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Construction and Characterization of a Recombinant Bioluminescence Streptomycetes for Potential Environmental Monitoring

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Abstract Bacterial bioluminescence has been known to be a highly valuable reporter system for its potential application as an effective and simple environmental monitoring method for toxic compounds. In this short report, we constructed a streptomycetes-*Escherichia coli* shuttle vector-containing bioluminescence system and evaluated its potential application for toxic compounds monitoring. The *luxAB* bioluminescence genes from *Vibrio harveyi* were cloned into a streptomycetes-*E. coli* shuttle vector (named pESK004) and functionally expressed in *Streptomyces lividans*. The recombinant *S. lividans* containing pESK004 exhibited an optimal bioluminescence at the optical density ($OD_{600\,nm}$) of 0.4–0.5 and aldehyde concentration of 0.005%. When the recombinant bioluminescence streptomycetes was exposed to a toxic compound such as heavy metals, chlorinated phenols, or pesticides, the bioluminescence was decreased proportionally to the concentration of toxic compound in the assay mixture. The EC_{50} (effective concentration to decrease 50% of the bioluminescence prior to exposure) values in the recombinant bioluminescence streptomycetes for mercury, 2,4-dichlorophenol, and malathion were measured at 2.2 ppm, 144.0 ppm, and 82.4 ppm, respectively. The degree of sensitivity and specificity pattern toward these toxic compounds characterized in this recombinant bioluminescence streptomycetes were unique when compared with previously reported bacterial bioluminescence systems, and this revealed that a recombinant bioluminescence streptomycetes might provide an alternative or complementary system for potential environmental monitoring.

Key words: Bioluminescence, streptomycetes, environmental monitoring

Streptomycetes are Gram-positive filamentous soil bacteria which undergo a unique morphological differentiation that was initiated by various environmental stimuli [5, 8]. Although streptomycetes are well known industrial microorganisms for their production of many valuable secondary metabolites, some streptomycetes species are also extremely valuable ecologically and environmentally for their superior capabilities of sensing and degrading diverse structures of many recalcitrant substances [1, 2, 16, 17, 20]. Several streptomycetes species have been isolated from various environments and characterized for the purpose of toxic compound biodegradation [6, 10, 14, 22]; however, the toxic compound sensing capability of streptomycetes has never been evaluated extensively in either the natural or the recombinant forms. Recently, public awareness regarding environmental concerns led to the development of more efficient and reliable environmental monitoring systems [11, 13, 21]. Among various kinds of environmental monitoring systems, a natural or recombinant bacterial bioluminescence system has been considered to be the most simple and quick-response biosensor system for the toxic compound monitoring [3, 4]. Due to the fact that the degree of sensitivity and specificity pattern toward toxic compounds are highly variable depending on the host bioluminescence microorganism, it is important to continuously develop and characterize various kinds of bioluminescence host systems to maximize the bioluminescence-based biosensor capability. Unlike previously-reported Gram-negative bioluminescence systems, the Gram-positive bioluminescence streptomycetes system lacks the outer membrane barrier for toxic compound to be taken in and is able to maintain stable protoplast, both of which should be advantages for further strain improvement to enhance monitoring sensitivity. Therefore, a novel recombinant bioluminescence system using a streptomycete species was constructed and its potential application for toxic compounds monitoring evaluated.

To construct and characterize a recombinant bioluminescence streptomycetes, a *Vibrio harveyi luxAB* locus was isolated from the pUT plasmid [7] by using *Bam*HI/*Hin*dIII double

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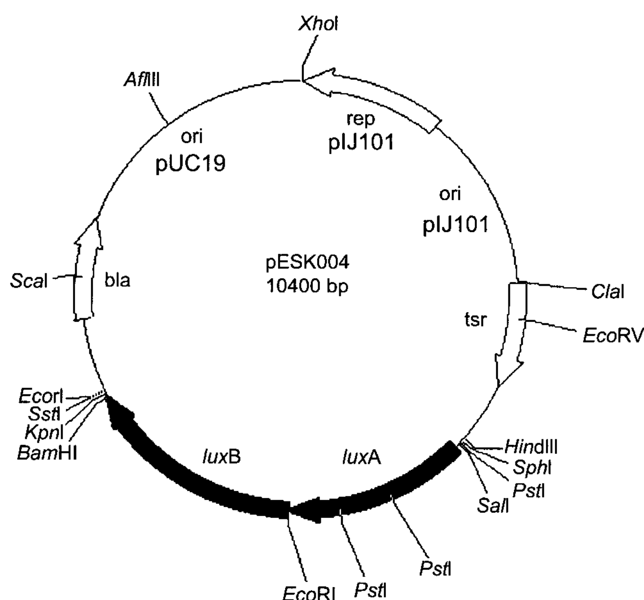

Fig. 1. A map of the streptomycetes-*E. coli* shuttle vector, pESK004.

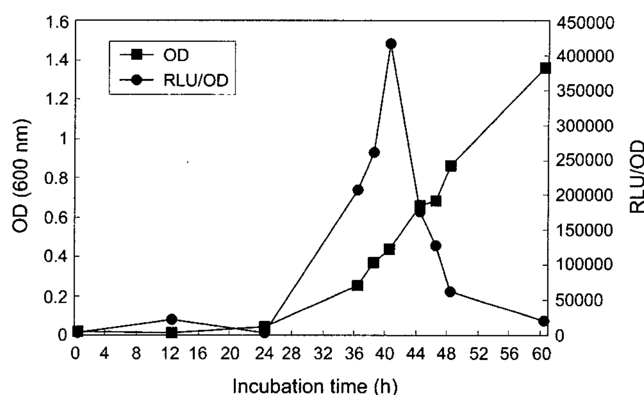

Fig. 3. Cell growth ($OD_{600\,nm}$) and specific bioluminescence ($RLU/OD_{600\,nm}$) by the recombinant bioluminescence streptomycetes, ESK004, during YEME culture.

digestion, and then cloned into the *Bam*HI/*Hin*dIII double-digested streptomycetes-*E. coli* shuttle vector, pWHM3 (Fig. 1). This recombinant plasmid, named as pESK004, was then transformed into *S. lividans* by using a typical streptomycetes protoplast-PEG transformation method [9], and this was confirmed to be stably maintained as a high-copy plasmid in *S. lividans*. The *S. lividans* containing pESK004, named as ESK004, was cultured in 25 ml of YEME media (yeast extract 3 g/l, Bacto-peptone 5 g/l, malt extract 3 g/l, glucose 10 g/l, sucrose 340 g/l, $MgCl_2{\cdot}6H_2O$ 2 ml/l) in the presence of thiostrepton (0.5 ug/ml) at 30°C while shaking at 200 rpm [9]. The ESK004 culture samples were harvested at various time periods to measure the optical density ($OD_{600\,nm}$) by spectrophotometery (Shimadzu UV-1601, Kyoto, Japan) and the streptomycetes bioluminescence by a luminometer (Bioscan LUMI-SCINT™, Washington D.C., U.S.A.). The bioluminescence (luciferase acitivity) was expressed as relative light units (RLU), for which the detailed assay method was reported elsewhere [3, 17, 19]. Since this recombinant *S. lividans* ESK004 contains only *luxAB* genes, n-decyl aldehyde (Sigma Chemical Co, St. Louis, U.S.A.) was added as a substrate to carry out the bioluminescence assay. A streptomycetes bioluminescence was proportionally increased with the amount of n-decyl aldehyde that was added in the assay mixture, exhibiting the highest RLU at 0.005% of n-decyl aldehyde (Fig. 2). However, streptomycetes bioluminescence was decreased at a level higher than 0.005% of n-decyl aldehyde, probably caused by cellular toxicity (Fig. 2). The $OD_{600\,nm}$ and total RLU values from the ESK004 culture showed a steady increase during the incubation period, but the $RLU/OD_{600\,nm}$

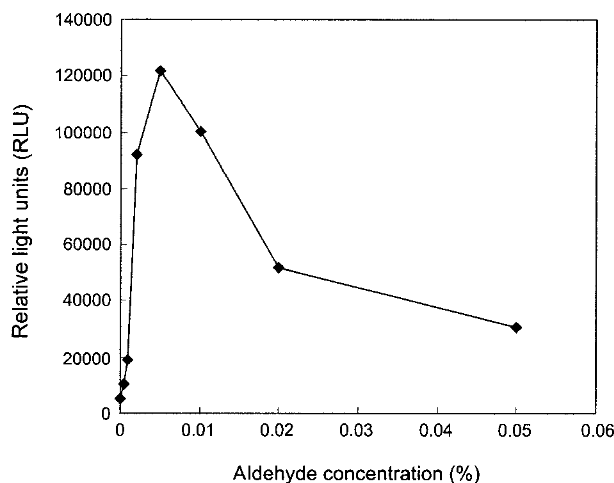

Fig. 2. Streptomycetes bioluminescence expressed as RLU depending on various n-decyl aldehyde concentrations.

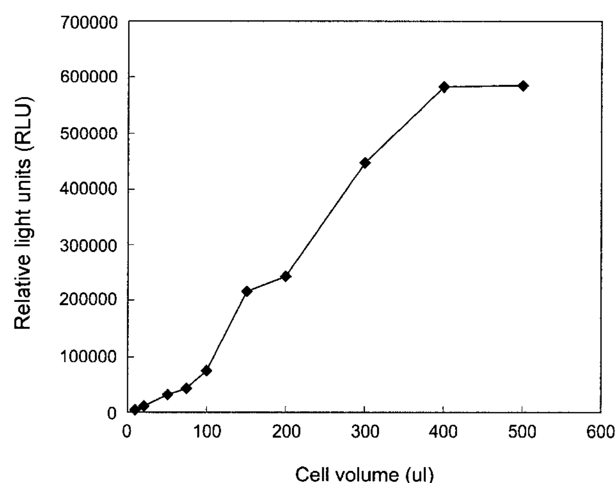

Fig. 4. Streptomycetes bioluminescence expressed as RLU depending on various cell culture volumes.

value started to decrease rapidly after 40 h (Fig. 3), suggesting that the optimal assay condition for a recombinant streptomycete bioluminescence took approximately 40 h of the ESK004 culture with $OD_{600\,nm}$ of 0.4–0.5. Although the streptomycetes bioluminescence was also proportionally increased with the amount of streptomycetes culture volume added to the assay mixture (Fig. 4), a culture volume of 100 μl in an assay mixture provided a sufficient bioluminescence without any mixing problem in an assay vial. Therefore, the optimum assay condition for the recombinant bioluminescence streptomycetes was determined to be a mixture containing 100 μl of approximately 40 h-grown (or $OD_{600\,nm}$ of 0.4–0.5) ESK004 cell that was cultured in a YEME medium at 30°C with 200 rpm, and 20 μl of n-decyl aldehyde (final concentration 0.005%) in a total assay volume of 200 μl. This optimum assay condition for the recombinant bioluminescence streptomycetes was highly reproducible with consistent RLU values.

A bacterial bioluminescence-based toxicity test has been well characterized in several natural and recombinant microorganisms [3, 4]. A basic concept of the bioluminescence-based toxicity test is to measure the RLU reduction after the bioluminescence microorganism that is exposed to a certain toxic compound. The EC_{50} value for an individual toxic compound was defined as an effective concentration to decrease 50% of the bioluminescence prior to any exposure. In order to evaluate a potential biosensor capability of a recombinant bioluminescence streptomycetes, three different classes of toxic compounds such as heavy metals (e.g. mercury, aluminum, cadmium, iron, lead, selenium, manganese, zinc), chlorinated phenolic compounds (e.g. 2-chlorophenol, 4-chlorophenol, 2,4-dichlorophenol), and pesticides (e.g. parathion, diazinon, malathion) were tested by using the optimally-cultured recombinant bioluminescence streptomycetes, ESK004. Each toxic compound that was properly diluted to a final volume of 80 μl was added to an assay mixture containing 100 μl of the optimally-cultured recombinant bioluminescence streptomycetes and 20 μl of n-decyl aldehyde, followed by measuring the bioluminescence after undergoing a 5 min incubation process at room temperature. Like other bacterial bioluminescence, the streptomycetes bioluminescence was decreased proportionally depending on the amount of all the tested toxic compounds. All the EC_{50} values for the toxic compounds tested are summarized in Table 1. As shown in Table 1, the EC_{50} values characterized in this recombinant bioluminescence streptomycetes are variable depending on the toxic compounds tested, and the degree of sensitivity as well as its specificity pattern toward these toxic compounds are unique when compared with other bacterial bioluminescence systems reported previously [12, 15]. Unlike other bacterial bioluminescence systems, a recombinant bioluminescence streptomycetes was found to be highly insensitive toward most of the chlorinated phenolic compounds (2,4-dichlorophenol EC_{50} values for *Vibrio* sp. and ESK004 are 3.9 and 144.0, respectively) [12, 15]. Comparing with other bacterial bioluminescence systems, however, the level of the sensitivity toward heavy metals tested in the recombinant bioluminescence streptomycetes was quite comparable (mercury EC_{50} values for *Vibrio* sp., UV2, and ESK004 are 3.5, 2.5, and 2.2, respectively) or even higher for some pesticides (malathion EC_{50} values for UV2 and ESK004 are 1146.5 and 82.4, respectively) [12, 15]. The biological significance of differential sensitivity toward a specific toxic compound observed in the recombinant bioluminescence streptomycetes needs to be further investigated. In conclusion, a luciferase gene, *luxAB* from *Vibrio harveyi*, was cloned into a streptomycetes-*E. coli* shuttle vector, which was stably maintained and functionally expressed in a heterologous host, *S. lividans*. Based on the unique features, which are not present in other bacterial bioluminescence systems, the recombinant bioluminescence streptomycetes reported in this article should provide an alternative or complementary system for providing a potential environmental monitoring application.

Table 1. EC_{50} values for various toxic compounds tested in a recombinant bioluminescence streptomycetes, ESK004.

Toxic compound	EC_{50} for ESK004 (R^2)[a]
Hg	2.2 (0.92)
Al	10.5 (0.94)
Cd	57.8 (0.96)
Pb	105.2 (0.97)
Se	77.9 (0.95)
Mn	74.4 (0.99)
Zn	9.8 (0.98)
Fe	15.2 (0.93)
Parathion	193.2 (0.97)
Diazinon	351.6 (0.91)
Malathion	82.4 (0.91)
2-Chlorophenol	98.2 (0.98)
4-Chlorophenol	120.7 (0.94)
2,4-Dichlorophenol	144.0 (0.98)

[a]R^2 value derived from the least square method.

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