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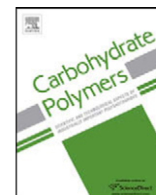

Structural characterization of bacterial cellulose produced by *Gluconacetobacter swingsii* sp. from Colombian agroindustrial wastes

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ABSTRACT

Bacterial cellulose microfibrils from non-conventional sources were produced by *Gluconacetobacter swingsii* sp. Agroindustrial residues such as pineapple peel juice and sugar cane juice were used as culture media. Hestrin and Schramm's medium was used as a reference. The production of bacterial cellulose from pineapple peel juice (2.8 g/L) was higher than that produced from Hestrin and Schramm's medium (2.1 g/L). The carbon and nitrogen resources in pineapple peel and sugar cane juice were sufficient for the microorganism development. Ribbon-like microfibrils with a width of 20–70 nm were observed in all media. Changes in crystallinity and mass fraction of the I_α allomorph were observed. The aggregation of cellulose chains into microfibrils was slightly hindered by other polysaccharides in the agroindustrial waste that adhered to the surface of the subfibrils. In conclusion, agroindustrial residues can be used as a culture medium to produce bacterial cellulose with low cost for large-scale industrial production.
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1. Introduction

Cellulose, the most abundant biopolymer in Nature, can be synthesized by plants, some animals and a large number of microorganisms, as is the case with *Gluconacetobacter* (formerly *Acetobacter*) (Brown, 1886a,b). This is a gram-negative bacterium, strictly aerobic, capable of producing cellulose extracellularly at temperatures between 25 and 30 °C and pH from 3 to 7 (Bielecki, Krystynowicz, Turkiewicz, & Kalinowska, 2005; Iguchi, Yamanaka, & Budhiono, 2000), using glucose, fructose, sucrose, mannitol, among others, as carbon sources (Ramanaka, Tomar, & Singh, 2000; Heo & Son, 2002). The bacteria synthesize cellulose as a primary metabolite. This synthesis mechanism helps the aerobic bacteria to move to the oxygen-rich surface. Moreover, the cellulose pellicle is produced to protect the cells from ultraviolet light and retain moisture (Klemm, Shumann, Udhardt, & Marsch, 2001).

Bacterial cellulose is synthesized in three stages. In the first stage, glucose molecules are polymerized (formation of β-1,4-glucosidic linkages) between the outer and cytoplasm membranes, forming cellulose changes. 10–15 parallel chains form a 1.5 nm-wide protofibril. In a second step, several protofibrils are assembled into 2–4 nm wide microfibrils, and, in a third step a bundle of microfibrils are assembled into a 20–100 nm-wide ribbon. A matrix of interwoven ribbons constitutes the bacterial cellulose pellicle (Iguchi et al., 2000; Klemm et al., 2001). The formation of the pellicle can be modified by strong aeration during agitated cultures or by the presence of certain substances that can affect the supramolecular organization of microfibrils by disrupting the formation of hydrogen bonds between cellulose chains (Bootten, Harris, Melton, & Newman, 2008; Hirai, Tsuji, Yamamoto, & Horii, 1998; Tokoh, Takabe, Fujita, & Saiki, 1998; Tokoh, Takabe, Sujiyama, & Fujita, 2002; Watanabe, Tabuchi, Moringa, & Yoshinaga, 1998; Whitney, Brigham, Darke, Reid, & Gidley, 1998; Yamamoto & Horii, 1994, 1996).

In terms of chemical structure, bacterial cellulose is identical to that produced by plants. However, it exhibits higher crystallinity, water-holding capacity, mechanical strength and purity. It contains no lignin, hemicellulose or other natural components. These features make it an interesting raw material for applications as nutritional component (Bielecki et al., 2005), artificial skin (Fontana et al., 1990), composite reinforcement, electronic paper (Jonas & Farah, 1998), flexible display screens (Nakagaito, Nogi, & Yano, 2010) and in traditional applications where plant cellulose is used. However, due to the high cost of carbon sources for

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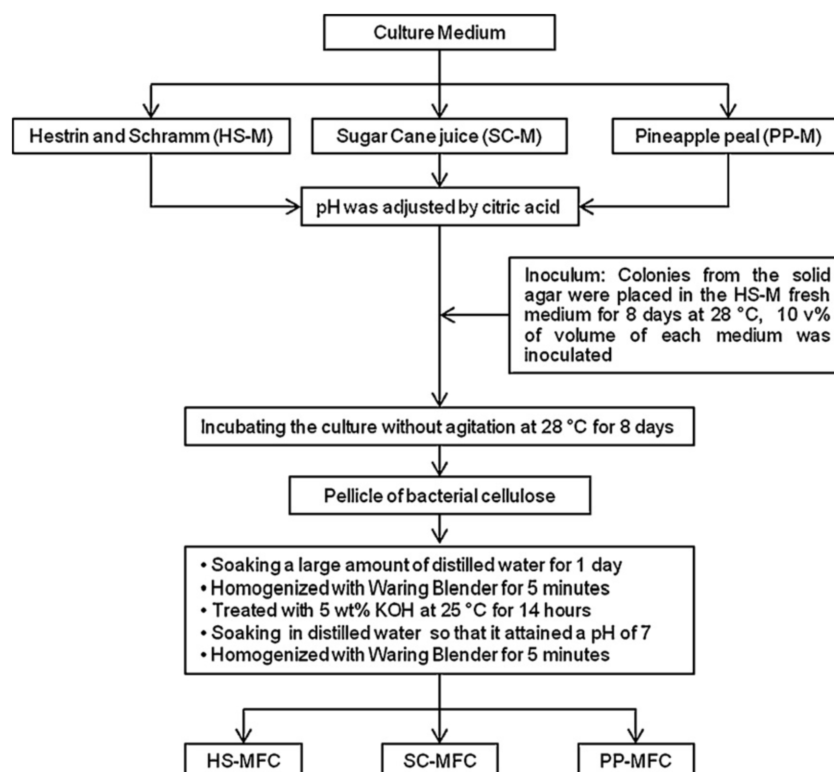

Fig. 1. Scheme for the production of bacterial cellulose from different culture media.

large-scale industrial production, the use of bacterial cellulose is limited (Vandamme, De Baets, Vanbaelen, Joris, & De Wulf, 1998).

In recent years, in order to decrease the costs of bacterial cellulose production, there has been a growing concern to develop culture media based on other sources of sugars like fruits and vegetables (Keshk, Razek, & Sameshima, 2006; Kongruang, 2008; Kurosumi, Sasaki, Yamashita, & Nakamura, 2009; Moon, Park, Chun, & Kim, 2006). In Colombia, there are a lot of organic wastes from different stages of agroindustrial productions that, in many cases, cannot be marketed due to their poor quality. However, they are rich in sugars such as glucose, fructose and sucrose, as well as nitrogen and vitamins that are useful for cellulose biosynthesis.

This work aimed at studying the morphology and structure of bacterial cellulose produced from Colombian agroindustrial residues like pineapple peel and sugar cane juice (*i.e.*, no refined sugar sources) as carbon sources. Bacterial cellulose produced into Hestrin and Schramm's medium (Hestrin & Schramm, 1954) was used for comparison. The cellulose microfibrils synthesized in different media were characterized by transmission electron microscopy (TEM), X-ray diffraction (XRD), attenuated total reflection Fourier transform infrared spectroscopy (ATR-FT-IR) and CP/MAS ^{13}C nuclear magnetic resonance (NMR).

2. Materials and methods

2.1. Materials

The *Gluconacetobacter* strain was first isolated from homemade vinegar culture and identified by 16S rRNA method (Arahal, Sánchez, Marcián, & Garay, 2008) as *Gluconacetobacter swingsii sp.* (Dellaglio et al., 2005)

Culture media used for bacterial cellulose production were sugar cane juice (0.008%, w/v, glucose, 0.066%, w/v, fructose, 8.57%, w/v, sucrose, 0.23%, w/v, total nitrogen), pineapple peel juice (2.14%, w/v, glucose, 2.4%, w/v, fructose, 2.10%, w/v, sucrose, 0.31%, w/v, total nitrogen) and Hestrin–Schramm (2%, w/v, glucose, 0.5%, w/v, peptone, 0.5%, w/v, yeast extract, 0.27%, w/v, Na_2HPO_4). Peptone and yeast extract are important in the HS medium as nitrogen source, the quantification of total nitrogen in sugar cane and pineapple peel juice showed that it was not necessary to add any source of nitrogen. The different culture media were filtered before being used to avoid the presence of fibers, acidified to pH 3.5 by the addition of citric acid and autoclaved at 121 °C. The cellulose samples produced from the three media above will be referred to as SC-MFC, PP-MFC and HS-MFC, respectively, in the following.

Experiments were prepared by adding 10 vol.% inoculums to the different media and statically incubating at 28 °C for 13 days. The collected pellicles were washed with water and homogenized in a Waring Blender for 5 min to obtain microfibrils. The microfibrils were treated for 14 h in a 5 wt% KOH solution, rinsed until pH 7, and homogenized in water for 5 min. The treatments and codification of the cellulose obtained from different media are summarized in Fig. 1.

Finally, films of constant thickness (0.1 mm) were prepared from a suspension of different cellulose microfibrils (0.3 wt%) by vacuum filtration and oven-dried at 70 °C for 48 h between glass plates.

2.2. Scanning electron microscopy (SEM)

SEM was used to observe the microorganism morphology and its distribution in the membrane. The membranes were removed from the homemade vinegar medium, dehydrated at room temperature in a graded ethanol series up to 100 vol.% and embedded in paraffin wax. Sections were cut using a rotary microtome until the membrane was seen on the surface. The sample was coated with gold/palladium using an ion sputter coater and observed with a Jeol JSM 5910 LV microscope operated at 20 kV.

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