ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Bacteria as Biosensor and Mercury Indicator for Control of Environmental Contaminants.

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ABSTRACT

Progresses increase in physical and chemical techniques for faster assessment of chemical toxin and pathogenic bacteria in the environment, there is an immediate demand for a bioassay that can report both the presence of an analyte and its biological properties. Biosensors are used for the detection of contaminants in the environment and can complement analytical methods by distinctive bioavailable from inert in addition to unavailable forms of contaminants. The indicator bacterium may be used when chemical toxins and pathogenic bacteria are present, as it has more specificity, harmless to humans and its level in water should have some direct relationship with pollution. In this study, a whole-cell bacterial biosensor has been deliberate to detect the presence of mercury in batch simulated water sample. Our results demonstrated that the viable bacterial biosensors developed could be valuable in mercury detection in the contaminated water samples at concentrations ranged from 10^{-4} to 10^{-8} M.

Keywords: Biosensors bacteria, Bio-indicator, Transducers, Environment, Water contamination

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ISSN: 0975-8585

INTRODUCTION

Environment is not only place for human but also landfill for their discarded materials which produced from their actions. Environmental contamination is becoming a foremost problem; designing a proper remediation tool requires an understanding about the pollutant sources and the available means of its detection [1-2].

Detection of heavy metals and toxicity within the context of environmental pollution monitoring is of a great importance. It refers to the manufacture and the proof of concept of a multi-channel bioluminescent bacterial biosensor in immobilized phase: Lumisens3. Is designed for the non-stop analysis of water pollution, enables the insertion of any bioluminescent strains (inducible or constitutive), immobilized in a multi-well removable card. The technical design of Lumisens3 has benefited from both a classical and a strong approach [3] The proof of concept of this biosensor was performed using a set of four bioluminescent bacteria (Escherichia coli DH1 pBzntlux, pBarslux, pBcoplux, and E. coli XL1 pBfiluxCDABE) in the online detection of CdCl2 0.5 µM and As 2O3 5 µM from an influent. When considering metals individually, the "fingerprints" from the biosensor were as expected. However, when metals were mixed together, cross reaction and synergistic effects were detected. This biosensor allowed us to demonstrate the simultaneous on-line cross detection of one or several heavy metals as well as the measurement of the overall toxicity of the sample.

Several common methods are used for detecting analytes in environment. The most common one is the conventional method, it is highly sensitive, accurate and based on physical and chemical analysis. However, reference chemical laboratories and a complete set of arrays of analytical experiments are necessary for such an extensive and costly analysis. A second approach is the use of biosensors, such as transgenic bacterial cells [4] that are able to detect the pollutant in complex environments. These biosensors bio-systems, produced by means of genetic engineering tools, can be used to detect and even analyze chemical compounds and pollutants such as mercury [5-9]. Heavy metals represent some of the most toxic ones not only for the environment, but also in many other areas like medicine and food industry. Society is learning to adjust to pollution by heavy metals, and is trying to remediate, control and minimize such pollution as much as possible. Mercury is one of the heavy metals with a chronic toxic effects which is impossible to notice at the initial stage of its presence in the environment [10].

The maximum allowable amount of mercury in surface and drinking water is less than 0.2 µg/L, which is much lower than standard concentrations of other metals in these water [10]. Bacterial biosensors, especially whole cells, have opened a new field in development of easy, cheap, quick, and user-friendly detection of mercury and other metals in the environment [4,12-13]. In this study, a whole-cell biosensor for the detection of mercury in a solution has been designed and evaluated. The recombinant plasmid construct was named pUMERG has been developed by insertion of the green fluorescence protein (GFP) gene downstream of the merR gene, which is controlled by the merR promoter.

MATERIAL AND METHODS

Biosensor *E. coli* Bacteria

The advantage of using *E. coli* as bio-sensing element is attributed to : its ability to metabolize a wide range of chemical compounds, its great capacity of adapting to adverse conditions and it also has the ability to degrade new molecules with time and also amenable for genetic modifications through mutation or through recombinant DNA technology [14]. Bacterial strains, media, and growth conditions. *E. coli* was used as a host in all experiments. LB medium and agar plates were prepared as described by Miller et al. [15].

Plasmid Construction

According to Sambrook *et al.,* [16] plasmids have been created and used in the current study, along with their abbreviations and descriptions, are listed in Table (I). The O/P region and the entire merR gene were amplified by polymerase chain reaction (PCR) from a viable plasmid containing the merR gene from Pseudomonas pBS228. Oligonucleotide primers used for PCRs were designed based on the nucleotide sequence of Pseudomonas pBS228. EcoRl and BamHI restriction endonuclease cut sites were merged at both ends of the sequence. Pfu DNA polymerase (Fermentas, USA) was used in PCRs. The obtained 3.21-kb construct (pUMER)

was transformed into E. coli DH5α (replicating host) and E. coli BL21 (DE3) (expressing host). PIVEX-GFP was used as the PCR template to amplify the GFP gene. The resulting PCR fragment was ligated to BamHl and Pstl sites next to merR in pUMER after predigestions with BamHl and Pstl enzymes. The final 3.96-kb concept was transformed into the E. coli strains DH5α and BL21 (DE3) [17].

Mercury assay conditions

For preparing the batch simulated sample mercury chloride ($HgCl_2$) with the following specifications has been used (molecular weight 271.5, assay ≥ 99.5 %, Segma Aldrich).

E. coli strains were grown in Luria-Bertani (LB) medium at 37 °C in an orbital incubator (BioTek, USA). *E. coli* strains carrying plasmids constructed from pUC19 were selected with ampicillin (100 µg/mL). For mercury sensitivity tests, culturing in test tubes began with 100-fold dilutions of fresh overnight culture of *E. coli* strains harboring pUMERG in 5 mL of medium. To assess cell growth, a spectrophotometer was used for measuring the absorbance at 600 nm (A600). When the A600 of a culture reached 0.4–0.6, cells were diluted 100fold in LB broth, and 0.1 mL of these dilutions was spread on LB agar plates that contained 100 µg/mL kanamycin and various concentrations of mercury ions. Plates were incubated overnight at 25–30 °C. The colonies of bacterial cells were counted after 18 hr [18].

Microscopy

To evaluate the function of the developed biosensor, the overnight culture was diluted 100-fold in fresh LB medium supplemented with 100 µg/mL of ampicillin and incubated at 37 °C using an orbital shaker at 220 rpm until the optical density at 600 nm reached 0.6. The propagated bacteria have been added to various mercury solution concentrations ranged from (10^{-8} to 10^{-4} M).

Assay mixtures contained 2 mL of diluted cell suspension and 2 mL of fresh LB medium containing Hg(II). Cultures were incubated at 30 °C in a rotary shaker (190–220 rpm).

For GFP measurements, culture samples of 200 µL (after 3 h) were centrifuged for 2 min at 15,000 ×g and the supernatant was poured. The cell pellet was washed 3 times with 200 µL of phosphate-buffered saline (PBS) and suspended in an appropriate amount of PBS [17]. *E. coli* strain BL21 (DE3), which was harboring pIVEX-GFP, was used as the positive control and was induced by IPTG. *E. coli* BL21 (DE3) with plasmid pUC19 which has been used as a negative control. Cells were magnified using a fluorescent microscope equipped with a mercury lamp (100 W), an excitation filter (385– 425 nm) [19].

Table (I) Plasmids and *E. coli* strains

Plasmid or Strain	Designation or genotype	Description
pUMER	**pUC19:P_{mer}- merR**	**MerR expression from P_{mer} by mercury induction**
pUMERG	**pUC19:P_{mer}- merR-gfp**	**MerR and gfp expression from P_{mer} by mercury induction**
E. coli DH5α	**F-gyrA96 (Nalr)recA1 relA1 thi-1 hsdR17 (rk-mk+) g1nV44 deoR Δ(lacZYA-argF) U169[ϕ80d Δ(lacZ)M15]**	**Replicating host**

Fluorometry

Activity of the biosensor cells was assessed by the GFP fluorescence of cells grown in LB medium containing different concentrations of mercury ions. Cell growth was measured by spectrophotometry (optical density at 600 nm). The excitation and the emission wavelength of the fluorimeter was set at 490/10 nm and 530/10 nm. *E. coli* BL21 carrying pUC19 was used as the baseline sample to zero the instrument. The signal produced were defined as the ratio of signal to noise (AFU/A600). Crude fluorescence amounts were measured using the instrument's arbitrary relative fluorescent units (AFUs). Specific fluorescence intensity is the crude

ISSN: 0975-8585

fluorescence's intensity, measured in relative fluorescence units, separated by the optical density at 600 nm assessed at each time point. Several measurements were obtained for each sample after 3 h of exposure [19].

RESULT AND DISCUSSION

Increasing environmental pollution caused by heavy metals lead to an urgent need to develop progressive analytical tools and methods for rapid detection of these types of contaminates in the environment. Heavy metals, including lead, mercury, copper, and chromium are important factors for health and environmental pollution. Contamination with these metals causes serious health problems, and thus fast and simple detection methods are vital for both human health and environmental protection. The biosensor provides several advantages as they are highly available, more economic approach, easy to work with in situ and can be easily regenerated and propagated. However, the main advantage of this biosensor is its ability to analyze biologic samples and in vivo application to detect trace amounts of mercury. In the present study we trying to use *E. coli* spps. as an indicator for in environmental mercury (Hg) pollution [20].

Fluorometry

As fluorescence is the indication of the presence of Mercury. Fluorescent strength was measured in different concentrations of mercury over 3 hr at room temperature as shown in **Figure (1)**. It was notice that, when mercury concentration is less than 10^{-8} M, appearance of GFP was very low and it was detected that there was no fluorescent emission. This could be due to merR binding to the related promoter, which suppresses the transcription and translation of GFP. Furthermore, in higher concentration of mercury ions (10^{-4} M), GFP expression was lowered by saturation of merR with mercury ion and the cytotoxic effects of these ions on bacterial cells. The mercury detection rate was measured between (10^{-8} to 10^{-4} M)

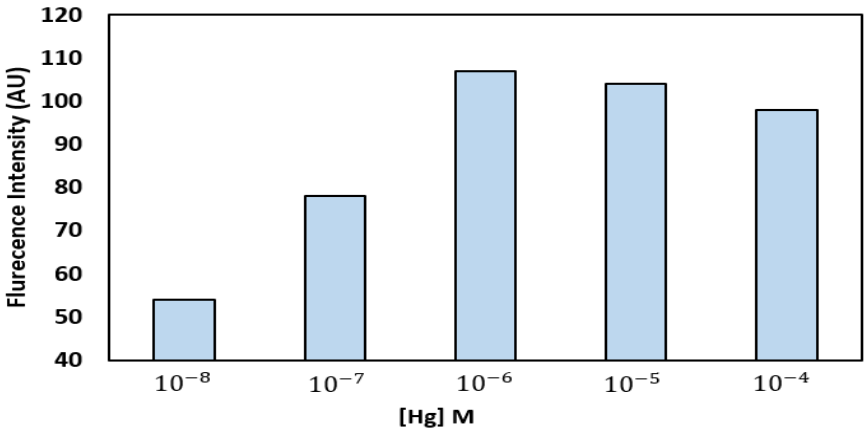

Figure (1) Effect of different mercury ions concentration on the fluorescence intensity.

Florescence microscopy

The florescent emission brought by mercury was detected via florescence microscopy **Figure (2)**. A direct relationship has been observed between mercury ion concentrations and fluorescent emission. Low mercury solution concentration could only induce transcription of GFP in a small number of bacteria.

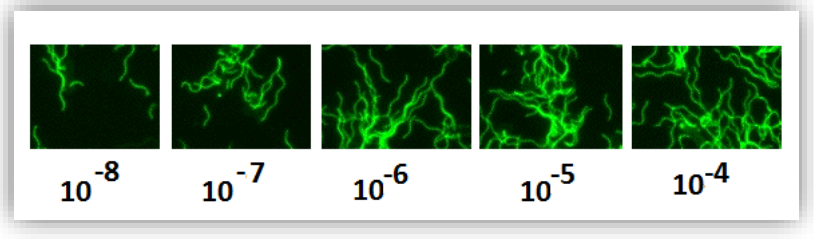

Figure (2) Fluorescence produced by biosensors after exposure to different concentrations of mercury.

Biosensors have many applications such as heavy metal detection. In this study, we have been used the merR gene (from Pseudomonas pBS228), pUC19 plasmid, and GFP reporter gene to develop a whole-cell biosensor capable of detecting mercury in samples. There are whole-cell biosensors that use reporter systems and upstream mercury-inducible promoters [21-23]. There are extensive reports of using bacterial biosensors since 1993; over time, the specificity and sensitivity of these biosensors have increased. These biosensors have a sensitivity lower than biosensors designed by Goddard [24]. The real sensitivity of the manufactured biosensors in the past decade were in the micro molar range, but sensitivities of newly planned biosensors are in the pico molar sorts. Maybe the advance of genetic manufacturing techniques and the use of more sensitive genes are the real reasons for this raise in sensitivity. Regarding the standard values of mercury in drinking water, this determination range is constant with the proposed use of the biosensor. Due to the decrease in the mercury-neutralizing merA gene in our biosensor cell, incompatible to expectations, a significant decrease in the number and strength of fluorescent bacteria was detected at high concentrations of mercury (10^{-4}) as indicated in Figure 1. One of the most appreciated benefits of these biosensors is distinguishing the bioavailable part of mercury in samples. In the occurrence of mercury ions, bacterial biosensors lead to signals that could be easily and in a very short time recognized and measured. Therefore, these systems are known as real time diagnostic systems. Although our biosensor cannot perform as strongly as advanced mercury biosensors, this simple biosensor is capable to react to our requests in the fields of metal contamination.

GFP was selected as a gene due to the ease of examining gene expression, sensitivity, ability to evaluate quality, stability, and absence of natural disturbances. Due to these advantages, as well as ease of access to this reporter gene, GFP was chosen for this study. The obtained data from our developed biosensor were evaluated. As can be observed in (Figures a and b), there is a direct relationship between increased concentrations of mercury ions and the number of cells expressing the GFP gene. Biosensor cells designed in this study were capable of detecting concentrations of mercury ions in a range of 10^{-4} and 10 M. Based on the standard values of mercury in water, this detection range is constant with the proposed application of the biosensor. Due to the lack of the mercury-neutralizing merA gene in our biosensor cell, opposing to expectations, a significant reduction in the number and intensity of fluorescent bacteria was observed at high concentrations of mercury (10^{-4}), Presence of mercury ions, bacterial biosensors lead to signals that could be easily and in a very short time recognized and measured.

CONCLUSION

It is hoped that further studies on the capabilities of this biosensor and enhancement of its accuracy will lead to its production on an industrial scale so that, in addition to water, it will be able to detect the exact quality and quantity of mercury in various samples, such as bodily fluids. Additional improvements can be applied to the whole-cell detection system and may include mercury elimination features.

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