國立屏東科技大學熱帶農業暨國際合作系
Department of Tropical Agriculture and International Cooperation
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博士學位論文
Ph.D. Dissertation

蛹蟲草菌絲體生長與子實體形成條件及萃取物抗氧化活性之研究

Studies on the Factors Affecting Growth of Mycelium and Fruiting Body Formation, and Antioxidant Activities of the Extracts of *Cordyceps militaris* L. ex St. Aman

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論文摘要內容：

蛹蟲草含有生物代謝產物，具有潛力作為中草藥。從遠古時代就有證據顯示蛹蟲草可用於活化人體的各種系統，除了早期廣泛應用於食補，在現代醫學蛹蟲草成分更是廣泛應用於各項研究中。目前相關研究利用蛹蟲草潛在的有效成分促進中草藥治療之功效，並能提升綠色生技革命發展，以建立安全、合理性之友善環境。

本研究目的在探討對蛹蟲草菌絲體、子實體與抗氧化物質產生的最佳培養條件，進行下列試驗：（1）探討不同培養條件（培養基、溫度、碳源、維生素源與穀物源）對蛹蟲草 *Cordyceps militaris* 兩個菌株 (AG-1、PSJ-1)菌絲生長和生產的影響；（2）探討不同液態培養方法（搖動和靜態培養）對菌絲體生產的影響；（3）探討不同菌絲體乾燥方法對生物量、胞外和胞內多醣生產的影響；（4）探討不同的液態培養方法（PVC 培養基）對子實體生長的影響；（5）探討不同溫度及濃度之蛹蟲粉對子實體生長的影響；（6）探討不同的液態培養方法（搖動，靜態）和菌絲體乾燥方法（烤箱乾燥和冷凍乾燥）對抗氧化物質性的影響。

結果顯示，在 MYPS 培養基和溫度 20-24 ºC 下，*C. militaris* 兩個菌株 AG-1 和 PSJ-1 的菌絲體生長最佳。添加葡萄糖濃度為 30 g/L 及維生素 B1 濃度為 0.03 g/L 可以促進菌絲生長。以黑糯米（越南產）作為培養基，可獲得兩種菌株的最佳產量。利用不同的液態培養基和不同培養方法（靜態，靜態+搖動，搖動）發現 MYPS、PVC 兩種是適合培養蛹蟲草的培養基，而靜態浸沒培養方法適合兩菌株 AG-1 和 PSJ-1 的菌絲生長。

以 *C.militaris* 兩個菌株的抗氧化能力來看，以 PVC 和 MYPS 培養基靜態培養，其萃取物對 1,1-二苯基的自由基清除率較高（DPPH）。採用靜態培養方法的液態培養基（PVC）降低 TPC，TFC，而 TPC 和 TFC 降低與 *C. militaris* 的抗氧化特性相關。PVC 浸沒式液態培養可以代替兩個菌株 AG-1 和 PSJ-1 某些培養成分，改善萃取物的抗氧化能力和活性 。在浸沒的液態培養基中培養的兩個菌株，其菌絲體以冷凍乾燥方法可提高 TFC 和抗氧化物含量。結果顯示，在所有浸沒液態培養處理條件下，*C. militaris* AG-1 和 PSJ-1 菌絲體都具有良好的抗氧化性能，尤其是 DPPH 自由基清除試驗和脂質過氧化作用。

初始培養基 pH 影響 *C. militaris* AG-1 和 PSJ-1 的生物量和多醣產生。在 24 ºC 以 PVC 培養基（pH 6.7）進行靜態培養 18 天後，具有最佳生物量（AG：12.92±0.3 g/L，PSJ-1：9.03±0.24 g/L）和細胞外與細胞內多醣（AG：209.70±1.56 mg/L，PSJ-1：198.16±0.85 mg/L；AG-1：32.62±0.87 mg/L，PSJ-1：30.63±1.96 mg/L）。持續搖動培養對於生物質和細胞外多醣的產生是最佳的，而在靜態條件下的培養對於細胞內多醣的產生是最佳的。測試不同的油脂添加對菌絲體生物量和多醣產生的影 響，結果顯示在菌絲生物量（AG-1：8.27±0.09 g/L，PSJ-1：8.01±0.0 g/L）的生產中添加椰子油 3.5％，胞外多醣（EPS）（AG-1：1208.00 ± 2.30 mg/L；PSJ-1：1110.40 ± 3.16 mg/L），胞內多糖(IPS)（AG-1：23.61 ± 1.31 mg/g，PSJ-1：20.39 ± 1.55 mg/g）迅速增加並達到最高水平。

本研究探討不同的液態培養方法、溫度、蛹蟲粉添加和光照條件，對 *C. militaris* (AG-1、PSJ-1)子實體生產之影響。結果顯示，子實體的菇原體萌發時間提前 (AG-1：5.80 ± 0.58 天; PSJ-1：6.20 ± 0.37 天），產量和生物性狀（長/寬（cm））存在明顯差異。在藍光條件下培養，AG-1 和 PSJ-1 獲得了最高產量（AG-1：14.35 ± 0.53 g/ 瓶；PSJ-1：12.54 ± 0.61 g/瓶）和

表現較佳之長與寬（cm）AG-1：5.04 ± 0.41，0.50 ± 0.03 cm；PSJ-1： 4.96 ± 0.36，0.44 ± 0.02 cm)。

關鍵字：抗氧化活性、黑糯米（越南產）、蛹蟲草、浸沒液態培養、維他命 B、蛹蟲粉、發光二極體

ABSTRACT

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Name of Student: Dang Ngoc Hung Advisors: Lay Horng Liang, Ph.D.
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The Content of Abstract in This Dissertation:

Cordyceps militaris is a potential harbor of bio-metabolites for herbal drugs and evidences are available about its applications for revitalization of various systems of the body from ancient times. Besides their popular applications for tonic medicine by the all stairs of the community, the constituents of *C. militaris* are now used extensively in modern systems of medicine. The current survey records the mysterious potentials of *C. militaris* are boosting up the present herbal treatments, as well as gearing up the green pharmacy revolution, in order to create a friendly environment with reasonable safety.

The objective of the study was evaluating the best culture conditions for mycelium, fruiting body, antioxidant substance production. The item were carried out as follows: (1) evaluate the effects of different factors (media, temperature, carbon sources, vitamins sources, grain sources) on the mycelium growth and production of *C. militaris* two strains; (2) evaluate the effect of different liquid culture method (shake and static culture) on the mycelial production; (3) evaluate the effects of different mycelium drying method on the biomass and extra and intra-cellular polysaccharides production; (4) evaluate the effect of submerged liquid culture (PVC media) with different methods on the fruiting body growth;

(5) evaluate the effect of temperature and pupa powder sources and concentration on fruiting body growth of *C. militaris*; (6) evaluate effect of different liquid culture methods (shake, static), and mycelium drying method (oven drying, and freeze drying) on antioxidant compound and activity of the *C. militaris*.

The results showed that the mycelium of *C. militaris* two strains AG-1 and PSJ-1 present the best growth with MYPS media and at 20-24 ^{o}C, Addition of glucose at 30 g/L, vitamin B1 at 0.03 g/L concentration could promote the mycelium growth. Black glutinous rice (Vietnam) was the best grain source to produce spawn of *C. militaris* two strains. Using different liquid culture media and different methods (static, static+shake, shake), MYPS, PVC were the suitable meida and static culture were suitable submerged method for mycelium growth of *C. militaris* two strains AG-1 and PSJ-1.

Regarding to antioxidant properties and contents of *C. militaris* two strains AG-1 and PSJ-1, the results showed that submerged liquid culture containing higher contents of PVC and MYPS media by static culture reached the higher values of Scavenging on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, Chelating on ferrous ions, Hydroxyl radical scavenging assay, Scavenging activity of $ABTS^{\cdot+}$ radical cation, Lipid peroxidation as well as higher value efficiency of total phenolic contents (TPC), total flavonoids content (TFC). Whereas, submerged liquid culture medium (PVC) with static culture method reduced TPC, TFC that directly linked to a decreased antioxidant properties of *C. militaris*. These results suggested that PVC submerged liquid culture can be used to replace some parts for *C. militaris* two strains AG-1 and PSJ-1 cultivation, which also improved antioxidant properties and antioxidant activity of *C. militaris*, extracts. With freeze, drying method of *C. militaris* two strains AG-1 and PSJ-1 cultivated in almost submerged liquid culture showed efficiency in improving the TFC as well as antioxidant contents in comparison with oven drying method. In general, *C. militaris* two strains AG-1 and PSJ-1 had good antioxidant properties, especially DPPH radical scavenging assay and Lipid peroxidation at all submerged liquid culture treating conditions.

Cultivation conditions (initial medium pH) affect biomass and polysaccharide production in *C. militaris* two strains AG-1 and PSJ-1. The static culture with PVC media (pH 6.7) at 24 ^{o}C, after 18 days obtained the best biomass (AG: 12.92±0.3, PSJ-1: 9.03±0.24 g/L) and extra- and intra-cellular polysaccharide (AG: 209.70±1.56, PSJ-1: 198.16±0.85 mg/L; AG-1: 32.62±0.87, PSJ-1: 30.63±1.96 mg/L, respectively). Submerged liquid culture constant aeration was optimal for biomass and extracellular polysaccharide production, whereas cultivation under static conditions was the best for intracellular polysaccharide production. In this research, different oils addition was studied on the production of mycelial biomass and polysaccharides of *C. militaris* strains AG-1 and PSJ-1. The results showed that with coconut oil 3.5 % addition in the production of mycelial biomass (AG-1: 8.27±0.09, PSJ-1: 8.01±0.0 g/L), extra-cellular polysaccharide (EPS) (AG-1: 1208.00±2.30; PSJ-1: 1110.40±3.16 mg/L), and the IPS (AG-1: 23.61±1.31, PSJ-1: 20.39±1.55 mg/g) increased rapidly and reached the maximum level.

Different the liquid culture methods, temperatures, pupa powder conditional, and light conditions were studied on the fruiting body of *C. militaris* two strains AG-1 and PSJ-1. The results indicated that the yield and biological properties (length/ width (cm)) of fruiting body exist distinct differences. The period of primordia appearance days of *C. militaris* two strains AG-1 and PSJ-1 had been shortened (AG-1: 5.80±0.58 days; PSJ-1: 6.20±0.37 days), yield (AG-1:14.35±0.53 g/bottle; PSJ-1: 12.54±0.61 g/bottle), and biological (length and width (cm)) AG-1: 5.04±0.41, 0.50±0.03 cm; PSJ-1: 4.96±0.36, 0.44±0.02 cm) were better under blue light conditions.

Keywords: Antioxidant activity, black glutinous rice Vietnam, *Cordyceps militaris*, submerged liquid culture, vitamin B, pupa powder, LED-light

LIST OF ABBREVIATION

$ABTS^{\cdot+}$	The 2,2 -azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
AG-1	*C. militaris* strain
BE	Biological efficiencies
BHA	Butylated hydroxyanisole
BHT	butylated hydroxytoluene
DETBA	1,3-diethyl-2-thiobarbituric acid
DPPH	1,1-diphenyl-2-picrylhydrazyl
EDTA	Ethylenediaminetetraacetic acid
EPS	Extra cellular polysaccharides
FD	Freeze drying
GAE	Gallic acid equivalents
HWE	Hot water extraction
IC_{50}	Half maximal inhibitory concentration
IPS	Intra cellular polysaccharides
LED	Light Emitting Diode
MYPS media	Maltose yeast extract peptone sucrose
OD	Oven drying
PDA media	Potatoes dextrose sucrose agar powder
PSJ-1	*C. militaris* strain
TPC	Total phenolic contents
TFC	Total flavonoid contents

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Dang Ngoc Hung

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