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Short communication

Amplification of Newly Isolated Luciferase Gene from Marine *Photobacterium* sp. strain MIE using PCR

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Abstract

The aim of this study is to isolate a new luciferase gene from *Photobacterium* strain MIE by using specific polymerase chain reaction (PCR) and to identify whether this new *Photobacterium* sp. is similar to *P. kishitanii* or *P. phosphoreum*. The *Photobacterium* strain MIE has been successfully isolated, characterized and identified. Analysis of its 16S rRNA through the BLASTN program shows that this strain was closely related to several *Photobacterium kishitanii* and *Photobacterium phosphoreum* strains. Primers were designed based on the nucleotide multiple sequence alignment of the luciferase gene from *Photobacterium* species deposited in Genebank. The luciferase genes from this bacterium amplified by using specific primers showed the expected sizes of between ~1.8 kbp and ~2.1 kbp that were reported from *P. kishitanii* and *P. phosphoreum*, respectively.

INTRODUCTION

Many literatures and findings had reported on the use of *Photobacterium* sp. in environmental applications such as a pollutant indicator towards zinc toxicity [1], microbial testing in industrial wastewater [2], biosensor to evaluate aquatic toxicity [3] and for heavy metals detection based on its tolerance [4]. Most of the detection system relies on the bioluminescence activity. Hence, amplification of the responsible gene(s) that contributed towards this phenomenon is important. The gene that is responsible for the bioluminescence properties in *Photobacterium* sp. is luciferase. Based on the luciferase gene from the NCBI database, the expected size of the luciferase gene from *P. phosphoreum* is ~2.1 kbp while the expected size in *P. kishitanii* is ~1.8 kbp. Although, bacterial luciferase protein had been crystallized by Fisher *et al*. as early as 1996 [5], the mechanism of luciferase has not been fully resolved. Thus, the isolation of more luciferase gene will offer new knowledge and insights in order to investigate the function of newly isolated luciferase gene in *Escherichia coli* rather than the wild type. The most famous and rapid system for toxicity test is by using *Vibrio fisheri* or the Microtox™ test [6]. However, this system has some limitation being of marine origin and need the presence of high salts concentration that could mask the effect of toxicants being tested or so called the matrix effects [7]. Furthermore, only the luciferase gene (luxAB) from Vibrio sp. especially *Vibrio harveyi* had extensively being studied in *E. coli* [12]. Two luciferase gene cassettes from *Photorhabdus luminescens* [8-11] and *Photobacterium* sp. have been cloned and expressed in *E. coli* [13]. Preliminary works have shown that the luminescence activity from this bacterium could rival the widely reported *V. fisherii* (Microtox™) due to its broader optimum temperature for luminescence activity from 20 to 40 ^{o}C [14]. Hence, the isolation and cloning of luciferase from this bacterium could reveal important underlying mechanism for temperature stability. In this work, a preliminary amplification of the luciferase gene from this bacterium is reported.

MATERIALS AND METHODS

Growth media

Photobacterium strain MIE was grown on nutrient agar supplement with 1.5 g of sodium chloride (NaCl) with 72 hour incubation in 28°C. Colonies that emit green fluorescence on the plate (Figure 1) after 72 hour were inoculated and grown in nutrient broth with same NaCl concentration [14]. Genomic DNA were extracted by using Wizard® Genomic DNA Purification Kit (Promega, USA)

PCR primer design

Primers were designed in order to amplify the luciferase luxAB genes. Primers were designed based on the sequence alignment of luciferase genes from several *Photobacterium* sp.

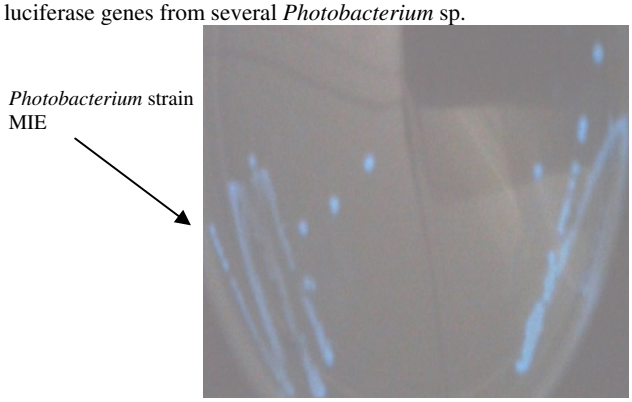

Figure 1: Green-blue fluorescent colonies of *Photobacterium* strain MIE on nutrient agar (NB) supplemented with 1.5 g of NaCl after 72 hours incubation at 28°C.

The nucleotide sequences of luciferase genes were taken from National Center for Biotechnology Information database (NCBI). Primers consists of two forward primers (F_luxAB_Phos-'GATGAAGTTTGGAAATATTTTCTCA', F_luxAB_Kish-'ATTTAATTTTGGTGTTGTGCG') and one reverse (R_luxAB_Both TTACGAGCTTTGTAAATTCTTTTG).

PCR conditions

Two PCR mixture with total of 25 µl each were prepared for amplification of lux AB gene differs in its forward primer used (F_luxAB_Phos with R_luxAB_Both and F_luxAB_Kish with R_luxAB_Both). The mixtures contain 200 ng *Photobacterium* strain MIE genomic DNA, 1X PCR buffer (Novagen, Germany), 0.2 mM dNTPs mixture (Novagen), 1 mM $MgSO_4$ (Novagen), 0.25 µM of each oligonucleotide primers (First Base, Malaysia).The initial or pre-denaturation step was carried out at 94°C for 4 min, the denaturation step at 94°C for 1 minute, the annealing step at 57.0°C for 1 min, the extension step at 72.0°C for 1 min for 30 cycles and the final extension step was carried out at 72.0°C for 4 min and cooling at 10°C. The PCR was run for another 29 cycles. The PCR product was electrophoresed on 1.0% (w/v) agarose gel (Figure 2).

RESULT AND DISCUSSION

The luciferase gene from *Photobacterium* strain MIE was successfully amplified with an expected size of about 2.1 kbp (Figure 2). The luciferase gene will be sequenced for further analysis and for comparison with established luciferase gene in the NCBI database. Lux A was ~1.1 kbp while luxB was ~1.0 kbp. This new luciferase gene will be cloned into *E. coli* for further study and for comparison study with the wild type *Photobacterium* strain MIE.

In the same figure (Figure 2) it also shown that both sets of primers were able to amplify both sizes of luciferase genes from *P. phosphoreum* and *P. kishitanii* (~2.1 kbp and ~1.8 kbp respectively). The nucleotide multiple sequence alignment conducted for both genes (result not shown) showed that the luciferase gene from *P. phosphoreum* covers the nucleotide sequence of luciferase gene from *P. kishitanii.*

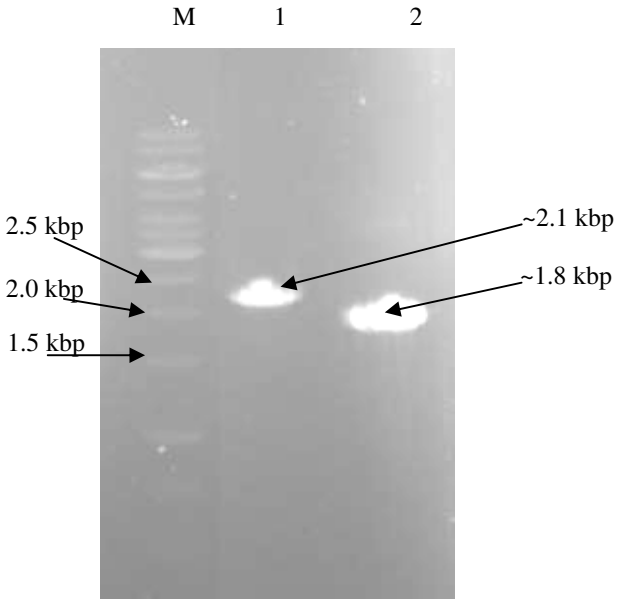

Figure 2: Amplification of lux AB gene from *Photobacterium* strain MIE. Lane M: 1 kb DNA ladder mix (Fermentas); Lane 1: PCR product by using forward primer: F_luxAB_Phos and reverse primer: R_luxAB_Both; PCR product by using forward primer: F_luxAB_Kish and reverse primer: R_luxAB_Both.

CONCLUSION

The PCR product obtained from *P. phosphoreum* luciferase gene which was approximately 2.1 kbp in size is 300 bp larger than the 1.8 kbp from *P. kishitanii*. Thus, it can be hypothesized that the new *Photobacterium* strain MIE probably belongs to the same class to *P. phosphoreum*, but this needs further identifications through gene sequencing.

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