bacterial suspension and incubated at 37 °C. The concentration of Fe^{2+} was determined by the ferrozine assay every 15 min and OD_{595} was measured.

2.4. Detection of toxic metals and assessment of toxicity in acidic water by using the acidophilic IOB biosensor

We further evaluated the potential for this color-based bacterial biosensor assay to detect toxic metals and determine their toxicity in acidic water. This is based on the inhibition of iron oxidizing activity in bacterial Strain Y10 by the metals' toxicity, with the inhibition of iron-oxidizing activity monitored by ferrozine assay. Thus, the toxicity of the metals in acidic water can be semi-quantitatively observed by eye or quantitatively measured by spectrophotometer. Bacterial Strain Y10 (OD_{600} = 0.3) was harvested and centrifuged at 5,000 rpm. The cell pellet was resuspended with solution A of TCM (pH 2.5) (Supporting Information, STable 1). Then, 1 mM Fe^{2+} and different levels (1x, 10x, 100x) of Taiwan EPA effluent standard concentration of toxic metals (As^{3+}, Cd^{2+}, Hg^{2+}, Pb^{2+}) was added individually or as a mixture into the bacterial suspension and incubated at 37 °C. The Taiwan EPA effluent standard concentration is 1x: As^{3+}, 500 μg L^{-1}; Cd^{2+}, 30 μg L^{-1}; Hg^{2+}, 5 μg L^{-1}; Pb^{2+}, 1000 μg L^{-1}. The concentration of Fe^{2+} was determined by the ferrozine assay every 15

154 min, the OD_{595} was measured and a digital camera was used to record the data.

155

156 *2.5. Data analysis*

157 The results were presented as the mean ± standard deviation (SD). Statistically significant

158 differences compared to the control were considered at $p < 0.05$ by the Student *t*-test.

159

160 ## 3. Results and discussion

161 *3.1. Isolation and identification of the acidophilic bacteria*

162 The bacterial strain designated Strain Y10 was isolated from a geothermal soil from the

163 region known for its highly acidic characteristics due to the effects of chemical weathering

164 from volcanic rocks and precipitation. The morphology of the colony grown on TCM plates

165 was observed as round, raised, and having a regular edge under the microscope. The 16S

166 rDNA sequence of bacterial Strain Y10 was deposited to the NCBI GenBank (accession

167 numbers:KU183497). BLASTN analysis of the 16S rDNA sequence of bacterial Strain Y10

168 showed that bacterial Isolated Y10 has 100% sequence similarity to *Acidibacillus*

169 *ferrooxidans* strain Y0010 (AY140235). Phylogenetic analysis based on the 16S rDNA of

170 bacterial Strain Y10 summarized the relationship of the bacterial species among the

171 sulfur-oxidizing or iron-oxidizing acidophile (Fig. 1).

172

3.2. Characterization of bacterial Strain Y10

To characterize bacterial Strain Y10, the growth curve, optimum pH, temperature, carbon, and sulfur utilization, and ferrous ion oxidation test were examined. For the growth curve, after inoculating a single colony into TCM (pH 2.5) and incubated at 37 ^{o}C, bacterial Strain Y10 culture reached the exponential phase at 48 h. After 72 h, the bacterial Strain Y10 culture grew into the stationary phase (Fig. 2).

For temperature characterization, bacterial Strain Y10 was inoculated into TCM liquid medium and the incubated condition was carried out at pH 2.5 with different temperatures at 30, 37, 45, or 50 °C. The results showed that bacterial Strain Y10 had the shortest generation time while growing at 45 °C (Fig. 3A). For pH characterization, the incubated condition was carried out at 37 °C with different initial pH values of 1.5, 2, 2.5, 3, or 3.5. The results showed that bacterial Strain Y10 had the shortest generation time when growing at pH 2.5 (Fig. 3B). This suggests that the optimum temperature and pH for the bacterial Strain Y10 in TCM medium are 45 ^{o}C and pH 2.5, respectively, with a generation time about 3.8 h (Fig. 3A).

Bacterial Strain Y10 only grew in the liquid TCM medium (without solution C and D) (pH 2.5) with both glucose and tetrathionate or glucose and sulfur supplement (Table 1). Bacterial growth did not occur in the medium with only either a carbon source (glucose, glycerol, citric acid, glutamic acid, and yeast extract) or a sulfur source (sulfur, thiosulfate, and tetrathionate) supplement (Table 1).

Since BLASTN analysis of the 16S rDNA sequence of bacterial Strain Y10 showed 100% sequence similarity to *A. ferrooxidans* strain Y0010 (AY140235), we further examined whether bacterial Strain Y10 is able to oxidize iron. The results showed that bacterial Strain Y10 was capable of oxidizing ferrous iron (Fe^{2+}) to ferric iron (Fe^{3+}) (Fig. 4). A bacterial culture of Strain Y10 (OD_{600} = 0.3) was harvested and mixed with 1 mM Fe^{2+}. After adding Fe^{2+} into the bacterial Strain Y10 suspension, the concentration of Fe^{2+} was observed to gradually decline (Fig. 4). Moe than half of the Fe^{2+} was oxidized by the bacterial Strain Y10 at 30 min and Fe^{2+} was completely oxidized to Fe^{3+} at 120 min (Fig. 4).

Our results indicated that the bacterial Strain Y10 is an acidophilic as well as mesothermophilic iron-oxidizing bacterium which can survive in both sulfur and glucose supplied TCM medium (without solution C and D) (pH 2.5). Other *Acidithiobacillus* bacteria, for example, *Acidithiobacillus caldus*, *Acidithiobacillus ferrivorans*, and *Acidithiobacillus thiooxidans* show similar characteristics of the phenotype to bacterial Strain Y10, including optimum temperature, pH, generation time, and function. *A. caldus* has the closest similarity to bacterial Strain Y10 in optimum temperature (45 °C) (Hallberg and Lindstrom, 1994) (Fig. 3A). The optimum temperature for *A. ferrivorans* and *A. ferrooxidans* is lower than that of bacterial Strain Y10 (approximate 30 °C) (Hallberg et al., 2010; Yang et al., 2008). Both of these bacteria are mesophiles, but the major difference between *A. ferrivorans* and *A. ferrooxidans* in temperature is that *A. ferrivorans* is a psychrotolerent mesophile that could

211 grow at a temperature lower than 5 °C (Hallberg et al., 2010).

212 Bacterial Strain Y10 shows an optimum at pH 2.5 for growth (Fig. 3B), which was
213 comparable with that of *A. caldus*, *A. ferrivorans*, and *A. Thiooxidans* (Hallberg et al., 2010;
214 Hallberg and Lindstrom, 1994; Yang et al., 2008). By comparison, under the optimal growth
215 conditions, the growth rate of bacterial Strain Y10 (3.8 h) was similar to that of *A. ferrivorans*
216 (3.2 h); *A. caldus* has a growth rate ~2.8 h and *A. ferrooxidans*has the highest growth rate (6 h)
217 (Hallberg et al., 2010; Hallberg and Lindstrom, 1994; Yang et al., 2008). Several studies have
218 used bacteria to conduct environmental applications, such as bioleaching, biomonitoring, and
219 bioremediation (Pathak et al., 2009; Van Ginkel et al., 2010; Zhou et al., 2007). Most of these
220 bacteria are acidophilic with similar phenotypes and function. However, the phylogenetic
221 analysis showed that bacterial Strain Y10 has only 80 - 82% similarity to *A. ferroxidans* or *A.*
222 *ferrivorans* (Fig.1).

223

224 *3.3. Detection of toxicity due to metals in acidic water by using the acidophilic IOB biosensor*

225 Since bacterial Strain Y10 is capable of completely oxidizing 1 mM Fe^{2+} to Fe^{3+} at 120
226 min at pH 2.5 (Fig. 4), different levels (0x, 1x, 10x, 100x) of Taiwan EPA effluent standard
227 concentration of multiple toxic metals (As^{3+}, Cd^{2+}, Hg^{2+}, Pb^{2+}) were used to evaluate the
228 iron-oxidizing inhibition of bacterial Strain Y10 at pH 2.5. Fig. 5 shows that without adding
229 toxic metals (0x), the initial purple color intensity (at 0 min) which represents 1 mM Fe^{2+}

gradually declined along with the decrease of Fe^{2+} concentration as time increased, in which the difference of purple color intensity could be readily distinguished by eye after 45-min incubation, indicating that Fe^{2+} was gradually being oxidized to Fe^{3+}. In contrast, it was observed that the Strain Y10 bacterial suspension containing multiple toxic metals (1x, 10x, 100x) showed deeper purple color than that of the control (0x) after 45-min incubation indicating lower rates of Fe^{2+} oxidation in the presence of toxic metals (Fig. 5). In addition, the purple color intensity shows a dose dependence manner in the presence of toxic metals (Fig. 5). The results suggest that the toxic metals might interfere the iron-oxidizing ability of the bacterial Strain Y10 in acidic water in a dose-dependent fashion (Fig. 5).

We further quantitatively evaluated the inhibition of the iron-oxidizing activity of the bacterial Strain Y10 by toxic metals in acidic water. In Fig. 5, the difference of purple color intensity caused by different levels of the toxic metals mixture could be readily distinguished by eye after 45 min incubation. Thus, the concentration of Fe^{2+} was determined by the ferrozine assay at 45 min and OD_{595} was measured. Fig. 6 showed that Cd^{2+} and Pb^{2+} even with 1x standard effluent concentration (Cd^{2+}, 30 μg L^{-1}; Pb^{2+}, 1000 μg L^{-1}) had more significantly inhibitory effects on the Fe^{2+} oxidation ability of bacterial Strain Y10. In contrast, As^{3+} and Hg^{2+} significantly inhibited Fe^{2+}oxidation of bacterial Strain Y10 only at 100x standard effluent concentration (Fig. 6). In the multiple toxic metals acidic water test, over 10x concentration of multiple toxic metals caused significantly inhibitory effect on

249 Fe^{2+}oxidation of bacterial Strain Y10 (Fig. 6).

250 Several toxicity biosensors have been previously developed to detect toxic metals by
251 inhibiting biological activity. Ishaque et al. (2006) reported that toxic metals can be detected
252 by the Microtox assay. Using the Microtox assay, the EC_{50} (concentration affecting 50%
253 reduction of bioluminescence) was As^{3+}: 768 μg L^{-1}, Cd^{2+}: 508 μg L^{-1}, Hg^{2+}: 109 μg L^{-1}, Pb^{2+}:
254 455 μg L^{-1}, respectively (Ishaque et al., 2006). In the multiple toxic metals environment, the
255 EC_{50} was reduced to As^{3+}: 49 μg L^{-1}, Cd^{2+}: 25 μg L^{-1}, Hg^{2+}: 10 μg L^{-1}, Pb^{2+}: 455 μg L^{-1}
256 suggesting that the toxicity of multiple toxic metals is greater than a single toxic metal
257 (Ishaque et al., 2006). Although the methods and concentration of toxic metals are different,
258 Fig. 6 shows that Pb^{2+} and Cd^{2+} inhibited iron oxidation more significantly than the multiple
259 toxic metals, which is slightly different to the findings of Ishaque et al., 2006. A
260 sulfur-oxidizing bacterial (SOB) biosensor has been used to detect toxic metals based on the
261 inhibition of SOB by toxic metals or other toxic chemicals in water (Hassan et al., 2012; Van
262 Ginkel et al., 2010; Van Ginkel et al., 2011), however, the studies did not use single isolated
263 sulfur-oxidizing bacteria but used a mixed culture of bacterial species, without
264 characterization of the bacteria. This might limit future application using the SOB.
265 Particularly, many toxic metals are hosted by extremely acidic industrial effluents (Sheoran et
266 al., 2012). Thus, most biosensors that can only be used to detect toxic metals at neutral pH are
267 not applicable for detecting toxic metals in extremely acidic water.

4. Conclusions

In this study, an iron-oxidizing acidophilic bacteria, Strain Y10, has been isolated and characterized. Based on the inhibition of iron-oxidizing activity, this color-based biosensor assay using IOB strain Y10 can be semi-quantitatively observed by eye or quantitatively measured by spectrometer to detect the toxicity of multiple toxic metals at pH 2.5 in 45 min. We have shown that toxicity due to metals in acidic water can be monitored using the iron-oxidizing acidophilic bacterial Strain Y10. Our study thus provides a novel approach for rapidly and cost-effectively detecting metal-based toxicity in acidic water that can not only superseded current chemical analysis methods by virtue of its greater specificity, but also increase the efficiency of screening large numbers of environmental samples.

Conflict of interest

The authors declare that no competing interests exist.

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376 **Table 1**

377 Carbon and sulfur sources utilization by bacterial Strain Y10.

Carbon source [a]	**Growth** [c]	**Sulfur source** [b]	**Growth** [c]
Glucose	-	S^0	-
Glucose + S_4O_6	+++	S^0 + glucose	++
Citric acid	-	S_2O_3	-
Citric acid + S_4O_6	-	S_2O_3 + glucose	-
Glycerol	-	S_4O_6	-
Glycerol + S_4O_6	-	S_4O_6 + glucose	+++
Glutamic acid	-		
Glutamic acid + S_4O_6	-		
Yeast extract	-		
Yeast extract + S_4O_6	-		

378 [a] Carbon source concentration: 2.5 mM; yeast extract concentration: 0.01%.

379 [b] Sulfur source concentration: 2.5 mM; S^0 concentration: 10 μg L^{-1}.

380 [c] OD_{600} < 0.05, no growth was observed; OD_{600} = 0.05 - 0.09 (+); OD_{600} = 0.1 - 0.3 (++);

381 OD_{600} > 0.3 (+++).

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Figure Legends

Fig. 1. Phylogenetic analysis of Strain Y10 based on its 16S rDNA gene sequence.

The neighbor-joining tree showing the phylogenetic relationships between the bacterial Strain Y10 (*) and other acidophilic species from GenBank.

Fig. 2. Growth curve of bacterial Strain Y10.

Bacterial Strain Y10 was inoculated into TCM medium (pH 2.5) and incubated at 37 °C. OD_{600} was determined after a period of incubation time. Data are presented as the mean ± standard deviation. Error bars represent the standard error of three independent experiments.

Fig. 3. Effects of (A) pH and (B) temperature on the growth rate of bacterial Strain Y10.

The bacterial Strain Y10 was inoculated in TCM liquid medium. (A). For pH assays, the incubated condition was carried out at 37 °C with different initial pH at 1.5, 2, 2.5, 3, or 3.5. (B). For temperature assays, the incubated condition was carried out at pH 2.5 with different temperature at 30, 37, 45, or 50 °C. The OD_{600} was measured at each period of time and the generation time was calculated. Data are presented as the mean value of two independent experiments.

Fig. 4. Ferrous oxidation test for bacterial StrainY10.

A bacterial culture of Strain Y10 (OD_{600} = 0.3) was harvested, the cell pellet was re-suspended by the solution A of TCM (pH 2.5) and Fe^{2+} (1 mM) was added. The concentration of Fe^{2+} was determined by the ferrozine assay at different time points. Data are presented as the mean ± standard deviation. Error bars represent the standard error of three independent experiments.

Fig. 5. Color-based acidophilic IOB biosensor by the inhibition of ferrous oxidation of bacterial Strain Y10.

A bacterial culture of Strain Y10 (OD_{600} = 0.3) was harvested and the cell pellet was re-suspended by the solution A of TCM (pH 2.5). To this, Fe^{2+}(1 mM) and different levels (0x, 1x, 10x, 100x) of Taiwan EPA effluent standard concentration of multiple toxic metals (As^{3+}, Cd^{2+}, Hg^{2+}, Pb^{2+}) was added into the bacterial suspension and this was incubated at 37 °C. Taiwan EPA effluent standard 1x: As^{3+}, 500 μg L^{-1}; Cd^{2+}, 30 μg L^{-1}; Hg^{2+}, 5 μg L^{-1}; Pb^{2+}, 1000 μg L^{-1}. The ferrozine assay was used to determine the Fe^{2+} concentration every 15 min and the digital camera was used to record the data.

Fig. 6. Quantitative detection of the toxicity of toxic metals by the inhibition of ferrous oxidation of acidophilic IOB biosensor.

Bacterial culture of Strain Y10 (OD_{600} = 0.3) was harvested and cell pellet was

re-suspended by the solution A of TCM (pH 2.5). Fe^{2+} (1 mM) and different levels (0x, 1x, 10x, 100x) of Taiwan EPA effluent standard concentration of toxic metals (As^{3+}, Cd^{2+}, Hg^{2+}, Pb^{2+}) was added individually or mixture into the bacterial suspension and incubated at 37 °C. The concentration of Fe^{2+} was determined by the ferrozine assay at 45 min and OD_{595} was measured. Data are presented as the mean ± standard deviation. Error bars represent the standard error of three independent experiments. Statistically significant differences compared to the control (0x) were considered at $p < 0.05$ by the Student *t*-test. $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), ns, not significant.

433 **Fig. 1**

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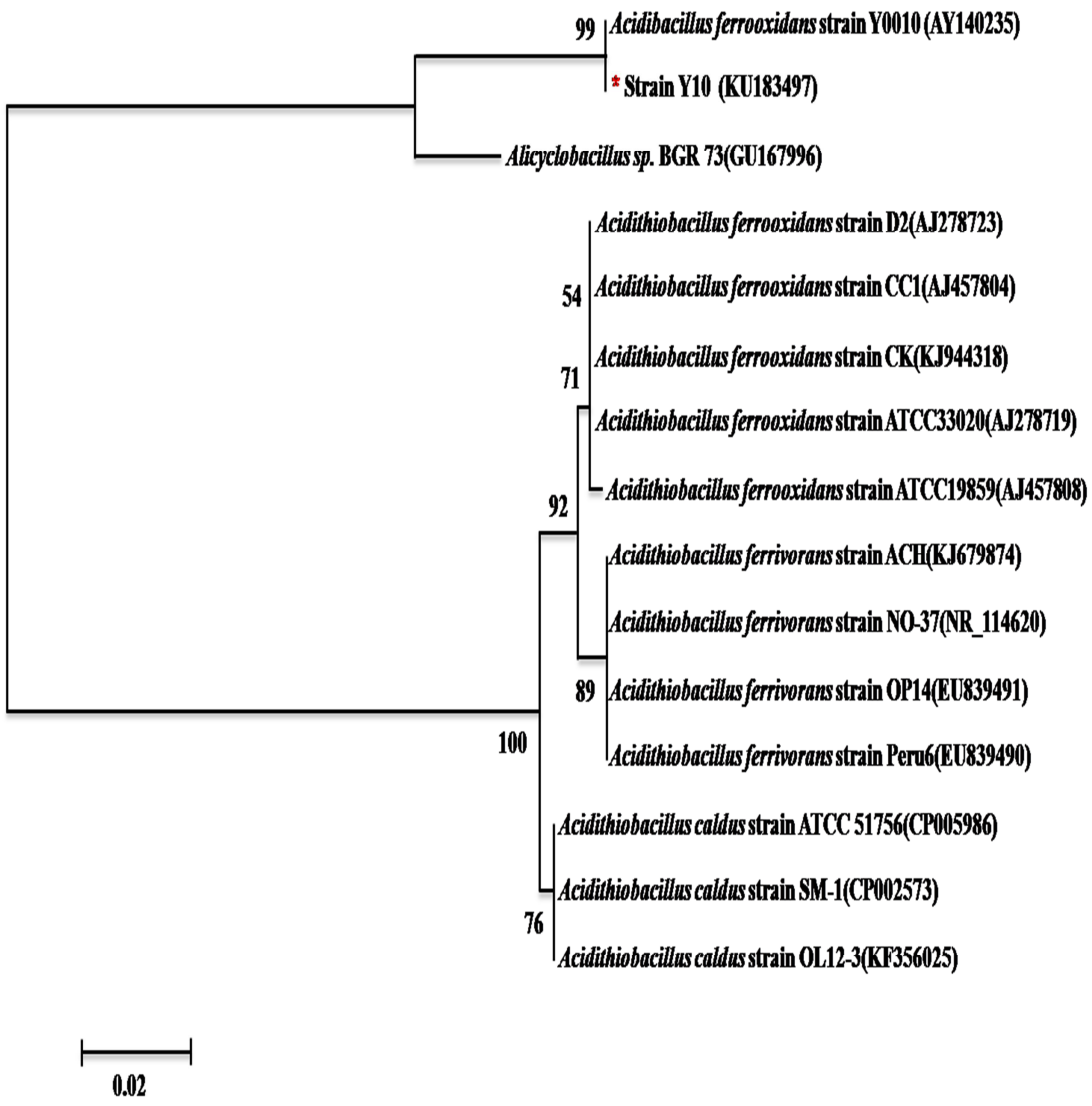

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441 **Fig. 2**

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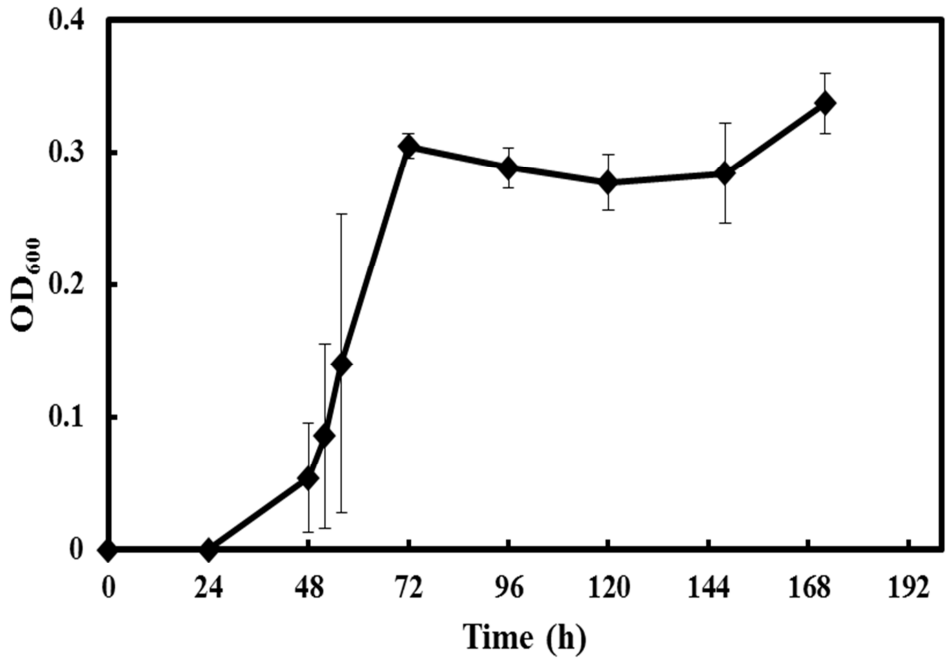

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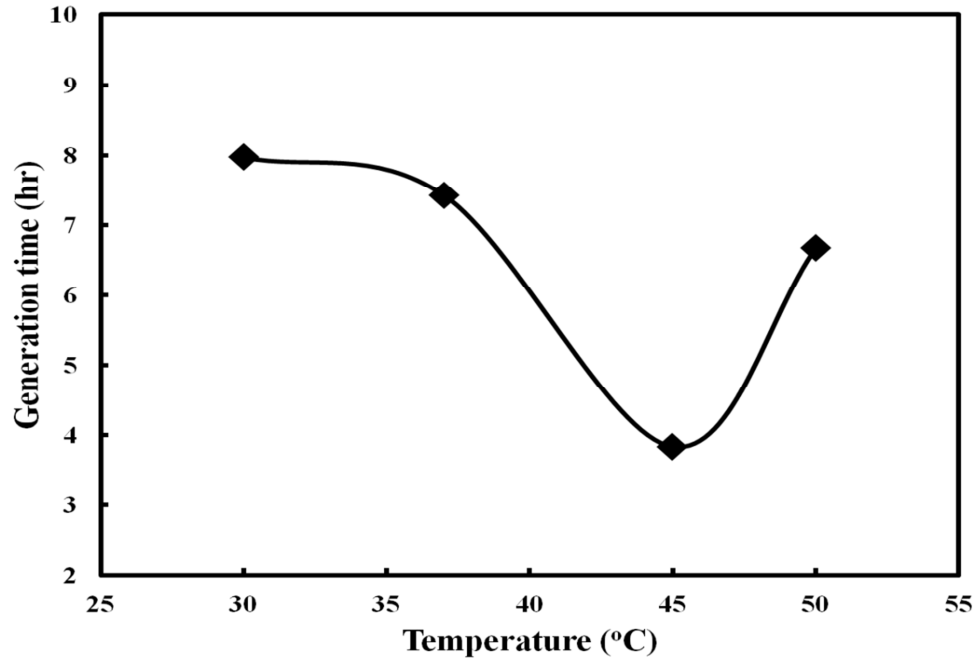
445 **Fig. 3**

446 **(A)**



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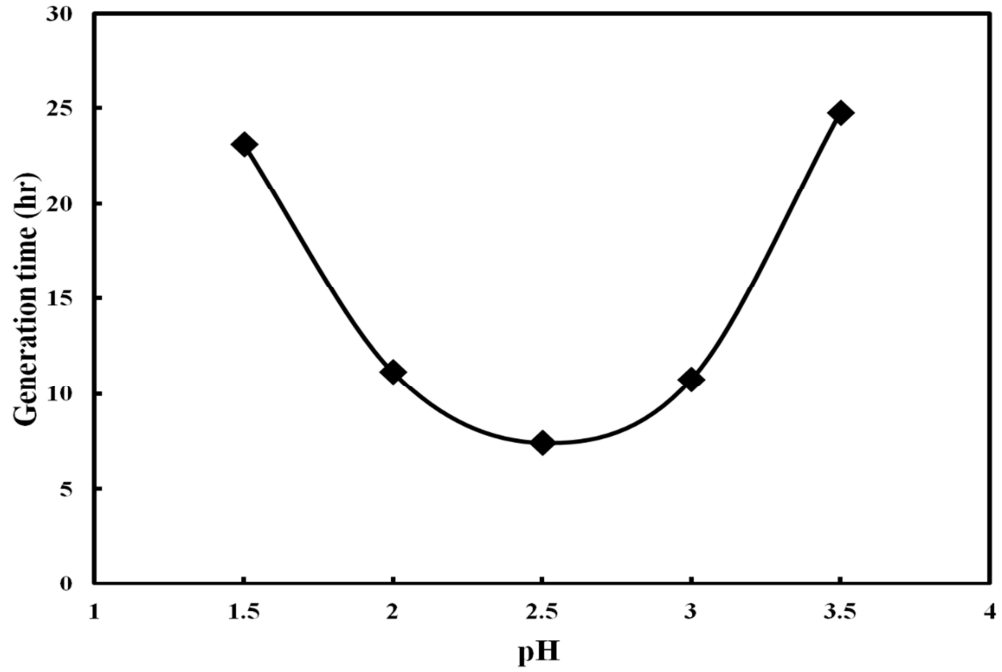
448 **(B)**



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451 **Fig. 4**

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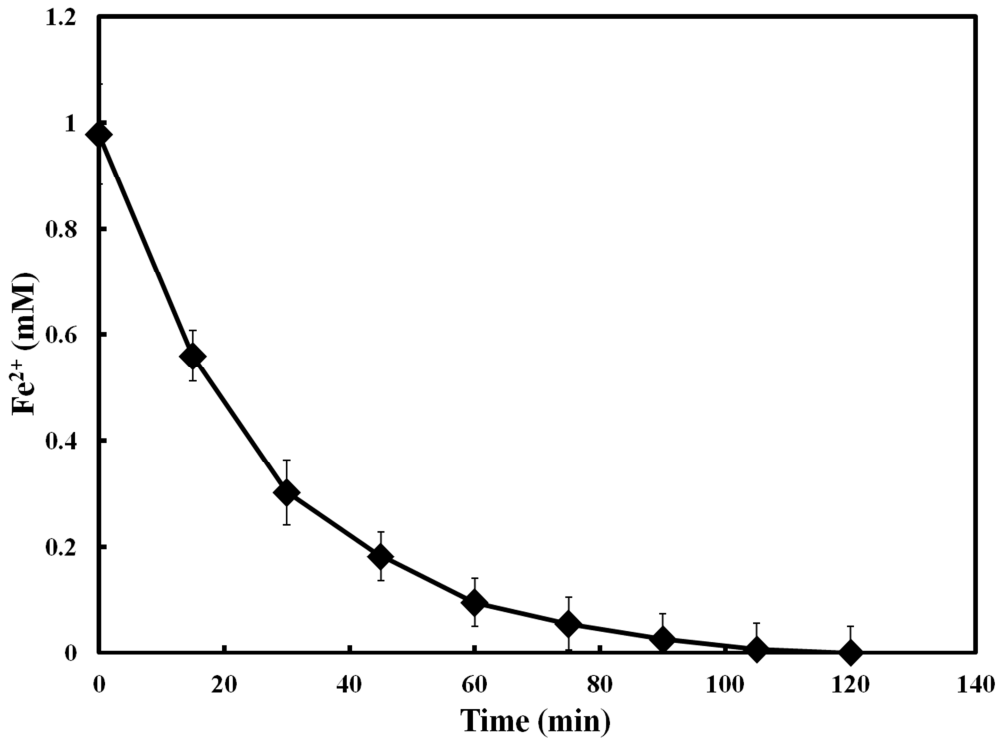

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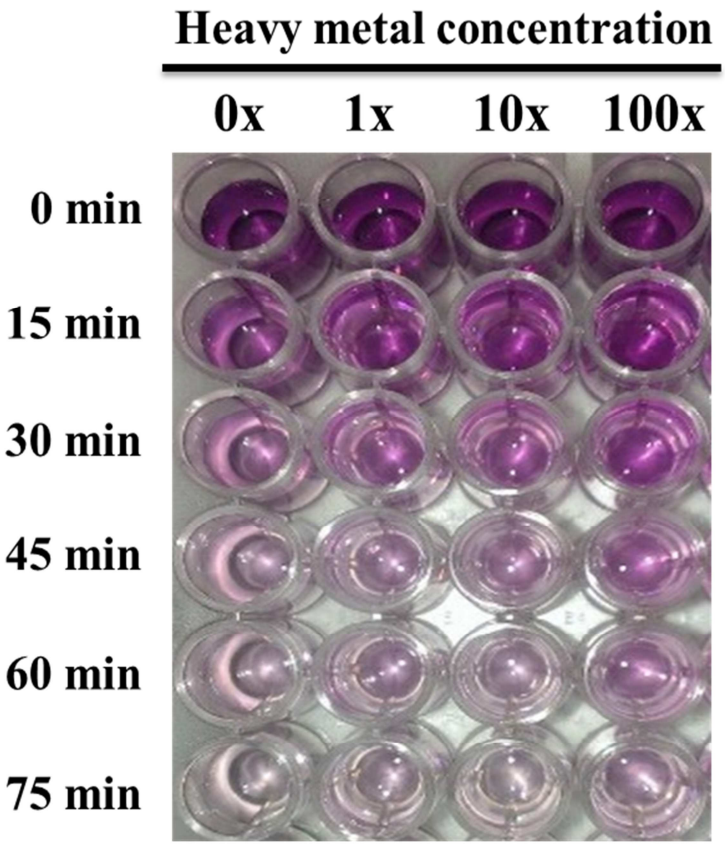
455 **Fig. 5**



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458 **Fig. 6**

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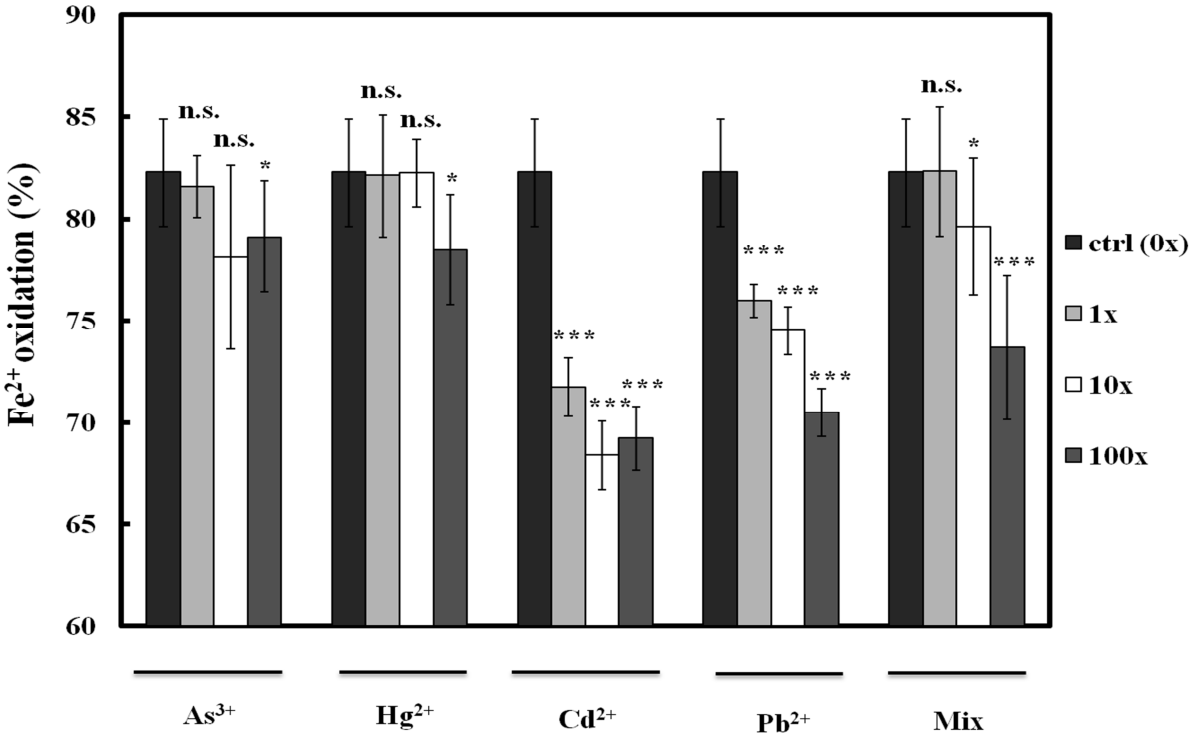

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Highlights

- 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.