การอยู่รอดของราที่ก่อให้เกิดไลเคนด้วยวิธีเก็บรักษาที่มีประสิทธิภาพ

SURVIVAL OF MYCOBIONTS IN AN EFFICIENCY PRESERVATION METHOD

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บทคัดย่อ: ลักษณะสำคัญของราที่ก่อให้เกิดไลเคนเมื่อนำมาเลี้ยงแบบปลอดเชื้อในห้องปฏิบัติการคือโคโลนีมีลักษณะแน่นและมีการเจริญที่ช้าอีกทั้งไม่สร้างสปอร์ในอาหารเลี้ยงเชื้อ ไม่มีรายงานที่กล่าวถึงวิธีที่เหมาะสมในการเก็บรักษารากลุ่มนี้ จากการศึกษาได้ทำการเก็บรักษาราที่ก่อให้เกิดไลเคนในน้ำกลั่นและ Malt Yeast Extract Broth (MYB)ที่ลดความเข้มข้นลงครึ่งหนึ่ง ในอุณหภูมิ 4-7 องศาเซลเซียส เป็นเวลา 12 เดือน ทำการตรวจสอบการอยู่รอดเป็นระยะ พบว่าราที่ก่อให้เกิดไลเคนในวงศ์ Arthoniaceae, Graphidaceae, Pyrenulaceae, Thelotremataceae และTrypetheliaceae สามารถอยู่รอดได้เป็นเวลา 12 เดือนทั้งในน้ำกลั่นและ MYB ที่เจือจาง ในขณะที่ราตัวแทนในวงศ์ Fuscidiaceae สามารถอยู่รอดได้เป็นเวลา 3 เดือน การเก็บรักษาราในน้ำกลั่นนี้เป็นวิธีที่สะดวกและช่วยป้องกันการปนเปื้อนจากการรบกวนของ mites

Abstract: Typically lichenized fungi or mycobionts in axenic culture are slow growing, they form compact colonies and are non-sporulating. There is at present no report of an appropriate technique to maintain cultures of these fungi. In this study, representative mycobionts from 6 families were kept in distilled water and half-strength Malt Yeast Extract Broth at low temperature (4-7^{O}C) for 12 months and periodically tested to determine their ability to survive. Mycobionts from families; Arthoniaceae, Graphidaceae, Pyrenulaceae, Thelotremataceae and Trypetheliaceae were found to survive for a period of 12 months whilst the representatives from the family Fuscidiaceae survived for only 3 months. Storage of fungi in water is an easy method and also reduces the risk of mite infestation of the cultures.

Introduction: Estimates for the global number of recognized lichens range between 13,500 and 20,000 (Feuerer and Hawksworth, 2007). The fungal partner or mycobiont can be grown in axenic culture and show potential sources of novel metabolites however these cultures has been considered to be too difficult to maintain in axenic condition regarding to their slow growing characteristics. It is essential that appropriate growth conditions and preservation techniques are used to ensure stability of these microorganisms for research and applications. Many filamentous fungi can be kept viable by periodic transfer, however some properties may be lost or genetic variation may be occurred. Therefore conditions for storage should be investigated to minimize the risk of such changes. The work on preservation of mycobionts was never written up fully. Data from The American Type Culture Collection (ATCC) shows that it uses only subculturing method for maintaining mycobionts in stock culture, this method has disadvantages as labor intensive, time consuming, risk of contamination and physiological characteristics lost. The aims of this study were to select appropriate methods and reliable for preserve mycobionts in axenic condition for future research uses.

Methodology: Tropical mycobionts were isolated and cultivated using the method previously described by Sangvichien et al (2008). The representative of mycobionts from 6 families of tropical ascomycetes fungi were chosen for studies on long term survival. The cultures from well-grown healthy stock agar slopes were transferred to fresh Malt-Yeast Extract Agar (MYA) medium and following 8 weeks at room temperature (25-30 ^{o}C) incubation resulting in well developed colonies which were then used as inocula. The colonies were cut into small pieces about 0.3 x 0.3 cm with a surgical blade and placed in sterilized screw cap vials containing deionized water and half-strength Malt-Yeast Extract Broth (MYB). These were stored under refrigeration at 4^{o}C for up to 12 months, to test viability and at monthly intervals pieces were removed from the fridge and transferred to fresh MYA Petri dishes. Plates were incubated at room temperature and observed colony diameter for growth up to 6 weeks and if no growth was observed then the cultures were recorded as non-viable (Figure 1 &2).

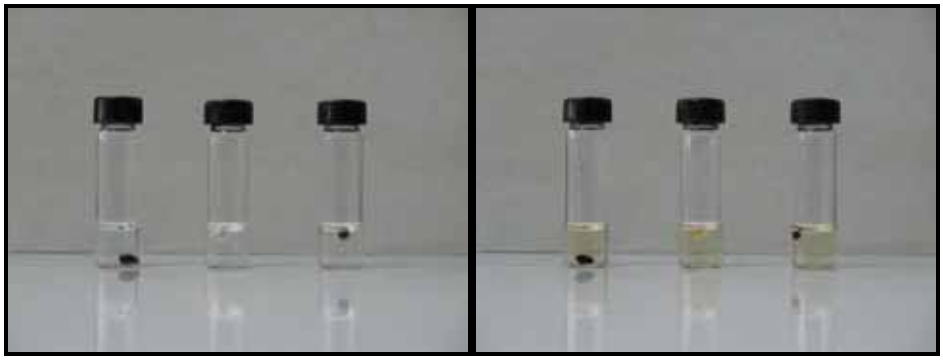

Figure 1 Mycobionts colonies in sterilized screw cap vials containing distilled water and half-strength Malt-Yeast Extract Broth (MYB).

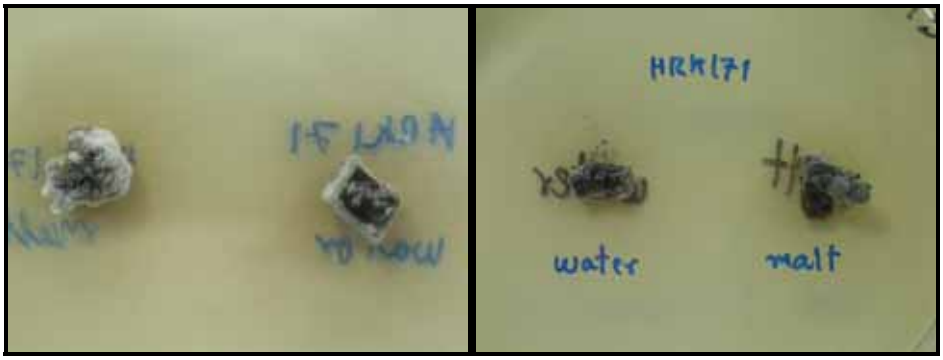

Figure 2 Formation of new colonies after 12 months storage on fresh media.

Result, Discussion and Conclusion:

From this study, the 13 representative of tropical mycobiont cultures in families Arthopyreniaceae, Graphidaceae, Pyrenulaceae, Thelotremataceae and Trypetheliaceae can be storage and survive for period of 12 months whilst only one represent from family Fusideaceae (*Maronea constance*) cannot survive longer than period of 3 months. Further study for preservation the rest of mycobionts in stock culture at Lichen Research Unit, Ramkhamhaeng University are on progress.

Table 1 Growth of selected mycobionts after period storage

Families	**Species**	Water			malt broth (half strength)		
		3 months	6 months	12 months	3 months	6 months	12 months
Arthopyreniaceae	*Arthopyrenia nieteriana* (HRK 171)	+	+	+	+	+	+
Fuscideaceae	*Maronea constance* (CM 126)	+	-	-	+	-	-
Graphidaceae	*Phaeographina chlorocarpoides* (KY 377)	+	+	+	+	-	+
	Phaeographis circumscripta (KY 473)	+	+	+	+	-	+
	Sarcographa labyrinthica (KY 240)	+	+	+	+	+	+
	Sarcographa actinobola (KY 440)	+	+	+	+	+	+
Pyrenulaceae	*Anthracothecium variolosum* (KY 208)	+	+	+	+	+	+
	Pyrenula sp. (KJB 17)	+	+	+	+	-	+
Thelotremataceae	*Ocellularia punctulata* (KY 408)	+	+	+	+	+	+
	Myriotrema muluense (HRK 141)	+	-	+	+	-	+
Trypetheliaceae	*Laurera benguelensis* (KY 534)	+	+	+	+	+	+
	Laurera meristospora (KY 472)	+	+	+	+	-	+
	Laurera keralensis (HRK 42)	+	+	+	+	+	+
	Trypethelium eluteriae (KY 64)	+	+	+	+	+	+

The technique that main culture collections (ATCC) uses for preservation of mycobionts in stock is only a subculturing method which has some disadvantages. Long term storage under liquid nitrogen was suggested by Honegger (1996) but there was no publishing for this protocols. Storage of fungi in the refrigerator at 4-7 ^{o}C slows down the rate of metabolism and increases the period between transfers to fresh media. The advantages of water storage are also including for economical cost of operation, less time consuming and prevent risk contamination from mite infestation which frequently occur during isolation of mycobiont from lichen samples.

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Key word: lichenzied fungi, mycobionts**,** preservation