Isolation of Bioluminescence Bacterium from Marine Fish and Amplification of Luciferase (lux AB) Gene

Muhammad Syukri Airil*, Mohd Yunus Shukor, Nur Adeela Yasid, Siti Aqlima Ahmad, Syahida Ahmad
Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia.
*E-mail: airilsyukri@gmail.com

ABSTRACT

Bioluminescence bacteria (BLB) are the most abundant and widely distributed light-emitting organisms that can be found in the marine environment. They are suitable for detecting pollution or integrated into bioluminescent imaging due to their ability to luminesce. However, there are limited studies regarding bioluminescent bacteria in terms of distribution and species present in Malaysia. This study aims to isolate BLB and amplify the luciferase (*lux*AB) genes. Bioluminescent bacteria were isolated from the guts of marine fish, *Selaroidesleptolepis* and streak onto luminescence agar (LA). The brightest luminous colony present in the dark was marked and streaked again obtain a pure colony. Then, the pure culture of the colony was subjected to genomic extraction before *lux*AB genes amplification and phylogenetic analysis. As a result, BLB were successfully isolated and identified to be *Photobacteriumleiognathi*s train SYA2 (MZ491870.1). Genes encoding for luciferase enzyme were also amplified and sequenced with the size of *lux*A and *lux*B were 767 bp and 943 bp, respectively. Pairwise distance showed that the isolate has the highest similarity to *P. leiognathi* (DQ790853) with 99.66% and the lowest similarity to *P. kishitanii* (AY642227) with 69.84%. The information about the isolate will contribute to the distribution of BLB in Malaysia as well as potential of BLB as a biosensor and bioreporter.

Keywords: Bioluminescence bacteria, *Photobacterium*, *luxAB*, luciferase, 16s rRNA

INTRODUCTION

Bioluminescence is a phenomenon that occur when an organism emits visible light as a byproduct of a chemical reaction. Among the bioluminescence organisms, bioluminescent bacteria (BLB) together with their mechanism of actions have been extensively studied over the past years [1-4].

The bioluminescent is regulated by several genes clustered together in a block called *lux* operon [5]. *Lux* operon consists of *lux* CDABE(G) as a core genes. Genes that encodes luciferase enzyme are *lux*A and *lux*B, while *lux*C, *lux*D, and *lux*E are part of the fatty acid reductase complex. Whereas *lux*G encodes for flavin reductase [6]. Bacterial bioluminescence reaction involves oxidation of reduced flavin mononucleotide ($FMNH_2$) to convert long-chain aliphatic aldehyde in the presence of molecular oxygen to produce acid, flavin mononucleotide, water, and light emission based on the following reaction [7]:

$$FMNH_2 + RCHO + O_2 \xrightarrow{Luciferase} FMN + RCOOH + H_2O + Light\ (490nm)\ (Equation\ 1)$$

Currently, there are limited studies on the BLB in Malaysia. Thus, this study aimed to isolate marine bacteria that generate bioluminescence from marine fish, *Selaroidesleptolepis*, obtained in Malaysia, and identify the species of isolate using molecular approach. The pairwise distance was calculated to determine the percentage of similarity between isolate and other related bacteria.

MATERIALS AND METHODS

Isolation of bioluminescence bacteria

Yellow-striped scad (*S. leptolepis*) obtained from the local market in Kajang, Selangor was halved laterally and swabbed from several parts of the body, which were eyes, guts, inner body and outer body. Modified luminescence agar (LA) and luminescent broth (LB) were used for bacterial cultivation [8]. The LB consisted of, per litre: 30 g NaCl, 1 g $MgSO_4$, 4 g K_2HPO_4, 10 g peptone, and distilled water, with the addition of 15 g bacteriological agar (Difco, USA) to produce LA. The swabbed LA plates were examined in pitch dark room for bioluminescence after incubation at room temperature of 25℃ for 12 hours. Positive colonies for bioluminescence were marked and isolated on a new plate to obtain a single colony.

Amplification of luciferase genes

Luciferase genes were made up of *lux*A and *lux*B genes. Forward and reverse primers for *lux*A were 5'-ATGAAATTTGGCAATATTTGTTTCTCA-3' and 5'-GCGCCACTTGTGTCATGAAG-3', respectively, while forward and reverse primers for *lux*B were 5'-CACAAGTGGCGCCATACCTA-3' and 5'-GGTCGATGACAGCGGCTTTA-3', respectively. Amplification of luciferase was done using a forward primer of *lux*A and a reverse primer of *lux*B. The 50 µL reaction mixture consisted of 10 µL of buffer, 0.5 µL of DNA polymerase, 2 µL of each primer, 1 µL of DNA template and 34.5 µL of distilled water. The PCR was optimised as followed: initial denaturation at 95℃ for 5 min, 34 cycles of (95℃ for 30 s, 55℃ for 30 s and, 72℃ for 1 min), further elongation at 72℃ for 5 min [9]. The reaction products were analysed by using 1% agarose gel electrophoresis.

Sequencing and molecular analysis

PCR product was sent to Apical Scientific, Malaysia for Sanger sequencing. The nucleotide sequences obtained were then compared with other *lux*AB sequences in GenBank using NCBI Basic Local Alignment Search Tool (BLAST) program and aligned with Clustal W. Maximum likelihood phylogenetic tree was constructed using Molecular Evolutionary Genetic Analysis (MEGA) [10] program version 10 with 1000 bootstrap. The pairwise distance was calculated using Mega X to determine the similarity of *lux*AB gene between isolate and other related reference bacteria using Tamura 3-parameter model with gamma distribution applied [11].

RESULTS

Isolation of bacteria

Gram staining showed that the isolate was Gram-negative with rod-shaped bacterium. The observed colony was circular, slightly raised, and white-cream in colour as well as opaque and smooth surface under the presence of light.

*Amplification of lux*AB

Amplification of *lux*A and *lux*B gene were done successfully and yield band with 766 bp and 941 bp, respectively. While *lux*AB amplification yields 1756 bp. Further phylogenetic analysis using sequence from *lux*AB(MZ568799) was done to confirm the isolate species (Figure **1**). Based on the phylogenetic tree, the isolate was clustered together with *P. leiognathi*with 82% confidence level.

Comparison of luciferase properties

Based on Table **1**, luciferase from this study showed the highest similarity to *P. leiognathid* (DQ790853) with 99.66% while the lowest similarity to *P. kishitanii* (AY642227) with 69.84% in *Photobacterium* genus. *Vibrioharveyi* was used as a representative of other BLB outside the genus of *Photobacterium*.

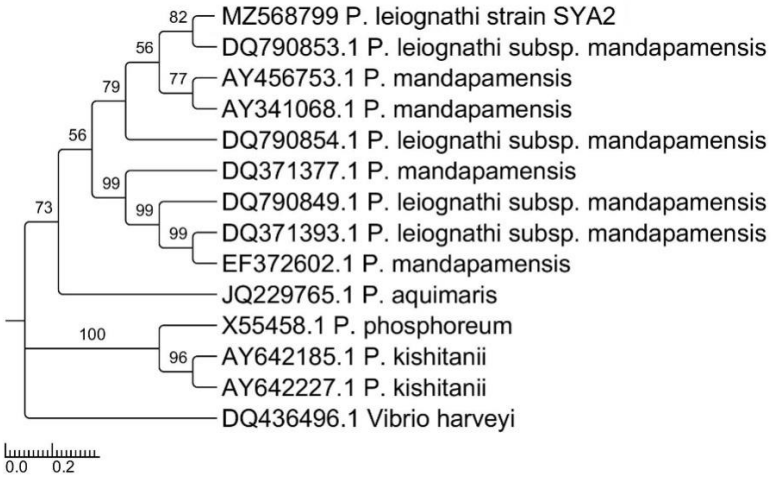

Figure 1. Phylogenetic relationship between isolate, *P. leiognathi* strain SYA2 and other related reference bacteria based on the *luxAB*gene sequence analysis with *Vibrio harveyi*as outgroup.

Table 1. Comparison of luciferase properties between isolate and other related luminescent bacteria.

Accession Number	Habitat and geographic origin	Similarity to luciferase of isolate (%)	Theoretical molecular weights (KDa)*	References
P. leiognathi subsp. mandapamensis(DQ790853)	*Acropomajaponicum*	99.66	63.11	[24]
P. mandapamensis (AY456753)	Sea water, Fort Lauderdale, Florida	99.48	62.88	[25]
P. mandapamensis (AY341068)	Sea water isolate, Mandapam, southern India	99.31	62.81	[25]
P. leiognathi subsp. *mandapamensis*(DQ790854)	*Acropomajaponicum*	99.37	63.00	[24]
P. mandapamensis (DQ371377)	*A. japonicum*, Taiwan	92.74	62.72	[24]
P. leiognathi subsp. *mandapamensis*(DQ790849)	*A. japonicum*	91.34	62.67	[24]
P. leiognathi subsp. *mandapamensis*(DQ371393)	*Photopectoralispanayensis*, Panay Gulf, Visayan Sea	89.67	62.01	[24]
P. aquimaris (JQ229765)	-	87.54	62.44	[26]
P. phosphoreum (X55458)	-	69.92	62.21	[2]
P. kishitanii (AY642185)	*Chlorophtalmusalbatrossis*, Kumano Sea	69.94	62.25	[27]
P. kishitanii (AY642227)	*C. albatrossis*, Kumano Sea	69.84	62.22	[27]
Vibrio harveyi (DQ436496)	-	38.69	80.68	-
*P. leiognathi*strain SYA2 (MZ568799)	*S. leptolepis*	100	63.63	This study

CONCLUSION

Bioluminescent bacterium was successfully isolated from marine fish and identified as *P. leiognathid* strain SYA2 based on *lux*AB gene sequence with 82% confidence level. The size of amplified *lux*A and *lux*B of the *P. leiognathid* strain SYA2 are 766 bp and 941 bp, respectively. Thus, the isolated bacteria can be utilised in many ways to benefit humans such as biosensors for pollution detection and integration of luciferase into reporter vector for bioluminescence imaging.

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REFERENCES

1. Ferri, Stefano R., et al. "Structure and properties of luciferase from Photobacteriumphosphoreum." Biochemical and biophysical research communications 176.1 (1991): 541-548.
2. STEWART, GSA B., and P. Williams. "Lux genes and the applications of bacterial bioluminescence." JGM. Journal of general microbiology 138 (1992): 1289-1300.
3. Heitzer, Armin, et al. "Specific and quantitative assessment of naphthalene and salicylate bioavailability by using a bioluminescent catabolic reporter bacterium." Applied and Environmental Microbiology 58.6 (1992): 1839-1846.
4. Iyer, Sweta Narayanan, et al. "Toward bioluminescent materials by plasma treatment of microfibrous nonwovens, followed by immobilization of one or both enzyme (s)(luciferase and FMN reductase) involved in luminescent bacteria." ACS Applied Bio Materials 3.5 (2020): 3401-3412.
5. Meighen, Edward A. "Molecular biology of bacterial bioluminescence." Microbiological reviews 55.1 (1991): 123-142.
6. Brodl, Eveline, Andreas Winkler, and Peter Macheroux. "Molecular mechanisms of bacterial bioluminescence." Computational and structural biotechnology journal 16 (2018): 551-564.
7. Dunlap, PAUL V., and Kumiko Kita-Tsukamoto. "Luminous bacteria." Prokaryotes 2 (2006): 863-892.
8. Nealson, Kenneth H., and John W. Hastings. "Bacterial bioluminescence: its control and ecological significance." Microbiological reviews 43.4 (1979): 496-518.
9. Xuan, Guanhua, et al. "Expression of genes encoding the luciferase from Photobacteriumleiognathi in Escherichia coli Rosetta (DE3) and its application in NADH detection." Luminescence 33.6 (2018): 1010-1018.
10. Kumar, Sudhir, et al. "MEGA X: molecular evolutionary genetics analysis across computing platforms." Molecular biology and evolution 35.6 (2018): 1547.
11. Tamura, Koichiro. "Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G+ C-content biases." MolBiolEvol 9.4 (1992): 678-687.