納豆菌與發芽糙米發酵組合物抗人類結腸癌細胞作用之探討 Effects of Combinatory Products Fermented by Bacillus natto with Germinated Brown Rice against Human Colon Cancer Cells

中文摘要

米是人類重要糧食之一，全世界一半以上的人口以米爲主食。近年來發現發芽糙米的營養價值更高於加工過之白米；而穀類經過發芽之後本身的營養價值又會大幅提高。納豆菌在發酵代謝過程中，會有活性物質生成，其中已有許多活性發酵代謝物被確認對人體具有保健功能。本實驗利用上述納豆菌和發芽糙米之發酵組合，針對抗菌抗氧化活性以及抗癌進行檢測。從先前的結果得知，本實驗室所分離的納豆菌 SYH-MT0379 與發芽糙米之發酵組合產物，在抗菌及清除自由基活性測試中之表現，皆優於同菌株與白米之發酵組合產物。針對納豆菌與發芽糙米發酵組合產物中之活性成分，進行乙酸乙酯萃取分層回收，再經矽膠薄層色層層析法純化製備以及高效能液相層析儀純度鑑定，可在薄層色層層析展開阻滯值 (retention factor; Rf) 爲 0.5 處，觀測到於紫外及可視光下皆可呈現吸收之活性單一物質，此物質經純化後被命名爲 PGBRNFL-I。PGBRNFL-I 可呈現廣效圖譜之抗細菌和抗真菌活性，亦可表現顯著清除自由基之能力。

由於 PGBRNFL-I 在抗菌實驗中被觀測到其抗菌活性會因含硫氫鍵化合物之拮抗而失效，硫氫鍵化合物遍存於細胞中，以維持細胞氧化還原之恆定，當此類分子的雙硫鍵及硫氫鍵受到攻擊時，細胞中的氧化壓力會干擾細胞 DNA 複製，間接造成了細胞毒性。針對此一發現，我們選擇了目前正逐年攀升，且已分別成爲台灣男女癌症死亡率第三位的結腸癌做爲抗癌篩選的研究對象，本實驗所選擇的癌細胞株爲人類結腸顏細胞株 HT29。從 MTT 試驗結果發現，PGBRNFL-I 對 HT29 具有相當程度之毒殺活性，且呈現明確之 dose-dependant 效果。對於纖維母細胞 L929，PGBRNFL-I 則不具有細胞毒性。此外，針對與腸癌細胞的轉移及惡化有關的 COX-2 蛋白活化指標，以西方轉漬法（Western blot）進行此酵素的抑制活性測試發現， PGBRNFL-I 確實能抑制 COX-2 的表現，且抑制調控 COX-2 基因的轉錄因子 NF-?耩的活化。由此可推論，納豆菌與發芽糙米發酵組合產物在日常飲食生活中對人體防癌保健及疫病之預防應有所助益。

英文摘要

Rice is one of the most important grains, and more than one half of populations in the world consume rice as the staple food. Recently, people found that nutritional value is higher in unprocessed brown rice than in processed white rice. After germinating, the nutritional value of rice will be largely raised. Thus in this experiment, fermentation of Bacillus natto SYH-MT 0379 was preformed by using germinated brown rice as a main functional substrate for biotransformation. After a

series of pretreatment steps were carried out to the fermentation broth, the fractionated portion of acidic ethyl acetate was detected to possess the active compounds with antimicrobial and antioxidant activities. Using TLC analysis followed by Diaion HP-20 and silica gel TLC separation, the active compound was purified at a mg level and thus named PGBRNFL-I. PGBRNFL-I exhibited a characteristic of fluorescence spot at UV 365 nm when analyzed by TLC plate. PGBRNFL-I possessed antimicrobial activities against all Gram positive and negative bacteria and also exhibited a significant scavenging effect on DPPH free radicals. Based on the fact that the antimicrobial activity of PGBRNFL-I was to abolish some compounds containing sulfhydryl (-SH), eg. reduced form of glutathione, which are ubiquitous existing in the cells and will accelerate the function of homeostasis balance to them. When disulfide bond or -SH group related to the intracellular macromolecules is attacked, DNA replication can be interfered which may cause cytotoxicity to some unlimited proliferate cells indirectly. Besides, colon cancer records a significant proportion of cancer deaths in Taiwan and is the third mortality. As a result, we chose human HT29 colon cancer cell line as our main target in representing the importance of colon cancer. The results of MTT together with the cytometric measurement of the intracellular ROS content to HT29 colon cancer cell line, indicated that PGBRNFL-I decreased cell growth in a dose-dependent manner via the involvement of ROS production which may induce cell death. As to fibroblast L929 cell, the MTT results indicated PGBRNFL-I had no cytotoxicity on it. The result of Western blot analysis showed that PGBRNFL-I suppressed the production of PMA-mediated COX-2 protein and also inhibited the expression level of nuclear factor-kappa B (NF-κB), all of which involved in the inflammatory disease. Altogether, this study presented a strongly support that the combinatory fermentation product of Bacillus natto and germinated brown rice is a promising source of functional food ingredient in application of cancer chemoprevention. Rice is one of the most important grains, and more than one half of populations in the world consume rice as the staple food. Recently, people found that nutritional value is higher in unprocessed brown rice than in processed white rice. After germinating, the nutritional value of rice will be largely raised. Thus in this experiment, fermentation of Bacillus natto SYH-MT 0379 was preformed by using germinated brown rice as a main functional substrate for biotransformation. After a series of pretreatment steps were carried out to the fermentation broth, the fractionated portion of acidic ethyl acetate was detected to possess the active compounds with antimicrobial and antioxidant activities. Using TLC analysis followed by Diaion HP-20 and silica gel TLC separation, the active compound was purified at a mg level and thus named PGBRNFL-I. PGBRNFL-I exhibited a characteristic of fluorescence spot at UV 365 nm when analyzed by TLC plate. PGBRNFL-I possessed antimicrobial activities against all Gram positive and negative bacteria and also exhibited a significant scavenging effect on DPPH free radicals.

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