

# 綠豆簧真菌 *Scopulariopsis flava* No. MZK-P01 菌株所生產新生理活性代謝物之探討

## New bioactive metabolites produced by a fungal strain of *Scopulariopsis flava* No. MZK-P01 from *Pracparatum mungo* origin

### 中文摘要

綠豆簧 (*Pracparatum mungo*) 為中國傳統民間藥方之一，因其具有清熱、解毒及消炎等功效，被認為是一種解毒聖藥。本實驗室過去曾從綠豆簧中分離出兩株真菌菌株 MZK-P01 和 MZK-P02，在以米糠為主要發酵營養來源時，可得到多個抗氧化活性代謝產物。經菌種形態觀察及發酵培養特徵分析結果，亦已鑑定出兩菌株皆為 *Scopulariopsis* 菌屬。本研究即是承接上述實驗成果，擬進一步進行此綠豆簧內生菌株之種源鑑定，並且藉由抗氧化及抗菌生理活性之初步篩檢平台，進行菌株活性代謝物篩檢及探索，接著進行其大量發酵生產及代謝物之分離純化，以期達到活性分子之結構分析鑑定及其生理效能綜合評估之目的。

本論文之內容可分為四個部份：

第一部分為菌種鑑定。進行菌株培養成長速率觀察，並以微生物外觀，配合光學、解剖和電子顯微鏡觀測菌株分生孢子、菌絲和分生孢子梗之結果，在與文獻比較下，判定菌株皆可被歸屬於 *Scopulariopsis flava* 菌種。菌種 rDNA 序列分析實驗的結果更可確立 MZK-P01 為 *Scopulariopsis* 菌屬。

第二部份以抗菌作用和清除 DPPH 自由基能力作為活性代謝物篩檢追索的平台。以 Czapek-Dox broth (CDB)、Sabouraud dextrose broth (SDB) 和 potato dextrose broth (PDB) 作為菌種發酵培養基的基礎，分成綠豆粉添加和未添加兩部分進行活性代謝物表現之評比。實驗結果可得以 CDB+ 3% 的綠豆粉作為發酵培養基，經往復式搖震連續發酵 7 天可產生抗 *Staphylococcus aureus* 最佳效果之活性代謝物。其後即以此模式進行生理活性物質之大量發酵生產。

第三部份以抗菌活性導向之酸鹼轉溶分層萃取，進行發酵液處理及活性代謝部分之回收。藉由矽膠正相薄層色層層析 (TLC) 和 RP-18 逆相高效能液相層析 (HPLC) 進行物質分離分析的結果發現，當矽膠 TLC 展開液為 benzene : ethylacetate = 50 : 50 時，藉由 UV 光 OD254nm 照射下，在 Rf 值 0.5 處可觀測一特定活性代謝物，且可由 2,6-dichlorophenol-indo-phenol (DPIP) 進行物質呈色。其次以 RP-18 管柱作為固定相配合移動相 100% methanol 分析的結果，可於 OD254nm 檢測波長下，在滯留時間 3 分鐘處觀測到單一物質吸收峰。此一特定活性代謝物最後經 n-hexane 溫熱再結晶處理，可得到無色針狀結晶之純化物，暫命名為 MZK-P01-A。

第四部份針對純化物 MZK-P01-A 進行 UV 光譜和 IR 光譜分析。結果可見，其 UV 光譜吸收峰於 287nm 附近有最大吸光值，而在 IR 光譜可見此物質分別在 3441 cm<sup>-1</sup> (broad, m, -OH)、1699 cm<sup>-1</sup> (sharp, s, C=O) 及 1406 cm<sup>-1</sup> (broad, m, C-O-H) 處各有一明顯吸收峰，而在 3032 cm<sup>-1</sup> (sharp, vw, aromatic C=C)，以及 1498、1450 cm<sup>-1</sup> (sharp, w, aromatic C-H) 可觀測到微弱但明顯吸收峰。綜上分析結果，再結合物質分層萃取轉溶之特性可推論，此一純化物應為具羧基(carboxylic acid)及含苯環(aromatic ring)之酸性化合物。從上述光譜資料可知，此真菌代謝物為新分子結構之天然物。

## 英文摘要

Pracparatum mungo is one of the Chinese traditional herbs. Since it shows the functions of heat-cleaning, detoxification and anti-inflammatory effects, it was popularly recognized as the saint medicine for detoxification. Our laboratory has previously isolated two fungal strains from Pracparatum mungo and designated the strains as MZK-P01 and MZK-P02, respectively. The fermentation products of either MZK-P01 or MZK-P02 in combination of germ brown rice exhibited a series of antioxidant activity. After the observation of their morphological and cultural characteristics, they were identified to belong to the genus of *Scopulariopsis* sp. Based on the above-mentioned background, the present study was setting at the discovery of their biological active metabolites as well as the strain identification. MZK-P01, a more promising one, was selected in this study for its superior biological ability

In summary, our study has achieved the experimental results and could be divided into four parts as follows:

Part 1 deals with the strain identification of MZK-P01. By the observation of morphological characteristics to the strain through light microscope, dissecting light microscope and scan electronic microscope, the hyphae, conidiophores, and conidia were clearly observed. The results led us to confirm the strain to belong to the species of *Scopulariopsis flava*. The evidence of the PCR product of strain MZK-P01 rDNA including its partial sequence also pointed out that the strain to be a species of *Scopulariopsis* sp.

Part 2 describes the bioactive activity to the target metabolites. The fermentation of MZK-P01 strain was carried out with a liquid culture of reciprocating shaking by using Czapek-Dox broth, Sabouraud dextrose broth, and potato dextrose broth as the cultivation source. When the fermentation condition was set at Czapek-Dox broth combined with 3% mung bean powder, the antimicrobial activity against *Staphylococcus aureus* could reach to the highest.

Part 3 discusses the isolation and purification procedure of the active metabolites.

Based on the acid-base conversion, an activity-guided fractionation was carried out to the fermentation filtrate. The acidic fraction containing the bioactive products was obtained accordingly. TLC and HPLC were successfully used as the main tools to isolate one of the target bioactive substances in a form of pure crystal needles. The substance was thus designated as MZK-P01-A.

Part 4 explains the analytical results of the spectroscopic data to the bioactive product. The UV spectrum showed a maximal absorption at 287 nm. The IR spectrum showed characteristic at 3441(broad, m), 1699(sharp, s), 1406(broad, m) and 1450 (sharp, w)  $\text{cm}^{-1}$  respectively. These results could be explained that the compound MZK-P01-A possesses an acidic C=O group and an aromatic group in its molecular structure. The spectroscopic data of the compound indicated that it is a novel natural product with the new molecular structure differing from the fungal metabolites being reported.

Keyword: *Pracparatum mung*, mung bean, antimicrobial activity, antioxidant activity, liquid fermentation, *Scopulariopsis flava*.