Study on production of bacterial cellulose by isolated Gluconacetobacter sp. WU2 and WU3 strains

張佩瑜、吳建一

E-mail: 322074@mail.dyu.edu.tw

ABSTRACT

Bacterial cellulose (BC), which is synthesized and secreted by the gram negative bacterium, Gluconacetobacter sp. WU2 and Gluconacetobacter sp. WU3, displays unique physical, chemical, and mechanical properties including a high crystallinity, a high water holding capacity, a well-developed surface area comprised of nanofibers, elasticity, mechanical strength, and biocompatibility.

Static batch fermentations for bacterial cellulose production were studied in carbon sources, nitrogen sources, pH, temperature, organic acid (citric acid, succinic acid and acetic acid), ethanol concentration (0-15%) in flask under 30℃ by isolated Gluconacetobacter sp. WU2 and Gluconacetobacter sp. WU3. These results showed that Gluconacetobacter sp. WU3 was the best BC producer without pH controlled, the WU3 strain could produce BC at a yield exceeding 1.92 g/L of glucose/peptone mixed medium, which was comparable to the yield by WU2 at 30 ℃. Structural changes in never-dried, disintegrated bacterial cellulose by various drying process were examined. The pretreated/treatmented bacterial cellulose were characterized by X-ray diffractometry (XRD), Fourier Transform Infrared spectroscopy (FTIR) and Scanning electron microscope (SEM). In additionally, Hunter lab colour parameters were determined to assess the effect of different alkali treatments on the colour characteristics of the bacterial cellulose. The overall quality of the freeze dried membranes had higher 'L' values.

Keywords: Gluconacetobacter sp. WU2, Gluconacetobacter sp. WU3, bacterial cellulose

Table of Contents

封面內頁 SIGNATURE PAGE 授權書iii 中文摘要iv 英文摘要v 致謝vi 目錄vii 圖目錄xii 表目錄xix 1. 前言1 2. 文獻回顧4 2.1 纖維素簡介4 2.2 細菌纖維素 (Bacterial Cellulose, BC)之介紹8 2.2.1 細菌纖維素之發展歷史8 2.2.2 細菌纖維素與植物纖維素之比較8 2.2.3 細菌纖維素之特性10 2.3 醋酸菌之簡介12 2.3.1 醋酸菌之型態12 2.3.2 醋酸菌生產細菌纖維素之生合成途徑及機制12 2.4 以微生物發酵生產細菌纖維素之研究22 2.4.1 生產細菌纖維素之微生物22 2.4.2 碳源對細菌纖維素產量之影響28 2.4.3 氮源對細菌纖維素產量之影響31 2.4.4 有機酸對細菌纖維素產量之影響33 2.4.5 培養條件對細菌纖維素產量之影響34 2.4.6 不同反應器對細菌纖維素產量之影響36 2.5 基因轉殖對細菌纖維素產量之影響41 2.6 細菌纖維素之應用44 2.6.1 食品方面48 2.6.2 藥品及醫學方面50 2.6.3 DNA分離50 2.6.4 分離混合物51 2.6.5 電子紙52 2.6.6 燃料電池薄膜52 2.6.7 其他方面53 3. 材料與方法55 3.1 實驗材料55 3.1.1 實驗藥品55 3.1.2 儀器設備56 3.2 菌種來源與菌種篩選、鑑定、生長條件58 3.2.1 可生產細菌纖維素之微生物篩選58 3.2.2 優勢菌株之16S rDNA 鑑定59 3.3 菌株培養61 3.3.1 影響細菌纖維素之生成之因子探討61 3.4 分析方法63 3.4.1 發酵液之分析63 3.4.2 还原糖定性與定量63 3.4.3 有機酸及酒精分析-高效能液相層析 (High Performance Liquid Chromatography, HPLC) 分析64 3.5 細菌纖維素之純化69 3.6 純化細菌纖維素之結構分析71 3.6.1 傅立葉轉換紅外線光譜 (Fourier Transform Infrared Spectroscopy, FT-IR) 分析71 3.6.2 掃描式電子顯微鏡 (Scanning Electron Microscopy, SEM) 分析71 3.6.3 X射線繞射光譜 (X-ray diffractometry, XRD) 分析72 3.7 純化細菌纖維素之物性分析72 3.7.1 色澤分析72 3.7.2 拉伸試驗73 3.7.3 保水性分析74 3.7.4 透光度分析75 3.7.5 含水率分析76 4. 結果與討論77 4.1 可生產細菌纖維素之菌株篩選與鑑定77 4.2 培養基組成之探討79 4.2.1 不同碳源對細菌纖維素之影響79 4.2.2 不同葡萄糖濃度對細菌纖維素之影響87 4.2.3 不同氮源對細菌纖維素之影響95 4.2.4 不同peptone濃度對細菌纖維素之影響103 4.2.5 不同葡萄糖酸添加濃度對細菌纖維素之影響111 4.2.6 Gluconacetobacter sp. WU2與Gluconacetobacter sp. WU3生產培養基之比較119 4.3 環境因子之探討120 4.3.1 不同初始pH對細菌纖維素之影響120 4.3.2 不同溫度對細菌纖維素之影響124 4.3.3 不同培養深度對細菌纖維素之影響128 4.4 添加不同酒精濃度對細菌纖維素之影響132 4.5 添加不同硫酸鎂濃度對細菌纖維素之影響137 4.6 純化後細菌纖維素之結構及物性之探討141 4.6.1 純化細菌纖維素之吸水性與保水性分析141 4.6.2 純化細菌纖維素之色澤分析143 4.6.3 純化細菌纖維素之強度分析147 4.6.4 純化細菌纖維素之透光度分析150 4.6.5 純化細菌纖維素之含水率分析155 4.6.6 純化細菌纖維素之FT-IR分析與XRD分析157 5. 結論161 參考文獻163 圖目錄 Figure 1-1 Schematic of this study procedure3 Figure 2-1 Biochemical pathway for cellulose synthesis by A. xylinum 17 Figure 2-2 Pathways of carbon metabolism in A. xylinum 18 Figure 2-3 Mechanism of BC formation by A. xylinum 19 Figure 2-4 Assembly of cellulose microfibrils by A. xylinum 20 Figure 2-5 The biochemistry of the biosynthetic process 21 Figure 3-1 The standard calibration curve of glucose64 Figure 3-2 The standard calibration curve of gluconic acid concentration 65 Figure 3-3 The standard calibration curve of citric acid concentration 66 Figure 3-4 The standard calibration curve of acetic acid concentration 67 Figure 3-5 The standard calibration curve of succinic acid concentration 68 Figure 3-6 The standard calibration curve of ethanol concentration 69 Figure 3-7 Bacterial...
Table 4-5 The Characteristics of biosynthesized BC film and Cellulose Triacetate (TAC)

Table 4-6 Physical properties of some native celluloses

Table 2-2 Properties of plant (PC) and bacterial (BC) cellulose

Table 2-3 The bacterial cellulose producer by various stains

Table 2-1 Degrees of crystallinity (Xc), crystallite sizes (D(hkl)), and lateral dimensions (d) of microfibrils obtained from bacterial cellulose production by various stains

Figure 4-40 XRD spectrograms obtained from bacterial cellulose

Figure 4-39 FTIR spectrograms between the transmittance and the thickness of bacterial cellulose from Gluconacetobacter sp.WU3

Figure 4-38 Water holding capacity of disintegrated wet bacterial cellulose thickness as a function of the centrifugal force

Figure 4-37 The relationship between the transmittance and the thickness of bacterial cellulose from Acetobacter xylinum WU1

Figure 4-36 The relationship between the transmittance and the thickness of bacterial cellulose from Gluconacetobacter sp.WU2

Figure 4-35 The relationship between the transmittance and the thickness of bacterial cellulose from Gluconacetobacter sp.WU3

Figure 4-34 Bacterial cellulose (HS media) in different dry methods. (A) wet, (B) freeze drying (-20℃), (C) room temperature, (D) oven (60℃), (E) oven (80℃), (F) oven (100℃)

Figure 4-33 Moisture absorption and retention behavior of dried bacterial cellulose with different drying methods in incubator with RH 95% and in dry cabinet with RH 35% at room temperature

Figure 4-32 Effect of various MgSO4 concentration on biomass and organic acids production by isolated strain Gluconacetobacter sp.WU3

Figure 4-31 Effect of various MgSO4 concentration on biomass and organic acids production by isolated strain Gluconacetobacter sp.WU2

Figure 4-30 Effect of various substrate depth on bacterial cellouse produce, cell growth and specific growth rate and sugar utilization by isolated strain Gluconacetobacter sp.WU3

Figure 4-29 Effect of various ethanol concentration on bacterial cellouse produce, cell growth and specific growth rate and sugar utilization by isolated strain Gluconacetobacter sp.WU3

Figure 4-28 Effect of various substrate depth on biomass and organic acids production by isolated strain Gluconacetobacter sp.WU3

Figure 4-27 Effect of various substrate depth on biomass and organic acids production by isolated strain Gluconacetobacter sp.WU2

Figure 4-26 Effect of various culture temperature on bacterial cellouse produce, cell growth and specific growth rate and sugar utilization by isolated strain Gluconacetobacter sp.WU3

Figure 4-25 Effect of various culture temperature on bacterial cellouse produce, cell growth and specific growth rate and sugar utilization by isolated strain Gluconacetobacter sp.WU2

Figure 4-24 Effect of various initial pH on bacterial cellouse produce, cell growth and specific growth rate and sugar utilization by isolated strain Gluconacetobacter sp.WU3

Figure 4-23 Effect of various initial pH on biomass and organic acids production by isolated strain Gluconacetobacter sp.WU2

Figure 4-22 Effect of various gluconic acid concentration on biomass and organic acids production by isolated strain Gluconacetobacter sp.WU3

Figure 4-21 Effect of variouse glucuronic acid concentrations on bacterial cellouse produce, cell growth and specific growth rate and sugar utilization by isolated strain Gluconacetobacter sp.WU2

Figure 4-20 Effect of various glucuronic acid concentrations on biomass and organic acids production by isolated strain Gluconacetobacter sp.WU2

Figure 4-19 Effect of variouse glucuronic acid concentrations on biomass and organic acids production by isolated strain Gluconacetobacter sp.WU2

Figure 4-18 Effect of peptone concentration on biomass and organic acids production by Gluconacetobacter sp.WU3

Figure 4-17 Effect of variouse peptone concentration on biomass and organic acids production by isolated strain Gluconacetobacter sp.WU2

Figure 4-16 Effect of peptone concentration on bacterial cellouse produce, cell growth and specific growth rate and sugar utilization by isolated strain Gluconacetobacter sp.WU3

Figure 4-15 Effect of variouse peptone concentration on biomass and organic acids production by isolated strain Gluconacetobacter sp.WU3

Figure 4-14 Effect of nitrogen concentration on bacterial cellouse produce, cell growth and specific growth rate and sugar utilization by isolated strain. Gluconacetobacter sp.WU2

Figure 4-13 Effect of nitrogen sources on bacterial cellouse produce, cell growth and specific growth rate and sugar utilization by isolated strain Gluconacetobacter sp.WU2

Figure 4-12 Effect of various nitrogen sources on bacterial cellouse produce, cell growth and specific growth rate and sugar utilization by isolated strain Gluconacetobacter sp.WU2

Figure 4-11 Effect of glucose concentrations on biomass and organic acids production by isolated strain Gluconacetobacter sp.WU2

Figure 4-10 Effect of glucose concentration on bacterial cellouse produce, cell growth and specific growth rate and sugar utilization by isolated strain Gluconacetobacter sp.WU3

Figure 4-9 Effect of glucose concentrations on biomass and organic acids production by isolated strain Gluconacetobacter sp.WU3

Figure 4-8 Effect of glucose concentration on bacterial cellouse produce, cell growth and specific growth rate and sugar utilization by isolated strain Gluconacetobacter sp.WU3

Figure 4-7 Effect of glucose concentration on bacterial cellouse produce, cell growth and specific growth rate and sugar utilization by isolated strain Gluconacetobacter sp.WU3

Figure 4-6 Effect of carbon sources on biomass and organic acids production by isolated strain Gluconacetobacter sp.WU2

Figure 4-5 Effect of carbon sources on biomass and organic acids production by isolated strain Gluconacetobacter sp.WU2

Figure 4-4 Effect of carbon sources on biomass and organic acids production by isolated strain Gluconacetobacter sp.WU2

Figure 4-3 Phylogenetic tree based on 16S rDNA sequence comparisons of strain WU3 and selected bacteria

Figure 4-2 Phylogenetic tree based on 16S rDNA sequence comparisons of strain WU2 and selected bacteria

Figure 3-8 Schematic diagram of tensile specimen

Figure 3-9 The relationship between transmittance and absorbance


Kai, A. 1984. The structure of the nascent fibril produced by Acetobacter xylinum:


