สารทุติยภูมิจากราที่ก่อให้เกิดไลเคนในสภาวะที่มีการกวนอาหารเลี้ยงเชื้อ

Secondary metabolites produced by the culturing of mycobiont in stir-culture condition

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บทคัดย่อ: การสร้างสารทุติยภูมิของราที่ก่อให้เกิดไลเคนขึ้นอยู่กับปัจจัยทางสรีรวิทยาวิทยาหลายประการ สภาวะการกวนอาหารเลี้ยงเชื้อเป็นปัจจัยหนึ่งที่สำคัญในการสร้างสารทุติยภูมิของราที่ก่อให้เกิดไลเคน จากการทดลองเลี้ยงราที่ก่อให้เกิดไลเคน 4 สายพันธุ์คือ *Graphina albissima*, *Ocellularia punctulata*, *Pyrenula kurzii* และ *Pyrenula* sp. ในขวดเลี้ยงเซลล์ที่มีการกวนอาหารเลี้ยงเชื้อ เป็นเวลา 15 นาทีทุกๆ 3 ชั่วโมง นาน 9 สัปดาห์ที่อุณภูมิ 30 องศาเซลเซียส นำเซลล์และน้ำเลี้ยงเชื้อมาสกัดสารด้วยตัวทำละลายเมทานอลแล้วนำไปแยกสารด้วยวิธีทินเลเยอร์โครมาโทกราฟี พบว่าราที่ก่อให้เกิดไลเคนเมื่อนำมาเลี้ยงในสภาวะที่มีการกวนอาหารเลี้ยงเชื้อจะมีอัตราการเจริญสูงกว่าและสร้างสารบางชนิดที่มีความแตกต่างไปจากการเลี้ยงในสภาวะตั้งนิ่งโดยส่วนที่สกัดจากเซลล์จะให้สารมากกว่าการเลี้ยงในสภาวะตั้งนิ่ง

Abstract: The production of secondary metabolites by lichen mycobiont depend on various physiological factors, shaking or agitation are important factors for production of secondary metabolites by the mycobionts. The representative mycobionts from 4 species of tropical lichens were cultivated in spinner flask and agitated for 15 minutes every 3 hours for 9 weeks at 30 ^{o}C. After time of incubation, the cultures were extracted by methanol and studies metabolites by Thin layer chromatography (TLC). Mycobiont cultures growing in the spinner flask systems showed higher growth rate and from TLC chromatogram, mycobionts growing in spinner flasks produced higher number of substances than static condition.

Introduction: Lichen and their mycobionts produce a wide range of secondary metabolites[1]that have biological activities for example, antimicrobial and pharmaceutical application. Recent studies suggested that the production of mycobiont substances depended on the composition of media and various ecological factors such as desiccation, temperature and aeration. Hamada (1996) described a large amount of crystalline and yield of usnic acid produced by the mycobiont *Lecanora pulverulenta* was found when 10% of sucrose were added to the media.[2] Xanthones were detected from some samples on the surface of the colonies and the surrounding agar of *Pyrenula pseudobufonia* after a few months of cultivation (Hamada, 2001).[3] Stocker-Wörgötter and Elix, (2002) examined cultural conditions and nutrient requirement to optimize production of secondary metabolites of cultured mycobiont *Lobaria spathulata*.[4] Later in 2009 Stocker-Wörgötter, Hager and Elix studied intraspecific chemical variation within the crustose lichen genus *Haematomma*:

anthraquinone production in selected cultured mycobionts as a response to stress and nutrient supplies.[5] Yamamoto, Kinoshita & Fujita (1998) cultivated 150 strains of mycobionts but only 30 of them grew well in liquid culture with orbital shake of 120 rpm at 15^{o}C for 4 weeks in dark condition.[6]This result suggested that shaking condition may not be a good condition for growing mycobionts, however static condition gave low yield of cell mass and metabolites. From experiment in our laboratory, cultivation of tropical lichen mycobiont on synthetic sponge in liquid media at slow speed (100 rpm) represented good cell mass but both of extracellular and intracellular metabolites production were varied depending on type of lichen mycobionts. Spinner flask systems designed for growth of animal cell culture suspension at low speed of stirring might be an alternative choice to study the effect of agitation in culturing the mycobionts. The aims of this study were to cultivate the tropical mycobionts under stir-culture condition and to investigate the metabolites produced by these cultured mycobionts.

Methodology:

Preparation and culture of mycobionts: All tropical lichen mycobionts in this study were obtained from ascospore discharged from lichen sample collected prior from Khao Yai National Park. The mycobiont were cultivated using the method previously described by Luangsuphabool, T. (2009).[7] The representative of these mycobionts *G. albissima, O. punctulata, P. kurzii* and *Pyrenula* sp. produced antibacterial activities were chosen for studies in stir-culture condition. 5 pieces of colonies of mycobionts from well-grown healthy in Petri dish were cut by a sterile 3.8 mm. diameter cork borer and transferred to 500 ml Bell-Flo™ Spinner Flasks containing 200 ml of Malt-Yeast Extract Broth (Figure 1). Inoculated spinner flasks were placed on stirrer and spinned for 15 minutes every 3 hours for 9 weeks at room temperature (Figure 2).

Figure 1. Bell-Flo™ Spinner Flasks containing 200 ml of Malt-Yeast Extract Broth.

Extraction of Intra and Excellular metabolites: After 9 weeks of incubation, the cell pellets and fluid were separated through filter paper. The intracellular substances were extracted by soaking cells in methanol for 24 hours. Meanwhile the culture fluid were extracted by ethylacetate in separating funnel. The solvent were then evaporated off by rotary evaporator at 40 ^{o}C and the concentrated crude extract were dissolved in 1 ml of methanol and kept in microtubes for further separation of metabolites by Thin layer chromatography (TLC).

Studies of mycobiont metabolites by Thin layer chromatography (TLC): The crude extract of sample were spotted on TLC plate and ran under mixed solvent system between dichloromethane and methanol at ratio 10:0.2 and then examined under daylight to observe visible colour and also under ultra-violet light at 254 nm and 365 nm respectively in the UV chamber. The substances present were then recorded on the basis of retention factor (Rf) value and colour under daylight and ultra-violet light.

Results, Discussion and Conclusion: From this study, the selected mycobiont cultures: *G. albissima, O. punctulata,, P. kurzii* and *Pyrenula* sp., grew well in the spinner flask systems and showed higher growth rate. The cell mass were higher than cultures on sponge at the static condition. After chemical extraction, substances produced by both intracellular and extracellular of these mycobionts were separated and detected by TLC, the results showed that the substances that occurred in both cells and broth of mycobiont when cultured in stir conditions were different from those cultured in static condition (Figure 3).

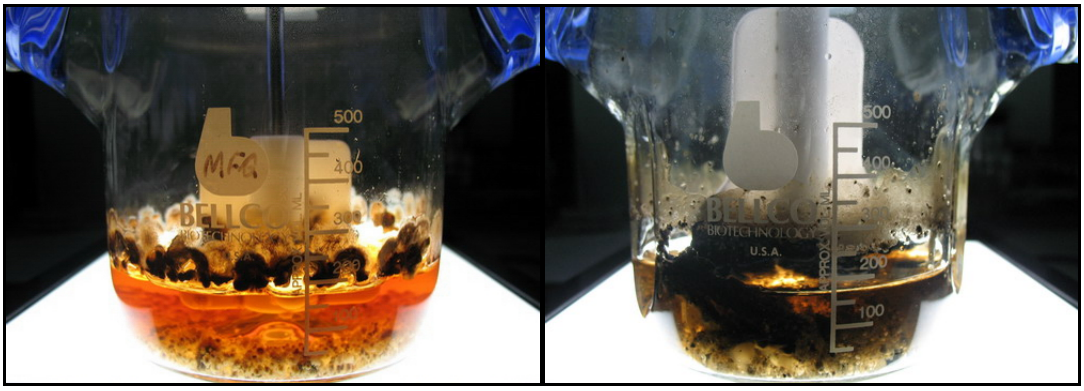

Figure 2. Mycobiont cultures growing in spinner flasks for 9 weeks. *O. punctulata* (left) *Pyrenula* sp. (right)

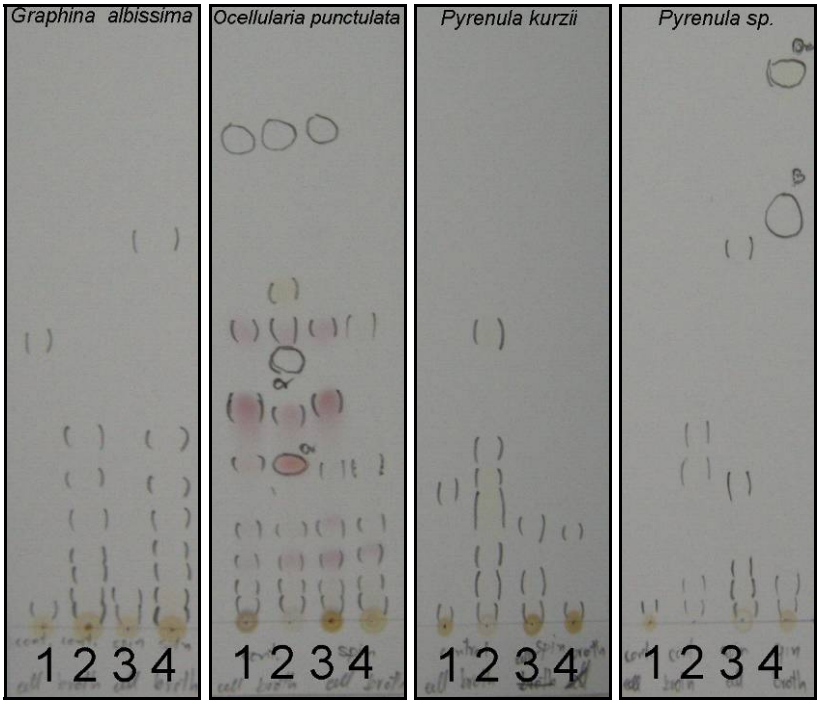

Figure 3. TLC of *G. albissima, O. punctulata, P. kurzii* and *Pyrenula* sp. respectively. lane 1 substances from cell in static condition, lane 2 substances from broth in static condition, lane 3 substances from cell in spinner flask, lane 4 substances from broth in spinner flask

From the figure 3, showed that intracellular metabolites from *P. kurzii* and *Pyrenula* sp. growing in spinner flasks were produced more substances than culture in static conditions and in case of *G. albissima* and *O. punctulata* both produced mainly the same substances as in static condition. Whereas extracellular metabolites from culture of *P. kurzii* and *O. punctulata* in spinner condition showed lower number of substances than static conditions. Further study of metabolites from these lichen mycobionts are under investigation for chemical structure analysis.

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Keywords: mycobionts cultures, stir-culture condition, secondary metabolites