SCREENING FOR INHIBITORS OF PROTEIN PHOSPHATASE (PP1), MAP KINASE (M KK1) AND MAP KINASE PHOSPHATASE (MSG5) USING ACTINOMYCETE STRAINS INVOLVED IN EUKARYOTIC SIGNAL TRANSDUCTION

MUHAMMAD NAIM BIN RAZALI

DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF BACHELOR OF SCIENCE WITH HONOURS

PERPU STAKAAN UNIVERSITI MALAYSIA SABA

BIOTECHNOLOGY PROGRAMME
SCHOOL OF SCIENCE AND TECHNOLOGY
UNIVERSITI MALAYSIA SABAH

2010
PENGESAHAN STATUS TESIS

JUDUL: SCREENING FOR INHIBITORS OF PROTEIN PHOSPHATASE (PDI), ALKALINE PHOSPHATASE (AKK1), AND INHIBITORS OF PROTEIN PHOSPHATASE-1 (MS02) USING ACTINOMYCETE STRAIN

IJAZAH: SARJANA MUDA SAINS DENGAN KEBIJAKAN (PETUKHNOLOGI)

SESII PENGAJIAN: 2009 / 2010

Saya MUHAMMAD NAIM BIN RAZALI

(HURUF BESAR)

mengaku membencarkan tesis (LPS/MASJNA/DOKTOR/FAISAH) ini disimpan di Perpustakaan Universiti Malaysia Sabah dengan syarat-syarat kegunaan seperti berikut:

1. Tesis adalah hakmilik Universiti Malaysia Sabah.
2. Perpustakaan Universiti Malaysia Sabah dibenarkan membuat salinan untuk tujuan pengajian sabaja.
3. Perpustakaan dibenarkan membuat salinan tesis ini sebagai bahan pertukaran antara institusi pengajian tinggi.
4. **Sila tandakan ( / )
   - [ ] SULIT
   - [ ] TERHAD
   - [ ] TIDAK TERHAD

(TANDATANGAN PENULIS)

Alamat Tepat: NO 3, JALAN MELOR 51,
DEPAN MELOR, 8200 KOTAH

ELANGOR, KELANTAN

Tarikh: 07/05/2010

(TANDATANGAN PUSTAKAWAN)

DR. IVY WONG NYET KUL

Nama Penyelidik

Tarikh: 07/05/2010

CATATAN:
- * Potong yang tidak bercakna.
- ** Jika tesis ini SULIT atau TERHAD, sila lampirkan surat daripada pihak berkuasa/organisasi yang memberi izin.
- @ Tesis dimaksudkan sebagai tesis bagi Ijazah Doktor Falsah dan Sarjana secara penyelesaian, atau disertasi bagi pengajian secara kerja kursus dan penyelesaian, atau Laporan Projek Sarjana Muda (LPSM).

PERPUSTAKAAN UMS

*100353898*
DECLARATION

I hereby declare that this dissertation is my own original work except for statements and summaries which have been duly acknowledged.

MUHAMMAD NAIM BIN RAZALI

(BS07110445)

(7 MAY 2010)
VERIFIED BY

SIGNATURE

1. SUPERVISOR
   (DR. IVY WONG NYET KUI)

2. EXAMINER
   (ASSOC. PROF. DR. JUALANG AZLAN GANSAU)

3. DEAN
   (PROF. DR. MOHD HARUN ABDULLAH)
ACKNOWLEDGEMENT

I would like to take this opportunity to thank my supervisor, Dr. Ivy Wong Nyet Kui for his continuous guidance, supports, ideas and encouragements until I finished my project and to my family as well for their support and opinions given during at home.

I also would like to express my appreciation to the Post-Graduate laboratory demonstrator, Wong Siak Chung and Hong Chea Yen for his willingness to sacrifice his time to guide me throughout this project until I finished my project. Not forget to mention the laboratory assistants as well, Mr. Asram, Pn. Radizah, and whoever helping me during my time at laboratory.

To all my dear friends, Baqaludin Amri, Norhanafi, Awangku Adam, Goh Yiu Yin, Nur Muhamad Asyraf, Fellino Sidin, Hamal Hakim and the people who have been helping me, thanks for your help, support, and encouragement. Last but not least, to all of you. Thanks.
ABSTRACT

The aim of this study is to search for potential inhibitor of Protein phosphatase-1 (PP1), Mitogen activated protein kinase kinase (MKK1), and also Mitogen activated protein kinase phosphatase (MSGS) from actinomycete strains that involved in eukaryotic signal transduction using yeast as a eukaryotic model. For PP1 screening, two types of yeast strains used are the mutant-type PAY 700-4 and the wild-type PAY 704-1. Mutant-type yeast carries the mutant-type gene of GLC7-10 while the wild-type yeast carries the wild-type GLC7 gene. For MKK1 screening, the mutant type yeast strain used was MKK1\textsuperscript{P386}. MKK1\textsuperscript{P386} gene which carried by the yeast was induced by the GAL1 promoter in response of glucose limitation. While the mutant type yeast strain, MKK1\textsuperscript{P386}-MSGS, was used in the screening of MSGS inhibitors. The yeast carries the MKK1\textsuperscript{P386}-MSGS gene that will suppress the growth inhibitory effects of over expression of MKK1\textsuperscript{P386} gene. There are 40 strains of actinomycete are used. They are H1001, H1006, H1036, H1045, H1032, H1002, H1004, H1017, H7323, H7434, H7456, H7478, H7998, H8565, H8598, H8601, H8632, H8637, H8641, H8648, H8651, H8654, H8667, H8688, H8691, H8693, H8698, H8700, H8724, H8961, H8970, H8991, H11448, H11464, H11526, H11661, H11670, H11783, H11821, and H11841. These strains of actinomycete were taken from soil in several Sabah regions. The secondary metabolites from actinomycete strains were extracted using equal volume of acetone and screened for PP1, MKK1 and MSGS inhibitors. In each screening methods, 20μl of acetone extract with the concentration of 50mg/ml were placed to each paper disks. Distilled water was used as a negative control. Acetone extracts of each actinomycetes strain do not show any activity when screening against PP1, MKK1, and MSGS inhibitions and each of them also are non-toxic. However, the possibility of potential inhibitors from actinomycete strains could not be excluded. Therefore, further investigation need to be done.
CONTENTS

DECLARATION ii
VERIFICATION iii
ACKNOWLEDGEMENT iv
ABSTRAK v
ABSTRACT vi
TABLE OF CONTENTS vii
LIST OF TABLES x
LIST OF FIGURES xi
LIST OF ABBREVIATION xii

CHAPTER 1 INTRODUCTION 1

CHAPTER 2 LITERATURE REVIEW 4
2.1 Signal Transduction and Cancer Disease 4
2.2 Actinomycetes and Its Secondary Metabolite Production 6
2.3 Yeasts as Eukaryotic Model 7
2.4 Protein Phosphatase-1 (PPI) 8
2.5 MAPK Kinase (M KK1) 10
2.6 MAP Kinase Phosphatase (MSG5) 14
### CHAPTER 3 METHODOLOGY

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>Culture of actinomycete strains</td>
<td>16</td>
</tr>
<tr>
<td>3.2</td>
<td>Agar Preparation</td>
<td>17</td>
</tr>
<tr>
<td>3.2.1</td>
<td>Mannitol-peptone agar</td>
<td>17</td>
</tr>
<tr>
<td>3.2.2</td>
<td>Oat meal agar</td>
<td>18</td>
</tr>
<tr>
<td>3.3</td>
<td>Extraction of Secondary Metabolites</td>
<td>18</td>
</tr>
<tr>
<td>3.4</td>
<td>Screening against PP1 inhibition</td>
<td>18</td>
</tr>
<tr>
<td>3.4.1</td>
<td>Screening Media for PP1 inhibitor</td>
<td>18</td>
</tr>
<tr>
<td>3.4.2</td>
<td>Screening System for PP1 inhibitor</td>
<td>20</td>
</tr>
<tr>
<td>3.4.3</td>
<td>PP1 screening expected result</td>
<td>20</td>
</tr>
<tr>
<td>3.5</td>
<td>Yeast Cultivation Media</td>
<td>21</td>
</tr>
<tr>
<td>3.6</td>
<td>Yeast Fermentation Media</td>
<td>22</td>
</tr>
<tr>
<td>3.7</td>
<td>Phosphate Buffered Saline (PBS)</td>
<td>22</td>
</tr>
<tr>
<td>3.8</td>
<td>Screening against MKK1 inhibition</td>
<td>22</td>
</tr>
<tr>
<td>3.8.1</td>
<td>Screening Media for MKK1 inhibitor</td>
<td>22</td>
</tr>
<tr>
<td>3.8.2</td>
<td>Screening System for MKK1 inhibitor</td>
<td>23</td>
</tr>
<tr>
<td>3.8.3</td>
<td>MKK1 screening expected result</td>
<td>24</td>
</tr>
<tr>
<td>3.9</td>
<td>Screening against MSG5 inhibition</td>
<td>25</td>
</tr>
<tr>
<td>3.9.1</td>
<td>Screening Media for MSG5 inhibitor</td>
<td>25</td>
</tr>
<tr>
<td>3.9.2</td>
<td>Screening System for MSG5 inhibitor</td>
<td>26</td>
</tr>
<tr>
<td>3.9.3</td>
<td>MSG5 screening expected result</td>
<td>27</td>
</tr>
</tbody>
</table>
CHAPTER 4 RESULTS

4.1 Actinomycete on Solid Media 28
4.2 PP1 screening results 30
4.3 MKK1 screening results 37
4.4 MSG5 screening results 42

CHAPTER 5 DISCUSSION

5.1 Extraction of Secondary Metabolites 47
5.2 Protein Phosphatase-1 (PP1) screening 48
5.3 Mitogen-activated Protein Kinase Kinase (M KK1) screening 50
5.4 Mitogen-activated Protein Kinase Phosphatase (MSG5) screening 51

CHAPTER 6 CONCLUSIONS 54

REFERENCES 56
<table>
<thead>
<tr>
<th>No. of Tables</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>10</td>
</tr>
<tr>
<td>The yeast strains used in the screening system against PP1 inhibitor</td>
<td></td>
</tr>
<tr>
<td>3.1</td>
<td>16</td>
</tr>
<tr>
<td>Source of actinomycete strains</td>
<td></td>
</tr>
<tr>
<td>4.1</td>
<td>29</td>
</tr>
<tr>
<td>Source of actinomycete strains, description of aerial and substrate mycelia and extracellular pigmentation</td>
<td></td>
</tr>
<tr>
<td>4.2</td>
<td>35</td>
</tr>
<tr>
<td>Screening of Actinomycete strains against PP1 inhibition</td>
<td></td>
</tr>
<tr>
<td>4.3</td>
<td>40</td>
</tr>
<tr>
<td>Screening of actinomycetes strains against M KK1 inhibition</td>
<td></td>
</tr>
<tr>
<td>4.4</td>
<td>45</td>
</tr>
<tr>
<td>Screening of actinomycete strains against MSG5 inhibition</td>
<td></td>
</tr>
</tbody>
</table>
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>No. of Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1 Structure of the ERK pathway</td>
<td>12</td>
</tr>
<tr>
<td>2.2 MAPK cascades of <em>Saccharomyces cerevisiae</em></td>
<td>13</td>
</tr>
<tr>
<td>3.1 The effect of extracts on mutant yeasts, M KK1\textsuperscript{P386}</td>
<td>24</td>
</tr>
<tr>
<td>3.2 The effect of extracts on mutant yeasts, M KK1\textsuperscript{P386} – MSG5</td>
<td>27</td>
</tr>
<tr>
<td>4.1 PP1 screening result</td>
<td>31</td>
</tr>
<tr>
<td>4.2 M KK1 screening results</td>
<td>38</td>
</tr>
<tr>
<td>4.3 MSG5 screening results</td>
<td>43</td>
</tr>
</tbody>
</table>
# LIST OF ABBREVIATION

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>PP1</td>
<td>Protein Phosphatase 1</td>
</tr>
<tr>
<td>M KK1</td>
<td>Mitogen-Activated Protein Kinase Kinase</td>
</tr>
<tr>
<td>MSG5</td>
<td>Mitogen-Activated Protein Kinase Phosphatase</td>
</tr>
<tr>
<td>RTK</td>
<td>Receptor Tyrosine Kinase</td>
</tr>
<tr>
<td>MPB</td>
<td>Mannitol-Peptone Broth</td>
</tr>
<tr>
<td>MAPK</td>
<td>Mitogen-Activated Protein Kinase</td>
</tr>
<tr>
<td>ERK</td>
<td>Extracellular Signal Regulated Protein Kinase</td>
</tr>
<tr>
<td>TGY</td>
<td>Threonine-Glycine-Tyrosine</td>
</tr>
<tr>
<td>TNY</td>
<td>Threonine-Glutamine-Tyrosine</td>
</tr>
</tbody>
</table>
CHAPTER 1

INTRODUCTION

Actinomycete can be either terrestrial or aquatic. Some of the most common soil life, fresh water and also marine life included as they are playing an important role in decomposing the organic materials and play a major role in carbon cycle and organic matter turnover. As the result, it is an important part of humus formation and also it helps replenishing the supply of nutrients in the soil.

Actinomycete can secrete both primary and secondary metabolites. They are well known as secondary metabolite producers. Actinomycete secondary metabolites are special compounds, which the possessing chemical structure is often quite different from the primary metabolites which they are produced such as sugars, amino acids and organic acids (Demain and Fang, 1995). The primary metabolite produced by actinomycete is crucial for their growth. They are the end products of catabolism, set up to form primary intermediates such as amino acids, nucleotides, vitamins, carbohydrates and fatty acids. These biosynthetic intermediates are subsequently assembled into the complex and essential metabolites that give structure and biological functions to the organisms.
Most of the organisms making secondary metabolites undergo complex scheme of morphological differentiation (Demain and Fang, 1995). The secondary metabolites production process, which is known as secondary metabolism, is brought on by exhaustion of nutrients and decrease in growth rate. This event generates signals which affect a cascade of regulatory events resulting in chemical differentiation (secondary metabolism) and morphological differentiation. The important classes of secondary metabolites are the antibiotics. The metabolic pathways of the primary metabolism often supply the precursors of the antibiotics. (Stegman et al., 2007).

There is a system in which all of organisms have it. The system called signal transduction pathway system. It is a process of converting of one kind of signal into another by a cell. It is involve in information processing and also a link to environmental cue for adaptive response. Signal transduction pathway involves ordered sequences of biochemical reactions inside the cell. The reactions is carried out by enzymes and activated by the second messengers. The level of signal transduction system is differed between prokaryotes and eukaryotes as the system components and their complexity are not the same. However, some of the input elements in prokaryotes and eukaryotes signal transduction have some similarities as they react into many similar signals from the environment or inside the cell.

Cancer is a disease causing uncontrolled growth or invasion which displayed by a group of cells. There are two types of genes which typically affected in cancer. The first one is cancer-promoting oncogenes which activated in cancer cells. It caused hyperactive growth and division of cells. The second gene is tumor suppressor genes which inactivated in cancer cells. As a result, cells losses its normal functions such as cell cycle regulation, accurate DNA replication and interaction with protective cells of the immune system.

These genes normally controlled cell process such as cell growth, cell division and cell repair. These processes are triggered by signals and associated genes will be
coded to react with certain responses. When all these processes are out of control or damaged due to mutation or other problems, the gene itself and the signal pathway that activates it will be suppressed or "switching it off". As the result, cell processes will be hindered or inhibited. Thus, it will leading to cancer.

The main objective of this research is to searching for protein phosphatase-1 (PP1) inhibitor, mitogen-activated protein kinase kinase (M KK1) inhibitor and also mitogen-activated protein kinase phosphatase (MSG5) inhibitor from actinonycete strains in eukaryotic signal transduction using yeast as eukaryotic model.
CHAPTER 2

LITERATURE REVIEW

2.1 Signal Transduction and Cancer Disease

Signal transduction pathway is a movement of signals from outside or inside the cell which the signals is sent to a certain pathway cascade resulting a correct response towards the signals. It is initialized by cell-surface proteins that respond to specific environmental stimuli. When these proteins are activated, it emanates a signaling cascade which involves a series of phosphorylation and dephosphorylation events (Supper et al., 2009). In other organism and bacteria, cells are capable of reacting towards the variety of signal transduction process in many way and respond to its environment. The more complex the organism, the more complex the repertoire of signal transduction process the organism must possess.

In eukayotes, a ligand or receptor interaction, which possesses an enzymatic activity, activated most intracellular proteins. It includes tyrosine kinase, G proteins, several of serine/threonine protein kinases, phosphatases, lipid kinases and hydrolases. The second messengers including cyclic nucleotides such as cyclic AMP
(cAMP) and cyclic GMP are created by some receptor-stimulated enzymes. Some activated proteins also interact with adapter proteins. Adapter proteins facilitate interactions between other signaling proteins and coordinate the formation of signaling complexes necessary to produce an appropriate cellular response to a particular stimulus.

Receptor tyrosine kinases (RTKs) are transmembrane proteins with an intracellular kinase domain and an extracellular domain that binds ligand. There are classified based on their structural properties and ligand specificity. RTKs need to form dimer in order to conduct their biochemical signals. The dimer is stabilized by ligand binding by the receptor. Autophosphorylation of tyrosine within the cytoplasmic tyrosine kinase domain of RTKs causing their conformational changes and it is activated by the interaction between the two cytoplasmic domains of the dimer. The kinase domain of the receptor activated and initiating signaling cascades of phosphorylation of downstream cytoplasmic molecules. They play a vital role in various cellular processes such as cell growth regulation, differentiation and metabolism (Li and Histrova, 2006).

Cancer is a disease arises as a result of a disturbance in signal transduction mechanism and also the breakdown in its pathway that control cell proliferation. It has been found that oncogenes causing the uncontrolled growth of cells and lead to the growth of cancer. Oncogene may encode defective component of a signaling pathway.

Signal transduction plays a vital role in signaling the cellular process and response in the cell. The disruption or over stimulation can lead to a various disease such as diabetes and immune disorder. Thus, the better understanding in signaling pathways and research done in finding the novel secondary metabolites from bacteria and other organisms is required and may help in the finding the inhibitor to inhibit the signal transduction (Stark, 2003). The finding of potential anti-cancer
agent to treat cancer disease in human can be achieved if the scientists continue to study the signal transduction system in eukaryotes.

2.2 Actinomycetes and Its Secondary Metabolite Production

Actinomycetes playing a vital role in decomposed an organic material in soil. They also play an important part in carbon cycle. Carbon cycle help in replenishing the nutrient supply in soil and formation of humus. Some of actinomycete form branching filaments which resemble the mycelia of fungi. They also grow in aerobic condition but some of them like Actinomyces israelii, can grow under anaerobic conditions. Most of them are Gram-positive bacteria with high GC-content DNA. In area which has a warmer climate, the actinomycetes are also responsible for produced the peculiar odor which come from the soil after the rain. Some of them resemble the smell of fruits. The can be found in the water, in the soil, in plants and animals residues. They also can be found in extreme north climate or in the tropics, on barrens rock, on top of the mountains and in the valleys.

They can utilize organic substances which cannot be utilized by other bacteria and fungi. It is essential for their survival. When all the nutrients in the soil are being fully utilized, decomposed and assimilated by other bacteria and fungi, they will begin to grow and other bacteria will stop growing. They will decompose unused and unutilized substances by other bacteria and fungi and used them as a source of nutrients for their growth.

Actinomycetes are best-known as secondary metabolite producers. When Selman Walksman have been granted a Nobel Prize for his discovery for actinomycin which is produces by soil bacteria in 1940, actinomycete has attracted many interest in researchers for its high pharmacological and commercial interest. Since then, huge number of naturally occurring antibiotics have been discovered. Most of them came from terrestrial microorganisms especially from the genus Streptomyces as their
contributed up to 75% of all described secondary metabolites produces by actinomycete (Demain and Fang et al., 1995). For example, strains of *Streptomyces hygroscopicus* produces over 180 different secondary metabolites.

The secondary metabolites products produce by actinomycetes have been widely used as antibiotics for many cancer diseases. For examples, chloramphenicol is produce by the *Streptomyces venezuelae* strain. It acts as bacteriostatic antibiotic, which can be used effectively against variety of bacteria. This shows that by understanding the relationship between the modes of action of secondary metabolites and the signal transduction, antibiotics which can be used against cancer diseases can be found. Nowadays, many researches based on secondary metabolites have been done in order to achieve this goal.

### 2.3 Yeasts as Eukaryotic Model

Yeast is a fungus which has been used for research since a long time ago. It is a unicellular organism which extremely important as a model organism in modern cell biology research as the genetic studies and the functions of yeast have been heavily studied. By using yeast, the information about the biology of the eukaryotic microorganisms can be gathered by the researchers.

Yeast has become the first eukaryotic organism that their whole genomic sequences have been discovered and available in molecular biology experiment (Goffeau et al., 1996). From the information found in yeast genome project, it is turned out to be used as a reference against sequences of human, animal or plant genes (Goffeau et al., 1996). Yeast has several advantages against other eukaryotes. For example, yeast can be produced in mass which other eukaryotes cannot. The nature of yeast that able to grow on defined media gives the researchers a complete control over experimental parameters.
2.4 Protein Phosphatase-1 (PPI)

Reversible protein regulation is a major regulatory mechanism of intracellular signal transduction. Protein serine/threonine phosphorylation and dephosphorylation is an important and significant component of the intracellular signaling machinery. It is directing such diverse functions as neurotransmission, muscle contraction, glycogen synthesis, T-cell activation, neuronal plasticity and cell proliferation (Aggen et al., 2000). Protein Phosphatase-1, or also called PP-1 is one of the four types of serine-threonine phosphatases mediating signaling pathways (Aggen et al., 2000). It can regulate diverse cellular processes such as cell-cycle progression, protein synthesis, muscle contraction, carbohydrate metabolism and so on.

It regulates variety of these cellular functions through its catalytic subunit (PP1c) with over fifty different established regulatory subunits. Means that it is regulated by its interaction with a variety of protein subunits that are differ from the inhibitor proteins and that appear to target the catalytic subunits (PP1c) to specific sub cellular compartments (Aggen et al., 2000; Cohen, 2002). The number of PP1c known targeting subunits is increasing rapidly. There are nearly 20 unique mammalian proteins have been identified. Also, it is been identified that homolog of several of these mammalian proteins found in yeast (Aggen et al., 2000).

One of the most generic signaling designs employed by the MAPK pathway, found in biological signal transduction, namely that of a cycle formed by a kinase phosphorylating a target protein and an opposing phosphatase that is in charge of dephosphorylating the target. This type of protein phosphorylation presents a fundamental mechanism which regulates the activities of numerous enzymes, receptors, transporters, docking and scaffolding protein.

When a phosphate group is transferred to a serine, threonine or tyrosine residue of a target protein (T), it is catalyzed by a kinase. In turn, the phosphatase
dephosphorylated the phosphorylated target protein (T-P) in order to complete the cycle. Protein phosphorylation often entails conformational changes which modulate the protein function. For example, the phosphorylation of MEK activates the kinases domain of the enzyme. Then, it will catalyze the phosphorylation and as a result, MAPK will be activated (Orton et al., 2005).

The study of the function of protein phosphatases has been revolutionized by the discovery of many-low-molecular-mass protein phosphatase inhibitors, which are able to penetrate living cells. Okadaic acid is the best and widely used protein phosphatase inhibitors. It is a polyether fatty acid which is produces by marine dinoflagellates and is the causative agent of diarrhoeic shellfish poisoning. Okadaic acid does not rapidly penetrate cell membranes, but accumulates slowly, making it difficult to control the actual concentration of the compound in vivo. It is a valuable tool used to distinguish between different phosphatases acting upon a given substrate (Wera and Hemmings, 1995). Previous studied showed that Okadaic acid has a various effects towards protein serine/threonine phosphatases on cell proliferation, cell transformation and cell cycle gene expression.

PP1c is a member of the PPP family of protein serine/threonine phosphatases. In most eukaryotes, multiple genes encode PP1c isoforms. However, in Saccharomyces cerevisiae, it posses only one gene encode PP1c isoforms, which is Gic7. The Gic7 involves in a diverse array of regulation such as protein translation, cell cycle, protein translation and cell wall integrity. It has a reduced function of phosphatases activity in a certain temperature in yeast cell. Yeast cell can only maintain its cell wall integrity with the presence of Sorbitol at 37°C. Sorbitol acts as an osmoprotectant (Andrew and Stark, 2000).
Table 2.1 The yeast strains used in the screening system against PP1 inhibitor

<table>
<thead>
<tr>
<th>Strains</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAY704-1</td>
<td>Mata ade2-1 his3-11-leu2-3, 112-trp1-1 ura3-1 Can1-100 ssd1-d2 glc7::LEU2 trp1::GLC7-10:: TRP1 Gal*</td>
</tr>
<tr>
<td>PAY700-4</td>
<td>Mata ade2-1 his3-11-leu2-3, 112-trp1-1 ura3-1 Can1-100 ssd1-d2 glc7::LEU2 trp1::GLC7-10:: TRP1 Gal*</td>
</tr>
</tbody>
</table>


2.5 MAPK Kinase (MKK1)

Cell responds to extracellular signals by transmitting intracellular instructions to coordinate appropriate responses. The highly conserved mitogen-activated protein kinase (MAPK) or extracellular signal regulated protein kinase (ERK) cascades is among the pathway which are often used to transduce these signals (Robinson and Cobb, 1997). MAPK cascades are signal transduction pathways that play pivotal regulatory roles in the biosynthesis of proinflammatory cytokines. This cascade contributes to the amplification and specificity of the transmitted signals that eventually activate several regulatory molecules in the cytoplasm and in nucleus in order to initiate cellular processes such as proliferation, differentiation, and development (Seger and Krebs, 1995). MAPK pathway is crucial for the maintenance of cell wall integrity through the regulation of cell wall and actin cytoskeleton dynamics.
MAPK cascade allow yeast cells to quickly adapt to changing environment as yeasts possess rapidly responding, highly complex signaling pathways. The MAPK pathways generally contain three protein kinases that act in series. They are MAPK kinase kinase (MAPKKK or MEKK), MAPK kinase (MAPKK or MEK) and MAPK. These three protein kinases can be found in animals, plants and also fungi. The activation or inhabitation of MAPK cascades will modulate transcription of specific genes by most of the extracellular and intracellular signals (Gustin et al., 1998). The activation of MAPK cascade was done by phosphorylation in both tyrosine and threonine residue.

Raf/ MEK/ ERK pathway starts with the activation of the small GTPase Ras, also possibly with Rap, by the receptor tyrosine kinases, G protein-coupled receptors and/ or integrins (Kolch, 2000). The activation will result in the membrane protein assemble large signaling complexes. It will recruit and activate Ras protein by inducing the exchange of Ras-bound GDP with GTP. Ras will be converted into its activated conformation. The interaction of Ras with GDP/GTP-exchange factors, such as SOS (son of sevenless), will mediate the process. On the other hand, GAPs (GTPase-activating proteins) controlled the deactivation of Ras, which significantly enhance the very low GTPase activity of GTP to GDP (Bernards, 2003). The small G-proteins recruit the MKKks c-Raf to the plasma membrane upon the activation, where Raf is activated in a complicated process that involves binding to Ras, phosphorylation and changes in conformation and binding interactions (Kolch, 2000). MEK-1/2 is activated by Raf through the phosphorylation of two serine residues.

Figure 2.2 show the structure of the ERK pathway. Tyrosine residues which serve as docking sites for adaptor and signaling molecules is autophosphorylated by RTK upon ligand binding. Ras and Rap1 are activated as the Guanosine-nucleotide exchange factors (SOS, C3G) are activated via adaptor proteins (Shc and Grb2; Crk). Ras can activate Raf-1 and B-Raf while Rap1 can activate B-Raf presumably. MEK-1/2 will be phosphorylated and activated by Raf protein and MEK-1/2 in turn will activate ERK-1/2. The induction of MKPs by ERK was included by negative-feedback loops as well as inhibitory phosphorylation of Raf-1 and SOS (Orton et al., 2005).
REFERENCES


