British Microbiology Research Journal
9(3): 1-13, 2015, Article no.BMRJ.18223
ISSN: 2231-0886

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The Characterization of Bacterial Cellulose Produced by *Acetobacter xylinum* and *Komgataeibacter saccharovorans* under Optimized Fermentation Conditions

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BMRJ/2015/18223
Editor(s):
(1) Alok K Upadhyay, Fox Chase Cancer Center, Philadelphia, Pennsylvania, USA.
Reviewers:
(1) Anonymous, Astronomy University of Missouri-St. Louis, USA.
(2) Anonymous, University of Sfax, Tunisia.
Complete Peer review History: http://sciencedomain.org/review-history/9779

Original Research Article

Received 10th April 2015
Accepted 12th May 2015
Published 15th June 2015

ABSTRACT

Aims: Studying the biological activities of *A. xylinum* ATCC 10245 *(as reference strain) and K. saccharivorans* PE5 for bacterial cellulose production (B.C) under optimized fermentation conditions and the structural characteristics of cellulose produced.
Study Design: The production of bacterial cellulose under optimal fermentation conditions by investigated strains then the purified cellulose was characterized by different techniques.
Place and Duration of Study: Agric. Microbiology Dept., Fac. of Agriculture, Ain Shams Univ., Cairo, Egypt.
Methodology: *A. xylinum* ATCC 10245 *and K. saccharivorans* PE5 strain were used as producers of bacterial cellulose. These strain were grown on productive medium under optimal fermentation conditions. The parameters of growth and B.C production were determined during the fermentation

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period. The specific growth rate, doubling time. Multiplication rate and generation number were calculated during the exponential phase of growth. Purified polymers were characterized and the properties of paper sheet were detected.

Results: Results indicated that the exponential phase of *Komagataeibacter saccharivorans* PE 5 and *Acetobacter xylinum* ATCC 10245 growth was detected during the first 48 to 144 hrs and during 168 hrs in productive media, respectively. The highest cellulose concentration and yield were obtained after 336 hrs by both tested strains being 11.11 gl^{-1} & 74.1% for *Acetobacter xylinum* ATCC 10245 and being 12.61 gl^{-1} & 84.11% for *Komgataeibacter saccharivorans* PE 5. The structure of BC produced from the tested strains was assayed by scanning electron microscope, fourier transform- infrared spectrum (FT-IR) and x-ray diffraction. It was revealed the diameter of thin ribbons ranged from 34.34 nm to 39.16 nm and the shape of FT-IR spectra for the two stains is similar to pure cellulose. The crystalinity index of *A. xylinum* ATCC 10245 is 54.14% and 52.76% for *K. saccharivorans* PE 5. Also, the properties of paper sheet from *K. saccharivorans* PE 5 cellulose increased about 1.19, 1.25, 1.33, 1.91 and 1.27 fold for wet tensile strength, dry tensile strength, degree of polymerization, brightness and opacity, respectively than *A. xylinum* ATCC 10245 cellulose.

Keywords: Bacterial cellulose production; A. xylinum, K. saccharivorans; scan electron microscope (SEM); fourier transform infrared (FT-IR); x-ray diffraction (XRD); bc sheet.

1. INTRODUCTION

Cellulose is one of the most abundant biopolymer on earth and most commonly harvested from plants [1]. This leads to the depletion in forest resources resulting in many environmental problems. Furthermore, the ever-increasing demand of industrialization has imposed extreme negative pressure on the delicate ecological balance of the plant world. It is, therefore, of the utmost importance for us to search an alternative for plant cellulose. One approach to reduce the demand from plants is cellulose production using a microbial system [2]. Plant cellulose (PC) and bacterial cellulose (BC) are chemically the same, β-1, 4- glucans, having same molecular formula $(C_6H_{12}O_6)n$, but their physical features are different [3]. As compared to plant cellulose, BC is chemically purer, i.e., free from lignin and hemicelluloses, has a higher degree of crystallinity and polymerization, greater tensile strength, high water absorbing capacity, extreme insolubility in most of the solvents, moldability and tendency to be modified during synthesis. Fibrils of BC are 100 times thinner than that of plant cellulose, making it more porous [4,5]. Because of all these unique properties, BC finds multifarious applications in various fields, particularly in medical field, where it can be used as an artificial skin, for making wound dressings, artificial blood vessels, and other biomedical devices [6-8]. Bacterial cellulose can also be used in many other fields like food, textile, paper, agriculture, and cosmetics, in the manufacture of audio products like ear diaphragms [9].

Cellulose is synthesized by Gram-negative species like *Acetobacter*, *Agrobacterium*, *Achromobacter*, *Aerobacter*, S*arcina, Azotobacter*, *Rhizobium, Pseudomonas and* Gram-positive bacterium such as *Sarcinaventriculi*, accounting for about 15 % of the total dry cell mass [10,11]. Several attempts have been made to isolate *Gluconacetobacter* sp. from fruits [12,13], flowers, fermented foods [14,15], beverages [16] and vinegar [17]. Although many more bacteria have been proven to synthesize bacterial cellulose, *Gluconacetobacter* sp. are known to have the highest cellulose production capacity [1,18]. Kim et al. [19] isolated *Gluconacetobacter* sp. RKY5 from persimmon vinegar and optimized the conditions for maximum cellulose production. In this optimized culture medium, *Gluconacetobacter* sp. RKY5 produced 5.63 g/L of cellulose after 144 h of shaken culture and 4.59 g/L were produced after 144 h of static culture.The most effective producers of cellulose are bacteria in the genus *Komagataeibacter* (formerly *Gluconacetobacter*) [20] such as *K. xylinus*, *K. nataicola, K. hanseniia*nd *K. swingsii* [21,22], *A. xylinum* [23,24], *A. hansenii* ([15,25] and *A. pasteurianus* [15]. In Egypt, bacterial cellulose is not commercially produced and only few researchers have been devoted in this respect.

In our investigations, we aim to study the growth behavior and structural characteristics of

bacterial cellulose produced by *Acetobacter xylinum* ATCC 10245 (reference strain) and *Komagataeibacter saccharivorans* (local isolate) under the optimized fermentation conditions to make paper sheet.

2. MATERIALS AND METHODS

2.1 Microorganisms and Culture Conditions

Acetobacter xylinum ATCC10245 and *Komagataeibacter saccharivorans* PE 5 were used as BC producers. The first one was obtained from international culture collections as a reference strain and the second one was isolated and identified by morphological, physiological and 16S rRNA gene sequence analysis [26]. These strains gave the maximum BC yield in GAM medium [27] which modified by Abdelhady et al. [26] under optimal coditions.

In this study, modified GAM medium No. 1 and No.2 were used as growth culture media for BC production by *Komagataeibacter saccharivorans* PE 5 and *Acetobacter xylinum* ATCC10245 respectively. Modified GAM medium No.1 containing (/l): mannitol, 15 g; tryptone, 6 g; ethanol, 7 ml; acetic acid, 2 ml; nicotinic acid, 0.0002 g and $CaCl_2.2H_2O$, 0.1 g while No.2 containing (/l): glucose, 15 g; yeast extract, 3 g; peptone, 3 g; ethanol, 5 ml; acetic acid, 3 ml; folic acid, 0.0004 g and NaCl, 0.1 g in 1000 ml distilled water at pH 3.5. Stoke cultures of these strains were maintained at 5°C on GAM medium after incubation at 30°C for 7days.

2.2 Standard Inoculum

Standard inoculum was prepared by inoculation of one test tube containing 5 ml of GAM medium with 1 ml of tested culture, then incubated at 30°C for 3 days. After incubation period, this tube was used as a standard inoculum ($O.D_{620nm}$ from 0.215 to 0.444) for static culture.

2.3 The Fermentation Process

This experiment was constructed to study the growth behavior, BC production and sugar consumption of tested bacterial strains grown on modified media during 17 days (408 hrs) at 30ºC as static culture (optimal conditions are recommended by Abdelhady et al. [26]). The propagation was carried as mentioned above. Samples (3 flasks for each sample) were taken periodically every 12 hrs to determine the optical density (O.D) of growth spectro-photometrically at 620 nm. The relation between incubation time and O.D was plotted using Excel program. All parameters of bacterial growth (specific growth rate, number of generation and multiplication rate and doubling time) and sugar consumption were calculated, pH values of bacterial culture were measured during the fermentation period. The purified pellicle obtained was weighed as fresh weight then dried at 60°C until a constant weight and expressed as gl^{-1} dry weight, then cellulose yield (%) and productivity were calculated, according to Gamal et al. [28] using the following equations:

$$\text{Yield}(\%) = \frac{\text{Dry cellulose production } (gl^{-1})}{\text{Original Sugar } (gl^{-1})} \text{ X100}$$

$$\text{Productivity } (gl^{-1}h^{-1}) = \frac{\text{Amount of BC produced } (gl^{-1})}{\text{Fermentation time (h)}}$$

Correlation coefficient and regression analysis between incubation time and some growth parameters were calculated.

2.4 Characterization of Bacterial Cellulose Produced

2.4.1 Microscopic study of bacterial cellulose

Scanning electron microscopy (SEM) analysis of BC was performed on BC dried sheets with a microscope (Hitachi S- 4200, Japan) under 60,000 magnifications to study the morphologyical characteristics. The surface and cross-section of the samples were sputtered with gold, and photographed. SEM

2.4.2 Fourier transform infrared spectrum (FT-IR) analysis

Fourier transform infrared (FT-IR) spectra were obtained using A JASCO 300-E Fourier transform infrared (FTIR) spectrometer. Each cellulose sample was air- dried on a glass slide in the form of a thin film. The samples were cut into very little particles for the evaluation of chemical structures using a potassium bromide pellet (KBr) technique. The IR specter membranes measured at wave number ranging from 4000 to 400 cm^{-1}.

2.4.3 X-ray diffraction analysis

X-ray diffraction spectra were recorded by using A philips x-ray diffracto meter at 40 kV and 30 mA (CuKα radiation) to analyze BC crystalline structure. Scans are performed over the 5°- 40° 2θ range using step 0.1° width. The crystallinity index is calculated [29].

2.5 Paper Sheet Making

The BC samples produced by tested strains were immersed in 0.5% (v/v) ammonium hydroxide overnight individually, and washed thrice with tap water. Subsequently, the BC pellicles were boiled for 30 min. to remove ammonium hydroxide and washed thrice again with tap water [30]. The pellicles were squeezed to remove water using a pressing machine and then dried at 65ºC for 3 hr.

2.6 Mechanical properties of Bacterial Cellulose Paper Sheet

The BC sheet were examined for tensile strength and Young's modulus using a universal testing machine (TA plus, Lloyd Instruments Ltd., England) at a test speed of 0.25 mm/min. Rectangular specimens for measurements were cut from samples with a gauge length of 30 mm. Five specimens were made to average the results.

2.7 Water Absorption Capacity

The water absorption capacity of BC sheet was examined using the procedure of ASTM D570-98. Each dried sample (2.54 × 7.62 cm) was immersed in distilled water at 23°C until equilibrium. The swollen sample was removed from water and excess water at the surface was wiped off. The sample was weighed and the water absorption capacity (%) was calculated (= (wet weight – dry weight)/dry weight× 100).

2.8 Determination of Degree of Polymerization

The chemical used in preparation process of paper sheet can cause considerable decrease in the degree of polymerization of cellulose to the point where the paper strength properties are adversely affected. It is therefore very important to estimate the extent of cellulose degradation after each process by measuring its viscosity.

2.9 Optical Properties of Bacterial Cellulose Paper Sheet

2.9.1 Brightness

The intensity of light designates the brightness of paper as compared to the intensity reflected by standard white body at the same wavelength. The apparatus used was Carl Zeiss ELREPHO Tester.

2.9.2 Opacity

It was measured according to TAPPI T519 standard method using a Hunterlab D25-5 color/difference meter.

2.10 Chemical Determination

Residual sugar in fermented culture was determined with glucose kits according to methods of Youngos [31].

2.11 Statistical Analysis

The collected data were statistically analyzed using SPSS computer analysis program [32] and the correlation coefficient and regression were analyzed with Microsoft Office Excel 2010.

3. RESULTS AND DISCUSSION

3.1 Biological Activity of Cellulose Producing Bacteria under Optimized Fermentation Conditions

Results illustrated by Fig. 1 clearly show that *Acetobacter xylinum* ATCC 10245 grew experimentally during the first 168 hrs (7 days) while it ranged from the first 48 to 144 hrs period of incubation for the growth of *Komgataeibacter saccharivorans* PE 5 in modified GAM medium No. 2 and No. 1, respectively. Also, it showed a highly positive correlation coefficient (R^2) between cell growth and fermentation period. The stationary phase was observed during 168 to 360 hrs and during 144 to 336 hrs for the first and second strains, respectively. The growth parameters calculated from exponential phase were specific growth rate (μ), number of generation (N) and multiplication rate (MR) and doubling time (td) were 0.01 h^{-1}, 1.73, 0.014 & 69.3 h for *Acetobacter xylinum* ATCC 10245 and were 0.008 h^{-1}, 1.39, 0.02 & 86 h for *Komgataeibacter saccharivorans* PE 5.The highest growth ($O.D_{620nm}$) being 0.928 and 0.954 were recorded

by *Acetobacter xylinum* ATCC 10245 and *Komgataeibacter saccharivorans* PE 5 after 360 and 336 hrs, respectively. Also, the consumed sugar was increased gradually to record the highest value at the end of the fermentation period (408 hrs) being 11.25 and 11.74 gl^{-1} for *Acetobacter xylinum* ATCC 10245 and *Komgataeibacter saccharivorans* PE 5, respectively. These results are in line with reported by Suwanposri et al. [33] who stated that *Komgataeibacter* sp. PAPI increased slowly during the first 2 days and then increased exponentially for 7 days that the beginning of stationary phase.

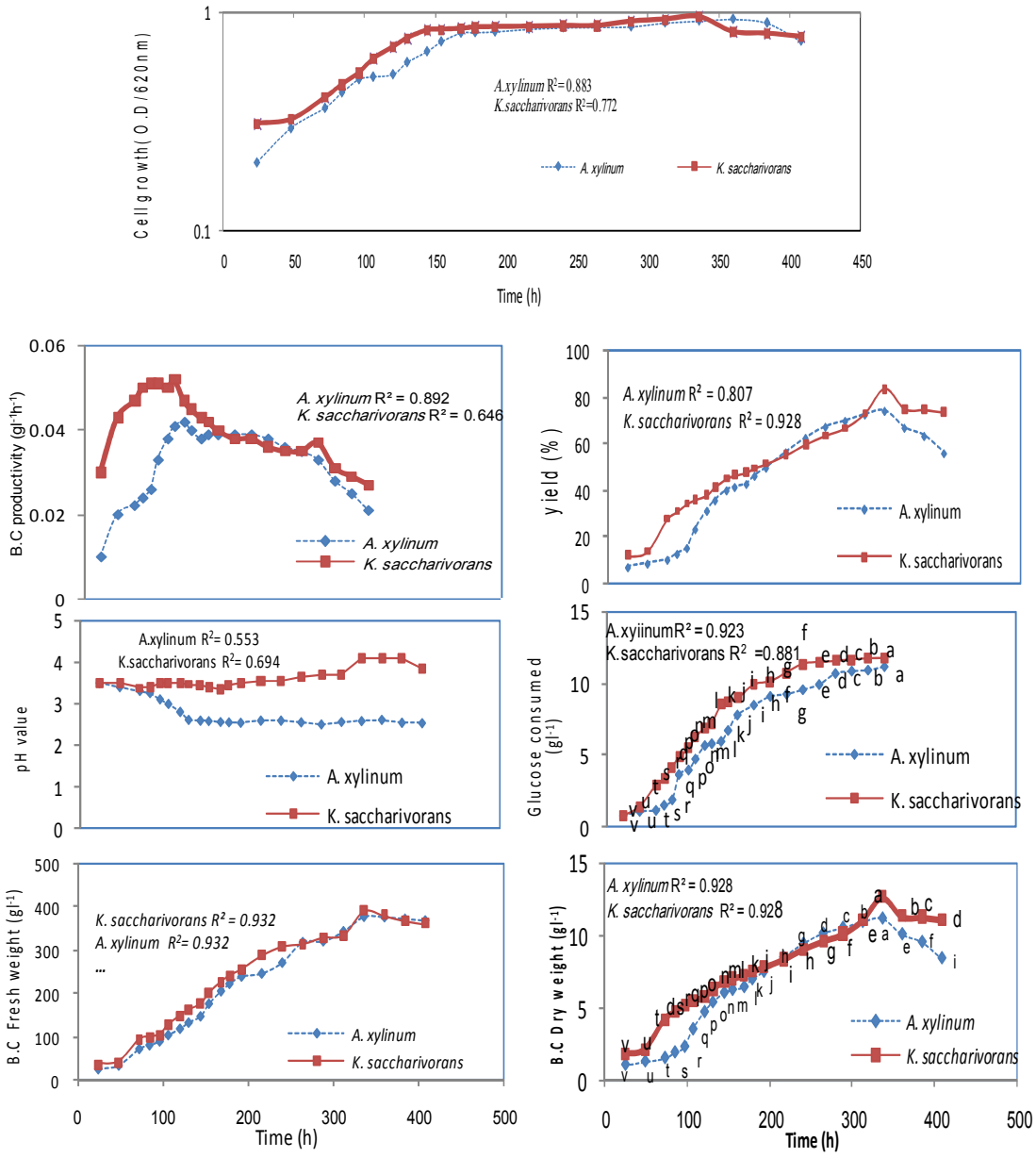

Fig. 1. Biological activity of *Acetobacter Xylinum* ATCC 10245 and *Komagataeibacter saccharivorans* PE5 for bacterial cellulose production on modified GAM media during 17 days of incubation period under optimized conditions

Values in the same line sharing the same letter do not differ significantly, according to Duncan's at 5 % level

With respect to BC production, it was found that both strains recorded the highest concentration and the highest percentage of cellulose yield after 336 hrs fermentation period being 11.11 gl^{-1} & 74.1% for *Acetobacter xylinum* ATCC 10245 and being 12.61 gl^{-1} & 84.11% for *Komgataeibacter saccharivorans* PE 5. Whereas the highest cellulose productivity was attained during the exponential phase by the first strain being 0.042 gl^{-1} h^{-1} after 144 hrs and by the second strain after 130 hrs being 0.052 gl^{-1} h^{-1}. This results shows that BC production by tested strains was growth- associated. Moreover, there are significant increased for B.C produced and consumed sugar during fermentation period. The pH was decreased from 3.5 to 2.54 after 408 hrs of cultivation by *Acetobacter xylinum* ATCC 10245 but it increased to 3.86 by *Komgataeibacter saccharivorans* PE 5. The positive correlation coefficient (R^2) was observed between fermentation period and optical density of cell growth, B.C dry and fresh weight, yield (%), B.C productivity, pH and glucose consumed with the tested strains. It could be stated that local strain *Komgataeibacter saccharivorans* PE 5 was the most efficient cellulose producing bacteria. The amount of cellulose produced by both strains after 6 days were higher than that reported (about 4.14 gl^{-1}) by Suwanposri et al. [33] on soya bean whey medium or on HS medium (1.15 gl^{-1}).

3.2 Characteristics of Bacterial Cellulose

3.2.1 Scanning electron microscope (SEM)

The morphological shape of B.C investigated samples show three dimensional porous network structure consisting of randomly arranged ribbon-shaped ultrafine fibers (Fig. 2). Saxena et al. [34] stated that cellulose biosynthesis is characterized by unidirectional growth and crystallization, where glucose molecules are linear bounded by β (1-4) glucosidic bond. The union of glucosidic chains forms oriented microfibrils with intramolecular hydrogen bonds. The diameter of thin ribbons arranged from 34.34 nm to 39.16 nm, which are smaller than fibrils from plant cellulose [35]. The results as in accordance with previously reported BC structure [33]. However, there are some morphological difference in the ribbons and reticulated structures of bacterial cellulose of *Acetobacter xylinum* ATCC 10245 and *Komgataeibacter saccharivorans* PE5. From cross-section images, the multiple layers structure can be seen in Fig 3 (b,d). The reticultated structure of *Komgataeibacter saccharivoran*sis PE5 tightly combined but loose with *Acetobacter xylinum* ATCC 10245 (reference strain). Fig (2) presents the surface view for *Komgataeibacter saccharivorans* cellulose, nanofiberils cann't be observed due to converge by a thick layers so it has well interconnected pore network structure. Kim et al. [36] reported that the large surface of B.C is necessary for cellulose attachment and vascularization. Youshinaga et al. [3] reported that cotton fibers, wood slurry fiber and synthetic fiber diameter is in range of 10-100 nm. Thus in comparison with these fibers, BC has unique paper- fine nano-structures.

3.2.2 Fourier transform infrared (FT-IR) spectroscopy

The FT-IR spectra of bacterial cellulose produced from *Komgataeibacter saccharivorans* PE 5, *Acetobacter xylinum* ATCC 10245 (reference strain) and pure cellulose are shown in Fig. 3 and its spectral band assignments are listed in Table 1. In this study, the FT-IR spectra of bacterial cellulose of *Komgataeibacter saccharivorans* PE 5 was compared with that of bacterial cellulose from reference strain and with that of pure cellulose powder (high purity grade reagents) according to previously reported by Ramírez-Flores et al. [37]. The pure cellulose spectrum, distinguish peaks of 3350 cm^{-1} and shouldering around 3400 cm^{-1} to 3500 cm^{-1} indicates O-H stretching, 2800 cm^{-1} 1 to 2900 cm-1 indicates C-H stretching, 1160 cm^{-1} indicates C-O-C stretching and 1035 cm^{-1} to 1060 cm^{-1} indicates C-O stretching. Other fingerprint regions for cellulose are peaks around 1300 cm^{-1} indicating C-H bending and around 1400 cm^{-1} indicating CH_2 bending [38]. The analysis of bacterial cellulose produced from *Komgataeibacter saccharivorans* PE5 showed peaks at 3340 cm^{-1}, 2855 cm^{-1}, 2920 cm^{-1}, 1319 cm^{-1}, 1157 cm^{-1}, 1425 cm^{-1} and 1023 cm^{-1}. The corresponding figure for B.C. from *Acetobacter xylinum* ATCC 10245, showed at 2894 cm^{-1}, 3340 cm^{-1}, 1314 cm^{-1}, 1053 cm^{-1}, 1426 cm^{-1} and 1160 cm^{-1}, thus confirming purity of the cellulose produced. Although, fingerprint peaks confirm the structure such as that of cellulose, the curve of peaks may vary, depending on the origin of cellulose. The shape of the curve is a signature of the origin of the cellulose. The comparison of the FT-IR spectra between bacterial cellulose spectra of reference strain and the bacterial cellulose of K. *saccharivorans* PE 5 (isolated from local rotten fruits) indicated that our bacterial cellulose produced is confirmed as pure cellulose synthesized from acetic acid bacteria.

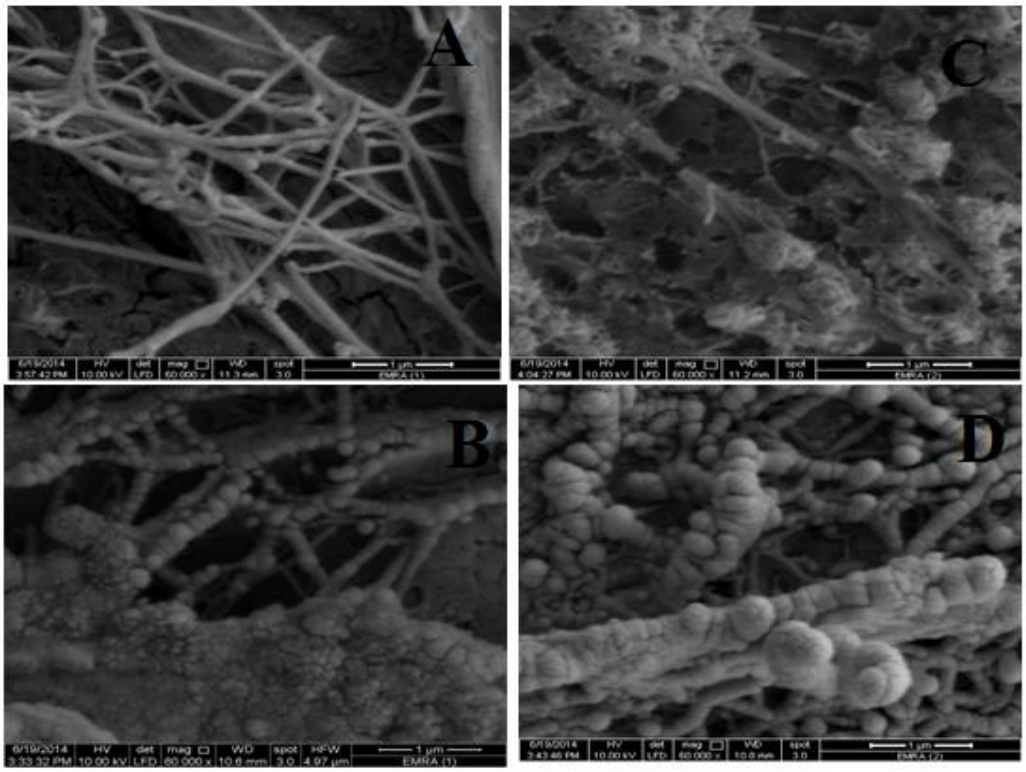

Fig. 2. SEM images of bacterial cellulose from *Acetobacter xylinum* ATCC 10245 A): Surface morphology, B):cross-section morphology and *Komagataeibacter saccharivorans* PE5, C):Surface morphology, D): cross-section morphology

3.2.3 X-ray Diffraction

X-Ray diffraction was used to compare the microstructural changes in two samples of bacterial cellulose especially to estimate the crystallization process. Two common crystalline forms of cellulose designated as I and II, are distinguishable by X-Ray diffraction. The metastable cellulose I, which is synthesized by the majority of plants and also by *A. xylinum* in static culture, parallel β-1,4 glucan chains are arranged uniaxially, whereas β-1,4 glucan chains of cellulose II are arranged in random manner. They are mostly non parallel and limited with a larger number of hydrogen bonds, which mostly in higher thermodynamic stability of cellulose [39]. Fig. 4. shows X-Ray diffraction patterns for bacterial cellulose synthesized from *Acetobacter xylinum* ATCC 10245 and *Komgataeibacter saccharivorans* PE 5 grown under optimum conditions after 7 days. Diffraction peaks appeard at 14.7º & 22.9º for *Komgataeibacter saccharivorans* PE 5 and at 14.5º& 22.7º for *Acetobacter xylinum* ATCC 10245. This result is in line with this obtained by Maeda et al. [40] who reported that diffraction peaks at 14.5º& 22.6º are assigned to cellulose I_α and I_β phase. Also Yamamoto and Horii [41] reveled that native cellulose iscomposite of two different crystalline phase called I_α and I_β. Crystalline parameter of crystal plats of BC of investigated strains are listed in Table 2. The Crystalline index of BC are 54.14% &52.76% for *Acetobacter xylinum* ATCC 10245 and *Komgataeibacter saccharivorans* PE 5, respectively. The values of d-spacing from studied bacteria are similar. It could be concluded from these results that the BC synthesized by the isolated bacteria have the similar structural characteristics of BC from *Acetobacter xylinum* ATCC 10245 (reference strain). Vanderhart and Atalla [42] reported that *G. xylinum* cellulose displays characteristics of highly crystalline, I_α – rich cellulose.

3.2.4 Mechanical and optical properties of paper sheet

Data of mechanical and optical paper sheets properties of *Acetobacter xylinum* ATCC 10245 and *Komgataeibacter saccharivorans* PE 5

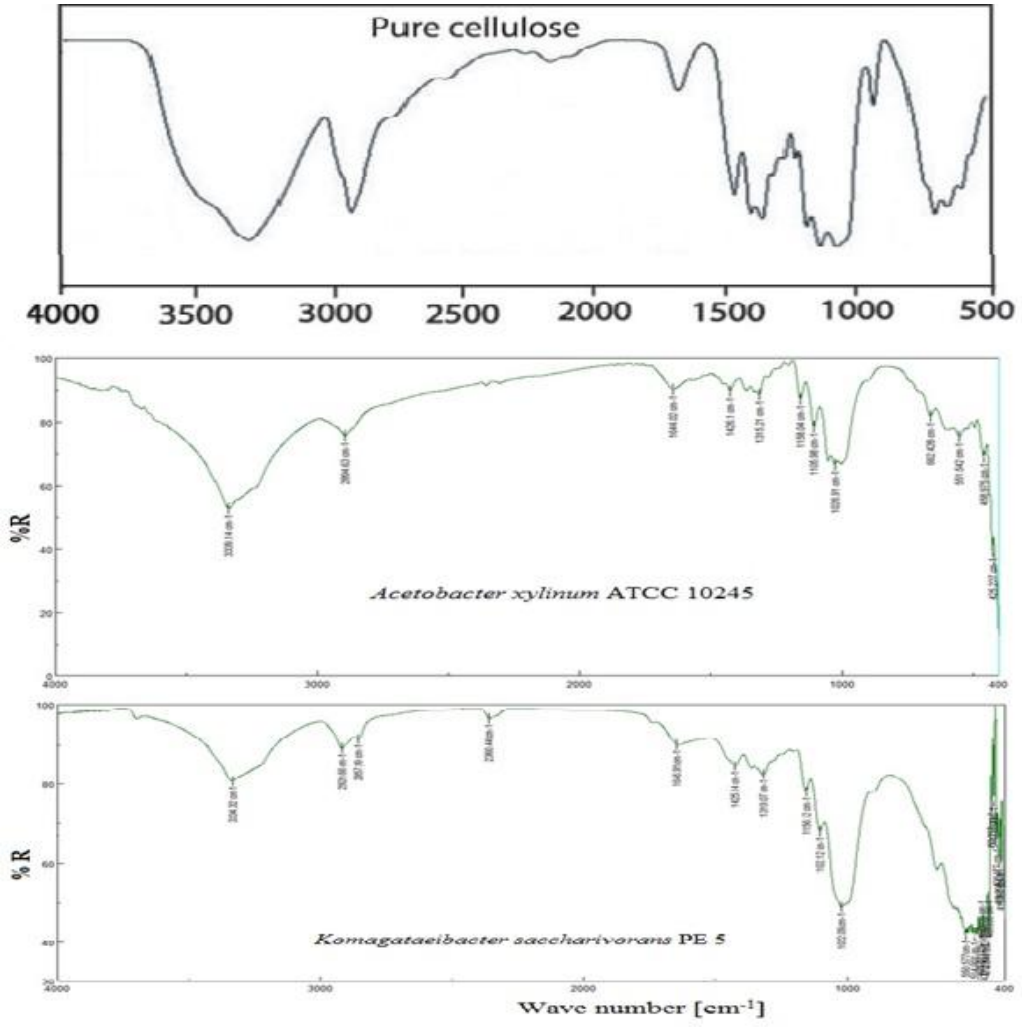

Fig. 3. FT-IR spectra of pure cellulose (micro granular cellulose from Sigma) by Ramírez-Flores et al. [37] , bacterial cellulose of *Acetobacter xylinum* ATCC 10245 (reference strain) and bacterial cellulose of *Komgataeibacter saccharivorans* PE 5

cellulose were recorded in Table 3 and shown in photo. 1. These data indicated that the properties of *Komgataeibacter* cellulose increased about 1.19, 1.25, 1.33, 1.91 and 1.27 fold for wet tensile strength, dry tensile strength, degree of polymerization, brightness and opacity respectively than *Acetobacter* cellulose. The high tensile strength of *Komgataeibacter saccharivorans* PE 5 cellulose might attribute to the unique in-twisting network structure with many super-fine nanofibers according to Dongping et al. [43]. Whereas the vice versa was true for wet young's modulus, dry young's modulus and water absorption capacity (%).In similar studies, Suwanposri et al. [33] found that the tensile strength value of BC film produced by *Komgataeibacter* sp. PAP1 on HS medium was 22.58 MPa. In this respect, Suwannapinunt et al. [30] stated that BC paper from the static tray showed the high wet tensile strength value than shaking flask and stirring bioreactor.

Also, it could be noticed that water holding ratio is 90.07 and 80.29 for *Acetobacter xylinum* ATCC 10245 and *Komgataeibacter saccharivorans* PE 5, respectively. These results showed that BC of *Acetobacter xylinum* ATCC 10245 has high water holding capacity. According to Dongping et al. [43], the results keep a close relation with BC's nanometer structure. Moreover, the more Iα crystalline component there is, the more water BC can

absorb because at the stage of water absorption the Iα crystalline component has a better water-absorption character than I_β crystals. It could be concluded that paper made from BC derived from optimized conditions, had good characteristics of special property paper named parchment paper. Yamanaka et al. [44] had reported that the native Acetobacter pellicle had mechanical properties including shape retention and tear resistance that were superior to many synthetic fibers.

They also observed that BC displays a Young's modulus value (a measure of shape retention) of 30 GPa, aproximately 4 times greater than any organic fiber. Also, Suwannapinunt et al. [30] produced BC fibrils from *Acetobacter xylinum* TISTR976 under static condition which have strong structure and parchment paper characteristics. So, BC sheet or film could be applied by industry, for example as biodegradable packaging for the food industry, home decorating goods as well as used as temporary skin for medical care and separation membrane [45].

Table 1. FT-IR spectra band assignments of pure cellulose and bacterial cellulose of investigated bacteria

Assignment, related bond	Wave number (cm-1)		
	pure cellulose	*A. xylinum* ATCC 10245 (reference strain)	*K. saccaharivorans* PE5
O-H stretching	3340 cm^{-1} to 3500 cm^{-1}	3340 cm^{-1}	3340 cm^{-1}
C-H stretching	2800 cm^{-1} to 2920 cm^{-1}	2894 cm^{-1}	2855 cm^{-1} & 2920 cm^{-1}
vibration of sugar ring			
C-O-C stretching	1160 cm^{-1}	1160 cm^{-1}	1157 cm^{-1}
C-O stretching	1035 cm^{-1} to 1060 cm^{-1}	1053 cm^{-1}	1023 cm-1
C-H bending	1300 cm^{-1}	1314 cm^{-1}	1319 cm^{-1}
CH2 bending	1400 cm^{-1}	1426 cm^{-1}	1425 cm-1

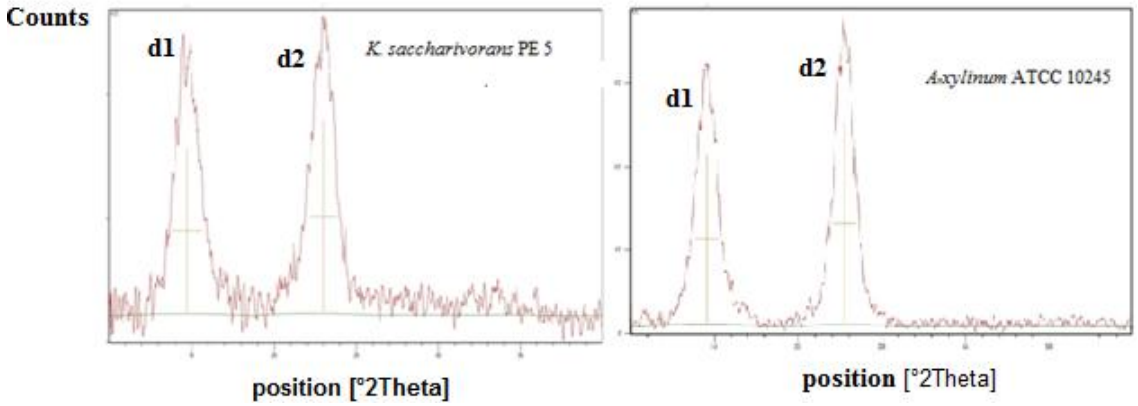

Fig. 4. X-Ray diffraction patterns of bacterial cellulose samples of *Acetobacter xylinum* ATCC 10245 and *Komgataeibacter saccharivorans* PE 5 grown in optimum conditions after 7 days

Table 2. Crystalline parameters of bacterial cellulose samples from *Acetobacter xylinum* ATCC 10245 and *Komgataeibacter saccharivorans* PE 5 determined from X-Ray diffractograms

Bacterial strain	d- spacings [Aº]		Diffraction angle, 2θ		Crystallinity index
	d1	d2	peak1	peak2	
Acetobacter xylinum ATCC 10245	6.08416	3.90395	1.43	1.33	54.14%
Komagataeibacter saccharivorans PE 5	6.01306	3.86468	1.9	1.62	52.76%

Photo. 1. BC pellicle of *Komagataeibacter saccharivorans* PE 5 and *Acetobacter xylinum* ATCC 10245 formed under optimized conditions, (K1,A1), fresh pellicle (K2,A2) and paper sheet (K3,A3)

Table 3. Mechanical and optical properties of paper sheet prepared from *Acetobacter* and *Komgataeibacter* cellulose

Propriety		*Acetobacter xylinum* paper sheet	*Komagataeiobacter saccharivorans* Paper sheet
Mechanical	Wet tensile strength (Mpa)	18.75	22.23
	Dry tensile strength (Mpa)	72.4	90.4
	Wet young's modulus (Mpa)	454	388.75
	Dry young's modulus (Mpa)	4021	3943.6
	Degree of polymerization(DP)	2181	2891
	Water absorption capacity (%)	90.07	80.29
Optical	Brightness (%)	24.4	46.5
	Opacity (%)	61.2	77.8

4. CONCLUSION

Bacterial friendly cellulose is produced from Komagataeibacter saccharivorans PE 5 and *Acetobacter xylinum* ATCC 10245 under optimized fermentation conditions. The first strain is the most efficient producer. The characterization of BC produced from two tested strains revealed that they are similar in chemical structure and like to pure cellulose powder. BC obtained were processed into paper called BC paper. Their properties suggested that BC paper could be applied by industry.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Brown RM. Cellulose structure and biosynthesis. Journal of Polymer Science: Part A: Polymer Chemistry. 2014;42:487–495.

2. Lynd LR, Weimer PJ, Vanzye WH, Pretonius IS. Microbiology and molecular biology reviews. 2001;66:506–577.
3. Yoshinaga F, Tonouchi N and Watanabe K. Research progress in production of bacterial cellulose by aeration and agitation culture and its application as a new industrial material. Biosci. Biotechnol. Biochem. 1997;61(2):219–224.
4. George J, Ramana KV, Sabapathy SN, Bawa AS. Physico-mechanical properties of chemically treated bacterial *(Acetobacter xylinum)* cellulose membrane, World J. Microbiol. Biotechnol. 2005;21:1323–1327.
5. Chawla PR, Bajaj IB, Survase SA, Singhal RS. Microbial cellulose: Fermentative production and applications. Food Technol. Biotechnol. 2009;47(2):107–124.
6. Fontana JD, de Sousa AM, Fontana CK, Torriani IL, Moreschi JC, Gallotti BJ. *Acetobacter* cellulose pellicle as a temporary skin substitute. Applied Biochemistry and Biotechnology. 1990;4: 253–264.
7. Alvarez OM, Patel M, Booker J, Markowitz L. Effectivness of biocellulose wonnd dressing for the treatment of chronic venous leg ulcers. Wounds. 2004;16:224–233.
8. Czaja W, Romanovicz D, Brown RM. Structural investigations of microbial cellulose produced in stationary and agitated culture. Cellulose. 2004;11:403–411.
9. Shah J, Brown RM. Towards electronic paper displays made from microbial cellulose. Appl. Microbiol. Biotechnol. 2005;66:352–355.
10. Bellamy WD. Single cell proteins from cellulosic wastes. Biotechnol. Bioeng. 1974;16:869–880.
11. Shoda M, Sugano Y. Recent advances in bacterial cellulose production. Biotechnology and Bioprocess Engineering. 2005;10:1–8.
12. Kojima Y, Tonouchi N, suchida TT, Yoshinaga F, Yamada Y. The characterization of acetic acid bacteria efficiently producing bacterial cellulose from sucrose: the proposal of *Acetobacter xylinum* subsp. *nonacetoxidans* subsp. nov. Bioscience Biotechnology, and Biochemistry. 1998;62(1):185-187.
13. Dellaglio F, Cleenwerck I, Felis GE, Engelbeen K, Janssens D, Marzotto M. Description of *Gluconacetobacter swingsii* sp. nov. and *Gluconacetobacter rhaeticus* sp. nov., isolated from Italian apple fruit. International Journal of Systematic and Evolutionary Microbiology. 2005;55(6): 2365–2370.
14. Lisdiyanti P, Kawasaki H, Seki T, Yamada Y, Uchimura T, Komagata K. Identification of *Acetobacter* strains isolated from Indonesian sources, and proposals of *Acetobacter syzygii* sp. nov., *Acetobacter cibinongensis* sp. nov., and *Acetobacter orientalis* sp. nov. Journal of General and Applied Microbiology. 2001;47(3):119-131.
15. Park JK, Park YH, Jung JY. Production of bacterial cellulose by *Gluconacetobacter hansenii* PJK isolated from rotten apple. Biotechnology and Bioprocess Engineering. 2003;8(2):83-88.
16. Jia S, Ou H, Chen G, Choi DB, Cho KA, Okabe M, Cha WS. Cellulose production from *Gluconobacter oxydans* TQ-B2. Biotechnology and Bioprocess Engineering. 2004;9(3):166-170.
17. Kadere TT, Miyamoto T, Oniango RK, Kutima PM, Njoroge SM. Isolation and identification of the genera *Acetobacter* and *Gluconobacter* in coconut toddy. African Journal of Biotechnology. 2008;7: 2963-2971.
18. Klemm D, Heublein B, Fink HP, Bohn A. Cellulose: Fascinating bio polymer and sustainable raw material (Review). Angew. Chem. Int. Edit. 2005;44:3358–3393.
19. Kim SY, Kim JN, Wee YJ, Park DH ,Ryu HW. Production of bacterial cellulose by *Gluconacetobacter* sp. RKY5 isolated from persimmon vinegar. Appl. Biochem. Biotechnol. 2006;131:705–715.
20. Yamada Y, Yukphan P, Vu HT L, Muramatsu Y, Ochaikul D, Tanasupawat S, Nakagawa Y. Description of *Komagataeibacter* gen. nov., with proposals of new combinations (*Acetobacteraceae*). J. Gen. Appl. Microbiol. 2012;58:397-404.
21. Lisdiyanti P, Navarro RR, Uchimura T, Komagata K. Reclassification of *Gluconacetobacter hansenii* strains and proposals of *Gluconacetobacter saccharivorans* sp. nov. and *Gluconacetobacter nataicola* sp. nov. Int.

J. Syst. Evol. Microbiol. 2006;56:2101-2111.

22. Castro C, Zuluaga R, Putaux JL, Caro G, Mondragon I, Gañán P. Structural characterization of bacterial cellulose produced by *Gluconacetobacter swingsii* sp .from Colombian agroindustrial wastes. Carbohydr. Polym. 2011;84:96-102.
23. Gromet-Elhanan Z, Hestrin S. Synthesis of cellulose by *Acetobacter xylinum*. VI. Growth on citric acid-cycle intermediates. J. Bacteriol. 1963;85:284–292.
24. Geyer U, Heinze T, Stein A, Klemm D, Marsch S, Schumann D, Schmauder HP. Formation, derivatization and applications of bacterial cellulose. Int. J. Biol. Macromol. 1994;16:343–347.
25. Jung JY, Park JK, Chang HN. Bacterial cellulose production by *Gluconoacetobacter hanseniiin* an agitated culture without living non-cellulose producing cells. Enzyme Microb. Technol. 2005;37:347–354.
26. Abdelhady Hemmat M, Hassan Enas A, Abd El-Salam Sohir S, Abdullah Sara M. Bacterial cellulose production as affected by bacterial strains and some fermentation conditions. Nature and Science. 2015; 13(3):30-40.
27. Hanmoungjai W, Chakeatiirote E, Pathom-aree W, Yamada Y, Lumyoung S. Identification of acid tolerant acetic acid bacteria isolated from Thailand sources. Research Journal of Microbiology. 2007; 2(2):194-197.
28. Gamal Rawia F, Nasser Fatma R, Abdelhady Hemmat M, EL-Sawy M. Glycerol production by osmotolerant yeast strain using fermentor as fed batch and continous culture techniques. Annals, Agric, Ain Shams Univ. Cairo. 1991;36(2): 319-321.
29. Mihranyan A, Llagostera A, Karmhag R, Strømme MR. Moisture sorption by cellulose powders of varying crystalinity. Int. J. Pharm. 2004;269:433-442.
30. Suwannapinun N, Burakorn J, Thaenthanee S. Effect of culture conditions on bacterial cellulose (Bc) production from *Acetobacter xylinum* Tistr976 and physical properties of Bc parchment paper. Suranaree J. Sci. Technol. 2007;14(4): 357-365.
31. Youngos. Effect of drugs on clinical lab. Pests, 4th ed AACC press; 1995.
32. Foster Jeremy j. Data analysis using SPSS for Windows Versions 8-10. Sage Publication Ltd., London; 2001.
33. Suwanposri A, Yukphan P, Yamada Y, Ochaikul D. Statistical optimization of culture conditions for biocellulose production by *Komagataeibacter* sp. PAP1 using soya bean whey. Maejo Int. J. Sci. Technol. 2014;8(01):1-14.
34. Saxena IM, Kudlicka K, Okuda K , Brown RM. Characterization of genes in the cellulose-synthesizing operon (acs operon) of *Acetobacter xylinum*: implications for cellulose crystallization. J. Bacteriol. 1994; 176:5735-5752.
35. Moon RJ, Martini A, Nairn J, Simonsen J, blood JY. Cellulose nanomaterials review: Structure, properties and nanocomposites. Chem. Soc. Rev. 2011;40:3941-3994.
36. Kim J, Cai Z, Lee HS, Choi GS, Lee DH, Jo C. Preparation and characterization of a bacterial cellulose/ Chitosan composite for potential biomedical application. J. Polym. Res. 2011;18:739–744.
37. Ramírez-Flores J, Rubio E, Rodríguez-Lugo V, Castaño VM. Purification of polluted waters by funtionalized membranes. Reviews on Advanced Materials Science. 2009;21:211-216
38. Marchessault RH, Sundararajan PR. Cellulose. In: Aspinall GO (ed.). The polysaccharides. New York: Academic Press, Inc. 1983;2:12-95.
39. Yu X, Atalla RH. Production of cellulose II by *Acetobacter xylinum* in the presence of 2,6-dichlorobenzonitrile. Int. J. Biol. Macromol. 1996;19:145–146.
40. Maeda H, Nakajima M, Hagiwara T, Sawaguchi T, Yano S. Bacterial cellulose/silica hybrid fabricated by mimicking biocomposites. Journal of Materials Science. 2006;41(17):564–565.
41. Yamamoto H, Horii F. CP/MAS ^{13}C NMR analysis of the crystal transformation induced for *Valonia* cellulose by annealing at high temperatures. Macromolecules. 1993;26:1313-1317.
42. Vanderhart Dl, Atalla RH. Studies of microstructure in native celluloses using solid-state ^{13}C NMR. Macromolecules. 1984;17:1465-1472.
43. Dongping SUN, Lingli ZHOU, Qinghang WU, Shulin YANG. Preliminary research on structure and properties of nano-

cellulose. Journal of Wuhan University of Technology-mater Sci. Ed. 2007;22(4): 677-680.

44. Yamanaka S, Watanabe K, Kitamura N, Iguchi M, Mitsuhashi S, Nishi Y, Uryu, M. The structure and mechanical properties of sheets prepared from bacterial cellulose. J. Materials Science.1989;24:141-3,145.

45. Lguchi M, Yamanaka S, Budhiono A. Bacterial cellulose—a masterpiece of nature's arts. J. Materials Science. 2000;35:261-270.

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