ISSN (Online) 2456 -1304

International Journal of Science, Engineering and Management (IJSEM)
Vol 2, Issue 12, December 2017

Microbial Association with Light Emission; Emitting Microbes

[1] Abha Tiwari , [2] U.K. Chauhan
[1] Dept. of Biotechnology and Microbiology Studies, A.P.S. University, Rewa (M.P.)
[2] Professor, Dept. of Biotechnology and Microbiology Studies, A.P.S. University, Rewa (M.P.)

***Abstract*: Mechanism of luminescence is a chemical reaction that produces brightness in firely. Living organisms including certain bacteria are capable of luminescence. Bacteria are the most abundant luminescent organisms in nature. Bacterial luminescence is due to the action of the enzyme called luciferase. Other interesting features of marine microorganisms are having their ability to survive at low temperatures and high salinity. The emission of light is a result of the enzymatic activity and biochemical of the living organism (Malave & Orengo et al.,2010). While the luminescence (lux) genes of symbiotic V. fischeri have been shown to be highly induced within the crypts, the role of these genes in the initiation and persistence of the symbiosis. We including genetic elements that allow recombination with the host, the lux DNA can also be integrated into the bacterial genome. Interaction of the two fusion proteins results in energy transfer from the bioluminescent molecule to the fluorescent molecule, with a concomitant change from blue light to green light (Angers et al. 2000). In this review we had discussed Bioluminescence emission of different patterns as flashes (Fireflies, Squids), continuous glow (Bacteria, fungi), blinking (Bacteria), wavy (sea pansy, Renilla), and switched on and off the system (Angler fishes). Although bacterial luciferases, particularly from V. harveyi and X. luminescens, are quite stable the fused luciferase cannot fold efficiently at 37°C (Ulitzurs, & Kuhnj, 2006).Bioluminescence property of these different microbes may also be used in different fields also.**

***Keywords*: Crypts, Luminescence, Wavy, Concomitant, Emission.Key words: Crypts, Luminescence, Wavy, Concomitant, Emission.**

I. INTRODUCTION

Bioluminescent organisms with their attractive beauty and ease of detection have drawn interest among scientists to study about them. It is the ability of organisms to release visible light by using natural chemical reaction. The emission of light is a result of the enzymatic activity and biochemical of the living organism (Malave & Orengo et al.,2010). Bioluminescence has been established in the diverse group of organisms ranging from microorganisms like bacteria and protists to the macro-organism such as fish and squid. Meighen et al.,1988, had studied that the light producing organisms can be found in terrestrial, lake and oceanic classes (Danyluk et al.,2007). The most abundant and widely distributed light emitting organism is luminous bacteria, and this will be found as free-living in the ocean, as gut symbionts in the digestive tracts of marine fish, as parasites in crustacean and insects, as light organ symbionts in teleost fish and also as saprophytes growing on dead fish or meat (Danyluk et al., 2007; A. Nawaz et al., 2011). These sets of species are diversely spread in terrestrial environment, freshwater and also in a marine ecosystem. These bioluminescent bacteria reported belonging to the class Gamma proteobacteria. Among the bioluminescent bacteria reported, 17 bioluminescent species were currently known (Kita-Tsukamoto et al., 2006). The luminous bacteria were also classified into three genera which are Vibrio, Photobacterium and Xenorhabdus (Photorhabdus) and the species within the genus Vibrio and Photobacterium usually exist in the marine environment and Xenorhabdus belong to the terrestrial environment. Bioluminescence is the phenomenon that consists of light emission from the results of biochemical and enzymatic activity of the living organism (Danyluk et al.,2007). It is a group of genes that are

responsible for this known as the lux operon found in the luciferase enzymes (Bluth et al.,2013). The reaction of bioluminescence involves the oxidation of a long-chain aliphatic aldehyde and reduced flavin mononucleotide (FMNH2). This mechanism needs oxygen and is catalyzed by enzyme luciferase. In this process, the excess energy is liberated and emitted as a luminescent blue-green light at 490nm (Kumar,2010; Malave-Orengo et al.,2010). The lux operon basically contains the genes lux ICDABEG. Lux A gene and LuxB gene are the luciferase gene which codes for α and β subunits respectively. On the other hands, lux CDE genes are fatty acid reductase genes that codes for polypeptides. It is important and necessary for the conversion of fatty acids into the long chain aldehyde (fatty acid pathway) (Malave-Orengo et al.,2010); E.A. Meighen.,1991). Bacterial luciferase is also the target gene in a mutagenicity assay and has been used to quantitate long-chain aldehydes protease activity and intracellular concentrations of NADH,

ISSN (Online) 2456 -1304

International Journal of Science, Engineering and Management (IJSEM)
Vol 2, Issue 12, December 2017

NADPH, and flavin mononucleotide (FMN) (Baldwin TO. et. al.,1983). Therefore, the reaction of bioluminescence emission of light can be written as follows:

$$FMNH_2 + RCHO + O_2 \longrightarrow FMN + H_2O + R\text{-}COOH + light\ (490\ nm)$$

2. MECHANISM OF BIOLUMINESCENCE IN DIFFERENT SPECIES:

Bioluminescence emission may be in different patterns as flashes (Fireflies, Squids), continuous glow (Bacteria, fungi), blinking (Bacteria), wavy (sea pansy, Renilla), and switched on and off the system (Anglerfishes).

2.1. Firefly: Bioluminescence is an enchanting process in which living organisms convert chemical energy into light. The light results from the oxidation of an organic substrate, a luciferin, catalyzed by an enzyme called a luciferase. In nature, there is an amazing diversity of organisms that emit light including bacteria, fungi, crustaceans, mollusks, fishes and insects (Hastings, 1995). While the specific biochemistries of bioluminescence are diverse, all include an enzyme-mediated reaction between molecular oxygen and an organic substrate. It is likely to that all bioluminescence processes involve the formation and breakdown of four-member ring peroxide or a linear hydroperoxide (Wilson, 1995; Wood, 1995). An overview of the chemical and mechanistic aspects of a major bioluminescence process that of the bioluminescent beetles will be presented here. The reader is also directed to another review of firefly luciferase (Inouye, 2010). Representing an estimated 2,000 species of luminous beetles (Coeleoptera), are three families: Lampyridae (the true fireflies), Phengodidae (click beetles), and Elateridae (glow-worms). Kang et al., 2006; Naylor,1999; Gelmini et al., 2000; Kalra et al., 2011; Dragulescu-Andrasi et al.,2011; Branchini et al., 2010; Kato et al., 1997)

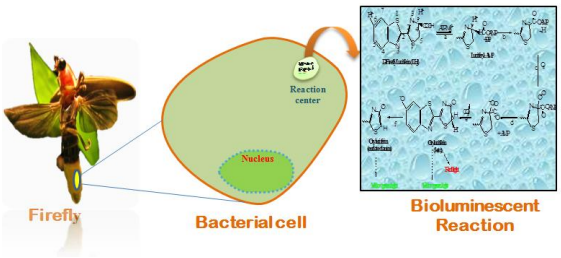

Figure.No.1. Showing mechanism of Bioluminescence in firefly.

Following the formation of the enzyme-bound luciferyl adenylate (step a), a proton is abstracted from the C-4 carbon of the adenylate by a basic side chain amino acid residue of luciferase (step b). Next, molecular oxygen adds to the newly formed anion (step c); and an electronically excited state oxyluciferin molecule and carbon dioxide are produced (step e) from a highly reactive dioxetanone intermediate (step d). According to the original mechanism based predominantly on chemo-luminescence model studies, red light emission (λ max 615 nm), which is observed at pH 6.0, results from the keto form of the emitter. At pH 8, the familiar yellow-green light emission (λ max 560 nm) is produced from the enolate dianion form of the excited state oxy-luciferin by a presumed enzymatic assisted tautomerization (step f). However, more recent experimental evidence with a firefly luciferin analog is consistent with the keto form of oxyluciferin alone being capable of producing all of the colors of firefly bioluminescence (Branchini et al., 2002). In nature, beetle luciferases display various colors of light from green (λmax ~535 nm) to red (λmax ~630 nm). Probably, luciferase modulates emission color by altering the resonance-based charge delocalization of the excited state, shown below in figure No.3 (Branchini et al., 2004; Viviani, 2002; Liu et al., 2008; Hirano et al., 2009; Navizet et al., 2010).

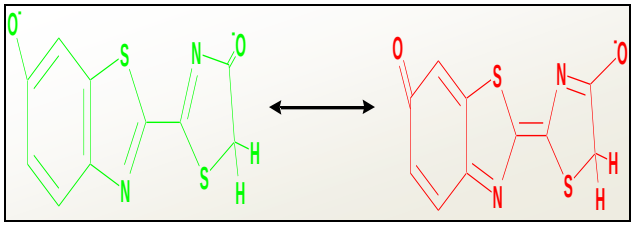

Figure no. 3. Showing the color change in compounds

2.2. Luminous Bacteria:

Those species that are involved into the light-emitting reaction are having an intracellular luciferase-catalyzed, oxidation reaction of reduced Flavin mononucleotide

ISSN (Online) 2456 -1304

International Journal of Science, Engineering and Management (IJSEM)
Vol 2, Issue 12, December 2017

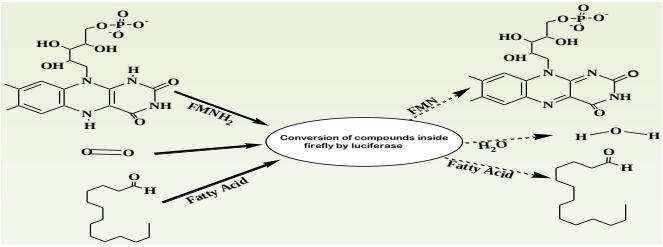

Figure no.2. Showing the reaction of bioluminescence in bacterial species

The Lumnicating nature of bacterial species requires oxygen, a source of energy, a luciferase enzyme and a long-chain fatty aldehyde. Bacterial luciferase reduced-FMN: oxygen oxidoreductase, 1-hydroxylating luminescent is a heterodimeric enzyme for which the subunits cc (~ 40 kDa) and p (x 37 kDa) is coded by the luxA and luxB genes respectively.

According to the observations finding by Foran & Brown (1988) that in both species of V. harveyi and V. fischeri, first four amino acid residues of the α and β subunits are identical. It has been suggested that luxA and luxB shows tandem duplication transferred from ancestors. The reactive conformation of luciferase is a αβ dimer but the active site is confined primarily to the α subunit (Cohn et al., 1985). Recently, LuxAB fusion proteins have been produced certain biological functions (Boylan et al., 1989; Escher et al., 1989; Kirchner et al., 1989. These fusion proteins have specific importance in improving expression in recombinant bacterial systems (Engebrech et al., 1983; Musa et al., 2008; Orengo et al., 2010; Kumar, 2010, Ransanga et al., 2012; Kannahi)
Maximum investigations of bioluminescent bacterial systems have been based upon two genera, Photobacterium and Vibrio. Enzymatic and regulatory functions required for expression of bioluminescent properties were firstly investigated (Engebrecht & Silverman, 1984). In addition to luxA and luxB, the lux operon characteristically contains three additional structural genes luxC, luxD and luxE that encode the subunits of a fatty acid reductase. The remaining lux genes, luxR and luxl, are involved in the regulation of lux expression (Meighen, 1988, 1991; Silverman et al., 1989). Maximum micro-organisms shows deficiency of genetic blueprint for luciferase and fatty acid reductase but they can supply FMNH2 , so for a dark bacterium such as Escherichia coli had the properties of bioluminescent, it only had the requirement of genetic transfer of luciferase genes and fatty acid reductase. With this addition, the genetic components necessary for bioluminescence are reduced to only 2 kb of DNA, comprising the luxA and luxB genes. Providing that these genes are expressed and that the luciferase is sufficiently stable, the potential exists to confer a bioluminescent phenotype on any prokaryotic organism. For eukaryotic micro-organisms the firefly luciferase (lux) (Tatsumi et al., 1988; Gould & Subramani, 1988; Alam & Cook, 1990) and other eukaryotic luciferases (Wood et al., 1989) are required.

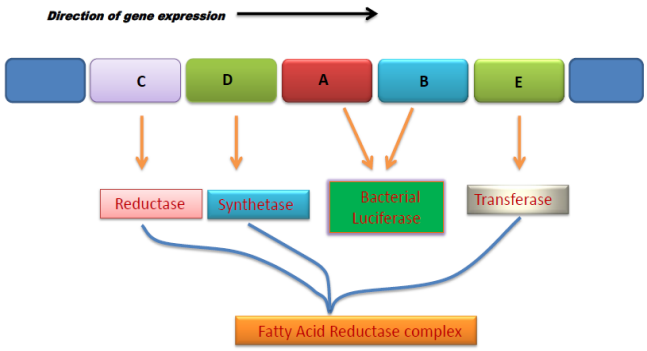

Figure no. 3. Showing the direction of gene expression

3. visualization of illumination:
The application of lux genes in any field of science requires the accessibility of instrumentation aimed for the visualization and quantification of photon emission. A scientific objective with appropriate instrumentation has possibly restricted the uptake of lux gene as a molecular biology tool. The most sensitive detector of photon emission which is available in most laboratories is the Scintillation Counter. A relatively unsophisticated instrument, without sample cooling, can be configured of less than 10 bioluminescent bacteria per ml of sample. To achieve this coincidence counting, a standard feature of scintillation counting must be disengaged, thus enabling the photon detectors to register every photon, not just those that arrive at the detectors with coincidence has been undertaken by Jago et al.1989; Engebrech et al., 1983; Musa et al., 2008;
Orengo et al., 2010; Ransanga et al., 2012; Kannahi and Shivshankari, 2014).

4. Gene transfer in species: The lux gene may be transferred to another species by the method of transformation by using different varieties of plasmids. The high expression of lux gene which requires a promoter on the plasmid. After the successful transformation, RNA polymerase RNA polymerase to recognize the transcription initiation sites in the lux DNA from other luminescent bacteria as a result of the absence of the appropriate regulatory lux genes and lux DNA has been transferred into a multitude of different prokaryotic species including many plant and mammalian pathogens. Transfer of DNA into the enteric bacteria, including E. coli, as well as into the gram-positive bacteria,

ISSN (Online) 2456 -1304

International Journal of Science, Engineering and Management (IJSEM)
Vol 2, Issue 12, December 2017

has been accomplished directly by transformation method. Transduction of lux DNA by means of bacteriophages. By including genetic elements that allow recombination with the host, the lux DNA can also be integrated into the bacterial genome. Interaction of the two fusion proteins results in energy transfer from the bioluminescent molecule to the fluorescent molecule, with a concomitant change from blue light to green light (Angers et al. 2000). A major limitation of many of these systems is the relatively long half-life of the reporter protein(s), which prevents monitoring both the initiation and the termination of transcription in real-time (Allen. et al., 2007)

Species	Method of Gene Transfer	Host Integration	Gene transferred
Citrobacter koseri	Transformation	-	Vf luxAB
Escherichia coli	Conjugation ,transformation ,transduction	±	Vf, Vh, Pp, P1, Xl, lux
Klebsiella aerogenes	transformation	-	Vf luxAB
Pseudomonas fluorescens	Conjugation	-	Vf luxABCDE
Salmonella typhimurium	Transformation, transduction	-	Vf luxAB
Shigella flexneri	transformation	-	Vf luxAB
Vibrio parahaemolyticus	Transduction	+	Vf luxABCDE
Xenorhabdus luminescens	Conjugation	-	Vf luxABCDE Vh luxABCD
Plant Pathogens			
grobacterium radiobacter	Conjugation	+	Vh luxAB
Agrobacterium tumefaciens	Conjugation	±	Vh luxAB, Vf luxABCDE
Bradyrhizobium japonicum	Conjugation	±	Vh luxAB, Vf luxABCDE
Erwinia amylovora	Conjugation	+	Vh luxAB
Erwinia caratovora	Conjugation	-	Vf luxABCDE
Pseudomonas glumae	Conjugation	-	Vf luxABCDE
Pseudomonas putida	Conjugation	-	Vf luxABCDE
Pseudomonas syringae	Conjugation	+	Vh luxAB
Rhizobium meliloti	Conjugation	-	Vf luxABCDE
Rhizobium leguminosarum	Conjugation	±	Vh luxAB, Vf luxABCDE
Xanthomonas campestris	Conjugation	±	Vh luxAB
Marine bacteria			
Vibrio harveyi	Conjugation	-	Vh.luxABCDEFGH, Pp luxABCDE
Vibrio fischeri	Conjugation	-	- Vh luxABCD

ISSN (Online) 2456 -1304

International Journal of Science, Engineering and Management (IJSEM)
Vol 2, Issue 12, December 2017

Photobacterium phosphoreum	Conjugation	-	- Vh luxABCD
Cyanobacteria			
Anabaena spp.	Conjugation	-	Vf luxAB, Vh luxAB
Gram-positive bacteria			
Bacillus megaterium	Transformation	-	Vf luxAB
Bacillus subtilis	Transformation	-	Vf luxAB,
Lactobacillus casei	Transformation	-	Vf luxAB
Lactococcus lactis	Transformation	-	Vf luxAB
Listeria monocytogenes	Transformation	-	Vf luxAB
Staphylococcus aureus	Transformation	-	Vf luxAB

Whereas: Vf =V. fischeri; Vh= V. harveyi; Pp= P. phosphoreum, PI= P. leiognathi, Xl=X. luminescens

5. Application of Bioluminescence:

•***Transformation:*** The primary function of bacterial luciferase is to catalyze the emission of light; this feature together with generation of the aldehyde substrate by fatty acid reductase can be successfully produced in other bacteria, by the transfer of the luxCDABE genes, which convert non-luminescent bacteria into light emitters.

•***Detection:*** It is also currently being practiced for the development of auto luminescent plants for light production and whole-cell biosensors for detecting various toxicants (Carmi, et al.,2009; Denyers. P et al.,1992 ; D. M. Close et al.,2011). Besides, cloning studies of luciferases of different luminescent organisms and their fluorescent proteins such as the well-known green fluorescent protein (GFP) have got a wide importance in bioluminescent Imaging (BLI) to study the interaction of infectious microorganisms with living cells (E.N. Harvey et al., 1992).

•***Screening:*** The ability to introduce the lux gene into different bacterial species provides a convenient method for rapidly screening for the presence of specific bacteria. Because very low levels of light can be measured, only a short period is required for transcription and translation of the lux genes before there will be sufficient synthesis of luciferase (Stewart, G et al., 1989, Ulitzur & Kuhn, Doyle et al. 2004; Shadel et al., 1990) for detection of light.

•***Recombinant preparation:*** Ulitzur & Kuhn (1987) described the engineering a 9kb DNA segment encoding the entire lux pathway from V. fischeri. Although the detection of specific pathogens such as Salmonella spp., Campylobacter spp. genes requires a committed programme for the genetic engineering of host-specific bacteriophage, this technology offers the potential for rapid, user-friendly microbial testing. A revolution in microbial testing can, however, is perceived with the near on-line detection of indicator micro-organisms (Kodikara et al., 1991). Recombinant lux bacteriophages can detect such indicator bacteria without recovery or enrichment, provided the bacteria are present in a food matrix at levels greater than l05 per gram .Since the assay requires only 30-50 min, this allows the evaluation of indicator strains in less than an hour.

•***Markers:*** The importance of in vivo bioluminescence as a reporter of cellular viability has been subject to several recent reviews (Branchini, B. R. et al., 2004: Hirano. T et al., 2009). Given that the production of light from recombinant bacteria containing the lux genes depends upon a functional intracellular biochemistry, it can be established that any substance or environment that impairs that biochemistry and thus compromises cellular viability which will lead to a reduction in light emission. In many cases there is a close interaction between the potential of lux to monitor gene expression and established as an environmental monitor of industrial effluents and toxicants. The most famous and rapid system for toxicity test is by using Vibrio fisheri. This system has some limitationbeing 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(Bulich A. A. et al.,1981; Kurvet I. et al.,2011). For example, the detection of aromatic hydrocarbons such as toluene and xylene could be achieved by constructing a lux gene fusion to the meta-pathway promoter from the TOL plasmids of Pseudomonas (Engebrecht, J et al.,1986). Dual-reporter assays can control for much of this variability, leading to more accurate and meaningful comparisons between samples (Hawkins et al. 2002; Hannah et al. 1998; Wood, 1998; Faridi et al. 2003). One of the earliest uses of luciferase imaging to detect microbial contamination involved eukaryotic firefly (Photinus) luciferase (Luc) not

ISSN (Online) 2456 -1304

International Journal of Science, Engineering and Management (IJSEM)
Vol 2, Issue 12, December 2017

bacterial luciferase samples. They suggested that this technology may also be used to distinguish genetically modified bacteria from indigenous bacteria in environmental samples (Levin GV, Chen CS et al.,1967).

6. CONCLUSION:

During the study of bioluminescent properties of bacterial species we are now concluded that phenomena of bioluminescence in nature have always been a focus of human attention. Prior to the identification of luminous organisms, the presence of even a dim luminous glow in the dark frightened countless numbers of individuals, who perceived the glimmer as ghosts or supernatural spirits. However, the curiosity of scientists to solve the mysteries of nature led to the identification of many luminous organisms responsible for the light emission in different environmental settings. The isolation of luminous bacteria from marine and terrestrial environments, and the characterization of their properties have resulted in the identification of a number of luminous bacterial species classified into several genera. All luminescent bacteria utilize FMNH2, O2, and long fatty aldehyde as substrates for the bioluminescence reaction catalyzed by luciferase (Lux-AB), with the fatty acid reductase complex (Lux-CDE) synthesizing the long chain aldehyde substrate of tetra decanal. A NADPH-dependent FMN reductase found in most if not all bacteria generates the FMNH2 substrate. We had found only the five genes in the lux operon, lux-CDABE, are needed to produce light emission, even in bacteria that normally do not emit light, and thus provide the opportunity to utilize the bacterial lux system as a light emitting sensor in many bacteria. The regulation of the induction of expression of the luxCDABE genes in luminescent bacteria at high cell density has led to the development of molecular prototypes for quorum sensing, an important new regulatory mechanism signaling bacterial crowding, and now found controlling key secondary metabolic pathways in many nonluminescent bacteria.

7. FUTURE PROSPECTS:

The high sensitivity, depend directly on the amount of luciferase over a very wide range and assay times of only seconds or minutes are unique features of luminescence assays. Besides, most assays for enzymes from non-luminescent organisms are relatively with narrow limits for the amount of enzyme and time of assay so that the response is directly proportional to the enzyme, conditions that may be difficult to achieve for routine analysis of samples expressed to different levels.

Currently, the major disadvantage in the use of the bacterial luciferase genes as reporter of gene expression resides in the necessity to use a monocistromic fused luxA-luxB gene for expression under a single promoter in eukaryotic cells. Although bacterial luciferases, particularly from V. harveyi and X. luminescens, are quite stable the fused luciferase cannot fold efficiently at 37°C (Ulitzurs,. & Kuhnj, 2006). This property would therefore limit the use of the fused bacterial lux gene in eukaryotic cells to plant, insect, and yeast cells grown at Temperatures at or below 30°C, which give very high levels of expression. Development of new luxA-luxB constructions coding for bacterial luciferases which can refold efficiently at 37°C will be necessary to obtain levels of expression in mammalian cells comparable to the expression of firefly luciferase. It is well established that luminescent bacteria (including V. harveyi) emit light efficiently only when they are at high cell density. This regulation is known as quorum sensing (Swift et al., 1998)

8. REFERENCE:

1. A. Nawaz, and N. Ahmed, Isolation and Characterization of Indigenous Luminescent Marine Bacteria from Karachi Coast. Academic Research International. Vol 1. Issue 2,2011, pp 74-83.

2. Almashanus, ., Musafiab, ., Hadar,R ., Suissa.M . & Kuhn. J. (1990). Fusion of Luxa and Luxb and Its Expression In E. Coli, S . Cerevisiae and D. Melanogaster. Journal Of' Bioluminescence and Chemiluminescence 5, 89-97.

3. Angers, S. et al. (2000) Detection of β2-adrenergic receptor dimerization in living cells using bioluminescence resonance energy transfer (BRET). Proc. Natl. Acad. Sci. USA 97, 3684–9.

4. Alam J & Cook, J . L. (1990). Reporter Genes: Application To The Study Of Mammalian Gene Expression. Analytical Biochemistry 188, 245-254.

5. Allen M, Wilgus J, Chewning C, Sayler G, Simpson M (2007) A destabilized bacterial luciferase for dynamic gene expression studies. Syst Synth Biol 1(1):3–9.

6. Ahmad, K. A., and G. S. A. B. Stewart. J. Appl. Bacteriol., Vol 2. Issue 2, 2013, pp 70-78.

7. A. R. Kumar, Isolation of Luminescent Bacteria from Bay of Bengal and their Molecular Characterization, Unpublished, 2010.

8. Baldwin TO, Berends T, Bunch TA, Holzman TF, Rausch SK, Shamansky L, Treat ML, Ziegler MM. Cloning

of the luciferase structural genes from Vibrio harveyi and expression of bioluminescence in Escherichia coli. Biochemistry. 1984 Jul 31;23(16):3663–3667.

9. Belas R, Mileham A, Cohn D, Hilman M, Simon M, Silverman M. Bacterial bioluminescence: isolation and expression of the luciferase genes from Vibrio harveyi. Science. 1982 Nov 19;218 (4574):791–793.

10. B. J. Bluth, S. E. Frew, B. McNally, "Cell-Cell Communication and the lux operon in Vibrio fischeri". (n.d) Retrieved December 5, 2013, from https://www.bio.cmu.edu/courses/03441/TermPapers/97TermPapers/lux/default.html

11. B. Danyluk, W. Uchman, P. Konicezny, and A. BilskaAn objective Method To Assess Bioluminescent Properties of Selected Bacterial Strains. Acta. Sci. Pol., Technol. Aliment., 2007, pp 5-16.

12. Beard SJ, Salisbury V, Lewis RJ, Sharpe JA, Mac-Gowan AP (2002) Expression of lux genes in a clinical isolate of Streptococcus pneumoniae: using bioluminescence to monitor gemifloxacin activity. Antimicrob Agents Chemother 46(2):538–542

13. Branchini, B. R., Murtiashaw, M. H., Magyar, R. A., Portier, N. C., Ruggiero, M. C., and Stroh, J. G. (2002) Yellow-green and red firefly bioluminescence from 5,5 dimethyloxyluciferin. J. Am. Chem. Soc. 124, 2112-2113.

14. Branchini, B. R., Southworth, T. L., Murtiashaw, M. H., Magyar, R. A., Gonzalez, S. A., Ruggiero, M. C., and Stroh, J. G. (2004) An alternative mechanism of bioluminescence color determination in firefly luciferase. Biochemistry 43, 7255- 7262.

15. Branchini, B. R., Ablamsky, D. M., Davis, A. L., Southworth, T. L., Butler, B., Fan, F., Jathoul, A. P., and Pule, M. A. (2010) Red-emitting luciferases for bioluminescence reporter and imaging applications. Anal. Biochem. 396, 290-297.

16. Blissett, S. J., And G. S. A. B. Stewart. 1989. In Vivo Bioluminescent Determination Of Apparent Km's For Aldehyde In Recombinant Bacteria Expressing Luxa/B. Lett. Appl. Microbiol. 9:149-152.

17. Boylan, M. O., Pelletierj,., Dhepagnon,S . , Trudel,S ., Sonenbergn, . & Meighene, . A. (1989). Construction Of A Fused Luxab Gene By Site Directed Mutagenesis. Journal Of Bioluminescence And Chemiluminescence 4, 3 10-3 16.

18. Bioluminescence In Soybean Root Nodules: Demonstrationof A General Approach To Assay Gene Expression In Vivo By Using Bacterial Luciferase. Proc. Natl. Acad. Sci. Usa 83:9080-9084.

19. Browning .D.F., and Busby, S.J.W. (2004) The regulation of bacterial transcription initiation. Nat Rev Microbiol 2: 57–65.

20. Bulich AA, and Isenberg DL. Use of the luminescent bacterial system for the rapid assessment of aquatic toxicity. ISA Trans. 1981; 20: 29-33.

21. Cohn,D . H., Milehama, . J., Simonm, . I., Nealsonk, . H. , Raush, S. K. , Bonamd, . & Baldwint, (1985). Nucleotide Sequence Of The Luxa Gene Of Vibrio Harveyi And The Complete Amino Acid Sequence Of The Alpha Subunit Of Bacterial Luciferase. Journal of Biological Chemistry 260, 6 1 39-61 46.

22. Campagna C, Dignani J, Blackwell SB, Marin MR. 2001. Detecting bioluminescence with an irradiance time-depth recorder deployed on southern elephant seals. Marine Mammal Science 17:402–14

23. Carmi, 0. A., G. S. A. B. Stewart, S. Ulitzur, and J. Kuhn. 1987. Use of Bacterial Luciferase To Establish A Promoter Probe Vehicle, Capable Of Nondestructive Real-Time Analysis Of Gene Expression In Bacillus Spp. J. Bacteriol. 169:2165-2170.

24. Doyle TC, Burns SM, Contag CH (2004) In vivo bioluminescence imaging for integrated studies of infection. Cell Microbiol 6:303–317.

25. Davidov Y, Rozen R, Smulski D, Van Dyk T, Vollmer A, Elsemore D, LaRossa R, Belkin S (2000) Improved bacterial SOS promoter::lux fusions for genotoxicity detection. Mutat Res Genet Toxicol Environ Mutagen 466(1):97–107.

26. Denyers,. P., Jassim, S. A. A. & Stewartg, . S. A. B. (1992). Engineering Microbial Bioluminescence and Biosensor Applications. In Molecular Biology In Clinical Research And Diagnosis. Edited By M. R. Walker. Oxford : Blackwell Scientific Publications (In The Press).

27. D. M. Close, T. Xu, G. S. Sayler, and S. Ripp, Sensors. 11(2011) 180-206.

28. Dragulescu-Andrasi, A., Chan, C. T., De, A., Massoud, T. F., and Gambhir, S. S. (2011) Bioluminescence

resonance energy transfer (BRET) imaging of protein protein interactions within deep tissues of living subjects. Proc. Natl. Acad. Sci. USA 108, 12060-12065.

29. E.N. Harvey, Bioluminescence, Academic Press, New York,1952.

30. E. A. Widder, Science. 328 (2010) 704-708

31. E.A. Meighen, Molecular biology of bacterial bioluminescence, Microbiol. Rev. 1991, 55(1):123

32. Eschera,., O'kaned, . J., Lee,J . & Szalaya, . A. (1989). Bacterial Luciferase Ap Fusion Protein Is Fully Active As A Monomer And Highly Sensitive In Vivo To Elevated Temperature. Proceedings of The National Academy Of Sciences Of The United States Of America 86, 6528-6532.

33. Engebrecht, J., M. Simon, and M. Silverman. 1985. Measuring Gene Expression With Light. Science 227:1345-1347.

34. Engebrecht, J., K. H. Nealson, and M. Silverman. 1983. Bacterial Bioluminescence: Isolation And Genetic Analysis Of Functionsfrom Vibrio Fischeri. Cell 32:773-781.

35. Engebrecht, J., and M. Silverman. 1984. Identification Of Genes And Gene Products Necessary For Bacterial Bioluminescence. Proc. Nati. Acad. Sci. Usa 81:4154-4158.

36. Engebrecht, J., and M. Silverman. 1986. Techniques For Cloning And Analyzing Bioluminescence From Marine Bacteria Methods Enzymol. 133:83-98.

37. Engebrecht, J., and M. Silverman. 1986. Regulation of Expression Of Bacterial Genes For Bioluminescence. Genet. Eng. 8:31-44.

38. Engebrecht, J., And M. Silverman. 1987. Nucleotide Sequence Of The Regulatory Locus Controlling Expression Of Bacterial Genes For Bioluminescence. Nucleic Acids Res. 15:10455-10467.

39. Engebrecht, J., M. Simon, and M. Silverman. 1985. Measuring Gene Expression with Light. Science 227:1345-1347.

40. Engebrecht J, Nealson K, Silverman M. Bacterial bioluminescence: isolation and genetic analysis of functions from Vibrio fischeri. Cell. 1983 Mar;32(3):773–781.

41. Escher, A., D. J. O'kane, J. Lee, and A. Szalay. 1989. Bacterial Luciferase Au Fusion Protein Is Fully Active As A Monomer And Highly Sensitive In Vivo To Elevated Temperature. Proc. Natl. Acad. Sci. USA 86:6528-6532.

42. Faridi, J. et al. (2003) Expression of constitutively active Akt-3 in MCF-7 breast cancer cells reverses the estrogen and tamoxifen responsivity of these cells in vivo. Clin. Can. Res. 9, 2933–9.

43. Fernández-Pinas F, Wolk CP (1994) Expression of luxCD-E in Anabaena sp. can replace the use of exogenous aldehyde for in vivo localization of transcription by luxAB. Gene 150(1):169–174.

44. Forand, . R. & Brownw, . M. (1988). Nucleotide Sequence Of The Luxa And Luxb Genes Of The Bioluminescent Marine Bacterium Vibrio Fischeri. Nucleic Acids Research 16, 777.

45. Fraga, H. (2008) Firefly Luminescence: a historical perspective and recent developments. Photochem. Photobiol. Sci. 7, 146-158.

46. Gelmini, S., Pinzani, P., and Pazzagli, M. (2000) Luciferase gene as reporter: comparison with the CAT gene and use in transfection and microinjection of mammalian cells. Methods Enzymol. 305, 557-576.

47. Gould,S . J. & Subramansi. , (1988). Firefly Luciferase As A Tool In Molecular And Cell Biology. Analytical Biochemistry 175, 5-1 3.

48. Hannah, R. et al. (1998) Rapid luciferase reporter assay systems for high-throughput studies. Promega Notes 65, 9–14.

49. Harveyi, E. N. 1952. Bioluminescence. Academic Press, Inc., New York.

50. Harms H, Wells M, van der Meer J (2006) Whole-cell living biosensors-are they ready for environmental application? Appl Microbiol Biotechnol 70(3):273–280

51. Hada, H. S., J. Stemmler, M. L. Grossbard, P. A. West, C. J. Potrikus, J. W. Hastings, And R. R. Colwell. 1985. Characterization Of Non-O1-Serovar Vibrio Cholerae (Vibrio Albensis). Syst. Appl. Microbiol. 6:203-209
.

52. Hawkins, E.H. et al. (2002) Dual-Gl Luciferase Assay System: Convenient dual-reporter measurements in 96- and 384-well plates. Promega Notes 81, 22–6.

53. Hill, P . J., Swift, S. & Stewart G, S. A. B. (1991). Pcr Based Gene Engineering Of The Vibrio Harveyi Lux Operon And The Escherichia Coli Trp Operon Provides For Biochemically Functional Native And Fused Gene Products. Molecular And General Genetics 226, 41-48.

54. Hirano, T., Hasumi, Y., Ohtsuka, K., Maki, S., Niwa, H., Yamaji, M., and Hashizume, D. (2009) Spectroscopic studies of the light-color modulation mechanism of firefly (beetle) bioluminescence. J. Am. Chem. Soc. 131, 2385-2396.

55. Inouye, S. (2010) Firefly luciferase: an adenylate-forming enzyme for multicatalytic functions. Cell. Mol. Life Sci. 67, 387-404.

56. Inouyes, ., Nakazawaa,. & Nakazawat,. (1984). Nucleotide Sequence Of The Promoter Region Of The Xyl Degf Operon On Tol Plasmids Of Pseudomonas Putida Gene 29, 323-330.

57. J.Malave-Orengo, E.N. Rubio-Marrero, & C. Rios-Velazquez, Isolation and characterization of bioluminescent bacteria from marine environments of Puerto Rico, Current Research, Technology and education Topics in Applied Microbiology and Microbial Biotechnology,2010, pp 103-108.

58. Jago, P. H., Simpson, W . J., Denyers,. P., Evansa, . W. , Griffiths, M. W., Hammondj., R. M., Ingramt, . P., Laceyr, . F., Macey,N. W., Mccarthyb, . J., Salusburyt,. T., Senior,P . S., Sizorowicfz., S., Smitherr, ., Stanfieldg, . & Stanleyp,. E. (1989). An Evaluation Of The Performance Of Ten Commercial Luminometers. Journal Of Bioluminescence And Chemiluminescence 3,13 1-1 45.

59. Jassim, S. A. A., A. Ellison, S. P. Denyer, And G. S. A. B. Stewart. 1990. In Vivo Bioluminescence: A Cellular Reporter For And Industry. J. Biolumin. Chemilumin. 5:115-122.

60. Kato, R., Hasegawa, K., Hidaka, Y., Kuramitsu, S. & Hoshino, T. (1997). Characterization of a thermostable DNA photolyase from an extremely thermophilic bacterium, Thermus thermophiles HB27. J Bacteriol 179, 6499-6503.

61. K, Kita-Tsukamoto, K. Yao, S. Yoshizawa, N. Uchiyama, K. Kogure, & M. Wada, Rapid identification of marine bioluminescent bacteria by amplified 16S ribosomal RNA gene restriction analysis. FEMS Microbial Lett 256, 2006, pp 298-303.

62. Kang, S. H., Cho, M.J. and Kole, R. (1998) Up-regulation of luciferase gene expression with antisense oligonucleotides: implications and applications in functional assay development. Biochemistry 37, 6235-6239.

63. Kannahi, M., Sivshankari, 2014. Isolation and identification of bioluminescent bacteria from marine water at Nagapattanam sea shore. Int. J. Pharm. Sci. Rev., 26(2): 346 351.

64. Kalra, J., Anantha, M., Warburton, C., Waterhouse, D., Yan, H., Yang, Y. J., Strut, D., Osooly, M., Masin, D., and Bally, M. B. (2011) Validating the use of a luciferase labeled breast cancer cell line, MDA435LCC6, as a means to monitor tumor progression and to assess the therapeutic activity of an established anticancer drug, docetaxel (Dt) alone or in combination with the ILK inhibitor, QLT0267. Cancer Biology & Therapy 11, 826-838.

65. Kirchnerg, Robertsj, . L., Gustafsong,. D. & Ingoliat . D. (1989). Active Bacterial Luciferase from A Fused Gene: Expression Of A Vibrio Harueyi Luxab Translational Fusion In Bacteria, Yeast And Plant Cells. Gene 81, 349-354.

66. Karp, M., and P. Meyer. 1989. Expression of Bacterial Luciferase Genes from Vibrio Harveyi In Bacillus Subtilis and In Escherichia Coli. Biochim. Biophys. Acta 1007:84-90

67. Kodikarac,. P., Crew, H . H. & Stewartg, . S. A. B. (1991). Near on-line detection of enteric bacteria using lux recombinant bacteriophage. FEMS Microbiology Letters 83, 261-266.

68. Kannahi, M., Sivshankari, 2014. Isolation and identification of bioluminescent bacteria from marine water at Nagapattanam sea shore. Int. J. Pharm. Sci. Rev., 26(2): 346 351.

69. Kumar, A. 2010. Isolation of bioluminescent bacteria from Bay of Bengal and their molecular characterization. PhD thesis. Dept of Biotech, AMET University, Chennai. India.

70. Kurvet, I., Angela, I., Bondarenko, O., Sihtmae, M., Kahru, A. 2005. LuxCDABE transformed constitutively bioluminescent Escherichia coli for toxicity screening: comparison with naturally luminous Vibrio fischeri. Sensors J., 11: 7865 7878.

71. Kurvet I, Iyask A, Bondarenko O, Sihtmäe M, and Kahru A. LuxCDABE-transformed constitutively bioluminescent Escherichia coli for toxicity screening: comparison with naturally luminous Vibrio fischeri. Sensors. 2011; 11(8):7865-7878.

72. Legocki, R. P., M. Legocki, T. 0. Baldwin, And A. A. Szalay. Molecular And General Genetics 226, 41-48.

73. Levin GV, Chen CS et al. Development of the firefly bioluminescent assay for the rapid, quantitative detection of microbial contamination of water. Amrl. Tr. 1967; 1–73.

74. Liu, Y. J., De Vico, L., and Lindh, R. (2008) Ab initio investigation on the chemical origin of the firefly bioluminescence. J. Photochem. Photobiol. A. 194, 261-267.

75. Meighene. A. (1988). Enzymes and Genes from The Lux Operons of Bioluminescent Bacteria. Annual Reviews of Microbiology 42, 15 1 -176.

76. Musa, N., Wei, L.S., Wee, W. 2008. Phenotypic and genotypic characteristics of vibrio harveyi isolated from Black Tiger shrimp (Penaeus monodon). World Appl. Sci. J., 3(6): 885 902.

77. Miyamoto, C. M., M. Boylan, A. F. Graham, and E. A Meighen. 1988. Organization of the Lux Structural Genes Of Vibrio Harveyi. Expression under the T7 Bacteriophage Promoter, Mrna Analysis And Nucleotide Sequence Of The Lux.d Gene. J. Biol. Chem. 263:13393-13399.

78. Navizet, I., Liu, Y. J., Ferre, N., Xiao, H. Y., Fang, W. H., and Lindh, R. (2010) Color-tuning mechanism of firefly investigated by multi-configurationally perturbation method. J. Am. Chem. Soc. 132, 706-712.

79. Neeraj K. Charmkar, Shrikant Kol and Rishabh D. Saket , 2017. “Associational study of fto gene polymorphism with obesity in vindhyan” International Journal of Current Research, 9,(09), 56902-56905.region”,

80. Orengo, M.J., Rubio-Marrero, N.R., Velazquez, R.C. 2010. Isolation and characterization of bioluminescent bacteria from marine environments of Puerto Rico. Current research, technology and educational topics in applied microbiology and microbial biotechnology.

81. O'kane, D. J., W. L. Lingle, J. E. Wampler, R. P. Legocki, And A. A. Szalay. 1988. Visualization of Bioluminescence As A Marker Of Gene Expression In Rhizobium Infected Soybean Root Nodules. Plant Mol. Biol. 10:387-399.

82. Park, S. F., D. A. Stirling, C. S. Hulton, I. R. Booth, C. F. Higgins, And G. S. A. B. Stewart. 1989. A Novel, Non-Invasive Promoter Probe Vector: Cloning Of The Osmoregulated Prou Promoter Of Escherichia Coli K12. Mol. Microbiol. 3:1011-1023.

83. Rodriguez-Mozaz S, Lopez de Alda M, Barceló D (2006) Biosensors as useful tools for environmental analysis and monitoring. Anal Bioanal Chem 386(4):1025–1041.

84. Ransanga, J., Lal, T.M., AI-Harbi, A. 2012. Characterization and experimental infection of Vibrio harveyi isolated from diseased Asian Seabass (Lates calcarifer). Malaysian J. Microbiol., 8(2): 104 115.

85. Saket, Rishabh Dev, et al. "Compliment receptor type-1 (CD35) gene polymorphism and Plasmodium falciperum Maleria." International Journal of Medical Research & Health Sciences 4.2 (2015): 422-431.

86. Shapiro E, Baneyx F (2002) Stress-based identification and classification of antibacterial agents: second-generation Escherichia coli reporter strains and optimization of detection. Antimicrob Agents Chemother 46(8):2490–2497.

87. Stock, J. B., Ninfa, A. J. & Stock, A. M. (1989). Protein Phosphorylation and Regulation of Adaptive Responses in Bacteria. Microbiological Reviews 53, 450-490.

88. Stewart, G., T. Smith, and S. Denyer. 1989. Genetic Engineering for Bioluminescent Bacteria. Food Sci. Technol. Today 3:19-22.

89. Shaw, J. J., and C. I. Kado. 1986. Development of a Vibrio Bioluminescence Gene-Set to Monitor Phyto-pathogenic Bacteria During The ongoing Disease Process In A Non-disruptive Manner. Biotechnology 4:60-64.

90. Shadel, G. S., Devine, J. H. & Baldwin, T. O. (1990). Control of the lux regulon of Vibrio ®scheri. J Biolumin Chemilumin 5, 99-106.

91. Schmetterer, G., C. P. Wolk, and J. Elhai. 1986. Expression of Luciferases from Vibrio Harveyi and Vibrio Fischeri in Filamentous Cyanobacteria. J. Bacteriol. 167:411-414.

92. Swift, S., Throup, J., Bycroft, B., Williams, P. & Stewart, G. (1998).Quorum sensing: bacterial cell-cell signaling from bioluminescence to pathogenicity. In Molecular Microbiology, pp. 185±207. Edited by S. J. W. Busby, C. M. Thomas & N. L. Brown. Berlin & Heidelberg: Springer.

93. T., P. M. Rogowsky, And C. I. Kado. 1987. Characterization of the Vire Locus of Agrobacterium Tumefaciens Plasmid Ptic58. J. Bacteriol. 169:1529-1536.

94. T. Wilson, And J. W. Hastings, Bioluminescence: Living Lights, Lights For Living, Harvard University Press, 2013.

95. Tatsumih ., Masuda, T . & Nakano, E . (1988). Synthesis of Enzymatically Active Firefly Luciferase in Yeast. Agricultural Biology and Chemistry 52, 1 123-1 128.

96. Tecon R, van der Meer J (2008) Bacterial biosensors for measuring availability of environmental pollutants. Sensors 8:4062–4080

97. Ulitzurs,. & Kuhnj, . (1987). Introduction of Lux Genes into Bacteria, A New Approach for Specific Determination of Bacteria and Their Antibiotic Susceptibility. In Bioluminescence and Chemiluminescence New Perspectives, Pp. 463-472. Edited By J. Schlomerich, R. Andreesen, A. Kapp, M. Ernst & W. G. Woods. Bristol: Wiley.

98. Viviani, V. R. (2002) the origin, diversity, and structure function relationships of insect luciferases. Cell. Mol. Life Sci. 59, 1833-1850.

99. Van der Meer JR, Belkin S (2010) where microbiology meets micro-engineering, design and applications of reporter bacteria. Nat Rev Microbiol 8(7):511–522.

100. Welham P, Stekel D (2009) Mathematical model of the lux luminescence system in the terrestrial bacterium Photorhabdus luminescens. Mol Biosyst 5(1):68–76

101. Yagur-Kroll S, Bilic B, Belkin S (2010) Strategies for enhancing bioluminescent bacterial sensor performance by promoter region manipulation. Microb Biotechnol 3(3):300–310.