以匍枝根黴菌液態培養菌膜作為傷口癒合生醫敷料之探討

Application of liquid cultured mycelial mattress from Rhizopus stolonifer for dressing of wound enhancement

中文摘要

根黴菌是自然界中常見真菌，多應用於食品發酵。本研究之目的為要尋找另一新的替代蝦蟹殼的幾丁質來源，作為生物醫學之傷口敷材應用。幾丁質是自然界第二大量資源，主要存在甲殼類及昆蟲的外骨骼和藻類及真菌細胞壁。利用根黴菌細胞壁具有幾丁質成分和安全及生長快速之特性，形成自然編織之菌膜，稱為 RHIZOCHITIN。取 5 mg RHIZOCHITIN 加 3 ml 三氟醋酸在 110℃水解 16 小時，在 N-acetyl-D-glucosamine 相同的位置出現一醣胺類。以薄層色層分析法定性並以 Elson-Morgan 呈色法進行醣胺定量，測得含有 27%醣胺，與市售 chitin 水解率比較，則含有 78.49%醣胺。菌絲成熟時出現黑色孢子囊不適作為醫療使用。經調整培養條件找尋生長最大產?及最少孢子囊之適合條件，以馬鈴薯葡萄糖培養基添加 2 %葡萄糖，29℃八天培養之結果最佳，每 100 ml 培養基可得約根黴菌菌絲 0.68 公克，鹼處理後得 RHIZOCHITIN 為 0.15 公克，回收率約 23%。為取得無孢子囊菌株，以致突變方式期能篩得理想之無孢子囊菌株，共篩得十六株突變株其孢子囊量較野生株少，其中以編號 NO.6 突變株，由於產量最高及孢子囊最少，選為後續放大實驗之菌株。以平盤取代錐形瓶進行大量培養，厚度減少，為增加厚度比較接種量與營養影響，接種量多寡無直接相關，但增加養分則能提高產?增加厚度。平盤培養單位面積平均產?較錐形瓶培養低，然而總產重並無明顯差異，且能藉由調整收成時間獲得無孢子囊之目的。進行大白鼠動物實驗計算傷口面積，使用 RHIZOCHITIN 癒合速度優於日本市售產品 BESCHITIN-W 與對照組，能促進生長因子-TGF-β與 VEGF 分泌，活化巨噬細胞與纖維母細胞及內皮細胞分泌更多細胞激素，吸引更多細胞修復組織並幫助血管新生作用，且能抑制 MMPs 避免細胞外間質及生長因子受過度破壞而延長傷口修補時間。RHIZOCHITIN 經證實具促進癒合效果且優於 BESCHITIN-W，癒合時間雖較 SACCHACHITIN 長，但基於經濟成本和取得來源及製程考量，RHIZOCHITIN 仍是具相當優勢的幾丁質來源。

英文摘要

Rhizopus stolonifer was a common fungus in nature and it was frequently used for food fermentation. The purpose of the present study was to use the cell wall of R. stolonifer as a new source of chitin for a potential biomedical material in wound dressing. Chitin was the second most abundant biopolymer on Earth and found mainly in crustaceans shells and insects as well as cell wall of fungi mycelia. The considerably safe and fast-growing properties of R. stolonifer was the key design in

the present study by culturing the mycelia to form an interwoven mattress named RHIZOCHITIN. The analyses for sugar composition of RHIZOCHITIN was carried out by hydrolyzed 5 mg of RHIZOCHITIN in sealed ampules with 3 ml trifluoroacetic acid at 110℃ for 16 hr and then the dried hydrolysate was analyzed by thin layer chromatography（TLC）. The results indicated that RHIZOCHITIN contained N-acetyl-D-glucosamine, the monomer of chitin, visualized by Elson-Morgan reagent. The glucosamine of RHIZOCHITIN analyzed by Elson-Morgan method was contained 27％, and compares with commercial chitin hydrolisis rate was contained 78.49 %. The glucosamine of RHIZOCHITIN analyzed by Elson-Morgan method was contained 78％. To avoid the formation of dark-colored sporangium produced in the late stage of culture, medium with various C/N ratio was tested. The desirable condition to obtain the maximal yield with the minimal sporangium was using PDB(potatoes dextrose broth)with additional 2% glucose at 29 ℃ for 8 days. Mutagenesis was also carried out to select strain of few sporangia formation. Sixteen mutant strains were selected with less sporangia formation and mutant strain No.6 was the lead candidate for the following study. Instead of flask culture, tray system was used for a scale-production of RHIZOCHITIN. Inoculation density of sporangial spore were found independent to the mass and thickness of RHIZOCHITIN ; in a range from 1?107 spores /ml to 1?108 spores /ml. However, the volume of medium was positively correlated with the thickness and total mass of RHIZOCHITIN. In average, the thickness of RHIZOCHITIN decreased in the tray system, but the total production was not significantly changed when the volume of medium was considered. Animal model with Wistar rats was employed for wound healing test. Full thickness excision wounds of 6 mm in diameter were created and identical size of RHIZOCHITIZ was applied to the wound with BESCHITIN-W and SACCHACHITIN as positive controls. The effect on the acceleration of wound was significant when compared to control groups. Growth factors including TGF-β and VEGF content were 3.62 folds on the 3rd day and VEGF was 1.68 fold on the 9th day of the untreated group. Matrix metalloproteinases analysis also indicated an attenuation effect of RHIZOCHITIN in the wound to a lower level that could enhance collagen and growth factor piling. All the results demonstrated that RHIZOCHIRIN could be a promising material for biomedical purposes