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MOLECULAR CLONING AND EXPRESSION OF THE LUX GENES OF *VIBRIO FISCHERI*

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ABSTRACT:

Vibrio fischeri is a symbiotic marine bacterium and a nonpathogenic member of the Vibrionaceae which produces luminescence by expressing the lux operon. Lux operon encoding luciferase and proteins. In this study, we amplified the chromosomal fragment contains *luxG* of *V. fischeri* and *V. harveyi*, amplified fragment cloned into the pUC18 vector and sequencing to confirmed the fragment. The sub cloning of *luxG* gene was carried out in the pUC18 expression vector and expression procedures were performed in *Escherichia coli* strain. As a result, a 2046 bp fragment which contains the whole fragment of luciferase coding genes and intergenic sequences were cloned in pUC18 expression vector. pUC18 *luxG* recombinant plasmid was confirmed by restriction analysis In this study, cloning of the luciferase coding genes was performed successfully, in which the synthesized construct can be applied as a reporter cassette in prokaryotic systems and as a marker or tag in the manipulation, and the control of gene expression in the fields of research, production, control of microorganism and other biotechnological applications.

KEYWORDS: *Vibrio fischeri, E.coli, pUC18 vector,* lux operon, luciferase, luminescence.

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INTRODUCTION

Vibrio fischeri is a marine bioluminescence bacterium and a nonpathogenic member of the Vibrionaceae, a large family of marine-proteobacteria, (Thompson *et al.,* 2004) it lives both as a free living organism and also as a symbiotic in the light-emitting organs of the Hawaiian bobtail squid, *Euprymna scolopes*, where it produces luminescence by expressing the lux operon (Nyholm and Mcfall-Ngai, 2004; Ruby, 1999; Visick and Mcfall-Ngai, 2000). In seawater and free-living form, *V. fischeri* exists at low cell densities and appear to be non-luminescent, while in light organ symbiosis with fish and squid, where the density of *V. fischeri* cells is high, it is usually luminescent (Sitnikov *et al.,* 1995). In the best studied luminous bacterium, *V. fischeri*, there are at least eight lux genes encoding the proteins essential for luminescence. The lux regulon is organized in two operons with a divergent transcription pattern. The seven genes (*luxICDABEG*), followed by a transcriptional terminator comprise the rightward operon, which encode the enzymes required for the synthesis of the autoinducer (*luxI*) and the alpha (*luxA*) and beta (*luxB*) subunits of the enzyme luciferase, the *luxC*, *luxD* and *luxE* genes encode the enzymes participating in the formation of the long-chain aldehyde, *luxG* is not essential for luminescence but is believed to increase the capacity of the cell to synthesize flavin mononucleotide (FMN). *luxG* from *Photobacterium leiognathi* TH1 was cloned and expressed in *Escherichia coli* in both native and C-terminal His_6-tagged forms. Sequence analysis indicates that the protein consists of 237 amino acids, corresponding to a subunit molecular mass of 26.3 kDa. Both expressed forms of LuxG were purified to homogeneity, and their biochemical properties were characterized. The leftward operon consists of a single gene, *LuxR*, encoding the transcriptional regulatory

protein (Visick *et al.,* 2000). In the luminescence reaction, luciferase converts aliphatic-aldehyde substrate, oxygen and reduced FMN (FMNH2) into the corresponding aliphatic acid, water and FMN, with the concomitant production of light. In the absence of the aldehyde substrate, luciferase catalyzes a reaction that yields no light and produces oxygen radicals rather than water (Nelson *et al.,* 2007). Luminescence in *V. fischeri* is controlled by a population density- responsive regulatory mechanism called quorum sensing (Dunlap, 1999). The aim of the current study was to clone the *luxG* subunits of the enzyme luciferase gene in a suitable prokaryotic expression vector in order to express and produce the desired protein in *Escherichia coli* and it can be used as a reporter gene construct for further biological and biotechnological applications. Moreover, purification and testing the biological activities along with comparison with other reporter gene systems or other constructs which carry luciferase genes will be the next goals of this research work.

MATERIALS AND METHODS

Bacterial strains and plasmids: *V. fischeri* strain, was isolated by scales of marine water fish (Angel fish) which was grown at 20 to 25°C. Luria Bertani media (7.2 to 7.4) used for the growth of *V. fischeri* was tryptone (10.0gm), yeast extract (5.0gm), sodium chloride (5.0gm), agar (18.0gm), distilled water (1000.0ml). Cloning and expression pUC18 (2.8 kb) vector were provided to niTza Biological Research Centre, Hyderabad respectively.

Genomic DNA extraction, PCR and sequencing: Genomic DNA extraction was carried out by Alkaline lysis method (Birnboim and Doly, 1979), purified and precipitated (Sambrook and Russell, 2001). Sense and antisense oligonucleotide primers were designed (Table 1) based on the nucleotide sequence data of *luxG* obtained from Gene Bank. PCR reaction mixture (30 µl) contained, 1µg DNA 0.1 mM dNTPs, 1.5 mM MgCl2, 1X PCR buffer, 20 pmol of each primers and 1.25 units Taq DNA polymerase. PCR amplification was performed under the following condition: denaturation step; 5 min at 94°C followed by 30 cycles of 40 s at 94°C, 60 s at 51°C in the annealing step and 60 s at 72°C for the extension step. The resulting PCR product was extended for a further 5 min at 72°C.

Table 1. Primers for amplifying *lux* genes by PCR.

Luxg	Primer
Forward	5' agactcgaatggcttttgagtca 3'
Reverse	5' tgaaggttacattgtcacgaaac 3'

The primers are presented from 5' to 3' ends; the introduced restriction sites are underlined.

The PCR product was analyzed on a 1.5% agarose gel and purified by using DNA extraction kit (Fermentase Lithuania) according to the manufacturer's instructions and subjected to sequencing using dideoxynucleotide chain termination method.

Construction of recombinant plasmids pUC18-*luxG*: To construct luminescent *E. coli*, purified PCR product of *luxG* gene was restricted by ECOR I enzyme and ligated to 1 µl T_4 DNA polymerase enzyme digested and transformed in *E. coli* (DH5α Strain) competent cells. Recombinant clones were confirmed by universal PCR and restriction enzyme digestion analysis. The *luxG* gene was released by BamH1 and Kpn1 digestion and subcloned in to the pUC18 expression vector.

Transformation of recombinant cell in LB media: 100 µl of competent cell was taken into the appendorf tube, then 40 µl recombinant samples were added in this tube. Incubated at 42ºC for 90 sec. to 2 minutes kept it in ice cold condition for 10 minutes. 1 ml of LB broth was added. Incubated at 37ºC for 1 hour. Five LB agar plates were taken in which two plates were with ampicilin and another two plates without ampicilin, and one plate was competent treated as a control. 200 µl of solution in each plate was taken and spread on the LB agar plates, incubated at 37 ºC for overnight (Heat Shock method).

RESULTS

A DNA fragment encoding the *luxG* part of lux operon was amplified by PCR using sense and antisense primers, specific restriction sites for BamH1 and Kpn1 were introduced into 5´ end of forward and reverse primer, respectively. The PCR product was analyzed in 1.5% agarose gel after electrophoresis (Fig. 1). The PCR product of *luxG* was purified from agarose gel using Fermentase kit and ligated into cleaved pUC18 cloning vector.

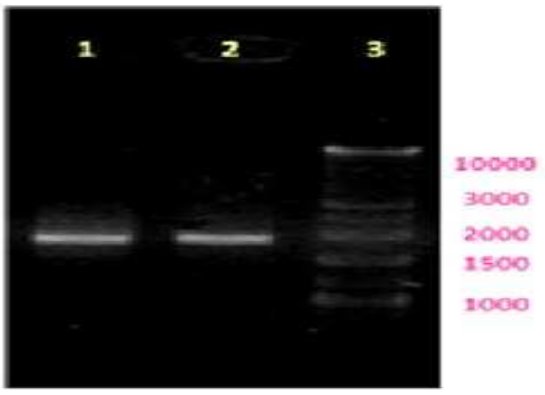

Fig. 1. PCR amplification of the *luxG* coding region using FluxG and RluxG primers. Lane 1 and 2 are the same PCR products; Lane 3 is a DNA ladder marker.

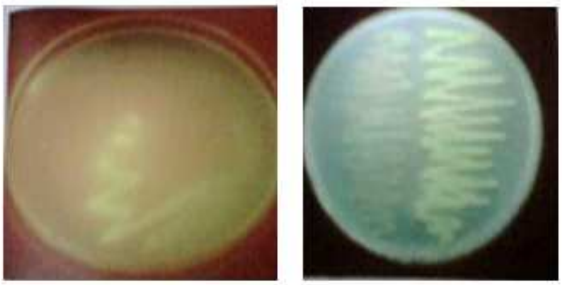

Fig. 2. Transformed plates to glowing the luciferase coding genes

Following the confirmation of the cloned luciferase coding genes by sequencing, using BamH1, Kpn1 restriction enzymes pUC18/*luxG* plasmid was digested, a 2046 bp DNA fragment purified, ligated into the ECOR I sites of an expression vector (pUC18) and transformed into *E. coli DH5α Strain*; recombinant plasmid was confirmed through restriction digestion using ECOR I enzymes and named pUC18/*luxG*; subsequently, transformation procedure continued into *E. coli* expression strains. Transformed plates with Ampicillin: which are capable to grow colony that is called transformed cell which is luminescent (light emitting cell), it can be seen in transilluminater. Non Transformed plates without Ampicillin: which are not capable to grow colony that is called non transformed cell which is not emit light.

DISCUSSION & CONCLUSION

Gene screening with an easily assayable product, reporter genes, amplify the signal from the cell surface to produce a rapid, highly sensitive, reproducible and easily detectable response. The variety of reporter genes available including β-galactosidase (*lac*Z), chloramphenicol acetyltransferase (*CA*T), insect luciferase (*luc*), bacterial luciferase (*lux*), alkaline phosphates (*phoA*), β lactamase (*bla*), β -glucuronidase *uidA (gusA, gurA)*, green fluorescent protein (*GFP*) and their applications are very broad in both *in vitro* and *in vivo* assays (Jiang *et al.,* 2008; Köhler *et al.,* 2000; Naylor, 1999). Among all studied reporter genes, bacterial luciferase are the most abundant, widely distributed, extensively studied and the best understood of all types of bioluminescent genes. It is G heterodimer, a flavine monooxygenize which is homologous in all bacterial luciferase, catalyzes the oxidation of a long-chain aldehyde and releases energy in the form of visible light. Yet luciferase coding genes have been isolated and cloned from different bacterial strains like *Vibrio harveyi*, *V. fischeri*, *Photobacterium phosphoreum*, *Photobacterium leiognathi* and *Xenorhabdus luminescens* in separate or fusion forms (Meighen, 1994; Wilson and Hasting, 1998). The lux genes have been transferred into *E. coli* and a multitude of different prokaryotic species by transformation, transduction, conjugation or even bacterial genome integration, using a variety of different plasmid vectors (Meighen, 1991). Previous studies indicate that, the genes encoding luciferase subunits can generate heterodimers in various forms (AB1, AB2, BA1, BA2 and A+ B), but the enzyme activity is decreased in comparison with wild type binary A+B construct, especially in the case of BA1 and BA2 forms which their activity reported to be about 2% of the wild type (Olsson et al., 1989). In contrast, the current synthesized construct pUC18. *LuxG* not only simplify the use of lux system as a reporter enzyme in prokaryotic cells, but also can provide a special condition in which both lux genes express simultaneously, from a same promoter (T7). Furthermore, it has been shown that obtaining the high expression of the lux genes requires a strong promoter and ribosome binding site (RBS) on the expression plasmid that can increase the amount of product, in which both are available in this effort using pUC18 vector. In the previous researches, expression of luciferase (*luxG*) component of the lux system using multiple plasmids (PB, pGMC12, pFIT001, Plx, pRS1105, pCVG) about *V. harveyi* and in the fusion form (pCK218) about *V. fischeri* have been reported (Greer and Szalay, 2002) and it was transformed in *E.coli*, By using the lux genes as reporters of gene expression, the strength and regulation of transcription from various promoters can be readily monitored and also light emission can easily be detected and measured. Furthermore, this reporter cassette can be used as a marker or tag in the manipulation and the control of gene expression in the fields of research, production, control of microorganism and other biotechnological applications.

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