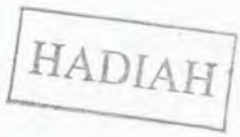
ISOLATION AND SCREENING OF ACTINOMYCETES ESPECIALLY *STREPTOMYCES* FROM LOWER SEGAMA, SABAH AGAINST MAP KINASE PHOSPHATASE (MSG5) IN SIGNAL TRANSDUCTION



LOO POOI ENG

THESIS SUBMITTED IN PARTIAL FULLFILMENT OF THE REQUIREMENT FOR BACHELOR OF SCIENCE WITH HONOURS

BIOTECHNOLOGY PROGRAMME
SCHOOL OF SCIENCE AND TECHNOLOGY
UNIVERSITI MALAYSIA SABAH

2004

UNIVERSITI MALAYSIA SABAH

BORANG PENGESAHAN STATUS TESIS@

JUDUL: ISOLATION AND SCREENING OF ACTINOMYCETES ESPECIALLY STREPTOMYCES FROM LOWER SEGAMA, SABAH AGAINST MAP KINASE PHOSPHATASE (MSG5) IN SIGNAL TRANSDUCTION

Ijazah: SARJANA MUDA BIOTEKNOLOGI

SESI PENGAJIAN: _____

Saya LOO POOI ENG

(HURUF BESAR)

mengaku membenarkan tesis (~~LPS/Sarjana/Doktor Falsafah~~)* 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 sahaja.
3. Perpustakaan dibenarkan membuat salinan tesis ini sebagai bahan pertukaran antara institusi pengajian tinggi.
4. **Sila tandakan (/)

☐	SULIT	(Mengandungi maklumat yang berdarjah keselamatan atau kepentingan Malaysia seperti yang termaktub di dalam AKTA RAHSIA RASMI 1972)
☐	TERHAD	(Mengandungi maklumat TERHAD yang telah ditentukan oleh organisasi/badan di mana penyelidikan dijalankan)
☑	TIDAK TERHAD	

Disahkan oleh

(TANDATANGAN PENULIS)

(TANDATANGAN PUSTAKAWAN)

Alamat Tetap: 3186-P, JALAN BAYAM, TAMAN SRI BAYAM, 15050 KOTA BHARU, KELANTAN.

Nama Penyelia

Tarikh: 12/3/2004

Tarikh: _____

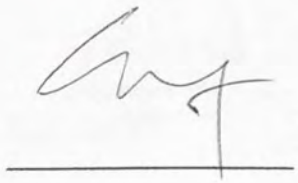
CATATAN: * Potong yang tidak berkenaan.

** Jika tesis ini SULIT atau TERHAD, sila lampirkan surat daripada pihak berkuasa/organisasi berkenaan dengan menyatakan sekali sebab dan tempoh tesis ini perlu dikelaskan sebagai SULIT dan TERHAD.

@ Tesis dimaksudkan sebagai tesis bagi Ijazah Doktor Falsafah dan Sarjana secara penyelidikan, atau disertasi bagi pengajian secara kerja kursus dan penyelidikan, atau Laporan Projek Sarjana Muda (LPSM).

DECLARATION

I hereby declare that the work in this final year project is my own except for quotations and summaries which have been duly acknowledged. The materials in this final year project are original except for quotations, accepts, summaries and references which have been duly acknowledged.



9th February 2004

(LOO POOI ENG)
HS2001-2647

CONFIRM BY

Signature

1. SUPERVISOR
   (PROF. DR. HO COY CHOKE) _____

2. EXAMINER 1
   (PROF. DR. PERUMAL RAMASAMY) _____

3. EXAMINER 2
   (DR. JUALANG AZLAN GANSAU) _____

4. DEAN OF SCIENCE AND TECHNOLOGY SCHOOL
   (PROF. MADYA DR. AMRAN AHMED) _____

ACKNOWLEDGEMENT

First of all, I would like to take this opportunity to express my sincere gratitude to all the people that have given me a lot of help and guidance along this accomplishment of my final year project. I would like to thank my supervisor, Prof. Dr. Ho Coy Choke for all the guidance and advices that he has given to me throughout this period.

Besides that, I would like to thank all the postgraduate students from Pascasiswazah Lab, Puah Seok Huah, Foo Sek Hin, Ong Si Mon, Hew Chaw Sen and Lai Ngai Shin, who really helped out a lot in solving all the problems that occurred. Not to forget my special thanks to my fellow course mates especially Tan ChiaYee, Lau Hui Chong, Jocques Galip who are so willing to share their knowledge with me during this final year project.

Last but not least, I would also like to thank every member of my family who have been standing behind and support me all along the way.

ABSTRAK

Sejumlah 20 sampel tanah telah dikutip pada ekspedisi saintifik yang diadakan di kawasan hilir Sungai Segama. Daripada 20 sampel tanah yang dikutip, 18 sampel tanah adalah dikutip sepanjang kawasan tepi Sungai Segama. Manakala 2 sampel tanah yang lain adalah dikutip dari kawasan tebing Sungai Tabin. Sebanyak 9 sampel tanah yang dikutip oleh saudari Tan Chia Yee dan 9 sampel tanah yang dikutip oleh saya sendiri telah digunakan untuk mengasingkan aktinomiset yang terdapat di dalamnya. Sejumlah 20 strain aktinomiset telah berjaya diasingkan dari sampel tanah tersebur pada HV agar media dengan menggunakan kaedah pencairan berperingkat. Strain aktinomiset yang diasingkan sererusnya ditulenkan dengan pengkulturan dalam Oatmeal agar. Fenotip morfologi bagi setiap strain aktinomiset diperhatikan berdasarkan warna aerial miselia dan substrat miselia pada media Oatmeal. Fermentasi aktinomiset kemudian dijalankan dengan menggunakan Mannitol-peptone media selama 5 hari pada suhu 28^{0}C. Hasil fermentasi dicampurkan dengan isipadu aseton yang sama dengan tujuan mengekstrakkan metabolit sekunder daripada aktinomiset. Ekstrak aseton kemudiannya digunakan untuk menguji dan mengesan perencat terhadap MAP kinase phosphatase, MSG5. Dalam kajian ini, terdapat dua sistem penyaringan yang digunakan. Dalam sistem penyaringan 1, terdapat 9 ekstrak yang menunjukkan aktiviti toksik terhadap yis mutan $MKK1^{P386}$-MSG5. Strain-strain aktinomiset ini termasuk H8807, H8908, H8918, H8919, H8930, H8961, H8996, H11074, dan H11082. Manakala dalam sistem penyaringan 2, terdapat 8 ekstrak yang menunjukkan kesan toksik. Strain-strain ini termasuk H8959, H8976, H8982, H11073, H11074, H11078, H11081 and H11084. Tiada strain yang menunjukkan potensi sebagai perencat bagi MSG5.

ABSTRACT

A total of 20 soil samples were collected during the scientific expedition at Lower Segama. Out of the 20 soil samples, 18 soil samples were collected along the riverside Segama River; as the other 2 soil samples were collected along the Tabin River. 9 soil samples that were collected by Miss Tan Chia Yee and 9 soil samples collected by myself were used for actinomycetes isolation. As a result of that, 20 strains of actinomycetes were successfully isolated on HV agar media at the pH of 7.2 after a serial dilution of 10^{-3} have been carried out. The isolated actinomycetes strains were further purified on Oatmeal agar at pH 7.2. The morphological characteristics such as the color of aerial mycelia and substrate mycelia of actinomycetes were observed. Purified strains of actinomycetes were then grown aerobically in Mannitol-peptone media, which contain 2%(w/v) Mannitol, 2%(w/v) peptone and 2%(w/v) glucose. The fermentation process was carried out for 5 days at 28^0C. Acetone was then used to extract the secondary metabolites from the fermented cultures. These acetone extracts were used to screen for MAP kinase phosphatase, MSG 5 inhibitors. There are two screening system being used. The difference of system 2 from system 1 is that the yeasts are grown in both glucose and galactose growth media separately. In the screening system 1, 9 extracts had shown some toxic activities against the mutant yeast strain MKK1^{P386}-MSG 5.These strains include H8807, H8908, H8918, H8919, H8930, H8961, H8996, H11074, and H11082. As for the screening system 2, there were 8 extracts that shown toxic activities, these include H8959, H8976, H8982, H11073, H11074, H11078, H11081 and H11084.

LIST OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS, UNITS AND ABBREVIATIONS

%	percentage
µL	microliter
^{0}C	degree Celcius
DNA	deoxyribonucleic acid
ERK	extracellular signal-regulated kinase
et.al	and friends
g	gram
G + C	guanine + cytosine
HV	humic acid B-Vitamin
L	liter
MAPK	mitogen-activated protein kinase
MAPKK	MAP kinase kinase
MAPKKK	MAP kinase kinase kinase
LSE	lower Segama
MEK	ERK-activating kinase
MEKK	MEK kinase
mg	milligram
ml	milliliter
OA	oatmeal agar
PKC	protein kinase C
r.p.m	round per minute
w/v	weight over volume

CHAPTER 1

INTRODUCTION

Malaysia, particularly in Sabah, possesses a wealth of biodiversity in natural forests and marine environment. The greatest potential of utilization of biodiversity in biotechnology lies in the rich microbial diversity. In this final year project, the research is focused on the filamentous bacteria, actinomycetes especially *Streptomyces*, which have produced several kinds of important bioactive secondary metabolites.

Actinomycetes are a large group of filamentous, gram-positive bacteria. Most of the actinomycetes can be found in soil and they are aerobic. Among all the various genera of actinomycetes, *Streptomyces* has yielded the largest number of bioactive secondary metabolites, acting both as antibiotics and anticancer drugs. Until today, more than 50 Streptomycete antibiotics have found practical application in human and veterinary medicine, agriculture and industry.

All cells have some ability to sense and respond to specific aspects of their environment. The binding of most signaling molecules to their receptors initiates a series of intracellular reactions that regulate virtually all aspects of cell behavior, including cell proliferation (Cooper, 1997).

The molecular study of cancer revealed that the causation of cancer is brought up by the breakdown of the signaling pathway that regulates normal cell proliferation. In other words, this involves protein kinases and phosphatases that carry out phosphorylation and dephosphorylation in signal transduction and cell cycle. Thus, protein kinases are the key targets in the discovery of inhibitors for cancer treatment.

In eukaryotes, a cascade of three protein kinases known as mitogen-activated protein kinase (MAPK) cascade is commonly found as part of the signaling pathways. The three protein kinases are: MAPK (mitogen activated protein kinase or known as extracellular signal-regulated kinase [ERK]), MAPK kinase (or known as MEK), and MAPK kinase kinase (MAPKKK) (Watanabe, 1995).

MSG5 (multicopy suppressor of *gpal*) is a novel gene that encodes a putative protein tyrosine phosphate (Doi *et al.*, 1994). It is a dual specificity protein phosphatase that phosphorylates the hydroxyl side chains of serine/threonine and tyrosine residues in their MAP kinase substrate (Kendall *et al.*, 1994). In the cell wall integrity pathway, MSG5 will dephosphorylate the Mpk1 gene, which is the homologue to MAP Kinase in mammalian cells.

In the mating pheromone pathway, MSG5 will inactivate phosphorylated FUS3, which is the homologue to the MAP Kinase in mammalian cells. Both of these pathways are the most well studied pathways in yeast. Presently, there is another homologue of MSG5 that is found in mammalian cells, Mkp3. Mkp3 is also a gene that encodes a dual specificity phosphatase that regulates MAPK pathway (Kawakami

et al., 2003). Hence, in this research, the main goal is to discover the inhibitor that will act against MSG5 in order to inhibit the MAP kinase pathway.

The objective for this final year project is to isolate actinomycetes especially *Streptomyces* from the soil sample collected from lower Segama River, using the selective HV media. Further on, secondary metabolites are extracted from the *Streptomyces*. Subsequently, the extractions are screened for the inhibitor of MAP kinase phosphatase (MSG5).

CHAPTER 2

LITERATURE REVIEW

2.1 ACTINOMYCETES

Actinomycetes are a large group of non-motile, filamentous gram-positive bacteria that form branching filaments (Madigan *et al.*, 2000). The successful growth of the filaments will form a ramifying network of filaments, called mycelium. The mycelium formed by actinomycetes is somehow much smaller than those of fungi, in fact they are spore-forming. When actinomycetes are grown in agar or a media, hyphae that grow on the upper part of agar are known as aerial mycelium and the growth in the agar are known as substrate mycelium.

The DNA base composition of actinomycetes consists of high G+C concentration, which falls in the range of 63-78% (Madigan *et al.*, 2000). Besides the G+C concentration, the type of cell wall peptidoglycan and the presence of isomer Diaminopimelic acid (DAP) are tested to separate the actinomycetes group into broad chemotaxonomic groups. The prevalent peptidoglycan type contains meso-diamonipimelic acid (meso-DAP) in position 3 of the peptide chains; and adjacent chains are cross-linked directly between the free amino group of meso-DAP and free carboxyl group of D-alanine (3:4 linkage). The principal variations concern the nature

of the diaminoacid in position 3; the presence, number and nature of the additional amino acids, which form interpeptide bridges; and the positions of the cross-link between peptides chains. However, these conventional identification tests are time-consuming and in most cases, cannot identify an isolate to a single genus.

In the recent discovery, a rapid method for identifying the filamentous actinomycetes genera is developed based on 16S-rRNA gene restriction fragment patterns (Cook and Meyers., 2003). The patterns are generated by using specific restriction endonucleases to perform in silico digestions on the 16S-rRNA sequences of all validly published filamentous actinomycetes species. Basically, this method is applied to identifying soil-inhabitant actinomycetes. Once the restricted 16S-rRNA of the soil actinomycetes is electrophoresed on agarose gels, the restriction patterns of the unknown isolates can be easily compared to the established patterns.

2.1.1 Habitats of Actinomycetes

Most of the actinomycetes are soil-inhabitants. Besides widely distributed in soil, actinomycetes also can be found in a wide variety of other habitats which include fresh water basins, stable manures and even in the atmosphere (Alexander, 1984). One of the modern approaches of discovering new bioactive substances is by isolating and screening for microorganisms from relatively unknown and unstudied areas In a recent study, actinomycetes have been isolated from the Antarctica soil. Molecular studies indicated that the strains belonged to the genera *Streptomyces*, *Actinomadura* and *Kitasatospora* (Mocheva *et al.*, 2002).

Generally, actinomycetes are aerobic heterotrophs. In other words, they require an organic carbon source and air to survive. Hence, actinomycetes are able to grow well at relatively low moisture surrounding, about 15-20% of the moisture holding capacity of the soil. Besides that, actinomycetes grow best in neutral soils at a pH of 6-7 and do not like soils with an acidic reaction.

As most of the actinomycetes are mostly soil inhabitants, they are the primary decomposer of the macromolecules complex in soil and also the tough plants materials like bark and woody stems. They are especially effective at attacking tough, raw plant tissues (cellulose, chitin, and lignin), softening them up for their less enterprising relatives.

2.1.2 Industrial Importance of Actinomycetes

Early in the year of 1888, Nocard first recognized the pathogenic potential of actinomycetes. Since then, several aerobic actinomycetes have been a major source of interest for the commercial drug industrial (Mcneil and Brown, 1994). In the 60's and 70's of the 20th century, 75-80% of all discovered antibiotics derived from the *Actinomycetales*, mainly from the *Streptomyces* species (Mocheva *et al.*, 2002). They have been proved to be extremely useful microorganisms for producing novel antimicrobial agents. Besides that, actinomycetes have also been well known as potential veterinary pathogens affecting many different animal species.

Among all the genera of actinomycetes, *Streptomyces* turned out to be the major producer of different antibiotics of great economic and medical importance. For an example, the synthesis of macrolide antibiotics like erythromycin and adriamycin, synthesis of aminoglycosides like streptomycin and neomycin and other groups of antibiotics such as tetracyclines are the major production of *Streptomyces* (Madigan *et al.*, 2000) Besides *Streptomyces*, other genera that involve in the production of antibiotics include actinomadura, norcardia, actinoplane, micromonospora, thermoactinomyces and rhodococcus (Palaniappan, 1995).

2.2 *STREPTOMYCES*

Streptomyces is the most successful genus in the actinomycetes with over 500 species that are already recognized by Bergey's manual (References). One distinct characteristic of *Streptomyces* is the production of spores from aerial filaments called sporophores. These rise above the colony and form spores called conidia by simple cross-wall divisions of the filaments. If the spores are being scrutinized under the electron microscope, the refractile spores can be seen. The differences in the shape and arrangement of aerial filaments and spore-bearing structures of various species are among the fundamental features that are used in classifying the *Streptomyces* groups. Besides the morphological characteristics, *Streptomyces* will produce a special fresh earthy smell, which arises from chemicals called geosmens.

As mentioned earlier, the most striking property of *Streptomyces* is the prolific ability of producing antibiotics. In some studies, it shown that over 500 distinct antibiotics substances are produced by *Streptomyces*. Particularly, the antibiotics that

are produced are applied in human and veterinary medicine and agriculture, as well as anti-parasitic agents, herbicides, pharmacologically active metabolites and several important enzymes in food and other industries. For examples, *Streptomyces griseus* produces streptomycin, *Streptomyces venezuelae* produces chloramphenicol, *Streptomyces fradiae* produces neomycin, *Streptomyces orientalis* produces vancomycin, *Streptomyces erythreus* produces erythromycin and as for *Streptomyces noursei*, it produces nystatin.

2.3 SECONDARY METABOLITES

Microbial secondary metabolites include antibiotics, pigments, toxins, effectors of ecological competition and symbiosis, pheromones, enzyme inhibitors, immunomodulating agents, receptor antagonists and agonists, pesticides, antitumor agents and growth promoters of animals and plants (Demain, 1998).

Secondary metabolites are normally produced at the end of vegetative growth and during stationary phase of the cell cycle (Madigan *et al.*, 2000). They have unusual structures and their formation is regulated by nutrients, growth rate, feedback control, enzyme inactivation and enzyme induction. However, the produced secondary metabolites are not essential for growth and reproduction.

Secondary metabolites are often produced as a group of a closely related structure. For instance, a single strain of *Streptomyces* has been found to produce over 30 related but different anthracycline antibiotics. Most secondary metabolites are complex organic molecules that require a large number of specific enzymatic

reactions for synthesis. In this case, we can refer to the synthesis of tetracycline, which require at least 72 enzymatic steps; and as for erythromycin, 25 steps are involved.

2.4 SIGNAL TRANSDUCTION

Signal transduction at the cellular level is a process where information or signal from the environment of cells is carried across the plasma membrane into the cell and finally transmitted to the cell nucleus, in order to stimulate coordinated changes in cell activity through a signaling molecule.

Basically, the signaling proteins will coordinate the precise orchestration of metabolism and other cellular processes in response to environmental cues. These signal transduction networks link receptors for extracellular mediators at the cell surface to appropriate effectors responses throughout the cell (Hancock, 1999).

In the simple movement of signals, they are associated with receptor molecules of the acetylcholine class: receptors that constitute channels that allow signals to be passed in the form of small ion movement. On the other hand, more complex signal transduction involves the coupling of ligand receptor interactions to many intracellular events. These events include phosphorylation change enzyme activities and protein conformations. Subsequently, an alteration will occur in cellular activity and changes in the program of genes will be expressed within the responding cells. Various of these coupling network will operate in each of the different types of cells in the

organism and these cells will monitor their environment and react appropriately to the benefit of the organism.

When the signal transduction process is scrutinized in exquisite detail, it is clear that the whole mechanism involve a chain linking of signaling molecules. The first extracellular signaling compound that is formed is known as first messenger. This first messenger will then lead to the production of small and transient molecule in the inside the cell, called second messenger. From the first messenger to second messenger and to the next molecule, the information or message will pass on, in order to activate or alter the activity of the next molecule in signal transduction process. The existence of this cell-signaling cascade will then allow the amplification of the original signal, so that the signal can act effectively on the target cell (Hancock, 1999).

However, real havoc will occur when the signaling molecules malfunction due to the mutations in the genes that encode them and nowadays, over 400 diseases have been linked to the breakdown of the signaling pathway that control normal cell proliferation and survival.

2.5 MAP KINASE PATHWAY

Mitogen-activated protein kinases (MAP kinase) are ubiquitous in all eukaryotes, ranging from yeasts to humans (Watanabe *et al.*, 1995). They are a group of serine-threonine protein kinases that regulate a variety of cellular processes in response to the extracellular and intracellular signals. These protein kinases are activated through a

kinase cascade known as the MAP kinase pathway. Basically, this pathway consists of three protein kinases that act in a module: a MAP kinase kinase kinase (MAPKKK or MEKK), a MAP kinase kinase (MAPKK or MEK) and a MAP kinase (MAPK) (Figure 2.1) (Gustin *et al.*, 1998).

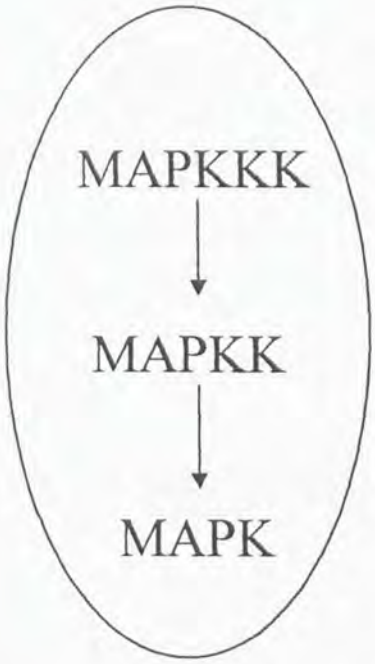


Figure 2.1: MAP Kinase module (Gustin *et al.*, 1998)

Once this MAP kinase pathway is activated, the MAPKKK will phosphorylate the MAPKK. The MAPKK will in turn activate the MAPK through the phosphorylation process. Consequently, the activated MAP kinase will phosphorylates a variety of intracellular targets including transcription factors, transcriptional adaptor proteins and other protein kinases and thus the signals can be transmitted into the nucleus. One unique thing about MAP kinase is that they become activated only when both tyrosine and threonine residues are phosphorylated.

REFERENCES

Alexander, M., 1984. *Introduction to soil microbiology.* New York.

Blackwell, E., Halatek, I. M., Kim, H. J., Ellicott, A. T., Obukhov, A. A. and Stone, D. E., 2003. Effect of the Pheromone-Responsive G_α and Phosphatase Proteins of *Saccharomyces cerevisiae* on the Subcellular Localization of the Fus3 Mitogen-Activated Protein Kinase. *Molecular and Cellular Biology* **23** (4), 1135-1150.

Choi, K. Y., Kranz, J. E., Mahanty, S. K., Park, K. S. and Elion, E. A., 1999. Characterization of Fus3 localization: active Fus3 localizes in complexes of varying size and specific activity. *Mol. Biol. Cell* **10**, 1553-1568.

Cook, A. E. and Meyers, P. R., 2003. Rapid identification of filamentous actinomycetes to the genus level using genus-specific 16S-rRNA gene restriction fragment patterns. *IJSEM Papers in Press.*

Cooper G. M., 2000. *The Cell, A Molecular Approach.* ASM Press, Washington D.C. Sinauer Associates, Inc.

Davenport, K. D., Williams, K. E., Ullmann, B. D., Gustin, M. C., 1999. Activation of the *Saccharomyces cerevisiae* filamentation / invasion pathway by osmotic stress in high-osmorilarity glycogen pathway mutants. *Genetics* **153**, 1091-1103.

Demain, A. L., 1998. Induction of microbial secondary metabolism. *International Microbiology* **1**, 259-264.

Denu, J. M., Zhou, G., Wu, L., Zhao, R., Yuvaniyama, J., Saper, M. A. and Dixon, J. E., 1995. The Purification and Characterization of a Human Dual-specific Protein Tyrosine Phosphatase. *JBC Online* **270** (8), 3796-3803.

Doi, K., Gartner, A., Ammeter, G., Errade, B., Shinkawa, H., Sugimoto, K. and Matsumoto, K., 1994. MSG 5, A Novel Protein Phosphatase Promotes Adaptation To Pheromone Response In S. cerevisiae. *The EMBO Journal* **13** (1), 61-70.

Elion, E. A., 2001. The Ste5p scaffold. *Journal of Cell Science* **114**, 3967-3978.

Ernsting, B. R. and Dixon, J. E., 1997. The *PPS1* Gene of *Saccharomyces cerevisiae* Codes for a Dual Specificity Protein Phosphatase with a Role in the DNA Synthesis Phase of the Cell Cycle. *JBC Online* **272** (14), 9332-9343.

Esch, R. K. and Errede, B., 2002. Pheromone induction promotes Ste11 degradation through a MAPK feedback and ubiquitin-dependent mechanism, *PNAS* **99** (14): 9160-9165.

Farley, F. W., *et al*, 1999. Relative Dependence of Different Outputs of the *Saccharomyces cerevisiae* Pheromone Response Pathway on the MAP Kinase Fus3p. *Genetics* **151**, 1425-1444.

Goodfellow, M. and Board, R. G., 1980. *Micromorphology and Fine Structure of Actinomycetes*. Academic press, London.

Goodfellow, M. and Minnikin, D. E., 1992. Prokaryotes. Balow, A., Truner, H. G., Schlegel, H. G., Starr, M. P. and Stolp, H. Second edition. Springer Verlag, New York.

Gustin, M. C., Albertyn, J., Alexander, M. and Davenport, K., 1998. MAP Kinase Pahtway in the Yeast *Saccharomyces cerevisiae*. *Microbiology and Molecular Biology Reviews* **62** (4), 1092-2172.

Hahn, J. S. and Thiele, D. J., 2002. Regulation of the *Saccharomyces cerevisiae* Slt2 Kinase Pathway by the Stress-inducible Sdp1 Dual Specificity Phosphatase. *JBC Online* **277** (24), 21278-21284.

Hancock, J. T., 1999, *Cell Signalling*, Pearson Education Limited, Longman, England.

Kaeberlein, M. and Guarente, L., 2002. *Saccharomyces cerevisiae MPT5* and *SSD1* function in parallel pathways to promote cell wall integrity. *Genetics* **160**, 83-95.

Kamada, Y., H. Qadota, C. P. Python, Y. Anraku, Y. Ohya, and D. E. Levin.1996 . Activation of yeast protein kinase C by Rho1 GTPase. *J. Biol. Chem.* **271:**9193 –9195.

Kawakami, Y., Rodriguez-Leon, J., Koth, C. M., Buscher, D., Itoh, T., Raya, A., Ng, J. K., Esteban, C. R., Takahashi, S., Henrique, D., Schwarz, M., Asahara, H. and Belmonte, J. C. I., 2003. MKP3 mediates the cellular response to FGF8 signalling in the vertebrate limb. *Nature Cell Biology* **5** (6), 513-519.

Levin, D. E., B. Bowers, C. Chen, Y. Kamada, and M. Watanabe.1994 . Dissecting the protein kinase C/MAP kinase signaling pathway of *Saccharomyces cerevisiae*. *Cell. Mol. Biol.* Res. **40:**229 –239.

Leza, M. A., Elion, E. A. 1999. *POG1*, a novel yeast gene, promotes recovery from pheromone arrest via the G1 cyclin *CLN2*. *Genetics* **151**, 531-543.

Madigan, M. T., Martinko, J. M. and Parker, J., 2000. *Brock Biology of Microorganisms*. Prentice Hall International, Inc, USA.

Martin, H., Rodriguez-Pachon, J. M., Ruiz, C., Nombela, C. and Molina, M., 2000. Regulatory Mechanisms for Modulation of Signaling through the Cell Integrity Slt2-mediated Pathway in *Saccharomyces cerevisiae*. *JBC Online* **275** (2), 1511-1519.

Mcneil, M.M. and Brown, J.M., 1994. The medically important aerobic actinomycetes: epidemiology and microbiology. *Clinical Mibrobiology Reviews* **7** (3), 357-417.

Miyadoh, S., Hamada, M., Hotta, K., Kudo, T., Seino, A., Vobis, G. and Yokota, A., 1997. Atlas of actinomycetes, The Society for Actinomycetes Japan, Asakura Publishing Co., Ltd.

Muda, M., Boschert, U., Dickinson, R., Martinou, J. C., Martinou, I., Camps, M., Schlegel, W., Arkinstall, S., 1996. MKP-3, a novel cytosolic protein-tyrosine phosphatase that exemplifies a new class of mitogen-activated protein kinase phosphatase. *JBC Online* **8**, 4319-4326.

Moncheva, P., Tishkov, S., Dimitrova, N., Chipeva, V., Antonova-Nikolova, S. and Bogatzevska, N., 2002. Characteristics of soil actinomycetes from Antartica. *Journal of Culture Collections* **3**, 3-14.

Palaniappan, N., 1995. Isolation and characterization of an antifungal agent from an indigenous *Streptomyces sp.*. University of Malaya.

Philip, B., and D. E. Levin., 2001 . Wsc1 and Mid2 are cell surface sensors for cell wall integrity signaling that act through Rom2, a guanine nucleotide exchange factor for Rho1. *Mol. Cell Biol.* **21:**271 –280.

Schmitz, H. P., Huppert, S., Lorberg, A. and Heinisch, J. J., 2002. Rho5p downregulates the yeast cell integrity pathway. *Journal of Cell Science* **115**, 3139-3148.

Sobering, A. K., Jung, U. S., Lee, K. S. and Levin, D. E., 2002. Yeast Rpi1 is a putative transcriptional regulator that contributes to preparation for stationary phase. *Eukaryotic Cell* **1** (1), 56-65.

Watanabe, Y., Irie, K. and Matsumoto, K., 1995. Yeast RLM1 Encodes A Serum Response Factor-Like Protein That May Function Downstream Of The Mpk1 (Slt2) Mitogen-Activated Protein Kinase Pathway. *Molecular And Cellular Biology* **15** (10), 5740-5749.