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## **OPTIMIZATION FOR SECONDARY METABOLITES PRODUCTION FROM TROPICAL LICHEN MYCOBIONTS**

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Lichens are resources for novel compounds with many of them having importance in medicine and other fields. Intact lichens cannot be cultivated but their mycobionts can be axenically grown in laboratory from ascospore discharge or tissue culture techniques. Frequency of spore discharge, germination and colony development were varied among tropical lichen species. Mycobiont cultures from stock at The Lichen Research Unit, Ramkhamhaeng University (RAMK) were screened for their abilities to produce biological activities. Among these four mycobiont species; Graphis sp., Graphina albissima, Ocellularia punctulata and Pyrenula kurzii were selected regarding their potential to produce metabolites with antimicrobial activities. From 7 different media for stimulation of growth of mycobionts, Malt-Yeast Extract medium was chosen for its superior performance and used for further experimentation. The mycobionts were grown on both solid and liquid media and the secondary metabolites produced under various conditions were examined. Static and shake liquid cultures with various supporting materials were examined for growth of mycobionts, however in these conditions growth rate were higher but the metabolites produced were lower in both number and quantities. Another way to culture the mycobionts was solid medium and was also considered an easier method and mycobionts were grown for period of 27 weeks at room temperature, cells and pieces of agar block were removed at intervals and extracted with

41-P methanol. Chemical profiles detected by Thin Layer Chromatography (TLC) indicated that the metabolites produced increased gradually during 9-15 weeks of incubation under these conditions. Comparison of pH between acidic, neutral and alkaline conditions for growth of these mycobionts showed that the optimize pH for all of them was at neutral pH. Some lichen substances absorb ultraviolet light and protect the algae from too intensive irradiation, in order to induce metabolite production in mycobionts cultures, both short and long wavelength UV light were investigated. The results indicated that the number of spots on TLC plates were decreased, however the conditions in this experiment were not entirely suitable. Scale up for high numbers of cell mass and secreted metabolites were done and further studied on the chemical structures of new chemical compounds were investigated and are discussed.