# MASTER'S THESIS

## Applications of the bacterial luciferin-luciferase system

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# Applications of the Bacterial Luciferin-luciferase System

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## Abstract

Bioluminescence is a common phenomenon found in many organisms, such as fireflies, squids and teleost fishes, these organisms depend on the bioluminescence as a light source or a lure to attract their prey. The production of the luminescence by these organisms depending on the specialized organ which is occupied and concentrated with one or more than one kinds of luminescent bacteria. The generation of the bioluminescence is a product from an oxidative reaction controlled by the Lux operon of the luminescent bacteria ($FMNH^- + RCHO + H^+ + O2 \rightarrow FMN + RCOOH + H_2O + h\nu$). The Lux operon usually contains LuxCDABE genes, where the LuxC, LuxD and LuxE genes encode the enzymes for the biosynthesis of the enzyme luciferase while the LuxA and LuxB genes encode the protein synthesis of the alpha and beta subunits of the bacterial luciferin.

In the present project, bioluminescent bacteria were isolated from squids (sp. *Loligo opalescens*). A pure culture of one luminescent bacterium was obtained and was subjected to identification by gram tests and analyses by polymerase chain reaction (PCR). A gram-negative bacterium, *Photobacterium leiognathi subspecies mandapamensis*, was identified through amplification of the recA gene by PCR and subsequent DNA sequencing.

In addition, the LuxA and LuxB genes in the *P. leiognathi* was amplified and cloned to produce different kinds of mammalian and bacterial expression for the expression of bacterial luciferin at the cellular level.

Transfection of the shrimp zygotes of *Macrobrachium lanchesteri* and cysts of *Artemia* were carried out by reagent-mediated trasnfection and electroporation and the expression of the bacterial luciferin was driven by the human cytomegalovirus promoter. Electroporation of the Artemia cysts succeeded but no observable or detectable bioluminescence upon induction. In contrast, growth of the the shrimp zygotes of *Macrobrachium lanchesteri* were found arrested after the reagent-mediated transfection.

In spite of making reporter organisms expressing bacterial luciferin, several bacterial expression constructs have been made in order to compare the efficiencies of expression in dual vectors, bicistronic vector and double promoter vector. Besides, a heavy metal biosensor was produced by utilizing the promoter ZntA (PzntA) upstream the bicistronic bacterial luciferin reporter gene. The PzntA is regulated and activated by the complex formed by a regulatory protein and heavy metal ions, the special affinity and selectivity of the PzntA gene sequence make it an essential

element in the detection of the availability of heavy metal, and the biosensor made in the present study was sensitive to Cd(II), Pb(II) and Zn(II) and the production of the bacterial reporter gene generated a dose-response in the reaction of the addition of the decanal which is a bacterial luciferin substrate .

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