利用核醣體 **DNA** 的朮轉錄區鑑定蟲草屬真菌

Identification of fungi in the genus Cordyceps by ribosomal DNA internal transcribed spacers (ITS)

中文摘要

近年來國內由於藥膳與食療風氣漸開，珍貴之中藥－冬蟲夏草（Cordyceps sinensis）其市場需求亦逐漸增大，目前冬蟲夏草之新種仍陸續被發現，其中已有記載者達 282 種 ；基本上蟲草屬（Cordyceps）之鑑定乃是依菌種之形態來分類的，然而菌種在一般培養基時不易產生子座等可作爲鑑定依據的特徵，且亦常有健康食品以乾燥的醱酵菌絲經研磨加工後之型式販售，因此鑑定此些菌種時會受到許多限制。因而本研究基於上述之原因，期能以 rDNA 之片段進行蟲草屬中各種（Species）之鑑定。 於本研究中已成功地建立了由蟲草屬真菌抽得高純度、高產量 Genomic DNA 的方法，且可順利地利用聚合脢連鎖反應法及兩套普遍性引子（ITS1 & ITS2、ITS3 & ITS4） 增幅得到 C. memorabilis CCRC（Culture Collection and Research Center）32218、C. militaris CCRC 32219、C. ophioglossoides CCRC 32220、C. sinensis CCRC 36421、C. sp.CCRC 32221、C. sinensis（西藏野外採集）、C. nutans（日月潭野外採集）、C. myrmecophila（日月潭野外採集）、傳統中藥店之冬蟲夏草藥材、Phytocordyceps ninchukispora CCRC 31900、Claviceps purpurea CCRC 31775、Ganoderma lucidum CCRC 36124、 Ganoderma pfeifferi CCRC 36159 等菌種的兩段以核醣體 DNA 之內轉錄區（Internal Tran scribed Spacer，簡稱 ITS）爲主的 DNA 片段（ITSI & ITSII），之後我們從所得到的 DNA 片段之大小來進行分析，發現到各菌種間的差異並不具有分類學上的意義，故以聚合脢連鎖反應產物之大小來作爲分類標準的方式並不可行；接著乃是將此些菌種的 ITSI 及 ITSII 之核酸序列互相比對，首先與發表於 GenBank 的 G. lucidum 及 G. pfeifferi 之 ITSI 及 ITSII 的核酸序列比對後，證實了經由本研究所建立的系統得到的菌種之核酸序列的可信賴度，且發現到屬於 C. sinensis 的同種菌株間其 ITSI 及 ITS 片段之核酸序列的相似性高達 99.5 %、99.7 %；且同時根據種源關係樹與自西藏 4000 公尺以上的草原中採集所得的 C. sinensis 比對後，我們發現食品工業發展研究所之菌種保存及研究中心的 C. sinensis 有可能是與 C. capitata 於種源關係上較近的菌種，而非其所聲稱的 C.s sinensis；同時我們亦利用此種方法發現到，自野外採集所得的 Wild specimen-3 可能即是 C. militaris 的無性世代，而我們也成功地將此套系統實際應用於了解如市售商品中所加入的菌絲成份其品種及真僞等方面。

英文摘要

Fresh specimens of Cordyceps sinensis collected from Tibet (Wild specimen-4) were compared with the special nuclear ribosomal DNA (rDNA) fragments to t

hat of other fungi including C. memorabilis CCRC (Culture Collection and Rese
arch Center) 32218、C. militaris CCRC 32219、C. ophioglossoides CCRC 32220、C
. sinensis CCRC 36421 、C. sp. CCRC 32221 、C. nutans (collected in the field;
Wild specimen-2)、C. myrmecophila (collected in the field ; Wild specimen -1
)、dried specimen of C. sinensis from Chine herb store (Dongchongxiachao-1 、
Dongchongxiachao-2)、Phytocordyceps ninchukispora CCRC 31900、Claviceps
purpu
rea CCRC 31775、Ganoderma lucidum CCRC 36124、Ganoderma pfeifferi CCRC
36159..
....etc. The method of DNA extraction from cultured mycelia or dried specimens
of thefungi was firstly studied to establish a stable procedure for high quan
tity and purity of genomic DNA. It was found that a modified rapidpreparation
method was the most efficient one. Then , the ITSI and ITSII DNAregions were f
urther amplified by polymerase chain reaction (PCR) with twopairs of fungal
universal primers (ITS1 and ITS2 , ITS3 and ITS4). Sequence analysis of the
amplified DNA were followed and the data from G.lucidum (CCRC 36124) and G.
pfeifferi (CCRC 36159) were confirmed as ITSIand ITSII of them in 99.5 - 100
% match by the published data of GenBank. Thepercentages of sequence similari
ty analyzed by DNASTAR revealed that ITSIand ITSII of C. sinensis derived from
fresh specimens and the dried specimensfrom chinese herb store were 99.5 % an
d 99.7 % , respectively. In addition , the results also indicated that the C.
sinensis (CCRC 36421) could be morerelated to C. capitata (ITSI→81.5 % , I
TSII→95.8 %) rather than C. sinensis(Wild specimen-4) (ITSI→71 % , ITSII
→81.5 %) and the specimen (Wild specimen-3) could be the anamorph of C. mi
litaris. According to the similarity of the ITSI and ITSII sequence , a prelim
inary phylogenetic tree was proposed to explain the possible distance of evolu
tion in the genus of Cordyceps when the key of Kobayasi (1982) was compared.
The methods and the results of present study can be useful in determining the
Cordyceps species in anamorphic state or the commercial Cordyceps product in
pulverized mycelial form , since two commercial products (CP-1 , CP-2) were
found to be C. sinensis (CCRC 36421) and C. militaris (CCRC 32219) , respe
ctively.