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Study on the development of bio-luminescence sensor for detection of mercurials using a light emission gene and a mercury-resistance operon .

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INTRODUCTION

Our study was focused on the mercury pollution that causes the most serious environmental problem. At the time of Minamata disease outbroken around Minamata Bay and second Minamata disease outbroken in the basin of Agano River, mercury poisoning was recognized as the very serious environmental problem. Therefore we considered that it is successive outbreaking , thought tremendous efforts to solve the problem have been done.

Especially in the case of the Minamata disease in the basin of Jintsuw River, Toyama, Japan, the investigation was carefully done, and mercury poisoning was accurately confirmed. Such finding of mercury in environmental water impressed the importance of the preventions of public mercury poisoning.

With present measuring methods of mercury in the environmental water, gas chromatography-mass spectrometry and atomic absorption spectrometry are adopted. These methods are effective and highly sensitive. However, pretreatment of samples to be purified and to be concentrated is very complicated, and it needs well-trained skill for the operation of these measuring equipments. recently, bacterial genes and operons which confer bacteria to resist and decompose physiological toxic substances were cloned and analyzed. Genetic engineering also allows the gene fusion technology to combine these special genes or operons with a bio-luminescence gene to react the targeted materials and report those existence by luminescence.

In this study, we constructed a fused gene operon to detect mercury. This paper reports the development process of the mercury bio-luminescence sensor and its performance.

EXPERIMENTAL METHOD

In this study, we constructed a ligated plasmid using a *mer*R, promoter/operator region of *Pseudomonas mer* operon, *mer*T,P whose products concern a transportation of mercury, and *mer*A encoding a mercury-reducing enzyme.

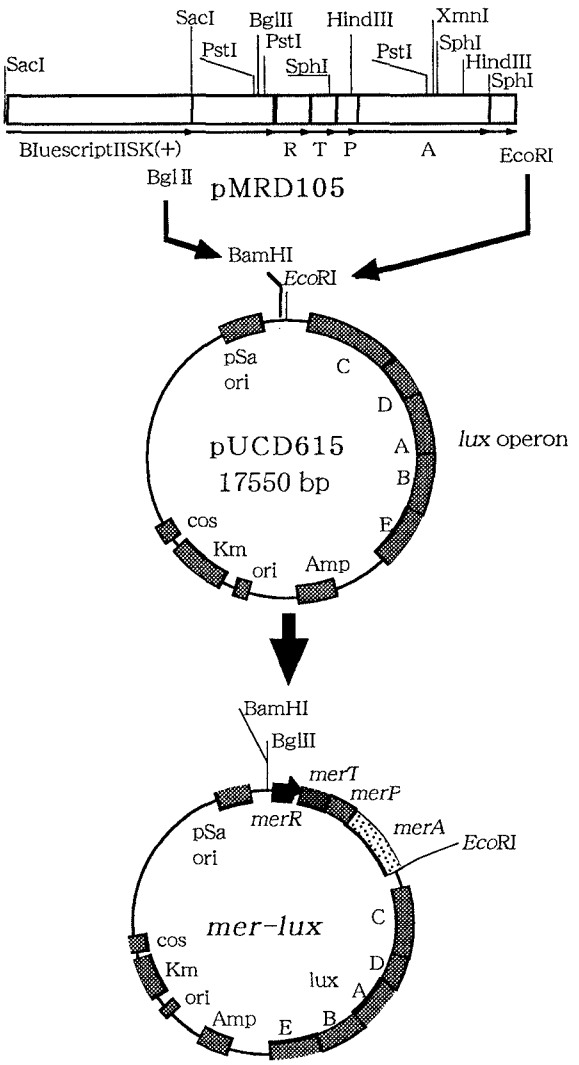

Fig.1. Strategy to construct a mercury bio-luminescence sensor.

Genetic engineering method employed in this study was shown in Fig.1. As shown in this figure, pUCD615 which possesses a luminescence gene was employed as a vector, and it was digested which *Eco*R I and *Bam*H I to be inserted with *mer* genes. pMRD105 was used as a donor of *mer* genes. A recombinant plasmid *mer-lux* was finally obtained to be used as a bio-luminescent sensor plasmid. Bio-luminescence induced by mercuric chloride and expressed in a transformed *E.coli* was measured by X-ray film or by a luminescence photometer. Affecting factors to express the fused genes in *E.coli* were examined with several conditions of bacterial cultivation.

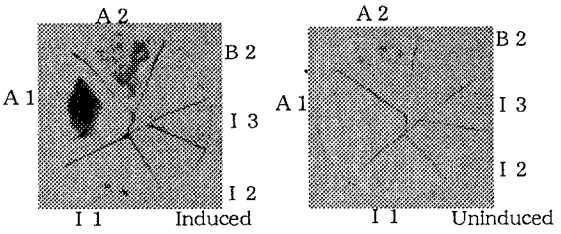

Fig.2. Result of bio-luminescence by mercury induction

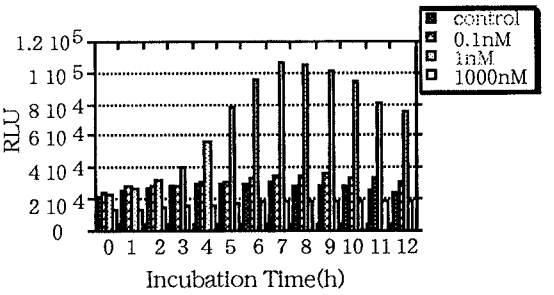

Fig.3. Sensitivity of mercury detection by *mer-lux* with a luminescence photometer.

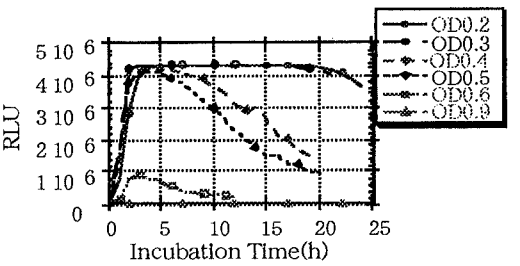

Fig.4. Comparison of light emission with the distinctions of OD600nm

RESULTS

①The recombinant plasmid constructed in this study did not emit light under uninduced condition with mercury, but that did it at induced condition. So we succeeded to develop the mercury bio-luminescence sensor. (Fig.2.)

②As a result, the limit of Hg detection was improved to 1nM. Bacterial response was inhibited at high mercury concentration over than 1,000nM. (Fig.3.)

③This bio-luminescence sensor was very stable and sensitive to mercury when the host *E.coli* was cultivated under 20℃. However, it did not show the same performance when the *E.coli* was cultivated under 37℃ at which the temperature is optimum to cultivate the bacteria.

④The transformed *E.coli* gave the highest emission intensity when the bacteria was cultivated between 0.2 to 0.9 of the optical density of 600nm(OD_{600}). Therefor logarithmic growth phase of the bacteria must be selected to use for the bio-luminescence sensor. (Fig.4.)

DISCUSSION

However, the development of the mercury bio-luminescence sensor has not completed, because many improvements are needed to develop more sensitive biosensor. Therefore, further study on the improved recombinant plasmid is necessitated to develop a sophisticated detection method of environmental mercury contamination. Bio-available mercury measurement in the environment and the measurement specificity of chemical from of mercury should be aimed as those future studies.

ACKNOWLEDGMENTS

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