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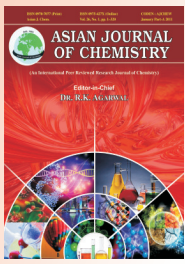

Biosynthesis Bacteria Cellulose by Strain of Bacteria Isolated from Native Corrupt Fruit†

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Bacterial cellulose (BC) is an interesting biomaterial for widespread applications. However, bacterial cellulose is still expensive compared with other popular organic products and limit its widespread commercial applications. We had isolated a high bacterial cellulose producing strain from local corrupt fruit successfully. The yields of bacterial cellulose from three strains isolated from native corrupt fruit were 3.5, 5.6 and 1.2 g/L, respectively. FT-IR and XRD results indicated the bacterial cellulose fabricated from local strain was typical cellulose I.

Keywords: Bacterial cellulose, Isolate, Yield, Crystal structure.

INTRODUCTION

Cellulose, a homopolymer of β-(1-4) linked glucose, is one of the most abundant polysaccharide produced in the biosphere. It can be synthesize by plant, animals and micro-organisms. Bacterial cellulose Produce by several bacteria as a highly swollen gel (static culture) at the air/surface interface which can be dried to form thin films[1,2]. Bacterial cellulose has many excellent properties, such as 100 % purity (free of lignin and hemicellulose), high surface based on nanostructured fibril (40 nm ± 6 nm) network, crystallinity (> 60 %), wet tensile strength, water-holding capacity(> 95 %) and biocompatibility[3]. These properties make it an interesting biomaterial for widespread applications in the cosmetics, textile, sewage purification, broadcasting, paper, food and medicine[4].

Bacterial cellulose, an exopolysaccharide, is produced by bacteria belonging to the genera *Acetobacter, Agrobacterium, Achromobacter, Aerobacter, Azotobacter, Sarcina, Salmonella, Escherichia, Rhizobium and Pseudomonas*[12]. However, bacterial cellulose is still expensive compared with other popular organic products and limit its widespread commercial applications. It is very important to develop methods to produce bacterial cellulose at the lowest cost possible. A significant point that lots of researchers have investigated is the culture conditions, alternative culture mediums or bacteria genetic engineering research.

This work aimed at isolate a high bacterial cellulose producing strain from local ecological system. The sample came from local corrupt apple, apricot and loquat. Bacterial cellulose was characterized by wide-angle X-ray diffraction (WXRD) and Fourier transform infrared spectroscopy (FT-IR).

EXPERIMENTAL

Three kinds of over ripen fruits (apple, apricot and loquat) were collected from local market in Huainan, China. the over ripen fruits were stored at 20-25 ºC until those fruits emitted the odor of ethanol and acetic acid. Strains came from apple apricot and loquat were named as APPs, APRs and LOQs.

The screening medium contained 2 % glucose, 0.5 % yeast extract, 2 % ethanol, 1.5 % magnesium 0.1 % sulphate potassium dihydrogen phosphate and 50 mg/L nystatin. Nystatin was used to inhibit the growth of mold. Rotten fruit were cut into small pieces and ground into paste, the prepared paste (10 g) was mixed with 100 mL sterile saline and shaken for 0.5 h at room temperature. The samples were diluted stepwise with sterile saline. Then 0.5 mL appropriate dilution was added to 30 mL of the screening medium. After 48 h of incubation at room temperature, 1 mL of enriched sample was inoculated into 100 mL of the screening medium and incubated at 30 ºC for 7 days. Flasks were observed for growth and pellicle formation. Strains producing thick cellulose-like pellicle was picked out for further analysis.

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The pellicles were harvested and washed with distilled water to remove residual medium and other impurities. Then the pellicles were rinsed in 1 mol/L NaOH solution at 90-95 ºC for 1 h to eliminate microorganism cells. The alkali treatment pellicles were put into dilute solution of acetic acid to neutralizing alkali. Finally the samples were washed with distilled water until the pH was nearly 7. The bacterial cellulose pellicles were dried at 50 ºC, the dry film were further used for FT-IR and XRD analysis.

The dry bacterial cellulose films were directly evaluated through Fourier transform infrared spectra (FT-IR) (Nicolet Spectrophotometer, Thermo Scientific Inc., USA). Spectra were recorded with 2 cm^{-1} resolution in the wave number region of 4000- 400 cm^{-1}.

The structure of bacterial cellulose was analyzed with a wide-angle powder X-ray diffractometer (Shimadzu XRD-6000). The X-ray diffraction pattern was recorded in a 2θ angle range of 10-60º with the scan speed 5.0º/min and sampling pitch 0.02º. The wavelength of the CuK_α radiation source used was 0.154 nm, generated at acceleration voltage of 40 kV and a filament emission of 30 mA.

The crystalline index (CI) of produced bacterial cellulose was determined by the following equation[5].

$$CI\ (\%) = \frac{I_c - I_{am}}{I_c} \times 100 \quad (1)$$

I_c represents the maximum intensity of the (002) lattice diffraction at 2θ 22.8º and I_{am} is the intensity of the baseline at 2θ 18º, which accounts for the amorphous part of cellulose.

RESULTS AND DISCUSSION

Bacterial cellulose bacterial were isolated from rotten fruit and was observed to form cellulose pellicle at air-liquid interphase. But bacterial strain isolated from different fruits produced various bacterial cellulose yield. In our experiments, the yields of bacterial cellulose from APPS, APRs and LOQs were 3.5, 5.6 and 1.2 g/L, respectively. The results indicated that local corrupt fruit could be potential bacterial cellulose bacterial carriers. The changes in the yields of bacterial cellulose might due to differences between strains.

FT-IR spectroscopic investigations evidenced the capability of different infrared adsorption bands to characterize the ordering degree of cellulose polymer. Two major allomorphs of cellulose I and II can be easily analyzed based on FT-IR bands and crystallinity ratios. FT-IR results showed that the characteristic vibrational modes of bacterial cellulose were almost same in the typical fingerprint regions as reported earlier[6]. The band at 898 cm^{-1} is typical of β-linked glucose polymers. The band at 1060, 1112 and 1160 cm^{-1}could be associated with C-O-C bond stretching, C-O bond stretching and C-O-C bridges, respectively[6]. The band at 1427 cm^{-1} could be associated with either CH_2 symmetrical bending or surface carboxylate groups. The band at 1650 cm^{-1} is due to the H-O-H bending vibration of absorbed water molecules[7]. Other band at 1367 cm^{-1} (C-H bending), 1336 cm^{-1} (OH in-plane bending), 1315 cm^{-1} (CH_2 wagging), 1282 cm^{-1} (C-H bending) and 1225 cm^{-1} (O-H in-plane bending) indicated the presence of crystalline regions within the structure[8]. As shown in Fig. 1.

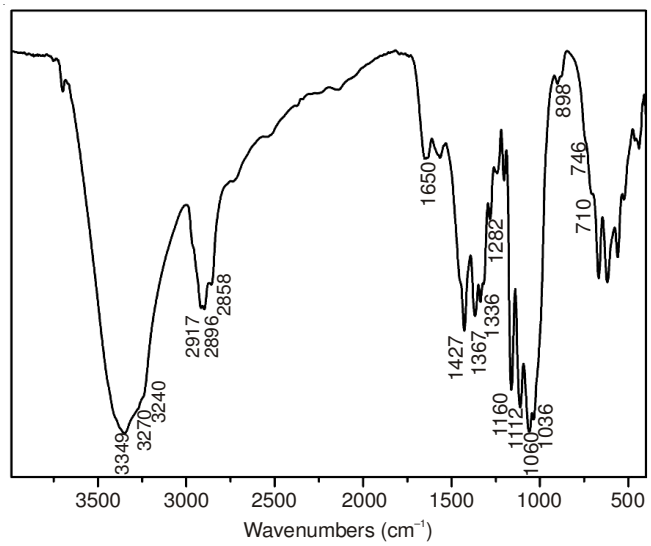

Fig. 1. FT-IR spectra of bacterial cellulose film

Bacterial cellulose, the bands centered at around 3240 cm^{-1} and at 746 cm^{-1} have been reported to the triclinic I_α allomorph, whereas bands centered at around 3270 cm^{-1} and at 710 cm^{-1} belong to the monoclinic I_β allomorph[9]. FT-IR spectra showed bacterial cellulose fabricated from local strain was typical cellulose I.

Fig. 2 shows the XRD pattern of bacterial cellulose film. Three distinct peaks appeared at 2θ = 14.76º, 16.86º and 22.92º, which correspond to the primary diffraction of the (110), (110) and (200) planes of polymorph cellulose I. No peaks, instead, are found at 2θ = 12.1º and 20.8º, which are characteristic of cellulose II. Cellulose I is the crystal structure with the highest axial elastic modulus. The crystalline index was calculated to be 79.8 % according to the formula (1).

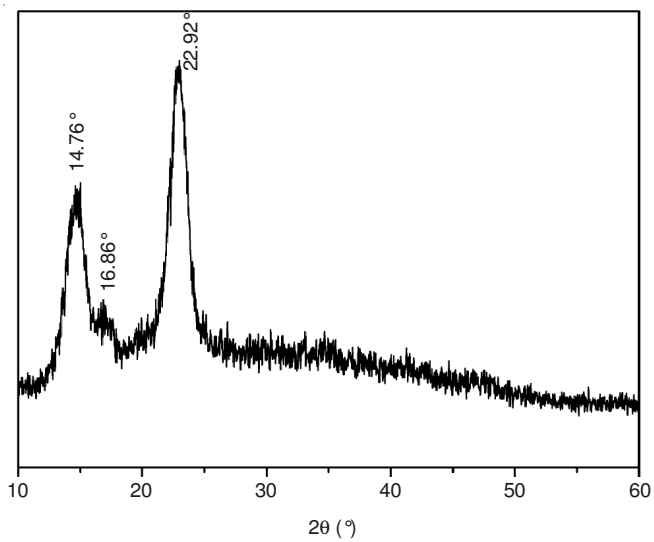

Fig. 2. XRD of bacterial cellulose film

Cellulose I is the form of cellulose found in nature composed of parallel chains and exist in two distinct allomorphs, I_α and I_β. The ratio of cellulose $I_{á}$ and Iβ depends on the organism producing it. Variations between the cellulose produced in different media were usually small[10]. Celluose I_α content is

known to be high in bacterial cellulose, whereas plant cellulose is rich in cellulose $I_{\beta,}$ which is the more stable of the two allomorphs. I_α content was calculated using the peak heights at 750 and 710 cm^{-1} by the equation determined by Yamamoto *et al.*[11]. According to our calculation, I_α and I_β content were 55.5 and 44.5 %, respectively.

Conclusion

The production on bacterial cellulose by a strain of bacteria isolated form native corrupt fruit was simply evaluated. The yields of bacterial cellulose from APPS, APRs and LOQs were 3.5, 5.6 and 1.2 g/L, respectively. FT-IR and XRD spectra showed bacterial cellulose fabricated from local strain was typical cellulose I. The optimum fermentation medium for the production of bacterial cellulose and strain identification was not investigated here, further studies will be done in future.

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