GENOMIC AND TRANSCRIPTOMIC ANALYSES OF *Jeotgalibacillus malaysiensis* TO PROVIDE INSIGHTS INTO ITS OSMOTIC ADAPTATION

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A special dedication to my lovely husband, parents,

family, and friends

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ABSTRACT

The genus *Jeotgalibacillus* under the family of *Planococcaceae* is one of the understudy genera. In this project, a bacterium strain D5 was isolated from Desaru beach, Johor. This study aimed to characterize strain D5 from morphological, physiological and biochemical aspects, in addition to examine the genomic feature and RNA expression response under saline stress. To achieve this objective, polyphasic analyses (phenotypic and genotypic), genome sequencing and RNA-Seq-based transcriptome analyses were performed. The result showed that strain D5 was mostly similar to *Jeotgalibacillus alimentarius* DSM 18867^T with 99.87% 16S rRNA sequence similarity, and lower in similarity to other species. Strain D5 is distinguished from the other *Jeotgalibacillus* type strains due to the differences in phenotypic and genotypic characteristics which include morphology, endospore position, and shape, acid production from carbohydrates, tolerance to NaCl, fatty acid composition, DNA G+C content and DNA–DNA relatedness values. The D5 was proposed as a new type strain with the name of *J. malaysiensis* (DSM 28777^T =KCTC 33350^T). The complete genome of *J. malaysiensis* (3.52 Mbp) was sequenced using PacBio RS II sequencing system and was compared with draft genomes of *J. alimentarius*, *J. campisalis*, and *J. soli*. In total, 224 complete metabolic pathways were detected in *J. malaysiensis* genome, which include glycolysis, Krebs cycle, TCA cycle, pentose phosphate and others. *J. malaysiensis* shared 1,158 orthologous genes with the other *Jeotgalibacillus* spp. Which are involved in central metabolism, such as genes for flagellar activity, amino acid transport, translation, ribosomal structure, and biogenesis. To understand the mechanism of osmotic adaptation in *J. malaysiensis*, cells were cultivated in marine broth supplemented with 2% NaCl (control), saline stress of 10% and 20% (w/v) NaCl. The pairwise differentially expressed gene (DEGs) detected during the osmotic upshift were analyzed and categorized according to eggNOG (evolutionary genealogy of genes: Non-supervised Orthologous Groups) groups. At 10% (w/v) NaCl, 195 genes were differentially expressed while at 20 % (w/v) NaCl, 166 DEGs were differentially expressed. Osmotic stress significantly affected carbohydrate, energy, and amino acid metabolism, as well as fatty acid biosynthesis. *J. malaysiensis* apply a combination of approaches which include utilization of the TRK system for the regulation of K^+ uptake, uptake and synthesis of various osmoprotectants especially proline and glutamate. As a conclusion, this study has provided new insights into the biology of this genus and showed that *J. malaysiensis* established halotolerance via a global cooperative mechanism, rather than by one single approach.

ABSTRAK

Genus *Jeotgalibacillus*, dari famili *Planococcaceae* merupakan salah satu genus yang kurang dipelajari. Di dalam projek ini, bakteria D5 telah dipencilkan dari Pantai Desaru, Johor. Kajian ini bertujuan untuk mencirikan bakteria D5 melalui aspek morfologi, fisiologikal dan biokimia, di samping mengkaji ciri genomik dan respon ekspresi RNA pada keadaan stress akibat kandungan garam yang tinggi. Bagi mencapai objektif ini, analisa melalui kaedah polifasik (fenotip dan genetik), penjujukan genom dan transkriptomik penjujukan-RNA telah digunakan. Keputusan menunjukkan, bakteria D5 didapati paling berkait rapat dengan *J. alimentarius* DSM 18867^T dengan 99.87% persamaan pada jujukan 16S rRNAnya berbanding spesis lain dalam genus ini. Bakteria D5 juga dibezakan dari spesis-spesis yang lain melalui perbezaan ciri-ciri fenotip dan genotip seperti morfologi koloni, kedudukan dan bentuk endospora, pengeluaran asid daripada karbohidrat, toleransi kepada NaCl, komposisi asid lemak, kandungan G + C di dalam DNA genomik dan nilai kaitan DNA-DNA. Justeru, bakteria D5 dicadangkan sebagai spesis baru yang dinamakan *Jeogalibacillus malaysiensis* (DSM 28777^T =KCTC 33350^T). Genom lengkap *J. malaysiensis* (3.52 Mbp) telah dijujukkan menggunakan sistem jujukan PacBio RS II dan dibandingkan dengan draf genom *J. alimentarius, J. campisalis*, dan *J. soli*. Keseluruhannya, 224 laluan lengkap metabolik dikesan di dalam genom *J. malaysiensis,* temasuklah glikolisis, kitaran Kreb, kitaran TCA, laluan fosfat pentosa dan lain-lain. *J. malysiensis* berkongsi 1,158 gen ortologous dengan spesis-spesis lain *Jeotgalibacillus*, gen-gen ini terlibat dalam metabolisma utama seperti aktiviti flagella, pengangkutan asid amino, translasi, struktur ribosom dan biogenesis. Untuk memahami mekanisma adapatasi osmotik *J. malaysiensis*, sel ini telah dikulturkan di dalam "*Marine broth*" yang mengandungi 2% NaCl (kawalan), 10%, dan 20% (w/v) NaCl. Gen-gen pasangan kelainan ekspresi (DEGs) yang dikenalpasti sewaktu peningkatan osmotik dianalisis dan dikenalpasti mengikut kumpulan "*eggNOG" (evolutionary genealogy of genes: Non-supervised Orthologous Groups)*. Pada 10% (w/v) NaCl, 195 gen telah menunjukkan kelainan pada ekspresinya manakala pada 20 % (w/v) NaCl, 166 DEGs menunjukkan kelainan pada ekspresinya. Tekanan osmatik, juga memberi kesan kepada metabolisma karbohidrat, tenaga dan asid amino, serta penghasilan asid lemak. *J. malaysiensis* menggunakan beberapa kaedah termasuk penggunaan sistem TRK untuk regulasi pengambilan K^+, pengambilan dan sintesis pelbagai *osmoprotectant* terutamanya prolin dan glutamate. Sebagai kesimpulan, kajian ini memberikan perincian yang baru dalam aspek biologi genus ini dan menunjukkan keupayaan *J. malaysiensis* untuk beradaptasi dengan kandungan garam yang tinggi (*halotolerant*) melalui pelbagai mekanisma berbanding hanya satu kaedah sahaja.

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LIST OF ABBREVIATIONS

BLAST	-	Basic Local Alignment Search Tool
Bp	-	Base pair
CO_2	-	Carbon dioxide
COG	-	Cluster of ortholog
DEG	-	Differential expression gene
dNTP	-	Deoxyribonucleotide triphosphate
E. coli	-	*Escherichia coli*
EDTA	-	Ethylenediaminetetra-acetate
eggNOG	-	Evolutionary genealogy of genes: Non-supervised Orthologous Group
EM	-	Electron microscope
EPS	-	Extracellular polysaccharides
FAME	-	Fatty acid methyl ester
FESEM	-	Field emission scanning electron microscope
H_2O	-	Water
H_3BO_3	-	Boric acid
HCl	-	Hydrochloric acid
HCO_3	-	Bicarbonate ion
HPLC	-	High Performance Liquid Chromatography
IPTG	-	Isopropyl β-D-1-thiogalactopyranoside
J.	-	*Jeotgalibacillus*
JAL	-	*Jeotgalibacillus alimentarius*
JCA	-	*Jeotgalibacillus campisalis*
JMA	-	*Jeotgalibacillus malaysiensis*
JSO	-	*Jeotgalibacillus soli*
K	-	Potassium
K^+	-	Potassium ion

KCl	-	Potassium cloride
KEGG	-	Kyoto encyclopedia of genes and genomes
KNO_3	-	Potassium nitrate
KOH	-	Potassium hydroxide
Lac	-	Lactose
LB	-	Luria-Bertani
MA	-	Marine agar
MB	-	Marine broth
Mb	-	Mega-base pairs
Min	-	minute
Na^+	-	Sodium ion
NaCl	-	Sodium Chloride
NADPH	-	Nicotinamide adenine dinucleotide phosphate-oxidase
NaOH	-	Sodium hydroxide
Ng	-	Nanogram
Nm	-	Nanometer
O_2	–	Oxygen
OD_{600}	-	Optical Density at 600nm
PCR	-	Polymerase Chain Reaction
Rpm	-	Revolutions per minute
rRNA	-	Ribosomal Ribonucleic Acid
RNA	-	Ribonucleic Acid
s	-	Second
TAE	-	Mixture of Tris Base, Acetic Acid and EDTA
TCA	-	The citric acid
TEM	-	Transmission electron microscope
TSB	-	Tryptic Soy Broth
v/v	-	Volume per volume
w/v	-	Weight per volume
X-Gal	-	5-bromo-4-chloro-indolyl-β-D-galactopyranoside

LIST OF SYMBOLS

%	-	Percentage
α	-	Alpha
β	-	Beta
σ	-	Sigma
°C	-	Degree celcius
d	-	Day
g	-	Gram
g/ L	-	Gram per liter
g/g	-	Gram per gram
h	-	Hour
h^{-1}	-	Per hour
lx	-	lux
m	-	Meter
min	-	Minute
mA	-	Milliampere
mL	-	Mililitre
mm	-	Milimeter
M	-	Molar
mM	-	Milimolar
nm	-	Nanometer
rpm	-	Revolutions per minute
µL	-	Microlitre
µm	-	Micrometer

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CHAPTER 1

INTRODUCTION

1.1 Background Information

Seventy percent of the earth surface is enclosed with water which carries 80% of entire life form (Soliev *et al.,* 2011). The marine environment comprises elevated level of salts; hence, many ambiguities connected with aquatic organisms are yet to be revealed. Marine environment has become an interesting research focus for numerous investigations because of its rich biodiversity for decades (Gerwick and Fenner, 2013; Mazalan *et al.*, 2012; Shah *et al.*, 2014; Trincone, 2013). The search for marine metabolites is difficult dut to the inaccessibility and non-culturability of the majority of microorganisms (Bhatnagar and Kim, 2012).

Marine microbiology emphasizes mainly on prokaryotes in particularly bacteria because of their easy dispersability and small size. Bacteria are virtually ubiquitous in marine environment. Additionally, natural populations of marine bacteria are physiologically diverse, and they can survive extensive periods of starvation and exhibit the ability to resume metabolic activities as soon as the substrates become available (Carrero-Colón *et al.*, 2006).

Marine environment also contains abundance of halophilic and halotolerant microorganisms. For these microorganisms, salinity and osmotic stress tolerance are prerequisites for survival. Marine bacteria become attractive to researchers because of their potential to produce compounds with unique biological properties (Teasdale *et al.*, 2009). Gene evolutionary, transferring, and mutagenesis events have diversified marine bacteria. To date, marine *Pseudomonas*, *Streptomyces*, *Bacillus*, *Pseudoalteromonas*, *Cytophaga* and *Vibrio* isolated from seawater, sediments, algae, and marine invertebrates are identified to produce bioactive agents. They are able to produce alkaloid derivatives (prodiginines and tambjamines), indoles (quinones and violacein), polyenes, peptides, macrolides, and terpenoids (Soliev *et al.*, 2011). Genomic analyses have become one of the tools to study in depth of bacteria potential, in addition to knowing their evolution and adaptations. By understanding the genome of the novel bacteria, it will help to discover more of its properties. The complete bacterial genomics allows scientists to obtain specific sequence that may be used to select potential compound or metabolites that can be further utilized (Arcus *et al.*, 2006; Sakharkar *et al.*, 2004).

Many halophiles and halotolerants are established and well characterized, however *Jeotgalibacillus* is one of the least-studied genera. *Jeotgalibacillus* spp. are bacteria that are taxonomy affiliated under *Planococcaceae* (family), *Bacillales* (order), *Bacilli* (class), and *Firmicutes* (phylum). *Jeotgalibacillus* genera were named due to its shape of bacilli and were isolated from a Korean feremented food '*Jeotgal*'. This food was made by adding 20-30% (w/w) salt to various types of seafood (Guan *et al.*, 2011). Many bacteria were isolated from this food; some of the bacteria have the ability to adapt with the high salt environment. Since jeotgal is a fermented food, facultative anaerobic bacteria isolated from it serves as a good starter for fermentation (Yoon *et al.*, 2001).

Currently, eight species are assigned to the genus, namely *Jeotgalibacillus alimentarius* (Yoon *et al.*, 2001), *Jeotgalibacillus marinus* (Yoon *et al.*, 2010), *Jeotgalibacillus campisalis* (Yoon *et al.*, 2004), *Jeotgalibacillus salarius* (Yoon *et al.*, 2010), *Jeotgalibacillus soli* (Cunha *et al.*, 2012), *Jeotgalibacillus malaysiensis* (this

study) and two recently added strain which are *Jeotgalibacillus alkaliphilus* and *Jeotgalibacillus terrae* (Srinivas *et al.*, 2016). Both *J. soli* and *J. terrae* were isolated from soil while the others *Jeotgalibacillus* spp. were isolated from salty environments.

Limited research was done on the ability of *Planococcaceae* to adapt high salt concentration in terms of genes regulation. Study on halotolerant bacteria usually focused on physiological and biochemical studies dealing with the mechanisms of haloadaptation and adaptability (Saum *et al.*, 2013a; Ollivier *et al.*, 1994; Zahran, 1997). Changes in salinity have been reported to trigger drastic changes of the transcriptome of the bacterial cells (Zajc *et al.*, 2013). Bacteria applied several mechanisms of adaptation in order to survive under high concentrations of salt in the environment. It is hypothesize that during the course of evolution and long process of adaptation, ability of each genus to response high salinity stress will be slightly different. It is therefore interesting to examine the type of genes that are expressed during high salinity stress to those at low salinity condition and the influence of high salt condition on *J. malaysiensis* physiologically.

1.2 Problem Statement

The findings of *J. malaysiensis*, a novel marine halotolerant bacterium from Desaru beach, Johor could provide insight into understanding this genus. *Jeotgalibacillus* is one of the least-studied genera in terms of the total number of strains, knowledge understood, and published reports. When this project was initiated, less than ten articles were related to this genus. The effects of evolution to this bacterium from various aspects have yet to be ascertained. Hence, by using genomic and transcriptomic approach, comprehensive analyses on this novel bacteria property and its adaptation to survive in high salt environment could be understood. The analyses can also provide a guideline for other researcher in understanding this genus and its survival especially in adapting to high salt environments such as marine.

1.3 Research Objectives

The objectives of this study are as follows:

i) To characterize *J. malaysiensis* using polyphasic approach.
ii) To analyze genome of *J. malaysiensis* and other *Jeotgalibacillus* spp.
iii) To examine the transcriptome changes of *J. malaysiensis* under high saline stress.

1.4 Research Scope

The research scope of the study covered full analyses of bacterium strain D5 as a new novel strain in *Jeotgalibacillus* genera and its ability to adapt the high salt condition are as follows:

i) To conduct 16S rRNA identification of bacteria isolated from marine seawater, sand, plants, rocks and sediment in Johor shore.
ii) To determine the novelty of bacteria based on molecular and biochemical identification.
iii) To characterize the isolated bacteria using polyphasic taxonomy, which included genotypic and phenotypic information, with the aim to name the bacteria as *J. malaysiensis*.
iv) To perform genome isolation, sequencing, assembly and annotation of type strains of *Jeotgalibacillus*.
v) To perform RNA isolation and purification of the bacteria grown in low saline (2% NaCl) and high saline (10% and 20% NaCl) medium. The total transcriptome was sequenced using Illumina MiSeq.
vi) To analyze the genome and transcriptome changes of *J. malaysiensis* RNA-Seq and bioinformatics software and databases.

vii) To determine the mechanism of salt tolerance in *J. malaysiensis* by studying differential gene expression (DEG) at low and high NaCl condition.

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