STRUCTURAL CHARACTERIZATION OF A NOVEL LUCIFERASE-LIKE MONOOXYGENASE FROM BACTERIA *Pseudomonas meliae*

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DEDICATION

This thesis is dedicated to my parents, who has supported me each and every moment, either good time or bad time, and my professor who has helped me throught the whole process.

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ABSTRACT

Luciferase is well known oxidative enzyme that produce bioluminescence. Since the discovery of luciferase, it has been used in many applications as emission of light during bioluminescent nature serves as a visual indicator for observation. In this study, the aim is to model and characterise a novelty of the luciferase-like monooxygenase protein/amino acid found in *Pseudomonas meliae* for its similarity to well established luciferase enzymes. The novel protein sequence was modelled and compared with established structures using bioinformatics methods. The *Pseudomonas meliae*, a plant pathogen that causes wood rot on nectarine, peach and *Platanus spp*. possess a luciferase-like monooxygenase that if activated, creates an intriguing prospect of using the pathogen's bioluminescent as a visual indicator of diseased plants. If the pathogen's own protein can be activated when the plant has been infected, its bioluminescent bacterial gall can be used to identify affected plants. In this study, the suitability of the luciferase like monooxygenase from *P. meliae* that infects chinaberry plants has to be modelled first, and then studied by comparing it with existing known luciferase. The sequence of *Pseudomonas meliae* (A0A0P9UTV8) was characterized and modelled using 3B9O as a template, using bioinformatics tools. Similarities between uncharacterized luciferase from *Pseudomonas meliae* and template from *Geobacillus thermodenitrificans* were analysed. The active site remains identical but with the exception of two amino acids; *P.meliae* Tyr138 instead of His138 and Leu311 instead of His311. All the other data 9on various properties of luciferase-like monooxygenase protein and its comparison between template alkane monooxygenase and model luciferase-like monooxygenase primary structure characteristics, similarities of amino acids sequences, binding sites, and predicted active sites of almost similar models. Both structures have similar key characteristics such as, high amino acid residue, Aspartic acid, and Glutamic acid. The results suggest that the absence of bioluminescence in *P.meliae* could be due to the evolutionary mutation in position 138 and 311. The Pseudomonas genera has been shown to react with light such as *Pseudomonas fluorescens* that emit luminescence under UV light as well as the application of bioluminescent *Pseudomonas aeruginosa* to assess the antimicrobial efficacy of wound dressings by monitoring light emission. Therefore, the *P.meliae* will have a potential future application, should the residues 138 and 311 be mutated to restore luciferase light emitting ability in future research. Suitability for further improvement, activation, and repurposing the luciferase from *Pseudomonas meliae* as a disease marker would depend on the outcome of this study.

ABSTRAK

Luciferase adalah enzim oksidatif yang terkenal yang menghasilkan bioluminescence. Sejak penemuan luciferase, luciferase telah digunakan dalam banyak aplikasi sebagai pemancaran cahaya oleh bioluminescent semulajadi berfungsi sebagai penunjuk visual untuk pemerhatian. Dalam kajian ini, matlamatnya adalah untuk memodelkan dan mencirikan sejenis gen monooxygenase seperti luciferase yang terdapat dalam *Pseudomonas meliae* kerana kesamaannya dengan enzim luciferase yang mantap. Susunan protein novel akan dimodelkan dan dibandingkan dengan struktur luciferase yang diketahui menggunakan kaedah bioinformatik. *Pseudomonas meliae*, patogen tumbuhan yang menyebabkan kayu membusuk nektarine, pic dan *Platanus spp*. mempunyai monooxygenase seperti luciferase yang jika diaktifkan, mewujudkan prospek yang menarik menggunakan bioluminescent patogen sebagai penunjuk visual tanaman berpenyakit. Sekiranya protein patogen itu dapat diaktifkan apabila tumbuhan itu dijangkiti, bakteria bakteria bioluminescent dapat digunakan untuk mengenal pasti tumbuhan yang terjejas. Dalam kajian ini, kesesuaian luciferase seperti monooxygenase dari *P. meliae* yang menjangkiti tumbuhan chinaberry perlu dimodelkan dahulu, dan kemudian dikaji dengan membandingkannya dengan luciferase yang diketahui. Urutan (A0A0P9UTV8) *Pseudomonas meliae* dicirikan dan dimodelkan menggunakan 3B9O sebagai templat menggunakan alat bioinformatik. Kesamaan antara luciferase yang tidak ditakrifkan dari *Pseudomonas milae* dan template dari *Geobacillus thermodenitrificans* dianalisis. Tapak aktif tetap sama tetapi dengan pengecualian dua asid amino; *P.meliae* Tyr 138 bukan His138 dan Leu311 bukan His311. Semua data lain mengenai pelbagai sifat luciferase seperti protein monooxygenase dan perbandingannya antara template alkane monooxygenase dan model luciferase seperti monooxygenase ciri-ciri struktur utama, persamaan urutan amino asid, mengikat laman web dan meramalkan laman-laman aktif yang hampir serupa. Kedua-dua struktur mempunyai ciri-ciri utama yang sama seperti, residu asid amino tinggi, asid aspartik, dan asid glutamat. Keputusan menunjukkan bahawa ketiadaan bioluminescence di *P.meliae* boleh disebabkan oleh mutasi evolusi di kedudukan 138 dan 311. Genera Pseudomonas telah ditunjukkan untuk bertindak balas dengan cahaya seperti *Pseudomonas fluorescens* yang memancarkan pencahayaan di bawah cahaya UV serta aplikasi daripada *Pseudomonas aeruginosa* bioluminescent untuk menilai keberkesanan antimikrob pembalutan/persalinan luka dengan memantau pelepasan cahaya. Oleh itu, *P. meliae* akan memiliki kemungkinan aplikasi masa depan, sekiranya residu 138 dan 311 dimutasi untuk mengembalikan keupayaan pemancar cahaya luciferase pada masa akan datang penyelidikan. Kesesuaian untuk penambahbaikan, pengaktifan, dan penggunaan semula luciferase dari *Pseudomonas meliae* sebagai penanda penyakit bergantung pada hasil kajian ini.

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ATP	-	Adenosine triphosphate
Fluc	-	Firefly Luciferase
AMP	-	Adenosine monophosphate
Msc	-	Masters of Science
Da	-	Dalton
GS	-	Ground State
DNA	-	Deoxyribonucleic acid
CFSSP	-	Chou & Fasman Secondary Structure Prediction Server

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CHAPTER ONE

INTRODUCTION

1.1 Research Background

The name of luciferase and luciferin were introduced by a scientist Emil Du Bois-Reymond-Reymond in 1885 (Fraga, 2008). Later on, in 1940, Dr Green and McElroy extracted and purified luciferase protein. By using this process, they have isolated the enzyme and determined its conformational structure (C. England *et al.*, 2016). He investigated the components required for the bioluminescence of the click beetle. He used only cold water and abdomens of *Elateridae* click beetle, and it was able to produced luminescence in the laboratory. Basically he named two extracted components, the molecule that was consumed in the reaction "Luciferine" and the enzyme which is responsible for the chemical reaction, he named Luciferase. The next essential discovery was by Marlene Deluca, who reported that the cloning of firefly luciferase (FLuc) in *Escherichia coli*, have a direct way for this technique to be widely utilized in many luciferase systems (Kricka *et al.*, 1989). Firefly luciferase is broadly used as a reporter gene for gene regulation and pharmaceutical screening. Firefly luciferase doesn't require subsequent processing activity because firefly luciferase's 61k Dalton protein is active. Firefly luciferase catalyzes an oxidative reaction including ATP (Baldwin, 1996). It catalyzes the oxidative decarboxylation of luciferin, a 6-hydroxybenzothiazole, to oxyluciferin in the ATP, Mg^{2+}, and O_2 (Koncz *et al*, 1990). Firefly luciferin and molecular oxygen yields an electronically energized oxyluciferin species. The excited species emits visible light, which is used by the firefly in its reproductive behavior. Firefly luciferase was the first enzyme for which the biochemical detail was investigated (Baldwin, 1996). Among other applications, the firefly luciferase has been used as reporter gene in living cell and organisms. For instance, the longer-wavelength light emission enhances the

animal's tissue penetration. For the substrate, ATP and D-luciferin would likely improve the glossiness of the light delivered by intracellular luciferase.

Luciferase, especially sourced from firefly (*Photinus pyralis*) has been utilized as a reporter protein in different assay systems including gene expression and was applied in the process of high-throughput screening for drug discovery (Inouye, 2010). Using bioluminescence as a visual cue to signal changes have been well established. This bioluminescence normally happens in nature in different green growths, microscopic organisms, parasites, and some oceanic creatures, for example, jellyfish (Thorne *et al.*, 2010). The luciferase gene is basically extracted and used as a reporter gene to visualize expression of various gene for a wide variety of organism. The first luciferase protein was successfully purified from fireflies in 1940s (Kirkpatrick *et al.*, 2019).

In this study, the aim is to examine the luciferase like monooxygenase gene found in *Pseudomonas meliae* for its similarity to well established luciferase enzymes. The novel protein sequence was modelled and compared with known structures using bioinformatics methods. Suitability for further improvement, activation, and repurposing as a disease marker would depend on the outcome of this study.

1.2 Problem Statement

Firefly luciferase has an established application in reporting gene regulation and pharmaceutical screening. The bioluminescent nature serves as a useful cue, creating a relatively easy visual indicator for parameters pre-determined by the applications. The *Pseudomonas meliae*, is a plant pathogenic bacteria which causes wood rot on nectarine. Peach, platanus, and platanus spp possess a Luciferase-like monooxygenase. The presence of luciferase like monooxygenase by a pathogen, in this case *Pseudomonas meliae*, creates an intriguing prospect of using the pathogen's bioluminescence as a visual indicator of

diseased plants. If the pathogen's own protein can be activated when the plant has been infected, its bioluminescent bacterial gall can be used to identify affected plants. In this study, the suitability of the luciferase like monooxygenase from *P. meliae* that infects chinaberry plants is to be first modelled and studied by comparing it with existing known luciferase.

1.3 Research Objectives

The objectives of this research are:

1. To investigate the primary and secondary sequence characteristics of a novel Luciferase-like monooxygenase protein from *Pseudomonas meliae*.
2. To model and analyse the three dimensional (3D) structure of the protein.
3. To compare the modelled 3D structure with established template structures in the database.

1.4 Significance of the study

Bacterial galls are plant tumor disease characterized by non-self-limiting tissue overgrowth. The gall poses a significant economic effect on plantations of nectarine, peach, and *platanus* species. The gall forming bacteria inhibit the soil and can survive for two years, making its total eradication a challenge. Galls have been shown to infect young trees or cause loss of productivity by having a dead top (spike top) (Hansen *et al.*, 1933). The affected plants would have slow growth and dieback of shoots and stems (Taghavi *et al.*, 2009). If the luciferase expressed by *Pseudomonas meliae* is suitable and subsequently introduced, it would create a novel reporting system pathogen, which uses the bacterial gall against itself. Infected trees can be quickly identified and corrective actions can be

taken. Thus, the economic effect of the disease on nectarine, peach, and *platanus spp* can be minimized.

1.5 Scope of Research

The research is purely computational work, using established bioinformatics tools to achieve the desired objectives. The chosen organism is a pathogenic species, named *Pseudomonas meliae*. All research work would be based upon data already present in existing sequence and structural databases. No laboratory work shall be performed at this stage of this research, as it will be beyond the scale and time available for this MSc dissertation.

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