

## Introduction-

**Bioluminescent bacteria** are light-producing bacteria that are predominantly present in sea water, marine sediments, the surface of decomposing fish and in the gut of marine animals. While not as common, bacterial bioluminescence is also found in terrestrial and freshwater bacteria. These bacteria may be free living (such as *Vibrio harveyi*) or in symbiosis with animals such as the Hawaiian Bobtail squid (*Aliivibrio fischeri*) or terrestrial nematodes (*Photobacterium luminescens*). The host organisms provide these bacteria a safe home and sufficient nutrition. In exchange, the hosts use the light produced by the bacteria for camouflage, prey and/or mate attraction. Bioluminescent bacteria have evolved symbiotic relationships with other organisms in which both participants benefit close to equally. Another possible reason bacteria use luminescence reaction is for quorum sensing, an ability to regulate gene expression in response to bacterial cell density.

## Bacterial groups that exhibit bioluminescence

All bacterial species that have been reported to possess bioluminescence belong within the families *Vibrionaceae*, *Shewanellaceae*, or *Enterobacteriaceae*, all of which are assigned to the class Gammaproteobacteria.

Family	Genus	Species
<i>Enterobacteriaceae</i>	<i>Photobacterium</i>	<i>Photobacterium symbiotica</i> <i>Photobacterium luminescens</i> <i>Photobacterium temperata</i>
<i>Shewanellaceae</i>	<i>Shewanella</i>	<i>Shewanella woodyi</i> <i>Shewanella hahneli</i>
<i>Vibrionaceae</i>	<i>Aliivibrio</i>	<i>Aliivibrio fischeri</i> <i>Aliivibriologei</i> <i>Aliivibriosalmonicida</i> <i>Aliivibriosifiae</i> <i>Aliivibrio "thorii"</i> <i>Aliivibriowodanis</i>
	<i>Photobacterium</i>	<i>Photobacterium aquimaris</i> <i>Photobacterium damsela</i> <i>Photobacterium kishitani</i> <i>Photobacterium leiognathi</i>

		<i>Photobacteriummandapamensis</i> <i>Photobacteriumphosphoreum</i>
	<i>Vibrio</i>	<i>Vibrio azureus</i> <i>Vibrio "beijerinckii"</i> <i>Vibrio campbellii</i> <i>Vibrio chagasii</i> <i>Vibrio cholerae</i> <i>Vibrio harveyi</i> <i>Vibrio mediterranea</i> <i>Vibrio orientalis</i> <i>Vibrio sagamiensis</i> <i>Vibrio splendidus</i> <i>Vibrio vulnicus</i>
	" <i>Candidatus Photodesmus</i> "	" <i>Candidatus Photodesmuskatoptron</i> "

### **Distribution**

Bioluminescent bacteria are most abundant in marine environments during spring blooms when there are high nutrient concentrations. These light-emitting organisms are found mainly in coastal waters near the outflow of rivers, such as the northern Adriatic Sea, Gulf of Trieste, northwestern part of the Caspian Sea, coast of Africa and many more. These are known as milky seas. Bioluminescent bacteria are also found in freshwater and terrestrial environments but are less wide spread than in seawater environments. They are found globally, as free-living, symbiotic or parasitic forms and possibly as opportunistic pathogens. Factors that affect the distribution of bioluminescent bacteria include temperature, salinity, nutrient concentration, pH level and solar radiation. For example, *Aliivibrio fischeri* grows favourably in environments that have temperatures between 5 and 30 °C and a pH that is less than 6.8; whereas, *Photobacteriumphosphoreum* thrives in conditions that have temperatures between 5 and 25 °C and a pH that is less than 7.0.

### **Genetic diversity**

All bioluminescent bacteria share a common gene sequence: the *lux* operon characterized by the *luxCDABE* gene organization. *LuxAB* codes for luciferase while *luxCDE* codes for a fatty-acid reductase complex that is responsible for synthesizing aldehydes for the bioluminescent reaction. Despite this common gene organization, variations, such as the presence of other *lux* genes, can be observed among species. Based on similarities in gene content and organization, the *lux* operon can be organized into the following four distinct types: the *Aliivibrio/Shewanella* type, the *Photobacterium* type, the *Vibrio/Candidatus Photodesmus* type, and the *Photorhabdus* type. While this organization follows the genera classification level

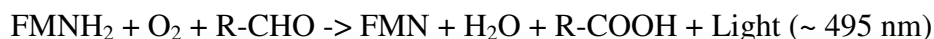
for members of *Vibrionaceae* (*Aliivibrio*, *Photobacterium*, and *Vibrio*), its evolutionary history is not known.

With the exception of the *Photorhabdus* operon type, all variants of the *lux* operon contain the flavin reductase-encoding *luxG* gene. Most of the *Aliivibrio/Shewanella* type operons contain additional *luxI/luxR* regulatory genes that are used for autoinduction during quorum sensing. The *Photobacterium* operon type is characterized by the presence of *rib* genes that code for riboflavin, and forms the *lux-rib* operon. The *Vibrio/Candidatus Photodesmus* operon type differs from both the *Aliivibrio/Shewanella* and the *Photobacterium* operon types in that the operon has no regulatory genes directly associated with it.

## Mechanism

All bacterial luciferases are approximately 80 KDa heterodimers containing two subunits:  $\alpha$  and  $\beta$ . The  $\alpha$  subunit is responsible for light emission. The *luxA* and *luxB* genes encode for the  $\alpha$  and  $\beta$  subunits, respectively. In most bioluminescent bacteria, the *luxA* and *luxB* genes are flanked upstream by *luxC* and *luxD* and downstream by *luxE*.

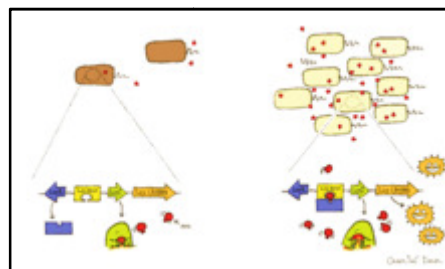
The bioluminescent reaction is as follows:



Molecular oxygen reacts with FMNH<sub>2</sub> (reduced flavin mononucleotide) and a long-chain aldehyde to produce FMN (flavin mononucleotide), water and a corresponding fatty acid. The blue-green light emission of bioluminescence, such as that produced by *Photobacterium phosphoreum* and *Vibrio harveyi*, results from this reaction. Because light emission involves expending six ATP molecules for each photon, it is an energetically expensive process. For this reason, light emission is not constitutively expressed in bioluminescent bacteria; it is expressed only when physiologically necessary.

## Quorum Sensing-

Bioluminescence in bacteria can be regulated through a phenomenon known as autoinduction or quorum sensing. Quorum sensing is a form of cell-to-cell communication that alters gene expression in response to cell density. Autoinducer is a diffusible pheromone produced constitutively by bioluminescent bacteria and serves as an extracellular signalling molecule. When the concentration of autoinducer secreted by bioluminescent cells in the environment reaches a threshold (above  $10^7$  cells per mL), it induces the expression of luciferase and other enzymes involved in bioluminescence. Bacteria are able to estimate their density by sensing the level of autoinducer in the environment and regulate their bioluminescence such that it is expressed only when there is a sufficiently high cell population. A sufficiently high cell population ensures that the bioluminescence produced by the cells will be visible in the environment.



A well-known example of quorum sensing is that which occurs between *Aliivibrio fischeri* and its host. This process is regulated by LuxI and LuxR, encoded by *luxI* and *luxR* respectively. LuxI is autoinducer synthase that produces autoinducer (AI) while LuxR functions as both a receptor and

transcription factor for the lux operon. When LuxR binds AI, LuxR-AI complex activates transcription of the lux operon and induces the expression of luciferase. Using this system, *A. fischeri* has shown that bioluminescence is expressed only when the bacteria are host-associated and have reached sufficient cell densities.

Another example of quorum sensing by bioluminescent bacteria is by *Vibrio harveyi*, which are known to be free-living. Unlike *Aliivibriofischeri*, *V. harveyi* do not possess the *luxI/luxR* regulatory genes and therefore have a different mechanism of quorum sensing regulation. Instead, they use the system known as three-channel quorum sensing system.

### **Symbiosis with other organisms**

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The symbiotic relationship between the Hawaiian bobtail squid *Euprymna scolopes* and the marine gram-negative bacterium *Aliivibriofischeri* has been well studied. The two organisms exhibit a mutualistic relationship in which bioluminescence produced by *A. fischeri* helps to attract prey to the squid host, which provides nutrient-rich tissues and a protected environment for *A. fischeri*. Bioluminescence provided by *A. fischeri* also aids in the defense of the squid *E. scolopes* by providing camouflage during its nighttime foraging activity. Following bacterial colonization, the specialized organs of the squid undergo developmental changes and a relationship becomes established. The squid expels 90% of the bacterial population each morning, because it no longer needs to produce bioluminescence in the daylight. This expulsion benefits the bacteria by aiding in their dissemination. A single expulsion by one bobtail squid produces enough bacterial symbionts to fill 10,000m<sup>3</sup> of seawater at a concentration that is comparable to what is found in coastal waters. Thus, in at least some habitats, the symbiotic relationship between *A. fischeri* and *E. scolopes* plays a key role in determining the abundance and distribution of *E. scolopes*. There is a higher abundance of *A. fischeri* in the vicinity of a population of *E. scolopes* and this abundance markedly decreases with increasing distance from the host's habitat.

Bioluminescent *Photobacterium* species also engage in mutually beneficial associations with fish and squid. Dense populations of *P. kishitanii*, *P. leiogathi*, and *P. mandapamensis* can live in the light organs of marine fish and squid, and are provided with nutrients and oxygen for reproduction in return for providing bioluminescence to their hosts, which can aid in sex-specific signaling, predator avoidance, locating or attracting prey, and schooling.

### **Role**

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The uses of bioluminescence and its biological and ecological significance for animals, including host organisms for bacteria symbiosis, have been widely studied. The biological role and evolutionary history for specifically bioluminescent bacteria still remains quite mysterious and unclear. However, there are continually new studies being done to determine the impacts that bacterial bioluminescence can have on our constantly changing environment and society. Aside from the many scientific and medical uses, scientists have also recently begun to come together with artists and designers to explore new ways of incorporating bioluminescent bacteria, as well as bioluminescent plants, into urban light sources to reduce the need for electricity. They have also begun to use bioluminescent bacteria as a form of art and urban design for the wonder and enjoyment of human society.

One explanation for the role of bacterial bioluminescence is from the biochemical aspect. Several studies have shown the biochemical roles of the luminescence pathway. It can function as an alternate pathway for electron flow under low oxygen concentration, which can be advantageous when no fermentable substrate is available. In this process, light emission is a side product of the metabolism.

Evidence also suggests that bacterial luciferase contributes to the resistance of oxidative stress. In laboratory culture, *luxA* and *luxB* mutants of *Vibrio harveyi*, which lacked luciferase activity, showed impairment of growth under high oxidative stress compared to wild type. The *luxD* mutants, which had an unaffected luciferase but were unable to produce luminescence, showed little or no difference. This suggests that luciferase mediates the detoxification of reactive oxygen.

Bacterial bioluminescence has also been proposed to be a source of internal light in photoreactivation, a DNA repair process carried out by photolyase. Experiments have shown that non-luminescent *V. harveyi* mutants are more sensitive to UV irradiation, suggesting the existence of a bioluminescent-mediated DNA repair system.

Another hypothesis, called the “bait hypothesis”, is that bacterial bioluminescence attracts predators who will assist in their dispersal. They are either directly ingested by fish or indirectly ingested by zooplankton that will eventually be consumed by higher trophic levels. Ultimately, this may allow passage into the fish gut, a nutrient-rich environment where the bacteria can divide, be excreted, and continue their cycle. Experiments using luminescent *Photobacterium leiognathi* and non-luminescent mutants have shown that luminescence attracts zooplankton and fish, thus supporting this hypothesis.

After the discovery of the lux operon, the use of bioluminescent bacteria as a laboratory tool is claimed to have revolutionized the area of environmental microbiology. The applications of bioluminescent bacteria include biosensors for detection of contaminants, measurement of pollutant toxicity and monitoring of genetically engineered bacteria released into the environment. Biosensors, created by placing a *lux* gene construct under the control of an inducible promoter, can be used to determine the concentration of specific pollutants. Biosensors are also able to distinguish between pollutants that are bioavailable and those that are inert and unavailable. For example, *Pseudomonas fluorescens* has been genetically engineered to be capable of degrading salicylate and naphthalene, and is used as a biosensor to assess the bioavailability of salicylate and naphthalene. Biosensors can also be used as an indicator of cellular metabolic activity and to detect the presence of pathogens.