納豆菌 Bacillus natto SYH-MT 0379 固態發酵所得豆科植物性異黃酮轉換生成物之生產性及抗氧化生物活性評估

Production and Antioxidant Evaluation of Leguminosae Isoflavonoids Derived from Microbial Conversion Products by Bacillus natto SYH-MT 0379 Solid-state Fermentation

中文摘要

「納豆」乃黃豆經由納豆菌（Bacillus natto）發酵而得之食品，富含多元性酵素及生理活性物質。本實驗利用枯草桿菌（Bacillus subtilis）的類緣菌--納豆菌（Bacillus natto）SYH-MT 0379，以盤式培養的方式進行納豆菌固態發酵。培養基之設計是結合豆科植物「黃豆」及生藥「葛根」，並且配合不同溫度之加熱前處理步驟而成。本研究之目的在於探討改良式固態發酵培養基之製程，對於納豆菌發酵生成之黏性物質，評估其 1)生產性功能，及 2)內含源自豆科植物性異黃酮經微生物轉換（Microbial conversion）生成物之抗氧化活性。

回收的黏性物質之「水可溶性部分」，經由納豆菌存活率、血栓纖維塊溶解能力（納豆激酶產量評估）及游離胺基酸總量，三項生產性功能分析結果證實，改良式固態發酵製程確實有助於提高水可溶性蛋白質含量與固態發酵物產量。然而「丙酮可萃取之濃縮液」，利用 HPLC 進行指標性異黃酮成分與含量追蹤，以 Diaion HP-20 樹脂吸附、Sephadex LH-20 膠體管柱和 TLC 分離，最終取得提供抗氧化活性機制評估之萃取分離物，命名爲 MT0379，經初步鑑定其內含有 Puerarin, Daidzein 和 Genistein 三種異黃酮成分。

由 Fenton 反應的實驗結果發現，MT 0379 具有清除過氧化氫、還原三價鐵但沒有螯合亞鐵的能力。對於清除 DPPH 自由基及超氧陰離子自由基亦有顯著效果。在進行脂質氧化反應，分別由硫丙二醯尿系統以及硫氰酸鐵法實驗得知，MT 0379 可抑制脂質最終氧化產物-丙二醛（Malondialdehyde, MDA）和氧化初期之氫過氧化物（Hydroperoxide）生成。

經 MTT 測試的結果顯示，MT 0379 對於非癌化細胞株，RAW 264.7 以及 293 細胞，具有 Dose- dependent 趨勢之細胞生長抑制作用，其細胞毒性 IC50 爲 100 μg/ml。以過氧化氫（H2O2）作爲模擬細胞遭受氧化壓力之模式，利用流式細胞儀偵測 RAW 264.7 細胞內 DCFH-DA 的氧化性產物之螢光表現，根據量化分析的結果證實，MT 0379 具有顯著性地清除 H2O2 之能力。在抑制 NF-kB 活化之篩選檢測中，由流式細胞儀偵測轉染 IkB-EGFP 螢光蛋白質體之 293 細胞及其胞內之 IkB-EGFP 螢光蛋白質的表現得知，MT 0379 具有促進 I-kB 磷酸化的可能。

英文摘要

Natto is a fermented food made of soybeans and is rich in digestive enzymes and biologically functional ingredients. In this study, we chose natto as our research subject, investigating Bacillus natto, its bioconversion ability on Leguminosae and isoflavonoid metabolites in antioxidant performance. A modified fermentation process of natto was designed by adding Pueraria radix and preheating the soybean-based culture medium. Our objective was to evaluate the productivity and antioxidant activity of the resulting bioactive metabolites, which was derived from a strain of Bacillus natto No. SYH-MT 0379 by solid-state fermentation.

First of all, we analyzed the fermentation harvest as well as its water-soluble extract by evaluation of their fibrinolytic effect (in term of nattokinase productivity) and total amount of free amino acids. The results suggested that the fermentation beer of Bacillus natto SYH-MT 0379 under the processes of cultural medium preheating, Pueraria radix addition, and solid-state fermentation, would significantly enhance the total fermentation yield and water-soluble hydrolysates. The 80% acetone extract (namely S-MT) was further subjected to purify its antioxidant components by a series of liquid chromatography column isolation (namely MT 0379) including Diaion HP-20, Sephadex LH-20 and TLC separation, and examine its antioxidant mechanisms and functions. Based on the results of HPLC analysis, six components containing in MT 0379 were further clarified to be a group of isoflavonoids.

In Fenton reaction, we examined the variation of ferric, ferrous iron and hydrogen peroxide in conducting the antioxidant mechanism of MT 0379. As to the free radical-scavenging properties of MT 0379, we evaluated the scavenging abilities of some typical ROS molecules by method of conventional colorimetry. In the results, we found

that MT 0379 had a catalase-like activity in reducing H_2O_2, however, no ferric iron chelating ability was observed in this experiment. In addition, MT 0379 also exhibited not only the reducing power but DPPH radical and superoxide anion scavenging activities.

According to the result of MTT assay, MT 0379 showed a relatively low cytotoxicity (IC_{50}=100μg/ml) toward RAW 264.7 macrophages and human kidney 293 cell lines in a dose-dependent manner.

In the cell level assay, we confirmed that MT 0379 has significant protective ability to RAW 264.7 macrophages under H_2O_2-induced oxidative stress. In the evaluation of the degree of lipid peroxidation, linoleic acid was chosen as the target for oxidation. The results showed that MT 0379 has the inhibition effects of lipid peroxidation by way of blocking the formation of TBARS, and also scavenging the hydroperoxides in an AAPH-induced system.

Evaluation of inhibitory activity of MT 0379 in NF-kB activation based on the mechanism of anti-I kB phosphorylation, we used pIkB-EGFP transfected 293 cells as the model to determine the level of fluorescence intensity by flow cytometry. According to the result of quantification analysis, MT 0379 may enhance the expression level of IkB phosphorylation