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PCR BASED GENE IDENTIFICATION OF LUX OPERON IN LUMINESCENT *VIBRIO CHAGASII* ISOLATED FROM KARACHI COAST

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ABSTRACT

The phenomenon of emitting visible light by an organism as a consequence of a natural chemical reaction that take place inside them is called Bioluminescence. Wide number of marine animals and microbes possess this extraordinary feature and it is reported to be the primary source of light in the dark ocean. In the current study, the luminescent bacterial strain *Vibrio chagasii* (DGU227) isolated from the coastal water of Arabian Sea. The strain DGU227 showed sharp luminescence in presence of Ampicillin. The four genes *DABE* of lux operon were identified through PCR and the order of genes confirmed through intergenic sets of primers i.e., DABE. This unique feature of luminescence makes the isolated luminescent strain a potentially advantageous reporter for performing various genetic engineering experiments.

Key words: Bioluminescent, *Vibrio chagasii*, *lux operon*, Gene Orientation, PCR.

INTRODUCTION

Light is an important element of many systems including the living system (Reeve *et al.*, 2014). Glowing organisms are present in almost all habitat. Among these bioluminescent organisms, luminescent bacteria are widespread and found almost everywhere including marine, freshwater, and terrestrial environment. They are usually distributed throughout the oceans having symbiotic, saprophytic, and parasitic relationships with other organisms, also occur as free-living in marine domain (Herring, 1987). Luminous bacteria mostly dwell in seawater, on body surfaces and inside the intestinal tract of large number of marine animals (Nealson and Hasting, 1979). Many studies on ocean bioluminescence reveal that there are innumerable luminous bacteria in sea water. Bioluminescence is a form of chemiluminescent reaction, resulting in the emission of visible light from living system (Hasting, 1983; Gitelson and Crompton, 1984). This phenomenon assists numerous invertebrates to communicate, attract mates and for defense as well. A bioluminescent flash sometimes can be seen from hundreds of meters away (Warrant and Locket, 2004; Turner *et al*., 2009)**.** Considerable variability has been reported at species level in metabolic patterns of luminescent bacteria and these differences may reflect ecologically important traits for individual species (Adin *et al*., 2008). During chemical reaction, visible light is generated because of energy released when luciferin (light emitting molecule) is oxidized. The rate of reaction of luciferin is controlled by an enzyme, either a luciferase or a photo protein (a luciferase variant), in which some co-factors for example Ca^{++} or Mg^{++} are required for light emission as these factors are responsible for conformational changes in protein, they bind together as a single unit and helps to control light emission precisely (Haddock *et al*., 2010).

Light emitting reaction in most of the bioluminescent organism is catalyzed by luciferase (a heterodimeric protein consists of two subunits i.e., α and β with molecular weight of ~43 and 38 kDa, respectively. As substrate, the reaction requires aldehyde (long chain), reduced FMNH, and molecular oxygen (Meighen, 1991). The products produced during luciferase catalyzed reaction includes a long chain fatty acid (RCOOH), oxidized flavin, water and light.

$$RCHO + FMNH_2 + O_2 \longrightarrow RCOOH + FMN + H_2O + light$$

The luminescent systems (lux operon) in marine bacteria consists of genes *lux A* and *lux B* codes for an enzyme luciferase and *lux C, lux D, lux E* codes for fatty acid reductase complex essential for light emission (Meighen, 1991). Lux system is easy to clone and can be transformed in variety of organisms. Firefly luciferase system is more suitable for expression in eukaryotes whereas in prokaryotes the bacterial system works ideally. However, eukaryotic cells when grow at lower temperatures can express considerable amount of bacterial luciferase indicates that the bacterial lux system can also be used in higher organisms (Ow *et al*., 1986).

Here, we report some molecular characteristics of bioluminescent bacteria *Vibrio chagasii*, obtained from Arabian Sea near costal area of Karachi, Pakistan.

The present research further aims to develop a device using genes from luminescent bacteria which can detect metal contamination in candidate samples. The molecular characteristics identified, can be used as a basic information to produce desired biosensor construct.

MATERIALS AND METHODS

Bacterial strain

The luminescent bacterial strain DGU227 having GenBank accession no. JF342691 of 16SrRNA gene (Badar *et al*., 2012) was selected from stock culture of Department of Genetics, University of Karachi, Karachi, Pakistan.

Growth Conditions

Luminescent agar (L.A.) medium was used to grow bioluminescent bacteria, composed of NaCl: 30g/L, Yeast Extract: 5g/L, Bactopeptone: 10g/L, dH_2O: 1L, 1.8 % Agar: 15g/L. Strain was grown best at 20˚C.

Isolation of Plasmid and Genomic DNA:

The isolated strain was cultured overnight at 20˚C in L.A. broth and cells were harvested by centrifugation at 8000 rpm in 1.5 mL centrifuge tubes (Eppendorf) for 2 minutes at room temperature. Plasmid DNA was isolated by Gene JET Plasmid mini prep Kit (Fermentas Life sciences). Phenol chloroform method was used to isolate genomic DNA of luminescent marine bacterial strain (Sambrook *et al*., 1989).

Primer Designing

The specific primers were designed for *vibrio chagasii* bacterial strain for the confirmation of location and Amplification of *Lux operon*. Primers were designed using NCBI *Primer Design Tool.* The sequences of primers are given in (Table 1).

PCR amplification of *luxA, luxB,* and *luxD* gene

The Polymerase Chain Reaction (PCR) was carried for 25 cycles with touch down range (each cycle consisting of 94°C for 1 minutes, 62°C-55°C for 30 seconds, and extension at 72°C for 1 minute). Initial denaturation was done at 94°C for 3 minutes and final extension at 72°C for 10 minutes. Plasmid and genomic DNA of the strain was used as a template to confirm *lux operon* location. Total of 50µl of reaction mixture was prepared with PCR master mix (2X) by Promega, 5µL of template DNA and Forward and reverse primer (each at a concentration of 10 pmol). A 10µL aliquot of the reaction mixture was examined visually on a 0.8 % (wt/vol) agarose gel stained with ethidium bromide.

Orientation of Lux operon:

The orientation of *Lux operon* was identified by overlapping PCR with intergenic sets of primers.

RESULTS

Bacterial strain

The bacterial strain DGU227 *Vibrio chagasii* (GenBank_Accession no: JF342691) was found to grow best at 20°C in a cooling incubator on Luminescent Agar (L.A) medium moreover, it showed luminescence on sharply when grown in the presence of ampicillin (500µg/ml) (Fig. 1).

PCR amplification of *lux genes A, B, D* and *E*

Both plasmid and genomic DNA were used as template to determine the location of the operon. Only the samples containing plasmid DNA as template shows positive results. The amplification of *lux* genes *A, B, D* and *E* yields 625bp, 678bp, 312bp and 130bp, respectively validates the presence of *Lux operon* on plasmid (Fig. 2).

Orientation of Lux Operon:

The orientation of *lux* genes was identified by using intergenic primers given in (Table I**)**, the order of the genes in operon appears to be *DABE* as PCR amplification was positive for DA, AB and BE set of primers giving a product of sizes 937bp, 1303bp and 815bp respectively, as shown in (Fig. 3). Orientation of Lux operon has been illustrated in (Fig. 4).

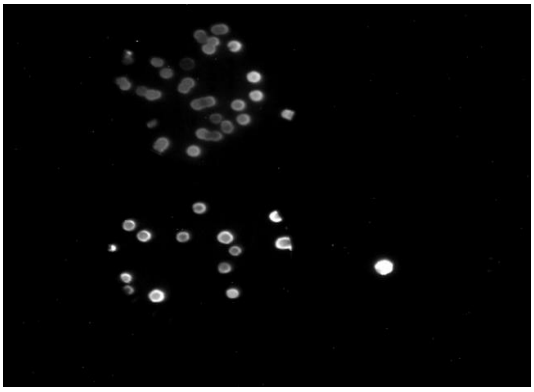
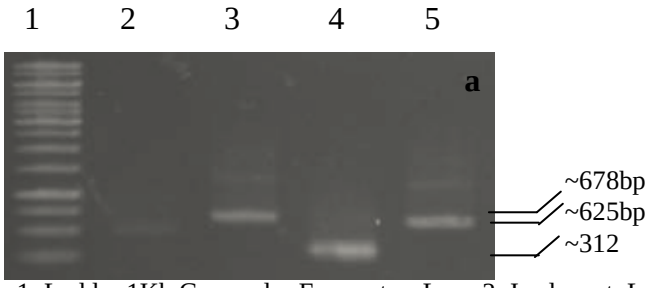
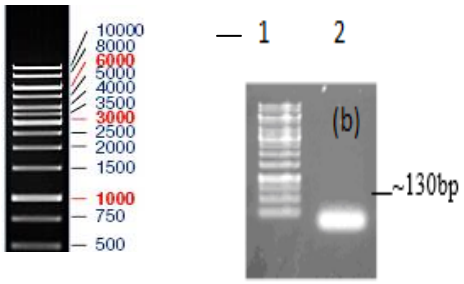


Fig. 1. Glowing colonies of *Vibrio chagasii* bacterial strain in L.A growth media supplemented with 500µg/mL Ampicillin.



(a) Lane 1: Ladder 1Kb Gene ruler Fermentas, Lane 2: Irrelevant, Lane 3: PCR product for gene *B*, Lane 4: PCR product for gene *D*, Lane 5: PCR product for gene *A*.



(b). Lane 1: Ladder 1Kb Gene ruler Fermentas; lane 2: PCR product for *luxE* gene.
Fig. 2. PCR Amplifications of *lux* genes, *D*, *A*, *B* and *E*.

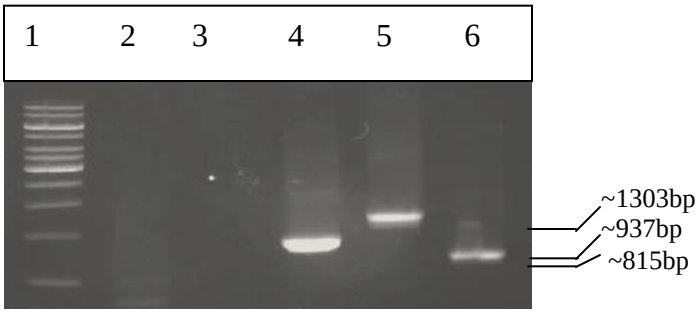


Fig. 3. Amplifications of *luxDA*, *AB*, and *BE* gene. Lane 1: Ladder 1Kb Gene ruler Fermentas; Lane 2 and 3 irrelevant; lane 4: PCR product for gene *DA*; Lane 5: PCR product for gene *AB*; Lane 6: PCR product for gene *BE*

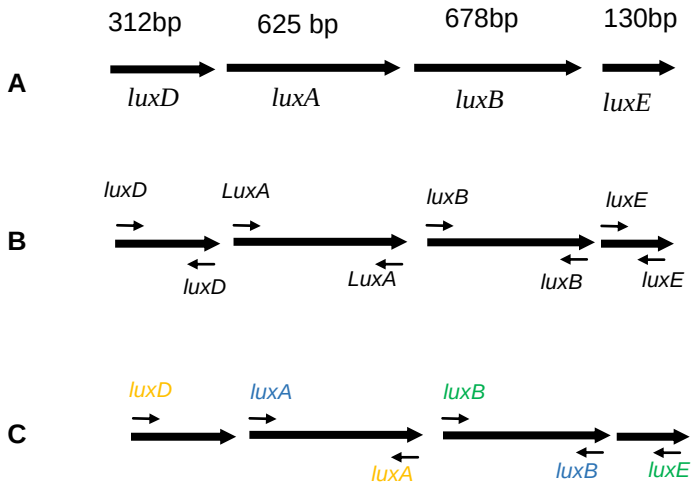


Fig. 4. Orientations of *lux operon* genes in *Vibrio chagasii* bacterial strain. A: showing *lux* genes DABE; B: showing positions of forward and reverse primer of *luxD, luxA, luxB* and *luxE*; C: showing position of forward and reverse primers for intergenic identifications of *luxDA* (937bp), *luxAB* (1303bp) *and luxBE* (815bp) genes.

Table I. Oligonucleotide sequence of *Lux operon* genes of *vibrio chagasii*.

Primers	Sequence (5'->3')	Length	Tm
LUXD			
Forward primer	CGCTTGCCAAGGTCGCTCAT	20	57.98
Reverse primer	CGGCTTTTAGACGGCGCTCA	20	57.74
LUXA			
Forward primer	TCCGCTTCGGTATCTGCCGC	20	59.29
Reverse primer	GCGATGCATTCTTCAGGCGTGC	22	59.69
LUXB			
Forward primer	CCTGTCCGCGTTGCCGAAGA	20	59.70
Reverse primer	TTGCTGCGTCGTGTCTGCGA	20	59.64
LUX E			
Forward primer	TCGGAGTCGGAGAGGTGGTCG	21	59.45
Reverse primer	GGTGCCACTAGTGCCGCTAC	20	57.44

Table showing forward and reverse Primer Sequences and respective melting temperatures of genes *luxD, luxA, luxB* and *luxE*.

DISCUSSION

These glowing creatures not only capture the attention of biologists, and researchers but fascinate general public as well. Physiology, biochemistry and genetic elements of bioluminescence are focus of researchers since ages. The use of luminescent genes as biosensors/bioreporters have brought revolution in environmental biology.

For current study, luminous bacteria isolated from sea water was selected from stock culture of department of Genetics, University of Karachi. Bacterial strain was long term preserved in glycerol. The glycerol preserves behaved perfectly and whenever required strains revived within 24 hours. We have selected luminescent bacteria *Vibrio chagasii*, identified through 16S rRNA gene sequencing (Badar *et al.*, 2012). The luminescence was sharper while growing in an antibiotic (ampicillin) supplemented medium. In bacteria, the expression of genes responsible for bioluminescence are clustered together and controlled by an operon called *Lux* operon. The orientation of *lux* genes were identified by using intergenic sets of primers. The bioluminescent genes *lux CDABE* are common to all known *lux* systems and are transcribed in that order (Meighen, 1994).

The ease of detection of *lux* gene organization (*lux* operon) has stimulated the use of bioluminescence genes for the construction of whole cell biosensors with numerous applications (Chatterjee and Meighen, 1995). Synthetic biology aims to design and model novel biomolecular components, networks and pathways, to rewire and reprogram organisms to provide solutions for various challenges. The applications of biosensors include detection and quantification of toxic contaminants in food, soil, water and air (Pititsyn *et al.*, 1997), detection of pathogens (Chatterjee and Meighen, 1995), monitoring of genetically engineered bacteria (Fleming *et al.*, 1994). Cellular metabolic activity can also be investigated through biosensors (Unge *et al*., 1999).

It is concluded that the bacterial strain DGU227 harbors a complete lux operon on a plasmid with the gene array DABE. The present research further aims to develop a devise using luminescent system that can detect metal contamination in variety of suspected samples.

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