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Effects of glucuronic acid oligomers on the production, structure and properties of bacterial cellulose

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ABSTRACT

The addition of certain supplementary carbon sources to the culture media can influence the production, structural features and mechanical properties of bacterial cellulose (BC). In this study, different concentrations (0, 1, 2 and 4%) of a by-product, single sugar α-linked glucuronic acid-based oligosaccharide (SSGO), were added to the culture media during the production of BC. Production with 1% (BC1), 2% (BC2) and 4% (BC3) SSGO led to increases in BC production of 10.45, 12.74 and 9.01 g/L, respectively, after 10 days of cultivation under static conditions, while it was only 7.4 g/L when no SSGO was added (BC0). The structures of BC0, BC1, BC2, and BC3 were confirmed by XRD and FT-IR analysis. FE-SEM micrographs showed increased fibril thickness and decreased pore size in the SSGO added samples. The tensile strength of the BC0 was 16.73 MPa, while it was 25.05 MPa for BC1. However, with further increases in the concentration of SSGO, the tensile strength decreased to 20.76 and 19.77 MPa for BC2 and BC3, respectively. The results of this study provide further insight into the additive role of SSGO and improvement of the physico-mechanical properties of BC.

1. Introduction

Bacterial cellulose (BC) is a pure form of cellulose that is free from impurities such as lignin and hemicelluloses. Its structural features and extra purity bestow BC with several advantages over plant cellulose. The ultra-fine compact fiber network structure and tensile strength of BC makes it an extra crystalline material with superior mechanical properties (Backdahl et al., 2006; Phisalaphong, Suwanmajo, & Tammarate, 2008). BC has also high water holding capacity, hydrophilicity, biocompatibility, polyfunctionality, and the ability to be molded into three-dimensional (3D) structures during synthesis (Backdahl et al., 2006; Czaja, Krystynowicz, Bielecki, & Brown, 2006; Phisalaphong et al., 2008). BC is considered an attractive biomaterial due to its potential for widespread applications in the food, paper, acoustic membrane, and pharmaceutical industries (Phisalaphong et al., 2008). BC has been used in drug delivery systems and enzyme immobilization and as a conductive material for various applications (Ciechanska, 2004; Wu & Lia, 2008; Yoon, Jin, Kook, & Pyun, 2006). Additionally, the never dried BC gels have high strength and are important in the biomedical field as wound dressing materials (Ciechanska, 2004; Czaja et al., 2006). BC has also been shown to play a pleiotropic role in artificial skin, artificial blood vessels, scaffolds for tissue engineering, treatment for skin injuries and severe body burns (Czaja et al., 2006), medical pads and dental implants (Czaja, Young, Kawechi, & Brown, 2007; Klemn, Schumann, Udhardt, & Marsch, 2001; Wan, Hutter, Millon, & Guhados, 2006).

Several attempts have been made to enable greater production of BC at a reasonable cost for commercial application. These include exploration of inexpensive sources, optimization of culture conditions, design of various bioreactors, and the addition of supplementary materials to the growth media (Bae, Sugano, & Shoda, 2004; Keshk, 2006; Kurosumi, Sasaki, Yamashita, & Nakamora, 2009; Park, Jung, & Park, 2003; Shah, Ha, & Park, 2010). One of the major causes contributing to the low BC yield is the generation of side products such as water soluble oligosaccharides (Khan, Khan, & Park, 2008). Accordingly, any strategy adopted to inhibit the production of such by-products could significantly enhance the BC yield. In our previous investigations, the addition of 1% water-soluble oligosaccharide (a single sugar α-linked glucuronic acid-based oligosaccharide (SSGO)) to the culture medium was found to enhance the production of BC (Ha, Shah, Ul-Islam, Khan, & Park, 2011). Additionally, a metabolic pathway was proposed which revealed that the formation of by-products could be blocked by their initial addition to the growth media (Ha et al., 2011).

Several studies have shown that the culture medium composition and fermentation conditions significantly affect the chemical structure and composition of microbial polysaccharides, including

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BC (Duta, França, & Lopes, 2006; Watanabe et al., 1998). The mechanical properties of BC depend on the fibril arrangements and density, which are affected by various factors including culture time, culture conditions, carbon source, inoculum amount, treatment and drying method (Guo & Catchmark, 2012; Tang, Jia, Jia, & Yang, 2010). In the presence of supplementary carbon sources, the production of BC occurs for relatively longer times, resulting in high micro fibril secretion and stronger BC fibers (Ul-Islam, Khan, & Park, 2012a). The relatively thick and dense fiber can ultimately modulate the mechanical properties of BC. The addition of 1% SSGO to the culture media was shown to produce higher BC with prolonged production time (Ha et al., 2011). However, further investigation of the effect of different SSGO concentrations on the production, structural variation, and mechanical properties of the formed BC is needed. In the present study, BC was produced with 0, 1, 2, and 4% SSGO added to the growth medium and the effects of the different SSGO concentrations on the relative production, structure variation and mechanical properties of BC were evaluated. The results of this study will provide further insight into the additive role of SSGO and enhanced physico-mechanical properties of BC.

2. Materials and methods

2.1. Microorganism and cell culture

The basal medium (MAE) was prepared by dissolving 10 g of glucose, 10 g of yeast extract, 7 g of peptone, 1.5 mL of acetic acid, and 0.2 g of succinate in 1 L of distilled water. The agar plates used to culture *Gluconacetobacter hansenii* PJK (KCTC 10505BP) were prepared from basal medium with 20 g agar/L. The pH of the medium was adjusted to 5.0 with 1 N NaOH, after which it was autoclaved for 15 min at 121 °C. *G. hansenii* PJK colonies were then inoculated into 50 mL medium in a 250 mL flask and incubated at 30 °C for 24 h while shaking at 150 rpm.

2.2. SSGO and BC production

The SSGO was produced according to a previously described method (Khan et al., 2008). Briefly, BC0 was produced in a static culture by inoculating 5.0% of the *G. hansenii* PJK broth into MAE medium at 30 °C at pH 5. BC1, BC2, and BC3 were produced by adding 10, 20 and 30 g SSGO/L to the basal media, respectively, before adjusting the pH. The rest of the process was the same as that for BC0. The produced BC was then harvested and treated with 0.3 N NaOH to remove the cells and debris, after which the BC sheets were washed thoroughly with deionized water until the pH became neutral and then freeze dried until used for various analyses.

2.3. Field emission scanning electron microscopy (FE-SEM)

Scanning electron microscopy (SEM) of the freeze-dried samples was performed using a Hitachi S-4800 & EDX-350 (Horiba) FE-SEM (Tokyo Japan). Samples were fixed onto a brass holder and coated with osmium tetra oxide (OsO_4) using a VD HPC-ISW osmium coater (Tokyo Japan) prior to FE-SEM observation.

2.4. Fourier transform infrared spectroscopy (FT-IR)

FT-IR spectra of all of the BC samples were recorded using a Perkin Elmer FT-IR spectrophotometer (Spectrum GX & Autoimage, USA, Spectral range: 4000–400 cm^{-1}; beam splitter: Ge coated on KBr; detector: DTGS; resolution: 0.25 cm^{-1} (step selectable)). For analysis, the samples were mixed with KBr (IR grade, Merck, Germany) pellets and processed further to obtain IR data, which were transferred to the PC to acquire the spectra.

2.5. X-ray diffraction (XRD) analysis

XRD patterns of the samples were recorded using an X-ray diffractometer (X'Pert-APD Philips, Netherlands) with an X-ray generator (3 kW) and anode (LFF Cu). The radiation was Cu Kα at 1.54 Å, the X-ray generator tension and current was 40 kV and 30 mA, respectively, and the angle of scanning was varied from 5° to 80°. The crystallinity indices of the BC samples were determined from the integrated areas of the crystalline and amorphous phases, as reported previously (Focher et al., 2001).

2.6. Mechanical properties

The tensile properties of the BC0, BC1, BC2 and BC3 were measured using an Instron Universal Testing Machine (Model 4465, USA) according to the procedure of the American Society for Testing and Materials (ASTM D 882) (Shezad, Khan, Khan, & Park, 2010). Two metal clamps were placed at either end of each 100 mm × 10 mm rectangular strip of dried sample. The clamps were then mounted on an Instron 4465 that measured both elongation and maximum tensile load before fracture. The experiment was repeated several times and the average values were taken.

3. Results and discussion

Various additives can significantly affect the production of BC by either enhancing the activities of the producing microorganisms or providing a supplementary nutritional source. The underlying mechanisms responsible for this enhancement may include variations in the viscosity, pH, and oxygen transfer rate of the media (Chao, Mitarai, Sungano, & Shoda, 2001). Two major factors influencing the production of BC are the quantity of the nutritional source (cellulose producing carbon sources) and the relative by-product formation (Ha et al., 2011). Various carbon sources such as glucose, fructose, sucrose, and ethanol are utilized in the production of BC (Çoban & Biyik, 2011), and its production decreases after complete consumption of these primary carbon sources. The presence of additives can supplement the primary carbon source and can keep up the BC production provided live cells are present in the media. Moreover, this strategy can inhibit the by-product formation, further enhancing BC production (Ha et al., 2011). SSGO is a by-product which is produced during the BC production by *G. hansenii* PJK and its addition to the culture medium can enhance the BC production by supplementing the primary carbon source and by inhibiting self-production (Ha et al., 2011). Therefore, the effects of various concentrations of SSGO on the production and productivity of BC, structural variations, and mechanical properties were investigated.

3.1. Effect of SSGO concentration on BC production

The relative production of BC0, BC1, BC2, and BC3 is shown in Fig. 1. The results indicate positive impacts of SSGO addition on BC production. Specifically, BC production increased significantly with the addition SSGO, from 7.4 g/L in BC0 to 10.45 and 12.74 g/L for BC1 and BC2, respectively, on tenth day of cultivation. However, a further increase in SSGO concentration to 4% did not have any significant effect on BC3 production. The total production of BC3 on the tenth day of cultivation was 9.01 g/L. Taken together; these results showed that the addition of 2% SSGO is the optimum quantity for the production of BC. As mentioned above, SSGO is produced as a by-product during BC production (Khan et al., 2008). The present study and our previous investigation (Ha et al., 2011) were based on the principle of blocking the production of side products by adding SSGO to the media so it could be utilized as a secondary carbon source for BC production. Accordingly, the amount of SSGO