Bacillus amyloliquefaciens V656 及 Monascus purpureus BCRC31499 所生產酵素於抗腫瘤物質之製備及抗腫瘤作用之探討

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摘 要

本論文探討了蛋白?、幾丁質?及其水解產物的生物效應。本研究的第一部分利用M. purpureus BCRC31499之蛋白?最適生產條件，進行大量醱酵，所得醱酵上清液經離心硫酸銨沉澱、透析後，再以DEAE Sepharose CL-6B離子交換層析進行分離純化，此蛋白?純化倍率為27倍，活性回收率為6%，比活性為10 U/mg，最終得到蛋白?量為1.6 mg，此酵素之分子量約40 kDa，等電點為pH 7.9，最適反應溫度在40℃，最適反應pH值為pH 7-9，在 pH 5-9穩定，但不具良好之熱穩定性，另外此蛋白?水解蝦蟹殼粉所產生之胺基酸及胜?可促進植物生長，未來可應用於生物肥料的生產。 在本論文的第二部分，我們探討了B. amyloliquefaciens V656所生產幾丁質?水解幾丁類物質 (水溶性幾丁聚醣、幾丁質及懸浮態幾丁質) 之水解條件，結果發現在一定粗酵素濃度下 (20 %, V/V)，最適當之幾丁類物質水解條件為水溶性幾丁聚醣、幾丁質濃度1%，懸浮態幾丁質濃度為3%、最適反應液之pH為5左右及最適反應溫度為40℃。利用HPLC分析水解產物之組成，發現在40℃反應12小時可得較多的N-乙醯幾丁六醣，隨著時間的增長可得較多更低聚合度的N-乙醯幾丁寡醣。 在本論文的第三部分，我們探討B. amyloliquefaciens V656及M. purpureus BCRC31499所生產酵素之水解產物，對小鼠大腸腺癌細胞株CT26增殖之效應及其作用機制。M. purpureus BCRC31499所生產之蛋白?粗酵素液水解酪蛋白、烏賊軟骨、鯖魚及鰹魚之水解產物，對小鼠大腸腺癌細胞CT26細胞株無生長抑制效果。但將小鼠大腸腺癌細胞與B. amyloliquefaciens V656所生產之幾丁質?粗酵素液水解幾丁類物質之水解產物 (100-500 ?g/mL) 培養不同時間後，利用MTT assay分析細胞增殖情形，以及將處理過水解產物之細胞以流式細胞儀觀察細胞週期的變化，並計算細胞凋亡之百分比。結果發現，三種幾丁類物質之水解產物在500 ?g/mL的濃度均明顯的抑制小鼠大腸腺癌細胞的增殖。以流式細胞儀分析，證實經此水解產物處理會導致小鼠大腸腺癌細胞之細胞週期滯留在S-phase並伴隨著sub-G1 比例的上升，導致小鼠大腸腺癌細胞進行細胞凋亡，具有劑量相關效應。利用電泳分析DNA fragmentation，證明了水解產物所造成的sub-G1比例是細胞凋亡的細胞，而此水解產物處理細胞所造成的細胞凋亡過程中，粒線體的功能會受到影響，粒線體膜電位有逐漸下降的趨勢。就結果而論，幾丁類物質之水解產物對小鼠大腸腺癌細胞有極強之抑制生長作用，且其機轉是透過細胞週期滯留在S- phase期以及導致細胞凋亡之程序。由這些結果可以瞭解B. amyloliquefaciens V656所生產幾丁質?水解幾丁類物質之水解產物對於小鼠大腸腺癌細胞生長之作用機轉，希望提供臨床另一種治療大腸癌症的方式，為病患提供更多的幫助。

關鍵詞 : 蛋白? ; 幾丁質? ; N-乙醯幾丁寡醣 ; 細胞凋亡 ; CT26細胞

目錄

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