Screening For Antimicrobial Activity Of Fungi In Soil Samples Collected From Kubah National Park

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Abstract: Antimicrobial agents including antivirals and antibiotics have saved millions of lives all over the world, but these drugs are losing their effectiveness due to the development of resistance of infectious disease agents towards them. The incidence of antibiotic-resistance towards current drugs has been rapidly increasing but fewer new antibiotics are being developed. This study was carried out on soil samples collected from Kubah National Park, Kuching, Sarawak, Malaysian Borneo, in order to discover novel antibiotics produced by soil microbes. Twenty one samples of soils were analyzed for antimicrobial producing fungi. Potential fungal isolates were tested against *Escherichia coli*, *Listeria monocytogenes*, *Salmonella enteritidis* and *Klebsiella pneumoniae.* Six fungal isolates labeled P550AIa, P550AIb, P550AIc, P550AId, P550AIIa, P550AIIb showed strong antibacterial activity against the test bacteria during the antimicrobial activity screening using agar overlay technique in the preliminary screening and secondary screening. Out of the 6 fungal isolates, 3 isolates P550AIa, P550AIb, P550AIc were selected to undergo antibiotics susceptibility testing and further characterization. The crude extract of the 3 fungal isolates were further characterized by MIC, TLC and bioauthography methods. The isolates showed MIC value and produced inhibition zone compared to the positive control (5×dilutions of penicillin-streptomycin solution). The characteristics of the spores produced by the three fungal isolates matched with the description for *Penicillium* spp.. Further confirmation by DNA sequencing of isolate P550AIb revealed its identity as *Penicillium verruculosum.* All the fungal isolates showing antimicrobial activity are potential to be used for producing antimicrobial compound for combating infectious bacterial agents as evidenced in this study.

Index Terms: Antimicrobial activity, Fungi, Identification, Soil.

1 INTRODUCTION

Antibiotic is a drug used to treat infections caused by bacteria that can cause illness to humans and animals. Antibiotic functions to inhibit or destroy the bacterial cells that cause certain disease [1]. In fact, antibiotic is secondary metabolite produced by bacteria [2] to maintain their niche and territory. There are few groups of microorganisms that can be used as sources for clinically useable antibiotics. As stated by Cooke and Gibson [3], only antibiotics that have an effect on bacterial cells but not the host cells are categorized as useful antibiotics. To date, over 100 different antibiotics are available to cure minor and life-threatening infections. Antibiotic resistance occurs when the effectiveness of drugs and chemicals designated to cure diseases are reduced [4].

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Scientists are continuously searching for novel antibiotic producing microbes because drug resistant strains of pathogen emerge more quickly than the rate of discovery of new drugs and antibiotics [5]. Consequently, a numbers of antibiotics that can fight against pathogenic bacteria had been discovered. According to Roberts [6], it is important to discover new antibiotics as the emergence of new diseases and reemergence of multiple-antibiotic resistant pathogens have caused current antibiotics ineffective. There are many sources where antibiotics can be discovered, however, soil is the most important source for the discovery of novel antibiotics. According to Dulmage and Rivas [7], soil microorganisms have continually been screened for their useful biological active metabolites, such as antibiotics since long ago. Therefore, this study was an attempt to discover novel antibiotics from microbes in soil samples from undisturbed area in Sarawak.

2 MATERIALS AND METHODS

2.1 Soil sampling, preparation and plating

Soil samples were collected at four different elevations; 50m, 350m, 450m and 550m above sea level along Ulu Rayu and Summit Trial at Matang Wildlife Centre-Kubah National Park, Sarawak. Samples were collected at 0-10cm using composite augering technique. Approximately 1g of the soil samples was dissolved in 9 ml of sterile Phosphate Buffer Saline (PBS) buffer (pH 7.4) to make soil suspension. The supernatant from the soil solution was pipette and spread over four PDA (BD Difco™, USA) plates. The plates were left at room temperature for 5 days to calculate and record the fungal colonies. Then, the plates were kept at 4°C for 2 days to delay the growth of soil microorganisms.

2.2 Test bacteria

The test bacteria were obtained from Microbiology Laboratory, Department of Molecular Biology, Universiti Malaysia Sarawak. The test bacteria used were gram-positive (Listeria