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Characterization of cellulose and other exopolysaccharides produced from *Gluconacetobacter* strains

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ABSTRACT

This study characterized the cellulosic and non-cellulosic exopolysaccharides (EPS) produced by four *Gluconacetobacter* strains. The yields of bacterial cellulose and water-soluble polysaccharides were dependent on both carbon source and *Gluconacetobacter* strain. The carbon substrate also affected the composition of the free EPS. When galactose served as an exclusive carbon source, *Gluconacetobacter xylinus* (*G. xylinus*) ATCC 53524 and ATCC 700178 produced a distinct alkaline stable crystalline product, which influenced the crystallization of cellulose. *Gluconacetobacter hansenii* (*G. hansenii*) ATCC 23769 and ATCC 53582, however, did not exhibit any significant change in cellulose crystal properties when galactose was used as the carbon source. Microscopic observation further confirmed significant incorporation of EPS into the cellulose composites. The cellulosic network produced from galactose medium showed distinctive morphological and structural features compared to that from glucose medium.

1. Introduction

Cellulose is a major component in plant cell walls, and is also produced by a variety of organisms such as algae, fungi and bacteria. Bacterial cellulose (BC) is an exopolysaccharide produced by various species of bacteria, such as the genera *Acetobacter* (reclassified as *Gluconacetobacter*), *Agrobacterium*, *Rhizobium* and *Sarcina* (Ross, Mayer, & Benziman, 1991). The *Gluconacetobacter* species are known to be one of the most effective cellulose producers. The chemical composition of BC is the same as that of plant cellulose, but some of its properties are quite unique such as high purity, water holding capacity, tensile strength and adaptability to the living body (Shoda, 2005). These characteristics give BC promising applications in a wide range of industrial fields including food additives, electronics (such as acoustic transducer diaphragms for headphones) (Iguchi, Yamanaka, & Budhiono, 2000), medical materials for wound healing and tissue regeneration (Fontana et al., 1990; Hu & Catchmark, 2011), and selective permeation membranes (Sokolnicki, Fisher, Harrah, & Kaplan, 2006).

BC has a significant advantage over higher plant cellulose in that it is free of lignins, hemicelluloses and pectins. However, cellulose is not the only product from the *Gluconacetobacter* system. In addition to cellulose, certain *Gluconacetobacter* strains have been reported to produce a variety of water-soluble polymers such as anionic acetan (Couso, Ielpi, & Dankert, 1987; Jansson, Lindberg, Wimalasiri, & Dankert, 1993; Valla, 1981) and neutral mannan (MacCormick, Harris, Cunning, & Morris, 1993). Those EPS produced have different levels of association to BC, and a small portion of them cannot be separated from BC pellicles by the routine purification procedures. Those EPS were defined as hard to extract EPS (HE-EPS) in our previous study (Fang & Catchmark, in press). Different from the free EPS, the presence of HE-EPS in the culture medium could interfere with the alignment of the BC crystals and modulate the bundling of cellulose ribbons.

BC synthesis in *Gluconacetobacter* strains also serves as a model system to study the basic principles that govern the biogenesis of cellulose as well as cellulose interaction with other polymers during synthesis. For example, a commonly used strategy to study plant cell wall assembly is to add purified hemicellulose to *Gluconacetobacter* fermentation medium. Other than hemicellulose, extensive work has already shown that BC fibril assembly and crystallization can be impacted by the adding a variety of biopolymers such as carboxymethyl cellulose, fluorescence brightener, etc. (Haigler, Brown, & Benziman, 1980; Haigler, White, Brown, & Cooper, 1982; Mondal, 2013) The addition of those polymers may modify the BC synthesis in a similar or distinct fashion through competing cellulose–cellulose hydrogen bonding during the early stage of ribbon assembly. The synthesis of EPS, especially those with strong association with cellulose, may impact the interpretation of this prior research. There is a need to explore different *Gluconacetobacter* strains and identify a pure cellulose production system.

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Gluconacetobacter can utilize a range of carbon sources to produce cellulose. The biosynthesis of cellulose in bacteria has traditionally been studied using glucose. Fructose, sucrose and complex carbon sources such as molasses have also been reported to provide constant high yields of cellulose (Bae, 2005; Mikkelsen, Flanagan, Dykes, & Gidley, 2009). Some of those studies demonstrated the composition of the media did not play a significant role in the structure of the cellulose produced. Galactose is also used as a carbon source for cellulose production. The structure of galactose is very similar to that of glucose and the only difference is the configuration of the hydroxyl group at the C4 position. However, previous reports have concluded that galactose is not an effective carbon substrate for BC production (Keshk & Sameshima, 2005; Mikkelsen, Flanagan, Dykes, & Gidley, 2009; Ramana, Tomar, & Singh, 2000). Those studies did not perform the characterization of any product formed using galactose as a substrate.

Further utilization of BC as a model system requires an understanding of *Gluconacetobacter* strains' ability to metabolize different carbon sources and their impact on cellulose and other related polysaccharide production. The main goal of this work is to understand the effect of galactose when used as a carbon source in place of glucose on cellulose structure and other EPS production. The association between BC and EPS produced on the crystal structure of cellulose was also evaluated in this study.

2. Method

2.1. Cell culture conditions

Four *Gluconacetobacter* strains (*G. hansenii* ATCC 23769, *G. xylinus* 53524, *G. hansenii* 53582, *G. xylinus* 700178) obtained from the American Type Culture Collection (Rockville, MD) were used in this study. Cells were cultured in a standard Hestrin–Schramm (HS) medium (Hestrin & Schramm, 1954) containing 2% (w/v) glucose, 0.5% (w/v) peptone, 0.5% (w/v) yeast extract, 0.27% (w/v) Na_2HPO4 and 0.12% (w/v) citric acid or in the modified media by replacing glucose with galactose. The final pH was adjusted to 5.0.

Primary inoculates were prepared by transferring a single colony from the HS medium working culture plate into 100 mL of each of the six modified HS media. Cells were grown in 100 mL of HS medium with 0.1% (vol/vol) cellulase (from *Trichoderma reesei*, Sigma, Lot#C2730) at 30 °C with shaking at 125 rpm for three days. Cells were harvested by centrifugation, then resuspended in the culture medium. The main cultures were grown in 250 mL Erlenmeyer glass flasks containing 100 mL medium for 7 days at 30 °C.

2.2. Purification of BC, free EPS and hard to extract (HE) EPS

At the end of incubation, the pellicles were collected and treated with 0.1 M NaOH aqueous solution followed by rinsing until a pH of 7 was achieved. The purified cellulose was lyophilized.

To harvest free EPS, the supernatant of the culture broth was obtained by centrifugation and KCl was added to a final concentration of 1% w/v to solubilize any protein present. The free EPS were precipitated with two volumes of ethanol. The ethanol precipitate was separated by centrifugation and then dissolved in water. This process was repeated at least three times. The HE-EPS were extracted from the BC films. The wet BC films were treated with 4 M NaOH aqueous solutions overnight at room temperature, and the alkali solution was neutralized. Salts were removed by dialysis (3.5 K molecular cut off) against DI water for 24 h. Protein concentrations were less than 1% for all the EPS samples, which were determined by the Bradford method.

2.3. NaOH extraction

BC composites produced from glucose and galactose medium were extracted with 4 M NaOH solutions for 12 h at 25 °C. After extraction, the residues were washed with DI water until neutral and freeze-dried.

2.4. Monosaccharide composition of EPS

The purified EPS were hydrolyzed in an aqueous solution of 2 N Trifluoroacetic acid (TFA) for 2 h at 120 °C in sealed glass vials. The resulting solution was evaporated at 40 °C to dry the EPS, and then the dried product was dissolved in distilled water. The analysis of monosaccharides of the hydrolyzates was performed on a Dionex ICS-5000 Capillary Reagent-Free IC System (Dionex, Sunnyvale, CA) and a CarboPac 20 column (Dionex). Monosaccharides were identified by comparison to the retention times with the monosaccharide standards.

2.5. X-ray diffraction (XRD)

X-ray diffraction diagrams were recorded using PANalytical X'Pert Pro multi-purpose diffractometer with Cu $K\alpha$ radiation generated at 45 kV and 40 mA. The diffractometer was used in reflection mode with the automatic divergence slit. The data was collected in the 2θ range 5–40° with a step size of 0.026°. Freeze dried BC samples were mounted onto a quartz sample holder.

Crystallinity has been used to describe the relative amount of crystalline material in cellulose. Two different approaches including peak height and peak deconvolution were used to estimate crystallinity of BC. In the peak height method, crystallinity was calculated from the intensity ratio between the (1 1 0) crystalline reflection and the amorphous background near 18.3° (Segal, 1959). In the peak deconvolution method, a pseudo Voigt function was used to profile the peak shape and area, assuming a linear background. A broad peak at around 21.5° was assigned to the amorphous contribution. Crystallinity is calculated from the ratio of the area of all crystalline peaks to the total area (Thygesen, Oddershede, Lilholt, Thomsen, & Ståhl, 2005):

The dimension of the crystal perpendicular to the diffracting planes with $h\,k\,l$ Miller indices, $B_{h\,k\,l}$, was evaluated by using Scherrer's expression (Nieduszynski & Preston, 1970):

$$B_{h\ k\ l} = \frac{K\lambda}{\sqrt{(\Delta 2\theta)^2 - (\Delta 2_{\text{inst}})^2}\cos\theta}$$

$B_{h\,k\,l}$ is the average crystalline width of a specific phase; K is a constant that varies with the method of taking the breadth ($K = 0.9$); λ is the wavelength of incident X-rays ($\lambda = 0.15418$ nm); θ is the center angle of the peak; $\Delta 2\theta$ is the full width at half maximum (FWHM) of the reflection peak and $\Delta 2\theta_{\text{inst}}$ is the instrumental broadening. The instrumental broadening was determined from the FWHM of four reflections of a silicon standard (NIST Si 640).

2.6. Field emission scanning electron microscopy (FESEM)

FESEM was conducted to observe sample morphology and microstructure. Purified BC samples were lyophilized and sputter coated with gold (approximately 5 nm). A LEO 1530 field emission scanning electron microscope (LEO Elektronenmikroskope, Oberkochen, Germany) was used operating at 5 kV.

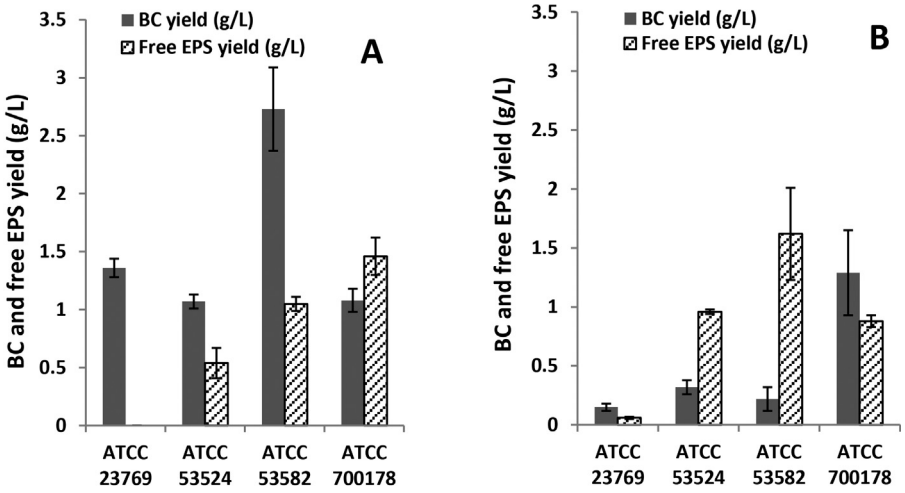


Fig. 1. BC and EPS productivity of four *Gluconacetobacter* strains in (A) glucose and (B) galactose media.

3. Results

3.1. BC, free EPS and hard to extract EPS production

Cellulose-negative (Cel^-) mutants accumulate after repeated transfer especially in shake-flask grown cultures, which became a well-known impediment to maintaining cell purity. These non-cellulose mutants have a mucoid (wet and flat) colony morphology on agar medium in contrast to cellulose-producing cells (MacCormick, Harris, Cunning, & Morris, 1993; Valla, Ertesvåg, Tonouchi, & Fjærvik, 2009). The Cel^- mutants of many *Gluconacetobacter* strains produce large quantities of another EPS, often referred to as acetan (Griffin, Morris, & Gasson, 1996; Valla, 1981, 1982). The mucoid nature of colonies formed by the mutants could be influenced by the presence of those water soluble EPS (Nguyen et al., 2010). The frequency of the mutation varies significantly depending on the strain and cultivation conditions, and may play a role in yields of cellulose and water soluble EPS. Therefore, cells used in the study were transferred no more than three times after culturing a single colony to ensure the purity of the starting culture and thus accurately access the production of cellulose and EPS.

Cellulose and EPS productivity in both glucose and galactose media in static culture were examined using four *Gluconacetobacter* strains. All the four strains were highly active producers of cellulose in the glucose medium. Among the four strains, *G. hansenii* ATCC 53582 gave the highest yield (approximately double that yielded by the other three stains) confirming other studies (Kawano et al., 2002) (see Fig. 1). The improved yield may have resulted from two factors. First, the synthesis of cellulose continued even after glucose was depleted, which suggests bacteria metabolize other substrates or byproducts for continuous BC production (Kawano et al., 2002). In addition, this strain is unique compared to the other strains because spontaneous mutation is absent (Brown & Lin, 1990; Brown, Roberts, & Saxena, 1990).

The results shown in Fig. 1 indicates that BC production of ATCC 700178 in the galactose medium was 20% higher than in the glucose medium. However, BC production of ATCC 23769, ATCC 53524 and ATCC 53582 in galactose was significantly lower than that of ATCC 700178, which agrees with the previous reports that galactose is not an effective carbon source for BC production in most *Gluconacetobacter* strains (Mikkelsen, Flanagan, Dykes, & Gidley, 2009). The fact that little cellulose is produced in those strains as compared to strain ATCC 700178 suggests that those strains may not express the sufficient amount of epimerase needed to convert UDP-galactose to UDP-glucose, the substrate required for cellulose synthesis.

Gluconacetobacter also produces the two types of water soluble EPS: free EPS and HE-EPS. In this study, we define the water soluble EPS that are suspended in the supernatant of the culture medium as free EPS. Controversial results were reported with regard to the relationship of BC and free EPS production. Ishida's work indicated that the presence of free EPS could stimulate the BC production by preventing the cells coagulation (Ishida, Sugano, Nakai, & Shoda, 2002). However, another group reported that the *G. xylinus* mutants that produced 83% less free EPS than the parent strain produced 65% more BC than that the parent strain (Watanabe et al., 1998). By comparing the cellulose and free EPS yield listed in Fig. 1, we concluded that the production of BC and EPS for the same strain was inversely related under different carbon sources. Since the synthetic pathways of both cellulose and free EPS start with UDP-glucose, they may compete for this substrate, which suggests an increase in free EPS production may reduce cellulose production. On the other hand, the yield of HE-EPS extracted from hydrated BC films was very low as compared to that of free EPS and BC. HE-EPS only made up 0.2–8.6% of the total BC dry weight, as shown in Table 1.

ATCC 23769 does not produce any significant amount of water soluble-EPS compared to the other strains. This may result from the organism lacking any of the genes associated with EPS synthesis which may include, for example, glycosyltransferases, epimerases, and other associated proteins or enzymes. We recommend it as the best model strain to study the interaction between cellulose and other polymers. For the other EPS producing strains, utilizing galactose rather than glucose as the carbon source can also either increase the EPS yield or the EPS association with cellulose.

3.2. Monosaccharides composition of free EPS and HE-EPS

We investigated the composition of EPS using acid hydrolysis. In this study, the yields of free EPS improved when the cultures were fed galactose, suggesting the carbon source influences EPS production. However, regardless of the source of carbohydrate, glucose, mannose and rhamnose were always the major monosaccharides measured after acid hydrolysis of EPS, as shown in Table 2. This composition relates to acetan previously reported in the literature (Couso, Ielpi, & Dankert, 1987). The synthesis of these heteropolysaccharides proceeds from different types of nucleotide sugars such as UDP-glucose, GDP-mannose and TDP-rhamnose, which are determined by the carbon sources availability and related enzyme activities (Ross, Mayer, & Benziman, 1991; Semino & Dankert, 1993). For examples, GDP-mannose pyrophosphorylase

Table 1
BC and EPS productivity of four *Gluconacetobacter* strains in glucose and galactose media.

Gluconacetobacter strain	Carbon source (glucose/galactose)	BC yield (g/L)	Free EPS yield (g/L)	HE-EPS yield (g/L) (percentage of BC)
ATCC 23769	Glucose	1.36 ± 0.08	0.00 ± 0.00	0.003 (0.2%)
ATCC 23769	Galactose	0.15 ± 0.03	0.06 ± 0.01	Not detectable
ATCC 53524	Glucose	1.07 ± 0.06	0.54 ± 0.13	0.01 (0.9%)
ATCC 53524	Galactose	0.32 ± 0.10	0.96 ± 0.02	0.04 (7.4%)
ATCC 53582	Glucose	2.73 ± 0.36	1.05 ± 0.06	0.11 (5.1%)
ATCC 53582	Galactose	0.22 ± 0.03	1.62 ± 0.39	0.02 (8.6%)
ATCC 700178	Glucose	1.08 ± 0.10	1.46 ± 0.16	0.03 (2.8%)
ATCC 700178	Galactose	1.29 ± 0.15	0.88 ± 0.05	0.05 (3.8%)

was found to play an important role in acetan production (Griffin, Morris, & Gasson, 1996).

The composition of HE-EPS was less diverse than that of the free EPS, in which no galactose or glucuronic acid groups were present. The acidic groups on the side chains give an anionic charge to EPS. Previous studies showed that the uronic acid in polysaccharides might prevent their adsorption onto cellulose (Tokoh, Takabe, Sugiyama, & Fujita, 2002). There was little association between polysaccharides with high galactose content and cellulose (Whitney, Brigham, Darke, Reid, & Gidley, 1998), which suggested that galactosyl substitution also presented a significant barrier to polysaccharide incorporation within the cellulose network. Mannose and glucose were the dominant monosaccharides components of the water-soluble EPS. Cellulose and EPS interaction may occur through the mannan or cellulosic backbone. Strong interactions between mannose-based polysaccharides and cellulose were predicted by Whitney, Wilson, Webster, Bacic, Reid, and Gidley (2006) because the mannose backbone shows similarities to cellulose in terms of stereochemistry, differing only in the configuration of the hydroxyl group at C2 position. A study by Chanzy, Grosrenaud, Joseleau, Dubé, and Marchessault (1982) showed that glucomannan extracted from ivory nut adopted a two-fold screw conformation closely matching that of cellulose, and which was further proved by the NMR analysis of glucomannan/cellulose composites (Whitney, Brigham, Darke, Reid, & Gidley, 1998). The findings from these studies indicated a positive selection of the incorporation of EPS into cellulose through the mannan and glucan backbones. The presence of side chain or substitution in those polymers will further modulate the strength of association.

3.3. Structure characterization of BC

Fig. 2 shows the XRD patterns of BC produced from the glucose and galactose medium. BC is a mixture of Iα and Iβ and the content of Iα is typically more than Iβ. For all BC samples, at least three diffraction faces, namely, (1 0 0), (0 1 0) and (1 1 0) could be recognized according to the triclinic indexation. The parameters calculated from the XRD profiles are summarized in Table 3.

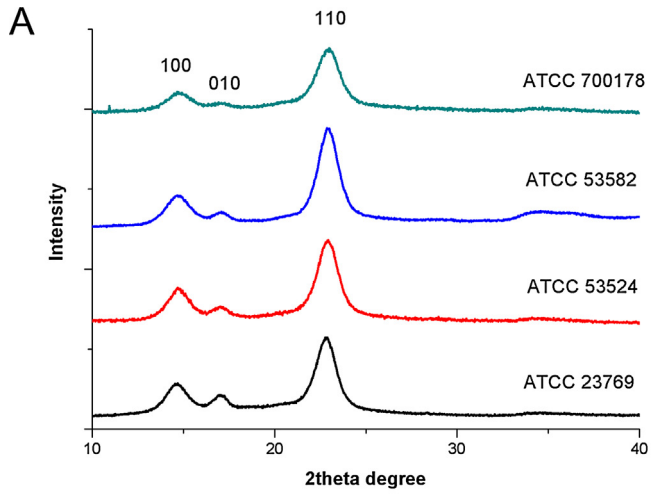
The crystallinity is one of the indicators of the relative ratio of crystalline component in a variety of cellulose sources. Peak height method always gives higher values compared to peak deconvultion method, as shown in Table 3. As pointed by Park, Himmel, Parilla, Johnson and Baker (2010) peak height method tends to underestimate the amorphous components, and therefore overestimates the crystallinity. The crystallinity values of control BC ranged from 76 to 85% in the peak height method and from 74 to 81% in the peak deconvultion method among four strains. Considering the reliability issue of the crystallinity measurement, these differences may not primarily come from the structure difference. However, switching the carbon source from glucose to galactose resulted in a decrease in crystallinity for the two strains ATCC 53524 and ATCC 700178. We believe the decrease in the overall crystallinity may relate to the increase in the amounts of HE-EPS incorporated into cellulose composites.

A major limitation of the crystal structure characterization is that the heavy focus on the measurements of absolute values of "crystallinity". Results from these measurements are highly variable and strongly dependent on a given measurement method. Crystal size is a more reliable source with less ambiguity. Weight average crystal width can be inferred from broadening analysis of XRD peaks. BC produced from the ATCC 23769 strain exhibited the largest crystal size. ATCC 23769 does not produce a significant amount of water soluble EPS compared to the other three strains. In plant system, lower amounts of noncellulosic components is usually a condition found associated to larger crystal. The rule may be applied in the *Gluconacetobacter* system.

The presence of galactose in the growth medium reduced the BC average lateral crystal dimensions. Averaging the data for (1 0 0), (0 1 0) and (1 1 0) reflections, showed that BC produced from galactose medium decreased the lateral dimensions over the control samples by 15% (ATCC 23769), 14% (ATCC 53524), 5% (ATCC 53582), 9% (ATCC 700178). The crystal size change on (0 1 0) is more significant than the (1 1 0) direction for all strains. Therefore, we proposed the (0 1 0) plane of the microfibrils was more subject to water soluble EPS modification, resulting in the significant decrease in the crystal size observed along that plane.

Table 2
Monosaccharides compositions of free EPS and HE-EPS.

Strain	EPS type	Carbon source	Glucose (%)	Mannose (%)	Rhamnose (%)	Galactose (%)	Glucuronic acid (%)
53424	Free	Glucose	27.4	69.1	1.7	1.8	–
53424	Free	Galactose	87.6	5.3	8.1	–	–
53524	HE	Glucose	76.1	10.5	13.4	–	–
53524	HE	Galactose	80.2	12.1	7.7	–	–
53582	Free	Glucose	32.6	56.2	7.4	–	3.6
53582	Free	Galactose	45.3	46.7	4.5	1.4	2.1
53582	HE	Glucose	24.6	75.4	–	–	–
53582	HE	Galactose	16.2	63.0	2.3	–	–
700178	Free	Glucose	34.3	57.3	3.8	2.4	2.3
700178	Free	Galactose	39.4	52.6	4.6	2.0	1.4
700178	HE	Glucose	79.6	13.3	7.1	–	–
700178	HE	Galactose	74.3	25.7	–	–	–



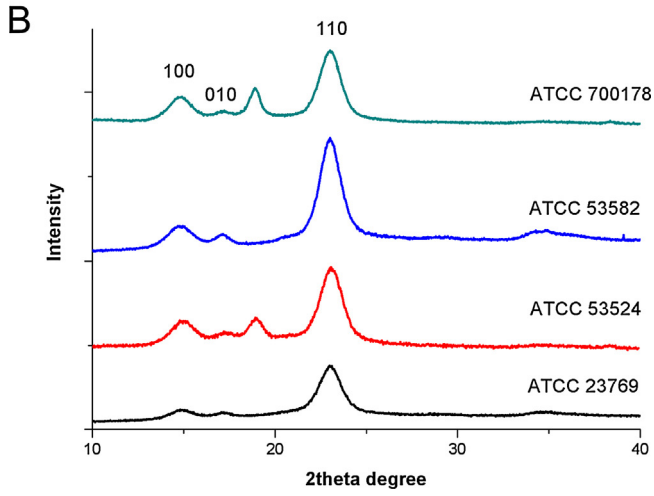


Fig. 2. XRD profiles of BC produced from (A) glucose and (B) galactose medium.

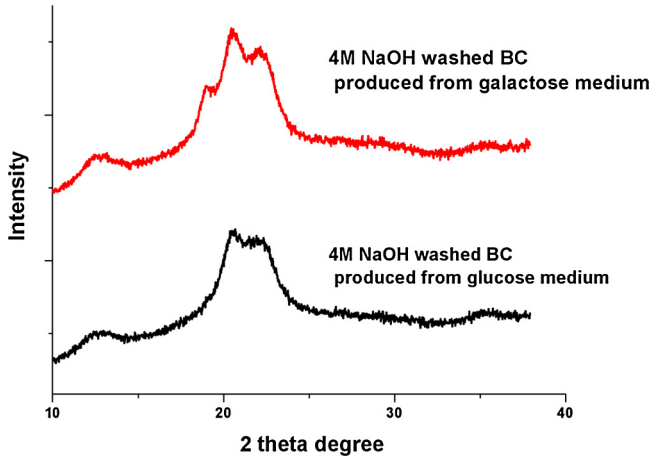


Fig. 3. X-ray diffraction data of BC samples produced from glucose and galactose medium each treated with 4 M NaOH solutions.

XRD results suggested that feeding galactose as an exclusive carbon source for ATCC 53524 and ATCC 700178 strains has changed crystal structure of BC. An additional peak around 18.8° is identified. The presence of this peak has been previously observed in the *in vitro* synthesized cotton product (Okuda, Li, Kudlicka, Kuga, & Brown, 1993). This unique signal is also found in the cellulose-like product produced from cyanobacteria (Nobles, Romanovicz, & Brown, 2001).

The appearance of this peak may be associated with either a highly crystalline EPS or co-crystallization between EPS and cellulose. The material used for XRD analysis was cleaned for at least 2 h in 0.1 M NaOH solutions and then washed in DI water for a week, periodically changing the water. After the purification treatment it is unlikely that any soluble compound remains in the material, unless highly associated with the cellulose network.

In order to obtain information about the material associated with the 18.8° peak, high concentration NaOH extraction was performed. Alkali solutions can partially disrupt the hydrogen bonding between cellulose microfibrils and other polymers. The intensity of the characteristic cellulose I peaks decreased significantly after 4 M NaOH extraction. Cellulose II peak can be observed in Fig. 3, a conversion from cellulose I to cellulose II was often reported in the literature for different cellulose sources during alkali treatment (Shibazaki, Kuga, & Okano, 1997). The unique signal around 18.8° was present even after 4 M NaOH extraction, which suggested that the structure is very stable. Highly crystalline cellulose microfibrils can be used as the substrate for co-crystallization of mannan to form "shish-kebab" structures, in which mannan adopts a two-fold screw conformation closely matching that of cellulose (Chanzy, Grosrenaud, Joseleau, Dubé, & Marchessault, 1982). Therefore, we hypothesized certain types of EPS could crystalize independently or co-crystallize with cellulose to generate that unique peak around 18.8°.

3.4. Morphology characterization of BC

FESEM was used to examine the surface morphology of lyophilized cellulose network. Micrographs obtained from BC produced from glucose medium (Fig. 4A) revealed a densely packed network of randomly oriented ribbons ranging from 30 to 60 nm in diameter. Such observations agree with the typical morphology reported for BC (Gu & Catchmark, 2012; Whitney et al., 2006). No morphological difference among the four strains was detected when glucose serves as an exclusive carbon source.

Table 3
Crystallinity and crystal size of BC produced from four *Gluconacetobacter* strains.

Strain	Carbon source	Crystal size ⟨1 0 0⟩ (nm)	Crystal size ⟨0 1 0⟩ (nm)	Crystal size* (nm)	Crystal size ⟨1 1 0⟩ (nm)	Cr (height) (%)	Cr (deconvolution) (%)
23769	Glucose	5.8 ± 0.3	8.5 ± 0.1	–	6.3 ± 0.1	78.3 ± 4.4	76.0 ± 4.3
23769	Galactose	5.2 ± 0.3	6.8 ± 0.2	–	5.8 ± 0.2	77.4 ± 2.9	76.6 ± 6.7
53524	Glucose	5.6 ± 0.3	7.1 ± 0.1	–	6.2 ± 0.1	75.7 ± 4.3	74.6 ± 3.0
53524	Galactose	4.9 ± 0.5	5.6 ± 0.2	11.6 ± 0.3	6.0 ± 0.1	67.2 ± 2.7	65.2 ± 2.3
53582	Glucose	5.1 ± 0.3	7.1 ± 0.3	–	6.3 ± 0.1	85.1 ± 0.9	81.2 ± 5.3
53582	Galactose	4.7 ± 0.3	7.4 ± 0.1	–	6.1 ± 0.1	78.4 ± 7.8	82.6 ± 5.4
700178	Glucose	4.9 ± 0.4	6.7 ± 0.1	–	5.7 ± 0.1	76.2 ± 3.5	74.4 ± 1.9
700178	Galactose	5.0 ± 0.1	5.2 ± 0.1	11.8 ± 0.1	6.0 ± 0.1	70.9 ± 4.2	68.4 ± 3.1

* Represents the crystalline reflection around 18.8°.

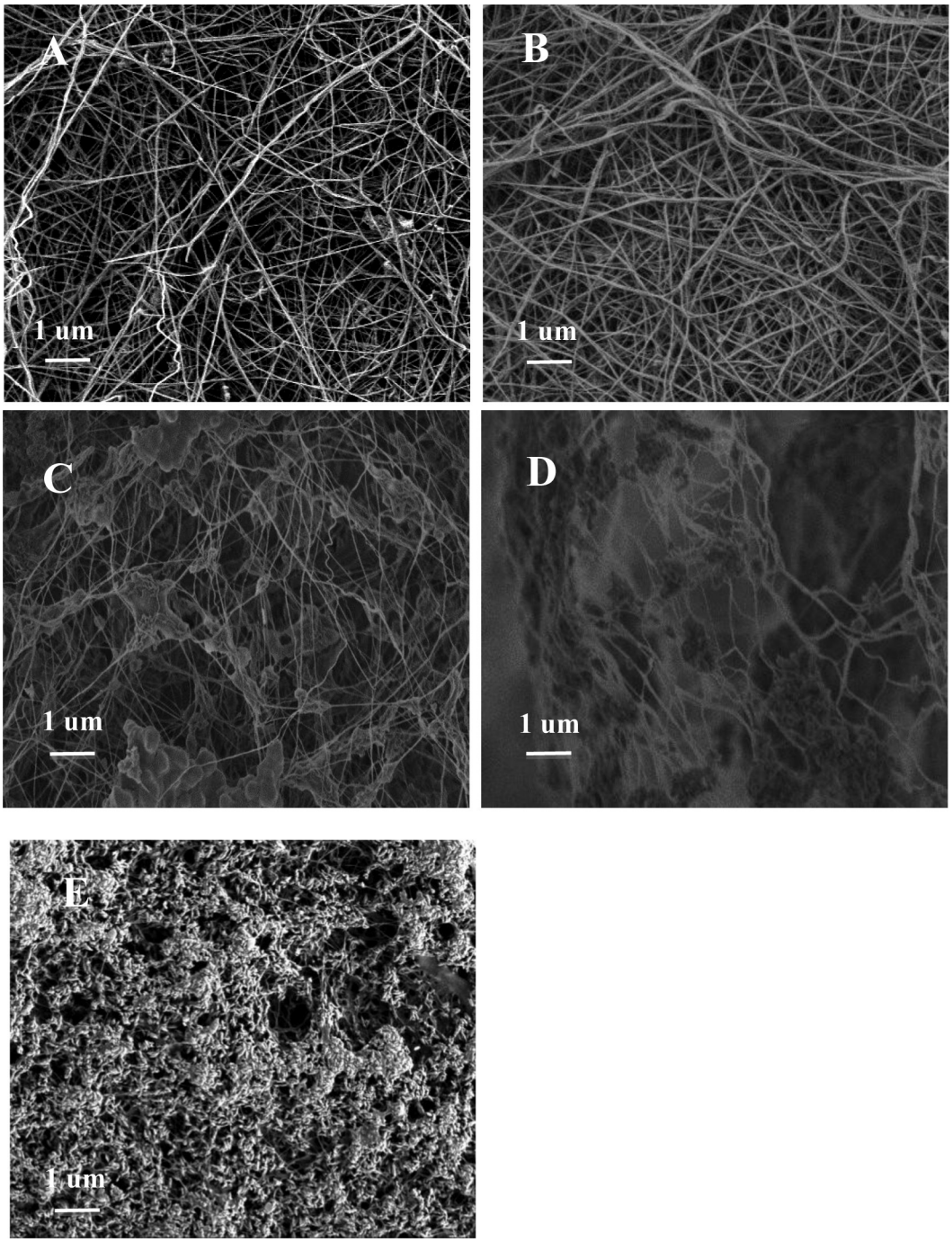


Fig. 4. Representative FESEM images of BC (A) BC with cells produced from glucose medium, (B) BC produced from galactose medium (ATCC 23769), (C) BC produced from galactose medium (ATCC 53524), (D) BC produced from galactose medium (ATCC 53582), (E) BC produced from galactose medium (ATCC 700178).

FESEM images in Fig. 4B–E showed that the surface of the BC composites produced from galactose medium were heterogeneous. Unlike the control samples in Fig. 4A, composites produced from galactose medium displayed different morphological features as shown in Fig. 4B–E, which suggested that the HE-EPS were not uniformly distributed in the cellulose network. In Fig. 4B, the morphological feature of the cellulosic network is very similar to that of the control. In Fig. 4C, cellulose network is dominant, where most HE-EPS is apparently associated with cellulose fibers. However, the EPS self-association is dominant in Fig. 4D and E. We believe the cluster-like structures are the large aggregates of EPS produced from galactose medium, since no similar regions were observed in regular BC samples. A few cellulose ribbons were also visible in that structure but became less dense compared to the regular BC in Fig. 4A. These results agree with the XRD data that the regular cellulose still is produced but another structure, possibly a distinct insoluble polysaccharide, is also present when galactose serves as an exclusive carbon source.

4. Conclusion

The effect of galactose and glucose as carbon sources on BC and water-soluble EPS production was studied. Galactose was not well metabolized into cellulose by ATCC 23769, ATCC 53524 and ATCC 53582 strains that exhibited high cellulose production in

glucose medium, while ATCC 700178 was the best cellulose producer in galactose medium. ATCC 23769 could serve as a good model system for cellulose synthesis and interaction with other polymers because it does not produce any significant amount of water-soluble EPS. The amount of cellulose and water-soluble exopolysaccharides produced varied based on the strains, which suggests different synthetic pathways or related enzyme activity among strains. This paper also described structural and morphological features of the cellulose composites produced from glucose and galactose medium. The presence of HE-EPS might modify the cellulosic network through surface coating or self-aggregating. The changes on crystallinity and crystal size were related to the amounts of HE-EPS incorporated into cellulose composites. The (0 1 0) plane of the cellulose microfibrils was more subject to HE-EPS modification as compared to the (1 0 0) and (1 1 0) planes. We also observed an additional peak around 18.8° in the XRD patterns of BC produced from galactose medium from ATCC 53524 and ATCC 700178, suggesting the production of a second crystalline polysaccharide when galactose serves as the exclusive carbon source.

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