UNIVERSITI PUTRA MALAYSIA

GENOMIC STUDY OF Photobacterium marinum STRAIN J15 AND DEVELOPMENT OF GENOME SCALE METABOLIC MODEL FOR CELLULAR METABOLISM IDENTIFICATION

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By

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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November 2017

Chairman : Suriana Sabri, PhD
Faculty : Biotechnology and Biomolecular Sciences

Photobacterium sp. strain J15 was originally isolated from marine water of Tanjung Pelepas, Johor. It was initially studied for its novel GDSL esterase and asparaginase production. With the production of the potential enzymes and secondary metabolites, it was realised that little is known about the general metabolism of this *Photobacterium* genus. Therefore, the aim of this study was to provide an understanding of cellular metabolism of strain J15 through genomics and phenomics analysis and characterization of the genome-scale properties. Genome scale metabolic model was also constructed in order to enhance the understanding of the cell's organization and its functionality as a whole.

In this study, the genome sequence of *Photobacterium* sp. strain J15 was determined using PacBio sequencing. The genome contained 5,684,538 base pairs comprised 3 contigs; 2 chromosomes and 1 plasmid. A total of 4,924 open reading frames (ORFs) were predicted and 4,811 proteins were annotated against NCBI database. There are altogether 3,238 proteins with GO assignment and 874 proteins assigned to KEGG pathways. Phylogenomics analysis showed that *Photobacterium* sp. strain J15 is indeed the same species with *P. marinum* AK15.

There were 535 subsystems represented in the draft genome scale metabolic model (GEM) of *P. marinum* J15. The *iSS1110* model consists of 1110 genes with 1495 biochemical reactions and 40 % of total number of the reaction was the sum of the three largest subsystems; amino acid metabolism, carbohydrate metabolism, and metabolism of cofactors as well as vitamins.

To investigate the connections between genome and phenome of *P. marinum* J15 and to validate the constructed GEM, BIOLOG Phenotype Microarray was performed. Also,

DuctApe program was used in order to further analyse metabolic functionality of this bacterium. Main phenotype microarray (PM) assays showed that *P. marinum* J15 was able to use: i) 93 of the 190 carbon sources tested, where 61 were used efficiently, among which there were 16 amino acids; ii) 41 of the 95 nitrogen sources tested, where 22 compounds were used efficiently, among which were 9 amino acids and 8 peptides, and iii) 3 of the 94 phosphorous and sulphur sources tested. Furthermore, resistance to antibiotics, high tolerance to osmotic stress, to basic pH and to toxic compounds was revealed by PM.

The complete genome, draft GEM and phenotypic data of *P. marinum* J15 in this study would be a resource for many subsequent studies of this genus, especially since there is only one published research on isolation of *P. marinum* AK15, but none on the genomic and phenotypic characteristic. This information will be essential for discovery of unique genes and secondary metabolite.

Abstrak tesis yang dikemukakan kepada Senat of Universiti Putra Malaysia sebagai memenuhi keperluan untuk Master Sains

KAJIAN GENOMIK *Photobacterium marinum* STRAIN J15 DAN PENGHASILAN METABOLIK MODEL BERSKALA GENOM UNTUK PENGENALPASTIAN METABOLISMA SEL

Oleh

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Photobacterium sp. strain J15 dipencilkan dari air laut Tanjung Pelepas, Johor. Ia pada mulanya dikaji untuk pengeluaran esterase GDSL dan asparaginase. Walaupun mempunyai pengeluaran enzim yang berpotensi dan metabolit sekunder, ternyata sedikit diketahui tentang metabolisme umum genus *Photobacterium* ini. Oleh itu, matlamat kajian ini adalah untuk memberi pemahaman mengenai metabolisme sel J15 terikan melalui analisis genom dan analisis fenomik dan pencirian sifat-sifat skala genom. Model metabolik skala genom juga dibina untuk meningkatkan kefahaman tentang organisasi sel dan fungsinya secara menyeluruh.

Dalam kajian ini, jujukan genom *Photobacterium* sp. strain J15 ditentukan menggunakan penjujukan PacBio. Genom mengandungi 5,684,538 pasang bes terdiri daripada 3 contigs; 2 kromosom dan 1 plasmid. Sebanyak 4,924 bingkai bacaan terbuka (ORF) telah diramalkan dan 4,811 protein telah dilengkapkan dengan pangkalan data NCBI. Terdapat 3,238 protein dengan fungsi GO dan 874 protein dipadankan dengan fungsi KEGG. Analisis phylogenomik menunjukkan bahawa *Photobacterium* sp. strain J15 merupakan spesies yang sama dengan *P. marinum* AK15.

Terdapat 535 subsistem yang diwakili dalam draf model metabolik skala genom (GEM) daripada *P. marinum* J15. Model iSS1110 terdiri daripada 1110 gen dengan 1495 reaksi biokimia dan 40% daripada jumlah reaksi adalah jumlah tiga subsistem terbesar; metabolisme asid amino, metabolisme karbohidrat, dan metabolisme kofaktor serta vitamin.

Untuk mengkaji hubungan antara genom dan fenom *P. marinum* J15, BIOLOG Phenotype Microarray telah dilakukan. Juga, program DuctApe digunakan untuk menganalisis lagi fungsi metabolik bakteria ini. Ujian microarray fenotip utama (PM)

menunjukkan bahawa ketegangan *Photobacterium marinum* J15 dapat digunakan: i) 93 daripada 190 sumber karbon yang diuji, di mana 61 telah digunakan dengan cekap, antaranya ada 16 asid amino; ii) 41 daripada 95 sumber nitrogen diuji, di mana 22 sebatian digunakan dengan cekap, antaranya 9 asid amino dan 8 peptida, dan iii) 3 daripada 94 sumber fosforus dan sulfur yang diuji.

Genom lengkap, draf GEM dan data fenotip *P. marinum* J15 akan menjadi sumber untuk banyak kajian seterusnya tentang genus ini, tertamanya kerana hanya terdapat satu kajian tentang isolasi *P.marinum*, tetapi tiada maklumat tentang genomik dan fenomik. Maklumat ini akan diperlukan untuk penemuan gen yang unik dan metabolit sekunder.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

°C	Degree celsius
%	Percentage
A_{600nm}	Optical density at wavelength 600 nanometer
µL	Microliter
µmoles	Micromoles
DSMZ	Deutsche Sammlung von Mikroorganismen und Zellkulturen
ATCC	American Type Culture Collection
ATP	Adenosine triphosphate
sp.	Species
bp	Base pair
DNA	Deoxyribonucleic acid
E. coli	*Escherichia coli*
EC	Enzyme Commission
g	Gram
kb	Kilobase
L	Litre
M	Molar
T	Transmission
SNP	Single nucleotide polymorphism
ANI	Average nucleotide identity

CHAPTER 1

INTRODUCTION

The genus *Photobacterium* was one of the earliest known bacterial taxa (Beijerinck, 1889). *Photobacterium* is a gammaproteobacteria from the *Vibrionacea* family. The genus is ubiquitous all over the world's oceans including coastal, open-ocean and deep-sea water habitat (Urbanczyk et al., 2011). Some species of this genus are capable of producing antibacterial compounds, industrial and medical important enzymes as well as essential polyunsaturated fatty acids. Meanwhile, some species are luminous, pathogenic, piezophilic, symbiotic with marine animals and some are opportunistic pathogenic to human (Labella et al., 2017).

To date, out of 23 species known, there are 20 species of *Photobacterium* genomes have been sequenced. The genome scale metabolic model (GEM) of this genus has yet to be constructed. The most studied member of *Photobacterium* is *P. profundum* strain SS9 and the majority of the studies focused on the genome sequence and the genome analysis in general (Vezzi et al., 2005). Since this genus is also known for its bioluminsence properties, most study also fixed on that proeprties, apart from its pathogenicity showed in some strains. The development of genome-scale metabolic network reconstructions for organisms with sequenced genomes would enabled a fast and systematic way to understand the physiology of cells and interpret experimental observations (Urbanczyk, Ast, and Dunlap, 2011) which is why it is necessary for a GEM of *Photobacterium* to be constructed.

Photobacterium sp. strain J15 is a marine organism isolated from Tanjung Pelepas, Johor (Shakiba et al., 2015). However, apart from the absence of GEM of *Photobacterium* species, there is also lack of information of phenotypic characteristic of this genus. This lack of data hinders better understanding on this bacterium, which is why the construction of GEM and phenotypic microarray data is warranted for this bacterium.

In regard to that, this study aims to construct the GEM of *Photobacterium* sp. strain J15 and also provide the phenotypic data by using BIOLOG Phenotypic Microarray. Hypothesis of this study is the reconstruction of GEM and BIOLOG Phenotypic Microarray could permit us to comprehend metabolism of the cell and the utilization of the cell in specific substrates. This information could help a lot in metabolic engineering especially in modifying certain genes to optimize the production of certain metabolites. The GEM of *Photobacterium* sp. strain J15 could serve as basic for the reconstruction of genome scale metabolic model of other strains of *Photobacterium*.

In order to construct the GEM, genome sequence of *Photobacterium* sp. strain J15 has to be acquired. Thus, the objectives of this study are as followed.

1) To acquire the genome sequences of *Photobacterium* sp. strain J15.
2) To construct the genome scale metabolic (GEM) based on the genomic sequences and annotation.
3) To validate the GEM with BIOLOG Phenotypic Microarray.

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