摘要

本研究分別探討乳酸菌 *Bifidobacterium longum* BCRC 14602、*Lactobacillus acidophilus* BCRC 10695、*Lactobacillus bulgaricus* BCRC 10696、*Streptococcus thermophilus* BCRC 13869、黑黴菌 (*Aspergillus niger*)及納豆菌 (*Bacillus natto*)對脫脂亞麻籽粉末、乙醇萃取物及鹼性甲醇萃取物中木酚素成分的轉換。將乳酸菌以脫脂亞麻籽乙醇萃取物培養，在 48 小時後 *B. longum*、*L. acidophilus*、*S. thermophilus* 對控制組分別增加 17.09%、15.65%及 13.65 %的 secoisolariciresinol diglucoside（SDG）含量，其中以 *B. longum* 能力為較佳。*A. niger* 及 *B. natto* 以脫脂亞麻籽粉末培養，個別以固態靜置與液態振盪培養方式發酵七天，其中 *B. natto* 以液態振盪培養得到較高之 SDG 釋放率（23.29 %）；然而所有菌株中皆未檢測到 secoisolariciresinol（SECO）的含量。從台灣挑選市售臭豆腐滷水分別為 STA、STG 和 STB。將臭豆腐滷水以脫脂亞麻籽粉末液態振盪培養 7 天後，STA、STG 和 STB 得到 SDG 釋放率分別為 46.97 %、33.86 %和 45.29 %，且 SECO 轉化率分別為 78.27 %、76.21 %和 82.27 %。從 STB 滷水樣品中挑選出單一株菌，以脫脂亞麻籽鹼性甲醇萃取物振盪培養 7 天後，挑選出僅需 48 小時內即可完全轉換出 SECO 的 HT 菌；此菌經初步生化鑑定為革蘭氏陰性菌（G^-），且外

型為短桿狀，且氧化酶試驗呈現陰性，經 API 20E Kit 初步鑑定為 *Proteus mirabilis*。本實驗期望能從 HT 菌中部分純化出將 SDG 轉換成 SECO 的酵素，而此酵素存在於胞外液，且反應以 pH 值 7.0 之 0.1 M 磷酸鉀為最適合之緩衝液，經由不同濃度之磷酸鈉緩衝液及磷酸水溶液探討結果証明 Na^+ 離子為影響 SDG 轉換成 SECO 之重要因子。將胞外液以不同濃度硫酸銨區分，結果顯示在 24 小時內 0-20%區分中殘存之 SDG 量比 HT 菌液及胞外液少，另外 0-10%區分更已經將 SDG 完全轉換成 SECO。在利用硫酸銨沉澱酵素以區分及利用 β-glucosidase 活性為依據，部分純化此酵素，發現各區分中所測得蛋白質含量與 β-glucosidase 活性成正比，但卻與轉換 SDG 能力不成正相關性，因為以鹼性甲醇萃取物之基質中，同時含 coumaric acid glucoside（CAG）及 ferulic acid glucoside（FAG），因此進行轉換試驗會面臨同一種酵素作用在不同基質之問題，即酵素對不同基質親和力不同，由上述實驗推測本菌胞外液對萃取液中 SDG 之 Km 值較大，而對 CAG 及 FAG 較小。為簡化酵素活性定量與區分，進一步以 SDG 標準品進行轉化，擬配合 HPLC 定量分析建立酵素活性單位，以建立此酵素純化過程之追蹤方法。

Abstract

In this report, we studied the conversion conditions of lignans in defatted flaxseed flour, and its ethanol extracts, or alkaline-methanol extracts by incubating with *Bifidobacterium longum* BCRC 14602, *Lactobacillus acidophilus* BCRC 10695*, Lactobacillus bulgaricus* BCRC 10696, *Streptococcus thermophilus* BCRC 13869*, Aspergillus niger*, and *Bacillus natto*, respectively. After the incubation of ethanol extracts from defatted flaxseed flour for 48 hours, the content of secoisolariciresinol diglucoside (SDG) released by *B. longum*, *L. acidophilus,* and *S. thermophilus* increased 17.09%, 15.65%, and 13.65%, respectively. Especially, the release ratio of SDG by *B. longum* was higher than the other Lactic acid bacteria. After the incubation of defatted flaxseed flour by *A. niger*, and *B. natto* with or without shaking for 7 days, the release ratio of SDG by *B. natto* (23.29%) under the shaking condition was the highest, however, the content of secoisolariciresinol (SECO) was not detected in any set of experiments. The stinky tofu soaking solutions were from Taiwan and were assigned as STA, STG, and STB, respectively. After the incubation of defatted flaxseed flour by STA, STG, and STB with shaking for 7 days, the release ratio of SDG were 46.97%, 33.86%, and 45.29%, and the conversion ratio of SECO were 78.27%, 76.21%, and 82.27%, respectively. After the incubation of alkaline-methanol extracts with shaking by single colony from STB for 7 days, the single colony which could convert SDG to SECO completely within 48 hours was assigned as strain HT. Strain HT, Gram-negative bacilli and oxidase test “negative”, was identified as *Proteus mirabilis* by the API 20E kit. In

this study, partial purification of the enzyme which can convert SDG to SECO from supernatant was further tested. We found that the enzyme activity existed in pH 7.0, 0.1 M potassium phosphate buffer. Besides, sodium ion was an important factor affecting SDG convertion. The 0-20% ammounium sulfate fraction from supernatant indicated less SDG residual than the whole cell culture and the supernatant. On the other hand, 0-10% fraction could convert SDG to SECO completely. The partial purification based on ammounium sulfate fraction and β-glucosidase activity for monitoring enzyme showed that the content of protein and β-glucosidase activity were positive correlation, but β-glucosidase activity and conversion ratio of SECO were not related. Because alkaline-methanol extracts also contained coumaric acid glucoside (CAG) and ferulic acid glucoside (FAG), a problem in conversion was induced that the β-glucosidase enzyme might react with different substrates. However, the enzyme had different affinities for different substrates. We purposed that the Km of enzyme from supernatant for SDG was larger than those for CAG and FAG. For simply monitoring the enzyme activity and fractionation, SDG standard and HPLC assay were used to set up for monitoring the enzyme activity to avoid the interference from CAG and FAG in alkaline-methanol extracts.