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JUDUL: FUNGI ISOLATION AND SCREENING FOR POTENTIAL INHIBITORS AGAINST THE TWO-COMPONENT SYSTEM AND THE SERINE/THREONINE PROTEIN KINASE IN ACTINOMYCETES.

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# FUNGI ISOLATION AND SCREENING FOR POTENTIAL INHIBITORS AGAINST THE TWO-COMPONENT SYSTEM AND THE SERINE/THREONINE PROTEIN KINASE IN ACTINOMYCETES

VUN SU CHIUN

THIS DISERTATION IS SUBMITED TO FULFILL THE PARTIAL REQUIREMENT TO OBTAIN THE DEGREE OF BACHELOR OF SCIENCE WITH HONOURS



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March 2005

## DECLARATION

I affirm that this paper is of my own effort, except for materials referred to as cited in the reference section.

25 March 2005

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## APPROVAL BY THE EXAMINERS

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## ABSTRACT

The aim of this research study was to isolate different strains of fungi and conduct a screening test by the fungi extract (secondary metabolites) against the two-component system and ser/thr protein kinase which can be applied in treating disease such as tuberculosis (TB) disease. A total 12 soil samples were collected from different sites of Danum Valley tropical rainforest, Lahad Datu under identified diterocarps trees. A total of 36 strains of fungi had been isolated by using the Potato Dextrose Agar (PDA) media (Booth, 1971; Johnston & Booth, 1983) with chloramphenicol and sodium chloride at pH 6.7 and pH 4.4. Chloramphenicol is a type of antibiotic which inhibit the growth of the unwanted bacteria. All the fungi cultures showed well and good growth on the PDA media which supplemented with rich required nutrients. The sufficient nutrients provide enough required energy and raw material source for its growth as well as its cell proliferation. The fungal cultures were then purified on the same media without chloramphenicol and also sodium chloride (NaCl). The morphology of the cultures were analyzed, observed and recorded. The morphology that was mentioned was such as the fungi's aerial mycelia, substrate mycelia and their extracellular pigment colour. Fermentation with aerobic condition was carried out after the fungi were inoculated on the PDA slant agar media and the silica gel stock for stock storage usage. The fungal colonies were inoculated onto the 10ml fermentation media inside the 125ml conical flask. This process was carried out for 120 hours (5 days) at $28^0$C on the shaker incubator machine that's rotated at 220 r.p.m. Cell harvesting was carried out after the period with the 10ml of acetone for the cell lysis. The fungi extracts were then poured inside McCartney Bottle. The acetone extracts were then tested against the Two-Component System (PhoP/PhoR) in *M.smegmatis* H8000 and Ser/Thr Protein Kinase (AfsK/AfsR) in *S.griseus* H10000. A total of 4 extracts showed as potential inhibitors against the PhoP/PhoR pathway in *M.smegmatis* (mc1255) strain H8000. The 4 extracts with strain number H9984, H9989, H9990 and H9995 showed inhibition zone surround the paper discs on 100µM $MgSO_4.7H_2O$ plate where the growth of the *M.smegmatis* was inhibited and not on the 1mM plate where none of the *M.smegmatis* growth was

inhibited by the extracts for both of the screening test which been conducted. A total of 5 extracts (H9386, H9387, H9407, H9409 and H9986) that showed activity on the 1.5% D(+)Glucose plate where inhibition zone seems to be appeared surround the paper discs which been soaked with the 5 extracts above but not on the 1.5% D-Mannitol plate. These 5 extracts expected to inhibit either only the sporulation or the entire growth of the *S.griseus* strain H10000 where none of the microscopic observation was been carried out to determine for the potential inhibitor among the 5 extracts.

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| PDA | Potato Dextrose Agar |
| TCS | Two-Component System |
| STPKs | Serine/Threonine Protein Kinase |
| e.g. | exempli gratis |
| Ser/Thr | Serine/Threonine |
| HPK | Histidine Protein Kinase |
| P | Phosphoryl group |
| DNA | Deoxyribonucleic acid |
| PknA | Protein kinase A |
| Sq. | Square |
| Km. | Kilometer |
| F. | *Fusarium* |
| B | Beta |
| RNA | Ribonucleic Acid |
| UV | Ultra Violet |
| psi | pound per square inch r.p.m. |
| HK | Histidine Kinase |
| RR | Response Regulator |
| ATP | Adenosine Triphosphate |
| D | Domain |
| ADP | Adenosine Diphosphate |
| *E.coli* | *Escherichia coli* |
| N-terminal | Nitrogen terminal |
| *sp.* | species |
| LPS | Lipopolysaccharide |
| TB | Tuberculosis |
| AIDS | Acquired Immune Deficiency Syndrome |
| μm | micrometer |
| *M.tuberculosis* | *Mycobacterium tuberculosis* |

| | |
|---|---|
| CFA | Freund's Complete Adjuvant |
| HIV | Human Immunodeficiency Virus |
| midTB | Multiple-Drug-Resistant TB |
| B.C. | Before Christ |
| LTBI | Latent Tuberculosis Infection |
| G+C | Guanosine plus Cytosine |
| $Mg^{2+}$ | Magnesium |
| $Ca^{2+}$ | Calcium |
| $Mn^{2+}$ | Manganese |
| *S.typhimurium* | *Salmonella typhimurium* |
| $Ba^{2+}$ | Barium |
| $Fe^{3+}$ | Ferum |
| *S.coelicolor* A3(2) | *Streptomyces coelicolor* |
| Act | Actinorhodin |
| Red | Undecylprodigiosin |
| CDA | Calcium-dependent antibiotic |
| Mmy | Methylenomycin |
| CM | Cell Membrane |
| C-terminal | Carbon-terminal |
| YMP | Yeast Mannitol Peptone |
| nM | nano Molar |
| IFO | Institute Fermentation of Osaka |
| NaCl | Sodium chloride |
| $^0C$ | Degree Celsius |
| YPD | Yeast Peptone Dextrose |
| µM | micro Molar |
| mM | mili Molar |

# CHAPTER 1

# INTRODUCTION

## 1.1 Fungi

Fungi are plant-like organisms that lack with chlorophyll organelle. Fungi are one of the five kingdoms of life. Many of the fungi are good as well as useful (e.g. edible mushrooms) while some of them causing problems (e.g. fungi that can injure other plants and people). There are over 100,000 species of fungi. Fungi usually absorbed their food from others; this was because that it doesn't have any chlorophyll pigment. They as well didn't use the light to make their own food and them also able to live in many of the damp and dark places.

Fungi are a group of organisms and micro-organisms that are classified within their own kingdom that is the fungal kingdom where they are neither plant nor animal. Fungi draw their nutrition from decaying organic matter, living plants and even animals. They do not photosynthesize as they totally lack with the green pigment chlorophyll it is present in many of the green plants. Many of the fungi play an important role in the natural cycle as decomposers it helps to return the nutrients to the soil back, they are not all destructive. Fungi are even used for medical purposes, such as species within the *penicillium* genus which provide antibiotics, e.g. penicillin.

**Figure 1.1(a)** *Penicillium notatum* is a species of fungus that was used as the original source of the antibiotic penicillin.


**Figure 1.1(b)** Species within the genus *Penicillium* produce flavors for blue and white cheeses, such as Gorgonzola.

## 1.2 Signal Transduction

### 1.2.1 Signal transduction and the two-component regulatory systems.

Two-component systems (TCS) and eukaryotic-like Ser/Thr protein kinases comprise major components of the signal transduction machinery of *Mycobacterium tuberculosis* (Tyagi and Sharma, 2004).One of the best understood systems among

the signal transduction protein is the DevR-DevS two-component system (Tyagi and Sharma, 2004).

Most all of the bacteria regulate cell metabolism in response to its wide variety of environmental fluctuations, these includes;

a) Temperatures changes.
b) Changes in pH.
c) Oxygen availability.
d) Changes in availability nutrients.
e) Changes in number of cells presents.

So, that's why there's must be a mechanisms by which the bacteria receive signals from the environment and then transmitted them to specific target to be regulated. However, in many cases the external signals is not transmitted directly to the regulatory protein. Instead, a signal is first detected by a sensor and transmitted in a changed form to the rest of the regulatory machinery. This process is called "Signal Transduction" (Madigan and Martinko, 2003).

### 1.2.2 Sensor kinases and response regulators.

The two-component system is the regulatory systems which cells sense and respond to the environmental signals. These two-component systems include two different proteins;

a) Sensor protein (located in the cell membrane).

b) Response regulator protein (Madigan and Martinko, 2003).

Sensor protein has a kinase activity and referred to as a sensor kinase. Kinase is an enzyme that phophorylates compounds. The mechanism of the two-component signal transduction system is showed as figure below;-

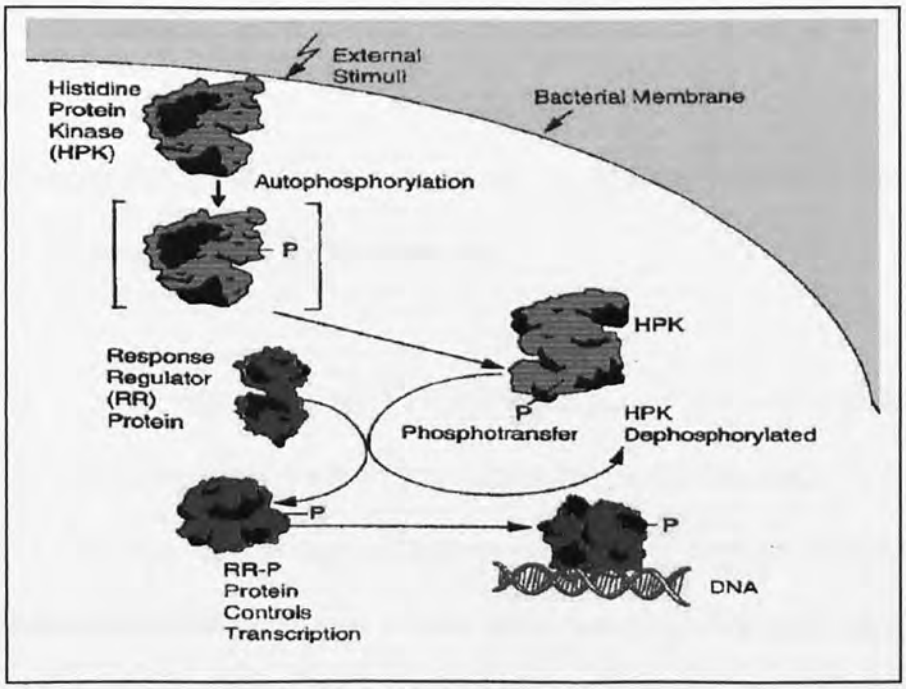


**Figure 1.2.2** Typical two-component signal transduction system (Barrett and Hoch, 1998).

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