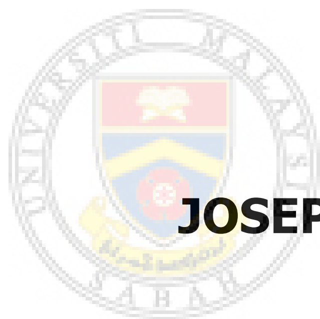
ANALYSIS OF THE COLD ADAPTATION STRATEGY OF ANTARCTIC YEAST *Glaciozyma antarctica* PI12



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BIOTECHNOLOGY RESEARCH INSTITUTE
UNIVERISITI MALAYSIA SABAH
2016

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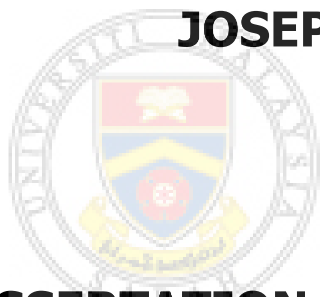
JOSEPH KOH SOON PENG



DISSERTATION SUBMITTED IN FULFILLMENT FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN BIOTECHNOLOGY

BIOTECHNOLOGY RESEARCH INSTITUTE
UNIVERISITI MALAYSIA SABAH

2016

DECLARATION

I hereby declare that the material in this project is my own except for quotations, excerpts, equations, summaries and references, which have been duly acknowledged.

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CERTIFICATION

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VIVA DATE	**:**	**4th APRIL 2016**

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ABSTRACT

Analysis of the cold adaptation strategy of Antarctic yeast *Glaciozyma antarctica* PI12

Psychrophilic yeast *Glaciozyma antarctica* PI12 was isolated from Antarctica. However, the information related to psychrophilic yeast and genus Glaciozyma is limited. Therefore, characterization of growth, cell doubling time, cell division, aerobic and partial anaerobic respiration system, morphology and the growth at -12°C, -7°C and -5°C were carried out. Our result showed that *G. antarctica* PI12 formed whitish creamy colony on PDA media, and has an optimal growth temperature of 12°C in YPD media. Its cell doubling time is 15.8 hours per generation, and the cell division occurs on either poles of the cell. *G. antarctica* PI12 can grow under both aerobic and partially anaerobic conditions, but with a faster growth at aerobic condition. Little is known about the other genes which are involved in the cold adaptation of *G. antarctica* PI12. Therefore, to understand the adaptation strategies of *G. antarctica* PI12*,* RNA-seq was carried out followed by a *de novo* assembly of *G. antarctica* PI12 transcriptome using the Trinity assembly package. Thermal stresses such as -12°C, 0°C, 16°C and 20°C were used to induce a maximum number of expressed genes by *G. antarctica* PI12. We have obtained approximately 465 million of reads using the paired-end Illumina sequencing platform. These reads was assembled into 6,301 unique genes, which comprised of a total of 46,196 unique transcripts (UT) sequences (mean sequence length ~1, 555 bp) including 29,885 UTs with coding sequence (CDS). Our data provide the first comprehensive sequence resource available for functional genomics studies in *G. antarctica* PI12*.* Besides, the gene expression patterns of *G. antarctica* PI12 in response to rapid temperature shifts were determined. 205 and 206 genes were affected when the cells were rapidly shifted from 12°C to 0°C or -12°C in minimal media, and YPD media. When the cells were rapidly shifted from 12°C to 16°C and 20°C, 116 genes were expressed. We grouped the genes obtained from minimal media and YPD into the early cold response (ECR, 0°C for six hours); late cold response (LCR, 0°C for 24 hours); early freeze response (EFR, -12°C for six hours); and late freeze response (LFR, -12°C for 24 hours). On the other hand, we grouped expressed genes in the heat shock response to the early heat response (EHR, 16°C for six hours); and late heat response (LHR, 16°C and 20°C for 24 hours); early heat response (EHR, 20°C for six hours); and late heat response (LHR, 20°C for 24 hours). Interestingly, there are groups of genes expressed consistently according to the time incubation at six and 24 hours. The result implies that the thermal specific early and late responses are mediated by a different and yet uncharacterized regulatory proteins. An adaptation model of *G. antarctica* PI12 which involved three components, namely the inactivation, the adaptive and the cell death was constructed based on the results, it indicates the complexity of the adaptation strategy of *G. antarctica* PI12 to adapt to a changing temperature.

ABSTRAK

Glaciozyma antarctica PI12 adalah sejenis yis basidiomycetes dan psikrofilik yang telah diasingkan daripada Antartika. Namun, maklumat yang berkaitan dengan yis psikrofilik dan genus Glaciozyma adalah terhad. Untuk memahami dengan lebih lanjut mengenai *G. antartica* PI12, siasatan terhadap ciri-ciri pertumbuhan, masa gandaan sel, pembahagian sel, system respirasi aerobic dan anaerobic seprara, morfologi dan kadar tumbuh semasa dieram pada -12°C, -7°C dan -5°C. Permehatian kami menunjukkan bahawa *G. antarctica* PI12 mempunyai permukaan berkrim putih pada media Potato Dextrose Agar (PDA), dan mempunyai suhu pertumbuhan optimum pada suhu 12°C dalam media C dalam media Yeast Peptone Dextrose (YPD). Masa sel mengganda adalah 15.8 jam setiap generasis, dan pembahagian sel yang berlaku pada kedua-due belah hujung sel. *G. antarctica* PI12 boleh bertumbuh bawah kedua-dua keadaan aerobik dan anaerobik, walaupun begitu, keadaan aerobik memberikan pertumbuhan yang lebih cepat. Maklumat terhadap gen-gen yang terlibat dalam adaptasi sejuk atau panas di dalam *G. antarctica* PI12 juga terhad. Oleh itu, kajian RNA-seq telah dilaksanakan dan diikuti oleh pemasanga RNA secara *de novo* menggunakan pakej pemasangan Trinity. Tegasan haba seperti -12°C, 0°C, 16°C dan 20°C telha digunakan untuk mendorong bilangan maksimum gen yang disalin oleh *G. antarctica* PI12. Sekira-kira 465 juta daripada penjujukan Illumina telah diperolehi. Termasuk 6301 gen yang unik, terdiri daripada sejumlah 46,196 transkrip unik (UT) urutan (min panjang urutan ~ 1,555 bp) termasuk 29,885 SUA dengan pengekodan urutan (CDS) yang diperlukan oleh *G. antarctica* PI12 semasa keadaan haba. Data kami merupakan sumber urutan komprehensif yang pertama yang ada untuk pelengkap data genomik *G. antarctica* PI12 yang sedia ada. Selain itu, corak ekspresi gen *G. antarctica* PI12 sebagai tindak balas kepada perubahan suhu pesat telah ditentukan. 205 gen dan 206 gen terjejas apabila sel-sel telah beralih secara pantas daripada 12°C kepada 0°C atau -12°C dalam YPD and MM. 116 gen telah terjejas apabila sel-sel telah beralih secara pantaas daripada 12°C hingga 16°C atau 20°C. Gen-gen diperolehi daripada semua eksperimen adalah reaksi sejuk awal (ECR, 0°C selama enam jam); reaksi sejuk lewat (LCR, 0°C untuk 24 jam); reaksi membekukan awal (EFR, -12°C selama enam jam); dan reaksi pembekuan lewat (LFR, -12oC untuk 24 jam). Kami juga mengkumpulkan gen dinyatakan dalam reaksi kejutan haba kepada reaksi hangat awal (EHR, 16°C selama enam jam); dan reaksi hangat lewat (LHR, 16°C dan 20°C selama 24 jam); reaksi haba awal (EHR, 20°C selama enam jam); dan reaksi haba lewat (LHR, 20oC selama 24 jam). Kami juga terkumpul gen bersalin konsisten mengikut masa pengeraman pada enam dan 24 jam. Keputusan kami menunjukkan bahawa reaksi tertentu berfungsi pada keadaan berubah awal dan lewat telah diantarai oleh protein-protein yang berbeza dan protein yang belum dicirikan. Selain itu, satu model penyesuaian *G. antarctica* PI12 mengandungi tiga komponen, iaitu komponen menyahaktifkan, komponen penyesuaian dan komponen kematian sel telah dibina berdasarkan keputusan yang didapati, model tersebut menunjukkan kerumitan strategi adaptasi daripada *G. antarctica* PI12 untuk menyesuaikan diri dengan suhu yang berubah-ubah.

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LIST OF SYMBOLS AND ABBREVIATIONS

G - Gram

Mg - Miligram

μg - Microgram

^oC - Degree Celsius

L - Liter

Ml - mililiter

μl - Microliter

Amp - Ampicilin

Kan^r - Geneticin Resistance Gene

Bp - Basepair

DNA - Deoxyribonucleic acid

dNTP - Deoxynucleotide triphosphate

et al. - *et alia* (and others)

i.e - That is

kb - Kilobase

Mb - megabase

kV - Kilovolt

Hr - Hour

Min - Minute

S - second

M - Molar

O.D - Optical density

PCR - Polymerase chain reaction

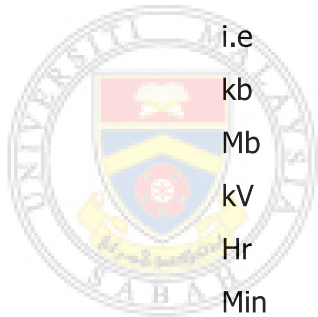
Rpm - Revolutions per minute

TBE - Tris borate EDTA

v/v - Volume per volume

w/v - Weight per volume

Nm - Nanometer



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CHAPTER 1

INTRODUCTION

1.1 Preamble

Cold adapted microorganisms are excellent candidates to provide the understanding of molecular adaptations of a cell towards extreme conditions. Psychrophilic and psychrotrophic microorganisms are first referred to those cold adapted bacteria (Morita, 1975). However, the term is now generally refers to those organisms capable to survive, and proliferate at extremely cold condition. Psychrophiles show metabolic fluxes, which are comparable with those exhibited by mesophiles at moderate temperatures (Mihaela *et al.*, 2009). Moreover, the enzymes produce by the pyschrophiles offer more novel opportunities for biotechnological applications (Zhao *et al.*, 2011).

In order to gain a better understanding of the cold adaptation of psychrophiles, more than 10 cold adapted microorganisms genome have been sequenced to achieve that purpose. Among the sequenced genomes are, *Colwellia psychrerythraea*, *Desulfotalea psychrophila*, *Methanococcoides burtonii*, *Methonagenium frigidum*, *Polaribacter filamentus*, *Polaribacter irgensii*, *Pseudoalteromonas haloplanktis* TAC125, *Psychrobacter arcticus* 273-4, *Psychrobacter cryohalolentis* K5 and *Psychromonas ingrahamii* (Auman *et al.*, 2006; Bakermans *et al.*, 2006; Corien *et al.*, 2009; Gosink *et al.*, 1998; Jeroen *et al.*, 1999; Medigue *et al.*, 2005; methe *et al.*, 2005; Rabus *et al.*, 2004 & Sauders *et al.*, 2003). The genome of the yeast used in this study, *Glaciozyma antarctica* PI12, also has been sequenced using the Roche, 454 and Illumina sequencing platforms. The genome size is about 2.2 million base pairs, sorted into 21 scaffolds, which consisted of a total of 7857 genes. About 10% of the genes found in the genome of *G. antarctica* PI12 are known to be novel genes. Some cold active and adaptation genes such as α-amylase (Ramli *et al.,* 2013), β-mannanse (Parvizpour *et*

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al., 2014), antifreeze protein 1 (Hashim *et al.,* 2013), and antifreeze glycopeptides (AFGP) (Shah *et al.,* 2012) have been identified and cloned.

Margesin (2009) highlighted several cold adaptation strategies that are common among psychrophilic microorganisms, including changes in amino acid copiousness that favor protein mobility; production of RNA and protein chaperones; desaturated membrane lipids; expression of cold shock protein; and increasing the cell wall elasticity. Generally, cold adaptations are grouped into three categories: 1) control of molecular motion, 2) resource efficiency, and 3) temperature-specific alleles (Margesin, 2009). However, there are many more genes that are involved in thermal adaptation that have yet been identified.

The genome sequence data per se will not provide information on genes that are expressed during cold adaptation. Therefore, RNA-seq or transcriptomic sequencing can provide further information of genes that are involved in cold adaptation. The recent RNA-seq, or known as deep RNA sequencing, is based on the NGS (next generation sequencing) technology. RNA-seq analysis can be carried out with or without the genome information (Feng *et al*., 2012).

The objectives of this study are to determine the adaptation mechanisms and strategies of *Glaciozyma antarctica* PI12 to thermal stresses, and to characterize the physiological profile of *G. antarctica* PI12. This thesis is divided into three chapters to address the above objectives. First, the characterization of *G. antarctica* PI12 will be determined based on the growth at its optimal growth temperature at 12°C, the growth at sub-zero temperatures -12°C, -7°C and -5°C, the doubling time of *G. antarctica* PI12 using cell counter, aerobic and anaerobic of *G. antarctica* PI12 and microscopic analyses based on fluorescent microscope and also Scanning Electron Microscope (SEM) to observe *G. antarctica* PI12 cell division and bud division. Moreover, the molecular techniques, such as genomic analysis and transcriptomic analysis will be applied to *G. antarctica* PI12. Transcriptomic analysis using RNA-seq is set to determine the genes that are involved during thermal stresses adaptation of *G. antarctica* PI12.

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1.2 Objectives

1. To determine the growth rate, doubling time, anaerobic and aerobic effects of *G. antarctica* PI12 at their optimum growth temperature at 12°C,
2. To characterize the morphological features of *G. antarctica* PI12 using Scanning Electron Microscope (SEM),
3. To establish a *de novo* transcriptomic database of *G. antarctica* PI12,
4. To identify the differential gene expression (DEG) patterns of *G. antarctica* PI12 in respond to various temperature shifts using Minimal Medium (MM),
5. To identify the differential gene expression (DEG) patterns of *G. antarctica* PI12 in respond to various temperature shifts using Yeast Peptone Dextrose (YPD).

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CHAPTER 2

LITERATURE REVIEW

2.1 Antarctica, the extreme niche

Antarctica is known to be the world's largest continent, with the area size around 14 million km^2. The continent is covered by two massive ice sheets, namely the East Antarctica, and the West Antarctica ice sheets. The two gigantic ice sheets are separated by a 3, 500 km long range, known as the Transantarctic Mountain, it is also known to be the largest ice-free area in the continent of Antarctica (~23, 000 km^2).

Antarctica is the coldest region on Earth, this is due to the rarefied solar radiation expose to the continent, only 16% of that solar radiation at equatorial region is exposed to Antarctica. Not only that, the high average surface elevation surface of the ice sheets, which in most of the places exceed 4, 000 m. To date, the lowest temperature recorded in Antarctica was -89.4°C at Vostok (Krause & Flood, 1997).

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Despite the fact it is the coldest region on Earth, some of the areas receive thermal increment based on the geographical differences in climate. It also depends on: 1) the length of the thaw period; 2) the length of the thaw period, and; 3) the number of the thaw day in summer.

2.2 Departure of the continent of Antarctica from the Supercontinent of Gondwana

Antarctica was a part of the supercontinent of Gondwana in more than 170 million years ago. The supercontinent of Gondwana consisted of the continents, which are known as Antarctica, Australia, New Zealand, South America, India and Africa. According to the continental shifting theory, the Gondwana broke apart into seven continents and shifting occurred. Fossils and rocks found in Antarctica also were found in other continents. The finding also suggests that Antarctica was once a much

warmer place before it separated from the Gondwana. It is because when Antarctica was still attached to Gondwana, West Antarctica was partially in the northern hemisphere, and East Antarctica was at the equator (Stonehouse, 2002).

The shifting of the Antarctica continent to the south is a lengthy process. The shifting slowly introducing coldness to the continent as it shifts toward the south. Therefore, all the living organisms that were once lived on this continent undergo multiple natural selections, especially natural selection based on the changing of temperatures. The idea of natural selection proposed by Charles Darwin in year 1859 is that, the organisms that successfully adapt or evolved with certain traits survived, whereas, those organisms failed to adapt were eliminated by the changing environment. Rogers (2007) also stated that a strong natural selection in Antarctica controlled by the environmental factors led to an adaptation of the Antarctic biota.

2.3 Endemism of microorganisms found in Antarctica

According to Cowan *et al.* (2011), even though Antarctica is geographically isolated, it has not been microbiologically isolated. This is because it constantly receives a population of non-indigenous microorganisms, mostly were transported from the southern hemisphere continents by a high altitude aeolian process (Pearce *et al.,* 2009; Hughes & Convey, 2010; Cowan *et al.,* 2011). Nevertheless, there is no quantitative method developed to measure the total inorganic, and organic inputs to the Antarctic, but Cowan *et al.* (2011) assuming the value would be larger per annum, with around 10^{10}-10^{12} cells for 1 m^2 x 1-cm deep soil profile of non-indigenous microorganisms can be found (Cowan *et al.,* 2011).

Non-indigenous microorganisms are possibly introduced to Antarctica as an aeolian particle, or the anthropogenic impact (human activity) (Cowan *et al.,* 2011). Aeolian particle capture experiments have demonstrated that most of the non-indigenous microorganism is introduced to Antarctica as an aeolian particle (Pearce *et al.,* 2009; Cowan *et al.,* 2011). For example, the southern oceans generate aerosols, which serve to be a vehicle for transport of marine microorganism and marine aerosol nutrient input in the near-coastal terrestrial of the Antarctic continent (Bokhorst *et al.*, 2007). Besides, a growing number of human visitations to Antarctica are also known as the factor the non-indigenous microorganisms were introduced to Antarctica.

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