การเจริญและฤทธิ์ในการยับยั้งจุลินทรีย์ของราที่ก่อให้เกิดไลเคนบางชนิดของประเทศไทย

GROWTH AND ANTIMICROBIAL ACTIVITY OF SOME LICHEN MYCOBIONT FROM THAILAND

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บทคัดย่อ: ราที่ก่อให้เกิดไลเคนเมื่อนำมาแยกและเพาะเลี้ยงในห้องปฏิบัติการพบว่ามีการเจริญได้ช้า อาหารเลี้ยงเชื้อเป็นปัจจัยที่สำคัญอย่างหนึ่งในการเจริญของราที่ก่อให้เกิดไลเคน จึงได้ทำการศึกษาอาหารเลี้ยงเชื้อชนิดแข็งที่ใช้เลี้ยงเชื้อราทั่วไป 5 ชนิด ได้แก่ Chemical Defined Medium (CDM), Corn Meal Agar (CMA) Malt Yeast Extract Agar (MYA), Oat Meal Agar (OMA) และ Potato Dextrose Aar (PDA) พบว่าราที่ก่อให้เกิดไลเคนสามารถเจริญเติบโตได้ดีในอาหาร MYA, PDA และ OMA ในขณะที่ CMA และ CDM ราที่ก่อให้เกิดไลเคน เจริญได้ไม่ดี และเมื่อนำน้ำเลี้ยงเชื้อมาทดสอบฤทธิ์ในการเป็นสารปฏิชีวนะ พบว่ามีราที่ก่อให้เกิดไลเคนบางชนิดสามารถสร้างสารที่ยับยั้งการเจริญของแบคทีเรีย *E. coli* และ *P. aeruginosa* ที่ใช้ทดสอบได้

Abstract: The aim of this work was to isolate axenic culture of tropical mycobiont (lichenized fungi) components and to observe growth conditions in laboratory. Common mycological media, Chemical Defined Medium (CDM), Corn Meal Agar (CMA), Malt Yeast Extract Agar (MYA), Oat Meal Agar (OMA) และ Potato Dextrose Aar (PDA) were used in solid culture for growth rate studied, MYA, OMA, and PDA were proved to be better medium for cultivation of these mycobionts than CMA and CDM. The antimicrobial effects of metabolites produced by cultivation of these mycobionts were also investigated. Culture broth from *Graphina albissima* and *Pyrenula* sp. represented inhibition of *E. coli* and *P. aeruginosa*.

Introduction: Culture of lichenized fungi or mycobionts has been considered to be too difficult to undertake mainly because it is time consuming and long term techniques are necessary for successful culture of the mycobionts. The fungal partner can grow on a range of common mycological media (1), in axenic culture the compact colonies are typically formed. Most of the research in lichenized fungi have focused on their secondary metabolites and molecular systematics (2, 3). The majority of isolated mycobionts is from temperate habitats with only a few exclusively from tropical countries. The aims of this study were to culture the tropical mycobionts in axenic condition and to investigate properties of metabolites from isolated mycobionts.

Methodology:

Growth in difference solid media: The lichens species *Graphina repleta*, *Laurera meristospora*, *Ocellularia* sp., *Phaeographina* sp.5, *Trypethelium eluteriae* were collected and mycobiont were isolated by ascospore discharge technique to obtain single and polyspores as described by Crittenden & Porter (1). Germination of their ascospores was investigated under stereozoom and compound microscope and transferred to 5 common mycological medium:

CDM, CMA, MYA, OMA and PDA to observe growth and colony development at 25 ^{o}C. The visual and image analysis was performed in week 3, 6, and 12 after inoculation.

Antimicrobial test: Malt Yeast Extract broth (MYB) was used for liguid culture of mycobionts since selected mycobionts were shown to have high growth rate in this medium. The inocula of culture were transferred to 250 ml Erlanmeyer flasks containing 50 ml of MYB and kept at room temperature. Observation was made daily and flasks were briefly swirled to circulate the medium. After 9 weeks of incubation, the culture fluid was filtered and lyophilized. The extracts were tested for antimicrobial activity with selected microorganisms: *Bacillus cereus, Norcardia asteroides, Escherichia coli, Pseudomonas aeruginosa, and Candida albicans* by paper disc diffusion method (4).

Result, Discussion and Conclusion: Growth of selected 5 different species represent from Thai tropical mycobiont on different solid media were followed over a 12-week period and the results were showed in Table 1 and colonies from after these incubation periods are illustrated in Figure1-3 (*Laurera meristospora*, *Ocellularia* sp., *Trypethelium eluteriae* respectively).

Table 1 Growth of selected mycobionts on difference solid media

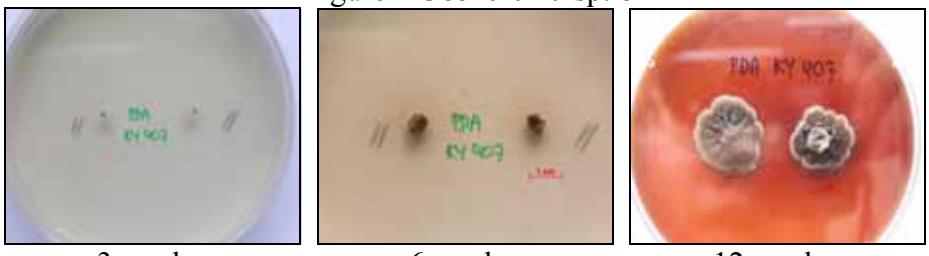
Species	MYA	PDA	OMA	CMA	CDM
Graphina repleta	++++	+++	++++	++	+
Laurera meristospora	++++	++++	++++	++	+
Ocellularia sp.	+++	++++	+++	++	+
Phaeographina sp.5	+++	++++	++++	++	+
Trypethelium eluteriae	++++	++++	++++	++	+

++++ best growth, +++ medium growth, ++,+ weak growth

Figure1 - *Laurera meristospora* on OMA

3 weeks 6 weeks 12 weeks

Figure 2 *Ocellularia* sp. on PDA



3 weeks 6 weeks 12 weeks

Figure 3 *Trypethelium eluteriae* on MYA

3 weeks 6 weeks 12 weeks

It can be seen from Table 1 and Figure 1 to 3 that overall for the majority of the mycobionts tested MYA, OMA and PDA proved to be the most suitable for cultivation of mycobionts in solid media. CMA and CDM generally resulted in poor growth.

The results of antimicrobial activity from culture fluid of mycobionts are given in Table 2

Table 2 Inhibition zones of antimicrobial activities from selected tropical mycobionts

Species	Clear zone diameter (mm)				
	Bacillus	*Norcardia*	*E. coli*	*Pseudomonas*	*Candida*
Arthopyrenia cf. consobriana	-	-	20.5	20	-
Graphina albissima	11.2	-	10.8	11.3	8.7
Laurera meristospora	-	-	-	-	-
Phaeographina pudica	-	-	19	8	7.8
Phaeographis circumscripta	11	8	24	27	-
Pyrenula sp.	13.8	13	10.2	7	21.7
Sarcographa actinobola	8	7	15	15	-
Trypethelium eluteriae	-	-	-	-	-

Extracts from *Graphina albissima* and *Pyrenula* sp. showed broad spectrum results to inhibit not only Gram positive, Gram negative bacteria but also *C. albicans*. The extract from *Pyrenula sp.* inhibited *B. cereus, N. asteroides*, and *C. albicans*. Substances from the extracts were isolated and purified for structure analysis, some of these compounds were identified as 8-methyldichlorodiaportin, ρ-hydroxybenzaldehyde and phenol. These selected tropical lichen mycobionts developed their colonies well on solid medium; MYA, PDA and OMA, and also produced their metabolites to inhibit tested microorganisms. Chemistry of metabolites from mycobionts and molecular based on phylogeny are underway for future studies.

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