UNIVERSITI TEKNOLOGI MARA

SECONDARY METABOLITES SCREENING AND ANTAGONISTIC STUDIES OF SOOTY MOULDS

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ABSTRACT

Sooty moulds are darkly pigmented, epifoliar, non parasitic, fungi feeding on sugary exudates secreted by sap feeding insects on plants. Very little is known on the secondary metabolites they may produce and their antimicrobial activities. As part of a collaborative programme jointly run with Mae Fah Luang University, Thailand, and University of Malaya, eleven strains of sooty moulds were cultured in various stress conditions by applying the OSMAC (one strain, many compounds) approach including the usage of epigenetic elicitors to awaken possibilities of any 'silent' biosynthetic gene that may not express under normal laboratory environment. For that purpose, an in-house protocol allowing parallel microscale fermentation in microtiter plates was implemented. Experiments carried out through the above protocol were disappointing as only one fungus (strain S02) showed significant secondary metabolite production. This happened when the said fungus was grown in presence of glycerol. When semi-solid fermentation on malt extract agar was used, secondary metabolite profiles obtained from HPLC analyses became more encouraging. Two extracts were specifically interesting and the corresponding strains MS34 and DPC052 were fermented on a large scale, their cultures extracted and their main components isolated by a combination of HPLC procedures. MS34 was thus shown to produce sulochrin, a compound known to inhibit eosinophil degranulation and possess weak antimicrobial activity. DPC052 extract yielded crystals that were determined as kojic acid by X-ray crystallography. Kojic acid is a well known inhibitor of the tyrosinase, thus preventing melanin biosynthesis. A third approach to the study of sooty moulds biosynthetic potentials utilised co-cultures with fungi isolated from some sooty mould infested phylloplanes. Three sooty mould strains, S01, S04 and DPC052 were observed to have some antimicrobial effect when co-cultured with fungi A015 based on growth diameter observation. However, HPLC-DAD analysis of co-cultured extracts did not show very significant changes in the metabolic profiles. The above exploratory work allowed defining study parameters for in-depth studies on sooty moulds. A major improvement may be provided by the addition to the growth media of water activity lowering agents such as glycerol to better mimic natural growth conditions. Further, the search for bioactive compounds should be expanded beyond small UV-active organic compounds of intermediate polarities. Eventually, co-culture experiments with xerotolerant or xerophilic bacteria, yeasts or filamentous fungi may lead to unsuspected results.

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CHAPTER ONE
INTRODUCTION

Sooty moulds are darkly pigmented, epifoliar, non parasitic, fungi that grow on plant surfaces. They negatively impact post-harvest products by interfering with plant photosynthesis. Sooty moulds feed on sugary exudates or honeydew secreted by sap feeding insects on plants. With sugar exudates as their nutrient source, there should be a strong competition for nutrient with other types of fungi. From observation, however, sooty moulds do not seem to be challenged for niche occupancy. This may be due to their ability to produce secondary metabolites that prevent the growth or kill other microorganisms.

Sooty mould unique habitats, usually leaf upper surfaces, are affected by microclimatic variations. Further, the ability of sooty moulds to withstand these conditions hints of their likely xerophily (Chomnunti *et al.*, 2014; Faull *et al.,*2002; Kwee, 1988; Nelson, 2008*)* Such xerophilic microorganisms possess potential in various areas. In spite of these potentialities, studies on their secondary metabolites are limited.

Considering the likely presence of cryptic biosynthetic genes in most fungal strains, the Microbial Metabolite Laboratory of Atta-ur-Rahman Institute, UiTM Puncak Alam, has recently developed the MECSUS protocol (Microtiter Plate, Elicitors in Combination, Solid Phase Extraction, UHPLC, Statistical Analysis) in order to elicit the production of secondary metabolites otherwise not detected. This unique protocol is based on the usage of deep-well microtiter plates as parallel microfermentors.