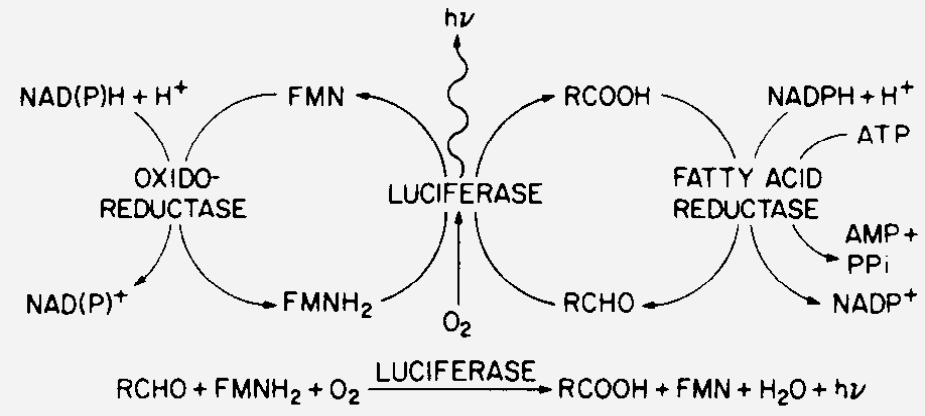
Experiment	**Bacterial bioluminescence of *Vibrio harveyi* (demonstration)**
Advisor	Munti Yuhana myuhana@botinst.unizh.ch
Textbook Chapters	BBOM 9th edition (1999): Chapters 7.6, (Fig. 7.21), and 13.11. BBOM 10th edition (2003): Chapters 8.7, 8.9, and 12.12. BBOM: Madigan M.T., J.M. Martinko and J. Parker: **"Brock: Biology of Microorganisms"**, Prentice Hall.
Objectives	To understand bacterial bioluminescence mechanisms. To observe changes of luminescence activity of *Vibrio harveyi* in the dark. To understand that luciferase is involved in the luminescence expression which is oxygen-dependent.
Background	Luminescent bacteria (e.g. genus *Photobacterium* and *Vibrio*), are wide-spread in the marine environment, where they exist as planktonic forms and in symbioses. Some physiological characteristics of luminescent bacteria are: Gram-negative, motile rods, aerobic (many of them are facultative anaerobes), capable of emitting light. They are associated with fish, squid, shrimp and other organisms, as parasites or as saprophytes. Luminesence can be used by the host organisms for a variety of purposes, like attraction of prey, intraspecies communication, or escape from predators. All luminous bacteria encode biochemically similar luminescence systems. Light production is catalyzed by the enzyme luciferase, a mixed function oxidase consisting of two different subunits, i.e. an alpha subunit approx. 42,000 Dalton, and a beta subunit approx. 37,000 Dalton. The *in vitro* light emitting reaction is coupled to aerobic oxidations:  **luciferase** **RCHO + $FMNH_2$ + O_2 ⟶ RCOOH + FMN + H_2O + blue green light ($\lambda \approx 490$ nm)** Fig. 1. Substrates, products, and pathways involved in the bacterial bioluminescence reaction (Engebrecht et al., 1983). Luciferase catalyzes the oxidation of a reduced flavin and a long-chain aldehyde, producing oxidized flavin and the corresponding long chain fatty acid. A fatty acid reductase has been implicated in the recycling of the fatty acid to the aldehyde. Expression of the genes for luciferase (*lux*) occurs in late log phase (Nealson, 1977) and has been shown to be induced by a small sensory molecule, called **autoinducer**. An autoinducer is a diffusable compound produced by the bacteria. It can accumulate in the environment during growth. This **autoinduction** system allows luminous bacteria to monitor their own population density.

Literature	Engebrecht, J., K. Nealson, and M. Silverman. 1983. Bacterial Biolumines-cence: Isolation and Genetic Analysis of Functions from *Vibrio fischeri*. Cell. 32: 773-781. Nealson, K.1977. Autoinduction of Bacterial Luciferase: Occurence, Mechanism, and Significance. Arch. Microbiol. 112: 73-79. Kuo, A., S. M. Callahan, and P. V. Dunlap. 1996. Modulation of Luminescence Operon Expression by N-Octanoyl-L-Homoserine Lactone in *ains* Mutants of *Vibrio fischeri.* Appl. Environ. Microbiol. 178: 971-976. Visick, K. L., J. Foster, J. Doino, M. McFall-Ngai, and E. G. Ruby. 2000. *Vibrio fischeri lux* Genes Play an Important Role in Colonization and Development of the Host Light Organ. J. Bacteriol. 182: 4578-4586. DeLoney-Marino, C. R., A. J. Wolfe, and K. L. Visick. 2003. Chemoattraction of *Vibrio fischeri* to Serine, Nucleosides, and N-Acetylneuraminic Acid, a Component of Squid Light-Organ Mucus. Appl. Environ. Microbiol. 69: 7527-7530.
www. Links	http://lifesci.ucsb.edu/~biolum/chem/ http://www.lifesci.ucsb.edu/~biolum/ http://www.biolum.org/ http://www.montegen.com/html/body_bacterial_bioluminescence.htm http://www.bio.cmu.edu/Courses/03441/TermPapers/99TermPapers/Quorum/default.html Evaluate these sites from a didiactic point of view.
Practical Work	The students will observe the dependence of luminescence of *V. harveyi* on oxygen.
Materials and Demonstration Protocols	Bacterial strain: luminous *Vibrio harveyi* isolated from brackish water shrimp (*Penaeus monodon* Fab.), off the coast of Java island. We will partially fill a long glass tube with a well grown culture of *V. harveyi,* go to a completely dark room, close our eyes for several minutes to get adapted to the darkness and observe the luminescence. We will turn the tube upside down several times to allow the oxygen present in the air bubble to diffuse into the medium. What will happen ?
Laboratory Rules & Precautions	There is no risk associated with this demonstration, but be careful in the darkness and do not drop the fragile glass tube with the liquid culture.
Goals & Experiences gained	Familiarity with one of the more unusual phenotypic characteristics of a microorganism: bacterial bioluminescence.
Timing	15 minutes
Reporting	Note observations in your lab book and use your notes for the report.
Questions to be answered	Why are some of the bacteria capable of emitting light in the dark? Why are these bacterial cells able to express their bioluminescence genes only after they reach a certain cell-density? Why does the luminescence fade away so quickly after we stop shaking?