

綠豆簧真菌菌株 No. MZK-P01 所生產抗氧化代謝物之研究

Study on the antioxidant metabolites produced by a fungal strain No. MZK-P01 from *Pracparatum mungo*

中文摘要

自古以來，綠豆簧即被稱為是一種“解毒聖藥”。其主要的功能為保肝解毒、減少疲勞感、退火降火氣、養顏美容等。綠豆簧 (*Pracparatum mungo*) 是由豆科 (Leguminosae) 植物屬的綠豆 (*Phaseolus mungo* L.)，經過自然發酵及繁複的中藥炮炙過程所煉製成的一種天然保健食品。

由於綠豆簧傳統的煉製過程耗費時日且步驟過於繁複，因此本研究即著眼於利用綠豆簧本身內生的菌株，結合現代發酵科學技術，選擇綠豆及與其保健功能類似或相當的天然食材來進行抗氧化活性代謝物的生產評估。同時更針對從綠豆簧所分離出具抗氧化活性代謝物生產能力的菌株 (分別編號 MZK-P01 及 MZK-P02)，進行菌種分類鑑定。

本研究所進行的抗氧化活性檢測項目包括: (1) 清除 DPPH 自由基能力之測定 (Scavenging of DPPH); (2) 還原力之測定 (Reducing power); (3) 脂質過氧化抑制能力 (Inhibition of lipid peroxidation) 之測定，此項目包括有: 硫丙二醯尿系統 (Thiobarbituric acid system) 與硫氰酸鐵法 (Ferric thiocyanate method) 兩項測定。

本研究內容共分四個部分，分別描述於下：

第一部分，以菌株 MZK-P01 或 MZK-P02 分別與綠豆、薏仁、黃豆、米糠、發芽糙米等五種食材進行液態發酵培養，以比較出能表現最佳抗氧化活性的培養組合。結果顯示，菌株 MZK-P01 與米糠的液態發酵組合所表現的抗氧化力較為顯著。此外亦探討以固態發酵取代液態發酵進行 MZK-P01 菌株與米糠的組合效果。結果顯示，固態發酵組合僅比液態發酵組合具稍佳之抗氧化力，然固態發酵因發酵培養及回收步驟均較為繁複，因此實驗中仍以液態發酵組合方式進行。

第二部分，以菌株 MZK-P01 與米糠的液態發酵組合為基礎進行抗氧化代謝物的量化生產。比較各酸鹼轉溶分層的抗氧化力之結果顯示，中性分層具有較佳的抗氧化活力。將中性分層經脫水減壓濃縮後，以矽膠薄層色層層析 (Thin layer chromatography; TLC) 方法，進行 DPPH 抗氧化呈色反應之測試；結果由液態發酵中性分層中，可分離出三個抗氧化活性物質，分別命名為 MZK-P01-A (以下簡稱 P01-A)、MZK-P01-B (以下簡稱 P01-B)、以及 MZK-P01-C (以下簡稱 P01-C)；而固態發酵中性分層中亦可分離出一個抗氧化活性物質，命名為 MZK-P01-D (以下簡稱 P01-D)。針對此四個物質進行抗氧化力檢測比較的結果顯示，依其抗氧化力的高低順序為: P01-C @ P01-D > P01-B > P01-A。

第三部分，針對上述四個抗氧化活性物質進行 UV-VIS 與 IR 光譜的檢測分析。結果顯示，P01-A 和 P01-B 的 UV 光譜吸收波峰均落在 $\lambda = 204, 206$ 及 320 nm

附近; 而且二者的 IR 光譜, 皆分別在 1389 cm⁻¹ 與 1366 cm⁻¹, 1458 cm⁻¹, 1231cm⁻¹, 及 728 cm⁻¹ 有顯著的吸收波峰, 因此推測 P01-A 和 P01-B 皆可能具有-OH 基, C=C 雙鍵的共軛 (Conjugated) 結構, C-O-C 的官能基, 以及具有一個置換取代或是對位 (Para-) 取代的苯環結構。而 P01-C 和 P01-D 的 UV 光譜, 則在 λ=205、207 及 259 nm 附近被觀測到有吸收波峰; 此外, P01-C 和 P01-D 的 IR 光譜, 皆在 3355cm⁻¹ 有吸收譜峰, 推測皆可能擁有-NH₂ 或=NH 基。由上述結果可推斷 P01-A 和 P01-B 的結構可能很類似而 P01-C 和 P01-D 的結構亦可能很類似, 惟此一部分仍待進一步確認。第四部分, 針對菌株 MZK-P01 與 MZK-P02 進行菌種分類鑑定。由解剖光學顯微鏡及掃描式電子顯微鏡的觀測結果可知, 兩菌株無論是在菌絲(Hyphae)、分生孢子柄 (Conidiophores)、以及分生孢子 (Conidia) 等均具有極為相似的形態特徵, 由此更可進一步推斷, 兩菌株皆屬於 Scopulariopsis 菌屬, 但為同屬不同種。

英文摘要

From time immemorial, Pracparatum mungo is called namely a kind of "The saint medicine to counteract poison". Its main function was for protect the liver to counteract poison、reduce the tired feeling、back the fire and to lower internal heat、keep and improve looks...etc.. Pracparatum mungo is the mung bean (Phaseolus mungo L.) belongs to the Leguminosae that was through natural fermentation and the complicated Chinese herbal medical dries by heat process to become the refining of a kind of natural health care food.

Because the traditional refining process of the Pracparatum mungo wastes lots of time and the steps were too complicated, so this research fixes attention on in make use of strains inside the Pracparatum mungo to combine with the modern fermentation science technique, and chooses the mung bean and the similar or rather natural food materials to carry on the production valuation of the antioxidative activity metabolites. Meanwhile even aims at the strains (the serial number was MZK-P01 and MZK-P02 respectively) were capable of producing the antioxidative activity metabolites that were isolated from the Pracparatum mungo to carry on the identification of bacterial species.

This research carries on the antioxidative activity examination items includes: (1) The measurement of the scavenging of DPPH free radicals ability; (2) The measurement of the reducing power; (3) The measurement of the Inhibition of lipid peroxidation, and this item includes: two measurements of thiobarbituric acid system and ferric thiocyanate method.

This research content is divided into four parts which was describing respectively in descend:

Part I, using the MZK-P01 or MZK-P02 strain to carry on liquid fermentation culture with five kinds of food materials such as mung bean · adlay · soybean · rice bran and germ brown rice respectively, and comparing with these culture combinations to choose the one which can express the best antioxidative activity. The result shows, the liquid fermentation that combines with the MZK-P01 strain and rice bran can express more obvious antioxidative ability. Besides, inquiring into the combination effect which was using the MZK-P01 strain and rice bran to carry on solid fermentation replaces liquid fermentation. The result shows, the combination of solid fermentation has slightly more antioxidative ability than the combination of liquid fermentation, but its step of fermentation and extraction were all more complicated. Therefore the experiment still carries on with the combination of liquid fermentation.

Part II, taking the combination of liquid fermentation with the MZK-P01 strain and rice bran as the foundation to turn the production in quantity. The result of the comparison between the antioxidative ability of each acid-base conversion layer shows the neutral layer has better antioxidative ability. After dehydrating · decompressing and condensing the neutral layer, then to carry on the DPPH antioxidative test of presenting the color reaction with the thin layer chromatography method; the result shows three antioxidative substances can be separated from the neutral layer of liquid ferments that were named the MZK-P01-A (call the P01-A as follows) · MZK-P01-B (call the P01-B as follows) and MZK-P01-C (call the P01-C as follows) respectively; and one antioxidative substance also can be separated from the neutral layer of the solid ferments that was named the MZK-P01-D (call the P01-D as follows). Aiming at the result of comparison between the antioxidative ability of these four substances shows the high and low sequence that depends on their antioxidative power one by one in order is: P01-C=P01-D > P01-B >P01-A.

Part III, to aim at the four antioxidative activity substances to carry on the examination analysis of the UV-VIS and the IR spectrum. As a result shows, the absorption wave peaks of the UV spectrum about the MZK-P01-A and the MZK-P01-B both were fall in the $\lambda=204, 206$ and 320 nm neighborhood; and the IR spectrum about the P01-A and the P01-B both have the obvious absorption wave peaks in 1389 cm^{-1} and 1366 cm^{-1} , 1458 cm^{-1} , 1231 cm^{-1} , and 728 cm^{-1} respectively, therefore predicting both of the P01-A and P01-B may have the —OH group, the conjugated structure of C=C double bond, the functional group of C-O-C, and the benzene ring structure which has one substituent or para- substituent. The UV spectrum about the P01-C and the P01-D near in the $\lambda=205, 207$ and 259 nm both were observed which have the absorption wave peaks; besides, the IR

spectrum about the P01-C and the P01-D both have the absorption wave peaks in 3355cm^{-1} , therefore predicting both of them may have the $-\text{NH}_2$ or $=\text{NH}$ group. From these results can predict the structure of P01-A and P01-B may be similar, and the structure of P01-C and P01-D may be similar, too. However this part still needs the further confirmation.

Part IV, to aim at the identification of bacterial species about the MZK-P01 and MZK-P02 strain. The result shows the two strains no matter at hyphae, the conidiophores, and conidia...etc. were all has the extremely alike appearance characteristic from the observations of the dissecting light microscope and the scan electronic microscope which can further predict both of the two strains belong to the *Scopulariopsis* sp., but were the different species of the same genus.