蛹蟲草之性別遺傳模式及利用單孢雜交育成高產量蟲草素品系之研究

The sexuality of Cordyceps militaris and
The sexuality of Cordyceps militaris and production of cordycepin by hybridization of monosporous strains

中文摘要

冬蟲夏草爲真菌中珍貴的藥用真菌，屬於子囊菌亞門，核菌綱，球殼菌目，麥角菌科，蟲草屬。於蟲草屬(Cordyceps)中又有 282 種。其中蛹蟲草(Cordyceps militaris)爲本屬之代表種，並於 1950 年中發現蟲草素(cordycepin)，並曾利用在抗腫瘤的治療。近年又將蟲草素利用於抗病毒的研究也發現有其價值。故本論文希望藉由育種的技術，找到具有高產量蟲草素的品系。爲找出含有高產量的品系，並了解蛹蟲草的交配型態，期能首先將子座上產生的有性孢子收集。使用 PDA (Potato Dextrose Agar)分別進行單一孢子培養，發現外觀菌絲的顏色可分爲三類,鮮黃色,淡黃色及白色。也觀察到其生長的情形似乎也與菌絲的顏色有關聯性，其中白色生長速度最慢，鮮黃色最快。從子座上取下的菌絲生長速度最快。經玻片培養並以 HCl-Giemsa stain 後觀察細胞核的型態,從染色的型態學上觀察並無法區分，觀察到均爲單一的細胞核。過去研究子囊菌均無法由交配反應判斷性別因子。本論文使用流式細胞儀，單染 Propidium Iodide (PI) 以及 Acridine Orange (AO)藉由 DNA 含量不同，來確定單倍體或是二倍體；再經由不同單孢的交配，收集交配的菌絲，使用流式細胞儀分析是否成爲二倍體，另外使用鏈鎖聚合?反應，經由放大 MAT 基因確定性別的控制因子爲 2 個基因，分別爲 MAT1-1-1 及 MAT1-2-1。因此證明了 C. militaris 爲 heterothallism 的性別遺傳模式。C. militaris 液態培養中蟲草素的含量，經層液相色層分析法(TLC)分析，結果顯示菌液中確實有蟲草素的成分。並發現在不同的天數產生蟲草素的產量有差異，進一步使用 High Performance Liquid Chromatography (HPLC)分析菌液中蟲草素的差異，顯示單性菌株蟲草素之產量高於交配後之兩性菌株，因此，在選種上可採用單子囊孢子菌株，進行篩選。

英文摘要

Many species in the genus of Cordyceps were used as traditional medicine for several hundred years. Among them, Cordyceps militaris was recently developed as functional food in Taiwan. C. militaris was characterizes with the production of cordycepin that was found to be functional in antitumor and antivirus. The purpose of the present study is to breed a high cordycepin production strain through mating

of mono-ascosporoes cultures and the mating types of C. militaris were detected in the same time. Ascospores discharged from cultivated stroma were also isolated individually from the surface of agar plate and grown in PDA slants at 25℃. The pigmentation of the monosporous strains can be separated into three types as white, light-yellow and deep-yellow. The growth rate were also different among these types, that indicated the meiotic segregation of the strains. HCl-Giemsa stain revealed to the hypha of slide culture revealed that all the strains before and after a all possible mating were single nucleated in hypha and phialoconidia. Flow cytometric analysis on the conidia suspension stained by acridine orange indicated that the mating occurred for some mating and the intensity of DNA demonstrated in two form which were suggested to be diploid and haploid strains. To confirm the mating type of C. militaris, primer of MAT1-1-1 and MAT1-2-1 were design for PCR. TLC and HPLC were employed to analysis the production of cordycepin of the strains before and after mating of the monoascosporous strains. However, all the mated strain produced less cordycepin in the liquid culture. This result indicated that high cordycepin production strain could be screened from monosporous isolation.