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Short communication

Gluconacetobacter sacchari: An efficient bacterial cellulose cell-factory

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ABSTRACT

The production of bacterial cellulose (BC) membranes by *Gluconacetobacter sacchari*, in Hestrin and Schramm (HS) based media containing glucose, sucrose, fructose, mannitol or glycerol as carbon sources, was studied for the first time. The highest BC production (2.7 g/L in 96 h) was obtained using glucose. The yields obtained are comparable with those obtained with other cellulose producing bacteria, and BC ultrastructure of the material was entirely similar. These results demonstrate the high potential of *G. sacchari* as a BC producing strain.

1. Introduction

Cellulose is the most abundant natural polymer and an inexhaustible source of novel materials for many applications (Klemm, Schumann, Udhardt, & Marsch, 2001). Although most cellulose available is produced by photosynthesis in plants, some microorganisms such as algae, fungi and bacteria also produce extra-cellular forms of cellulose. Bacterial cellulose (BC), produced by several bacteria as a highly swollen gel (~90% water) which can be dried to form thin films, has DPs of 2000–8000 and forms a 3D network of highly crystalline nano- and microfibrils with 10–100 nm width (Klemm et al., 2001). Its high purity, the unique physical properties, and its biocompatibility triggered considerable interest on BC, particularly in the biomedical area (Helenius et al., 2006), but also in high technology applications (Shah & Brown, 2005).

BC is produced by bacteria belonging to genus *Gluconacetobacter*, *Sarcina*, and *Agrobacterium*, etc. (Klemm et al., 2001). The *Gluconacetobacter* genus comprises several species like *Gluconacetobacter xylinus*, *Gluconacetobacter hansenii* and *Gluconacetobacter nataicola* (Dutta & Gachhui, 2007), that are well known BC producers. Among them, *G. xylinus* is considered the most efficient and the most studied BC producer.

In recent years, several attempts were made to isolate efficient BC producing bacterial strains, and many sources of these bacteria were reported (Aydin & Aksoyb, 2010), namely fruits, fermented foods, beverages and vinegar.

However, the search of high productive bacterial strains is still a challenge to increase the availability of BC and the development of new applications of this singular material. Following this goal, we report for the first time, the production of BC from *Gluconacetobacter sacchari* isolated from Kombucha tea (Nguyen, Flanagan, Gidley, & Dykes, 2008).

2. Experimental

2.1. Materials and reagents

Glucose (96%), sucrose (99.5%), fructose (99%), mannitol (98%) and glycerol (99.5%) were purchased from Sigma Chemicals. Bacteriological Agar, Yeast Extract and Bacteriological Peptone were purchased from Himedia. All other chemicals were of analytical grade and used as received.

2.2. Isolation of cellulose producing bacteria from Kombucha tea

The BC producing bacteria were isolated from Kombucha tea. 1 mL of the tea was diluted in physiological solution (serial dilution) and spread in Petri dishes with HS medium (20 g/L glucose, 5 g/L peptone, 5 g/L yeast extract, 2.7 g/L Na_2HPO_4, 1.15 g/L citric acid, 15 g/L agar, pH 5) (Hestrin & Schramm, 1954). After 5 days of incubation, at 30 °C, each grown colony was isolated in a Petri dish containing HS agar and tested for BC production in test tubes con-

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taining 10 mL of HS liquid medium. The culture was kept at 4 °C on HS agar.

2.3. Pre-inocula

The pre-inocula were prepared by growing the bacteria at 30 °C, during 48 h, in static conditions, in HS liquid medium. After this period, the flask was vigorously agitated and 5 mL of the culture media was withdrawn and inoculated into a 45 mL liquid production medium in 250 mL Erlenmeyer flasks.

2.4. BC production using different carbon sources

To assess the BC productivity of *G. sacchari*, several sugars (glucose, sucrose and fructose) and alcohols (glycerol and mannitol) were used as carbon sources (20 g/L) in HS based media, instead of glucose.

The initial pH of the media was adjusted to 4.5 (Embuscado, Marks, & Bemiller, 1994) and it was not controlled during cultivation. All the experiments were carried out under sterile conditions. The flasks were kept at 30 °C, in a static incubator, for 96 h.

2.5. BC purification and production

After the incubation time, BC membranes were withdrawn from the culture medium and treated three times with a 0.5 M NaOH solution, at 90 °C, for 30 min in order to eliminate all attached cells (Bae, Sugano, & Shoda, 2004). Then, the membranes were washed with distilled water to remove components of the culture media and other residues until its whitening and reaching pH 7.0. Purified BC membranes were dried at 105 °C and the concentration was determined in g/L (g of BC/L of culture media).

2.6. Characterization of BC membranes

FTIR-ATR spectra were taken with a Perkin Elmer FTIR System spectrometer; the resolution was 4 cm^{-1} after 32 scans.

XRD patterns were measured with a Phillips X'pert MPD diffractometer using Cu Kα radiation.

Scanning electron microscopy (SEM) of BC surfaces was performed using a SU-70 instrument operating at 4 kV.

3. Results and discussion

3.1. Isolation of Gluconacetobacter sacchari

Cellulose producing bacteria were isolated from Kombucha tea, a traditional oriental beverage (Nguyen et al., 2008). After the incubation period, tea samples were inoculated in HS agar and incubated at 30 °C. After 5 days, several distinct colonies growing randomly on the surface of the agar were observed. Microorganisms were differentiated based on the morphology of the colonies on the Petri dish agar. Only a circular shape transparent colony (Fig. 1) was able to produce BC as a thin membrane at the air/liquid interface after 24 h of incubation in test tubes. A pure strain was obtained after several streaks on HS agar. The bacterial strain was identified as *G. sacchari* by Nadicom-Gesellschaft für angewandte Mikrobiologie mbH laboratoty (Germany), based on the 16S rRNA gene sequence.

G. sacchari is a member of *Acetobacteriaceae* family (i.e. acetic acid bacteria), described after isolation from the leaf sheath of various sugar cane varieties (Franke et al., 1999). However, *G. sacchari* has never been reported in literature as a BC producer.

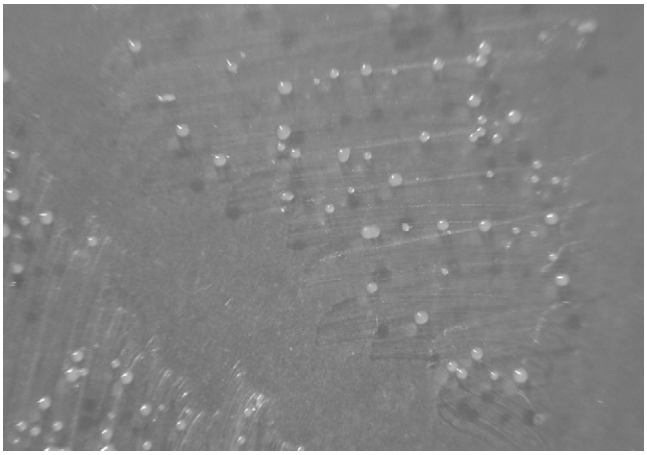

Fig. 1. Visual aspect of the BC producing colonies isolated from Kombucha tea.

3.2. Production of BC by Gluconacetobacter sacchari from different carbon sources

The efficiency of the isolated bacteria for cellulose production was accessed using several pure carbon sources, namely glucose, sucrose, fructose, mannitol and glycerol. The BC productions (g/L) after 96 h of incubation are shown in Fig. 2.

All the carbon sources studied supported the BC synthesis by *G. sacchari*, with the highest productions observed for glucose, (2.7 g/L), followed by mannitol, fructose, glycerol and sucrose (2.4, 2.28, 2.0 and 1.5 g/L, respectively).

In general, these BC productions are comparable with those obtained with common cellulose producing bacteria, using several carbon sources. For example, productions ranging from 2.81 to 3.83 g/L of BC for glucose, mannitol, glycerol, fructose and sucrose, after 96 h of incubation by *G. xylinus* were reported (Mikkelsen, Flanagan, Dykes, & Gidley, 2009). The use of a rich culture medium containing glucose as carbon source yielded 2.31 g/L of BC by *G. hansenii* (Park, Jung, & Park, 2003), after 100 h of incubation.

3.3. Characterization of BC membranes

All BC membranes produced by *G. sacchari*, from culture media containing the studied carbon sources, were characterized in terms of chemical structure, crystallinity and morphology by FTIR, XRD and SEM, respectively. BC membranes, presented the typical FTIR

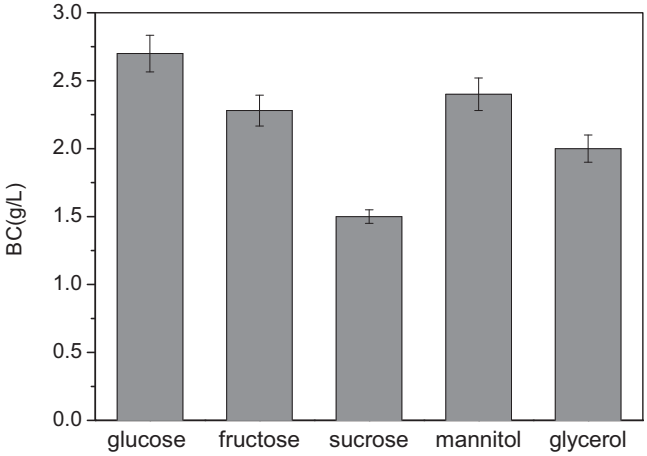

Fig. 2. BC productions (g/L) by *G. sacchari*, for 96 h of incubation using different carbon sources.

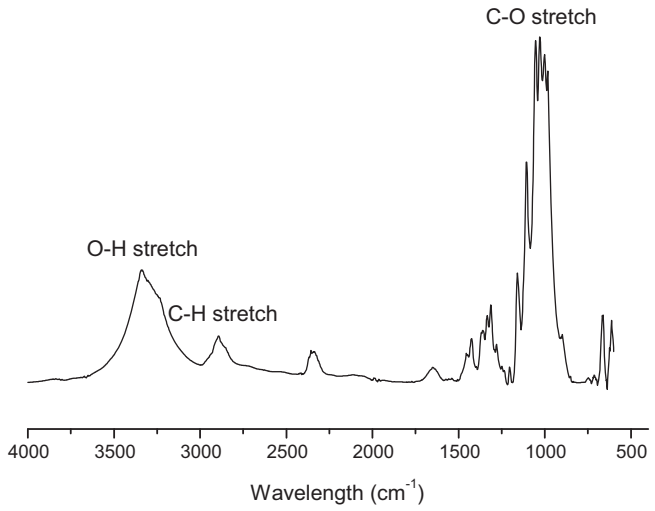

Fig. 3. FTIR spectrum of BC produced by *G. sacchari* in culture medium with glucose.

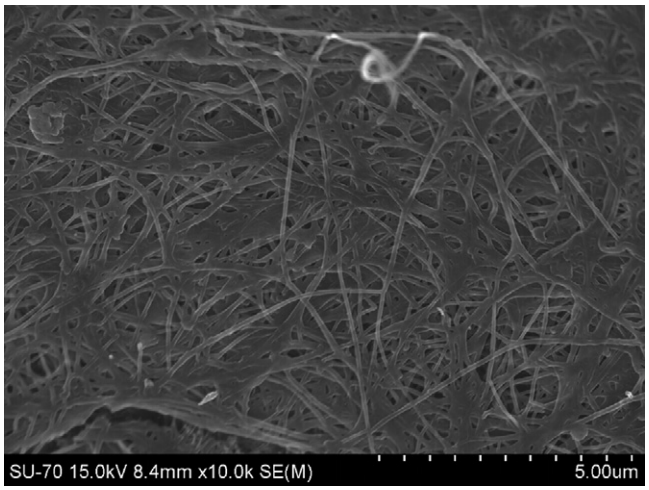

Fig. 5. SEM micrograph of BC produced by *G. sacchari* in culture medium with glucose.

spectra of cellulosic substrates (Fig. 3), with strong bands at around 3300, 2880 and 1100 cm^{-1}, associated to the vibrations of the O–H, C–H and C–O–C groups of cellulose, respectively, typically reported for this material obtained from *G. xylinus* (El-Saied, El-Diwany, Basta, Atwa, & El-Ghwas, 2008). All BC membranes presented the X-ray diffraction profile of cellulose I (Fig. 4), with the main diffraction peaks at around 2θ 14.9, 16.3, 22.5, and 34.6, normally assigned to the diffraction planes 1 0 1, 1 0 $\bar{1}$, 0 0 2, and 0 4 0, respectively. The crystallinity degree of these samples was around 79–84% and the composition of the carbon sources did not played an important role on the crystalline structure of BC. All these results were in tune with published X-ray data for BC produced by other bacteria (Jung et al., 2010).

Finally, all the studied BC samples presented the typical homogeneous tridimensional network of nano- and microfibrils of cellulose (Fig. 5), similar to those commonly reported for BC (Mikkelsen et al., 2009).

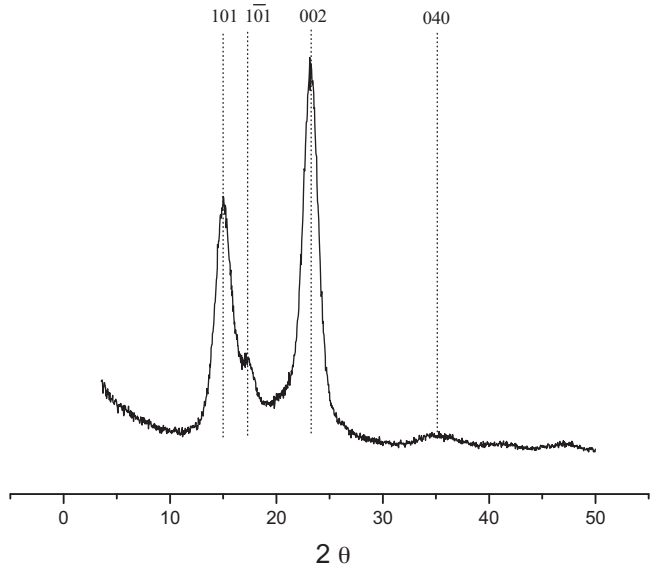

Fig. 4. X-ray diffractogram of BC produced by *G. sacchari* in culture medium with glucose.

4. Conclusions

The effectiveness of *G. sacchari* for the production of BC was demonstrated for the first time in this study. The production yields of BC obtained with this bacterium, although slightly influenced by the different carbon sources, are at the same level, or even better than those reported for common BC producing bacteria. Furthermore, the membranes formed were indistinguishable in structural and microscopic features.

These results demonstrate that *G. sacchari* can be used to efficiently produce BC, increasing its availability, and opening new perspectives for the development of new applications for this unique polysaccharide.

Acknowledgements

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