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Isolation and identification of a bacterial cellulose synthesizing strain from kombucha in different conditions: *Gluconacetobacter xylinus* ZHCJ618

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Abstract A bacterial cellulose (BC) synthesizing strain (*Gluconacetobacter xylinus* ZHCJ618) was isolated from kombucha and selected as the species for commercial applications owing to its high phenotypic stability and sustainable production capacity of 7.56 ± 0.57 g/L under static culturing conditions and 8.31 ± 0.79 g/L under shaking conditions. The morphological, physiological and biochemical characteristics of the strain were similar to those of *Gluconacetobacter* genus. The 16S rDNA sequence homologies with *G. xylinus* NCIB 11664 reached 99%, showing that the isolated strain can be identified as *G. xylinus*. The material properties of BC were studied by fourier transform infrared spectroscopy, scanning electronic microscopy, X-ray diffraction, thermogravimetric analysis, and tensile test. The results showed that BC synthesized under static conditions exhibited stronger tear strength, higher crystallinity, superior waterhold and rehydration rate than BC synthesized under shaking conditions.

Keywords Bacterial cellulose · *Gluconacetobacter xylinus* · Kombucha · 16S rDNA sequence

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Introduction

Bacterial cellulose (BC) is a gel substance synthesized by gram-negative bacteria in a liquid sugar matrix. It has the same molecular formula as plant cellulose but unique and sophisticated three-dimensional porous net-work structures. With the unique structure, BC demonstrates many excellent features such as high degree of polymerization (up to 8000), high crystallinity (of 70–80%) and water content(up to 99%), superior mechanical stability, which distinguishes it from plant cellulose [1]. These amazing physicochemical properties have aroused great interest from both researchers and industrialists. So far, BC has been widely used in various fields including food, advanced acoustic diaphragms [2–4], biosensors, tissue scaffolds, packaging, protective coatings, antimicrobial materials and flexible electronics [5, 6]. These researches and exploration have led to the emergence of more diverse potential applications exploiting the functionality of BC nanomaterials. In order to expand the scope of BC applications, it is necessary to produce BC with different structures and properties to develop novel BC nanomaterials with ground-breaking new features. Recently, many researchers have paid their attention to develop novel BC-based nanomaterials with ground-breaking new features through modifying BC by chemical methods [3]. As is known, the specific parameters of BC are determined by the biofabrication approach of BC [3], and the properties of cellulose largely depend on the specific assembling and supramolecular order controlled by the origin and treatment of cellulose [7]. So it is possible to obtain BC with different structures and properties different culturing approaches.

BC is mainly produced by microbial culturing, and many microbial species have the ability to synthesize BC,

including *Acetobacter*, *Agrobacterium*, *Rhizobium*, *Sarine*, *Pseudomonoas*, *Aerobacter*, *Achromobacter*, *Alcaligenes* and *Azotobacter* [8, 9]. Stapleton and Dobson [10] reported that *A. xylinum* produced an extra-cellular gelatinous substance, presumably BC, but in low yields. Serafica et al. [11] observed cellulose synthesis in the cell extract when *A. xylinum* was cultured in the presence of glucose and ATP. Mormino [12] used *A. xylinum* as a model bacterial system to study the synthesis of BC, and showed that static cultures and lyophilized *Acetobacter* cells could synthesize BC in the presence of glucose and oxygen. BC has many potential applications, but low yields and single properties are bottlenecks for industrial applications. Currently, studies on BC have mainly focused on screening for strains with improved BC production and optimizing of the BC synthesizing process.

Kombucha is a plaque biofilm, like jellyfish skin, called "sea treasure", which contains yeast, lactic acid bacteria and acetic acid bacteria. So far, several types of acetic acid bacteria including *A. xylinum* and *A. xylinoides*, as well as a variety of yeast and lactic acid bacteria have been found in kombucha [13]. In this study, kombucha was used to isolate strains with the ability of synthesizing BC. The isolated strains were identified through the study of physiological and biochemical properties and analysis of 16S rDNA sequence. Meanwhile, the isolated strain was cultured under static conditions and shaking conditions respectively, and products under different conditions were also identified with fourier transform infrared spectra (FT-IR), scanning electronic microscopy (SEM), X-ray diffraction (XRD), thermogravimetric analysis (TGA), and tensile test.

Materials and methods

Materials and chemicals

Kombucha was obtained from local farmers (Hanzhong, China). Celluclast (700 EGU/g) was purchased from Novozymes Biotech Co., Ltd (Beijing, China). Taq DNA polymerase, dNTPs, DNA Marker DL2000, and Wide Range DNA Marker were purchased from Takara Co., Ltd, (Dalian, China); methanol (HPLC grade) was bought from Kemiou Co., Ltd, (Tianjin, China). Other chemicals are of analytical grade and biochemical reagents obtained from factories at home.

Isolation of BC-producing strains

Kombucha film was first minced under sterile conditions, then placed in a conical flask, with sterile saline and glass beads, and shaken at 30 °C for 18–24 h. The resulting cell suspension was serially diluted and streaked at 30 °C for 2–3 days on solid medium (sucrose 5 g/L, beef extract 1.5 g/L, Na_2HP0_4 0.44 g/L, citric acid 0.08 g/L, agar 1.8 g/L, and ethanol 1 mL/L, pH 6.0) [14]. Individual transparent gel granular colonies were streaked repeatedly until single colony morphology was observed. The obtained single colonies on solid medium were preserved at 4 °C for rescreening. Then 200 μL fermentation medium (sucrose 5 g/L, beef extract 1.5 g/L, Na_2HP0_4 0.44 g/L, citric acid 0.08 g/L, and ethanol 1 mL/L, pH 6.0) was added to each well in a 96-well plate, and the prescreened strains were inoculated at 30 °C for static culture of 10 d in the wells [14]. The nutrition solution was observed in the gas–liquid contact area. The sample with gel film in the gas–liquid contact area was used as seed solution for BC production. BC yield was calculated to identify strains with the highest BC production capacity.

Identification of isolated strains

The identification of isolated strains was based on its morphological, physiological and biochemical characteristics, as well as 16S rRNA gene sequence. During the morphological characteristics study, Gram staining was applied for fresh cultures of all screened strains. The cell morphology, size, movement and presence of spores were determined by microscopy. The physiological and biochemical characteristics of the strains were tested according to methods in the Manual of Systematic Bacteriology [15] such as sugar fermentation, catalase and so on. Then the bacterial cells were collected [14], and genomic DNA was extracted for PCR on 16S rDNA with the forward primer: 5′-AGAGTTTGATCCTGGCTCAG-3′, and the reverse primer: 5′-TACGGCTACCTTGTTACGACTT-3′. PCR products were purified and sequenced by Sangon Biotech Co., Ltd. (Shanghai, China) The sequencing results were submitted to BlastN for sequence alignment and homology comparisons against the NCBI GenBank database. The 16S rDNA of representative species were used for multiple sequence alignment with ClustalX software, and the phylogenetic tree was constructed by MEGA 6.0 based on the Neighbor-Joining method with bootstrapping 1000 times.

Culture conditions

The isolated strains was inoculated into 30 mL of seed medium (sucrose 5 g/L, beef extract 1.5 g/L, Na_2HP0_4 0.44 g/L, citric acid 0.08 g/L, and ethanol 1 mL/L, pH6.0) in a 250 mL flask and incubated at 30 °C for 24 h. After incubation, 20 mL of broth was transferred into 200 mL of fermentation medium in a 500 mL flask at 30 °C for static culture for 10 d. The synthesized BC film was immersed

into 0.10 mol/L NaOH solution, soaked at 80 °C for 30 min, then boiled for 2 h, followed by repeated washing with distilled water until pH reached 7.0. Then the wet BC film was lyophilized [2]. The water holdup, rehydration rate and yield of BC film were measured by weighing according the following equations [2]:

$$\text{water holdup rate } (\%) = \frac{M_w - M_d}{M_w} \times 100;$$

$$\text{rehydration rate } (\%) = \frac{M_{wr} - M_d}{M_w - M_d} \times 100$$

where M_w represents the mass of the wet film, M_d represents the mass of the dry film, and M_{wr} is the mass of the wet file after rehydration.

The strains with the highest BC production capacity were not only cultured under static conditions, but cultured under shaking conditions at 150 rpm as well.

BC identification

The BC film was subjected to Infrared Spectra, TLC and HPLC for identification. The dried BC film was measured by infrared spectrometer using a VERTEX 70 Fourier transform IR spectrometer (Germany) at 450 mW, with a scan range of 400–4000 cm^{-1}, a resolution of 4 cm^{-1}, and a scanning speed of 0.2 cm/s at room temperature. Then the dried BC film was chopped with scissors, suspended in citrate buffer with pH 5.0, and hydrolyzed at 55 °C for 24 h with cellulase Celluclast (40 EGU/g). Hydrolyzed products were filtered with filter paper, 0.45 μm filter membrane, followed by thin layer chromatography in developing solvent (eluent ethanol: aqueous ammonia = 5: 1, v/v) with 2 mg/mL analytically pure glucose as a control. After being launched, the thin layer board was taken out for air drying and fumigated with iodine vapor. BC hydrolysate was identified based on R_f values against the control. Meanwhile, BC hydrolysate was analyzed by HPLC detection. During the testing, the parameters in the test are as follows: the HPLC was Waters 1525/2414, the column was Waters Sugar Pak I column (300 mm × 6.5 mm, 6.5 μm) with the column temperature 80 °C; the detector was differential refractive index detector of with temperature of 40 °C; the mobile phase was mixed solvent (acetonitrile: water of 77: 23, v/v) with the flow rate of 1.0 mL/min and the injection volume of 20 μL.

BC properties

The properties such as water holdup, rehydration rate, mechanical properties, thermal properties, microstructure, and crystallinity of BC synthesized by isolated strains under static conditions and shaking conditions was studied using modern instruments. The surface morphology of BC film was determined by SEM [2]. After treatment, the dried BC film was fractured in liquid nitrogen and sprayed with platinum, then observed and photographed on a Hitachi S-4800 scanning electron microscope with a voltage of 25 kV. The mechanical property of BC film was determined by tensile test using a TY8000 tensile testing machine [3]. The BC wet film was placed on release paper and cut into certain shape as required with a gauge of 50 mm and a width of 10 mm, then the tensile strength was measured with a test speed of 100.000 mm/min. The thermal property of BC film was determined by the thermal gravimetric method [16]. The dried BC film was placed in TG/DTA7300 thermogravimetric analyzer, at a heating rate of 10 °C/min under the temperature between 20 and 800 °C in N_2 atmosphere, and the mass change was measured at sample temperature to get TGA curve. The crystallinity was determined by XRD spectrum using a D/Max2200PC diffractometer. The dried BC film was fixed on a copper target on a sample holder with the test voltage of 40 kV, current of l00 mA, rate of 5°/min, step width of 0.02° and scan range of 0–80°. The crystallinity (Xc) and the crystal particle diameter of bacterial cellulose (L) could be calculated according to the following equations:

$$X_c\ (\%) = \frac{I - I_{am}}{I} \times 100; \quad L = \frac{k\lambda}{\beta \cos\theta}$$

where I represents diffraction intensity of diffraction peak, I_{am} represents diffraction intensity in the amorphous region, β represents half-value width (rad); k represents a constant, usually 0.89, λ represents X-ray wavelength (0.15406 nm), and θ represents the Bragg diffraction angle.

Results and discussion

Strain isolation and identification

After the isolation, it was observed that 50 strains were able to produce gel film at gas–liquid interface in 96-well plates. Further static culturing in flask shows that most of them could only produce small amount of film. Among them, 5 strains were tested to be able to produce more than 2 g/L. The strain with a yield of 7.56 g/L was singled out to be the target strain, and was named as ZHCJ618. When strain ZHCJ618 was cultured on solid plate, transparent gelatinous colonies with smooth moist and neat edges were obtained. The colony was difficult to be dispersed with an inoculation loop [Fig. 1(A)–(C)]. With increased growth, the center of the colonies gradually projected [Fig. 1(C)]. As is shown in Fig. 1(D), under 400× magnification, the bacterial cells appeared in short rod shape and were Gram-negative. Colony and cell morphology were consistent with

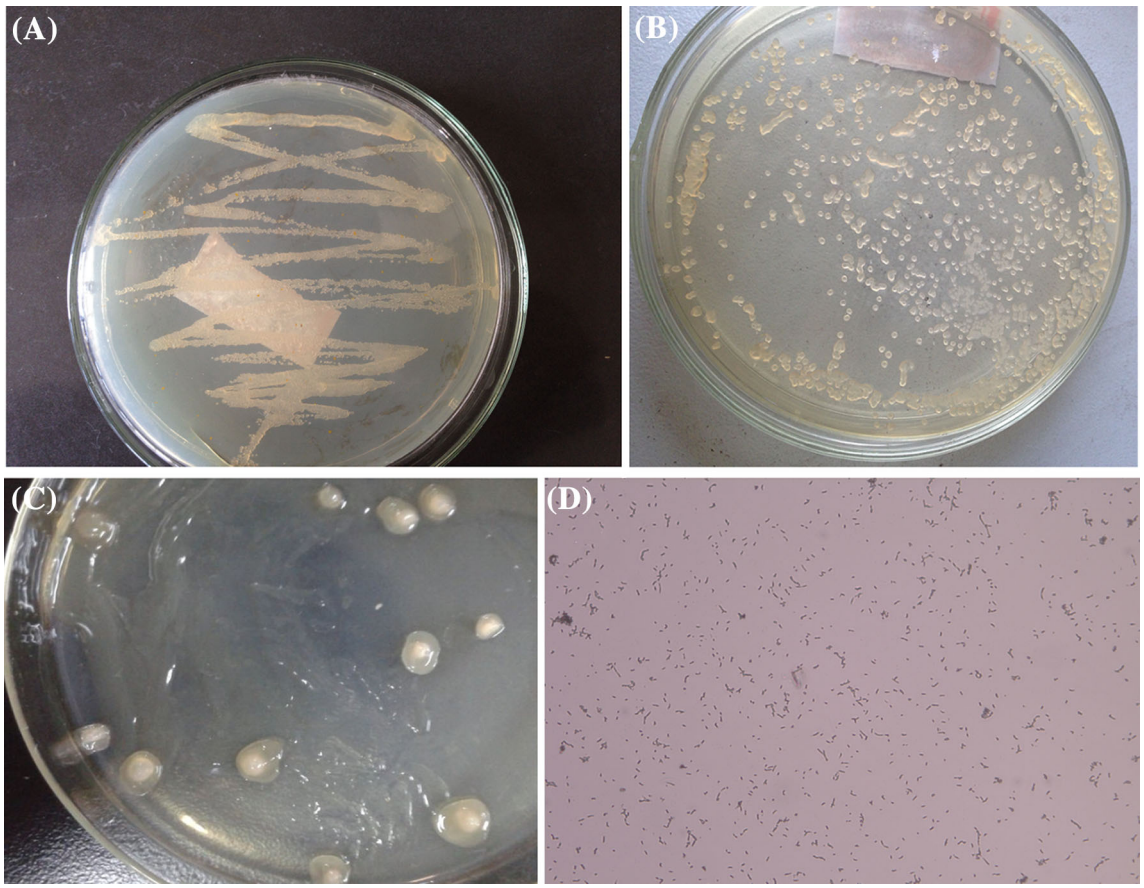

Fig. 1 Colonial morphology (**A**)–(**C**) and cell morphology (**D**) of strain ZHCJ618

the *Gluconobacter* in Manual of Systematic Bacteriology [15], indicating that they should be classified as *Proteobacteria*, *Alphaproteobacteria*, *Rhodospirillales*, *Acetobacteraceae*, *Gluconacetobacter*. The physiological and biochemical test results of strain ZHCJ618 are shown in Table 1. Based on Manual of Systematic Bacteriology [15] and in combination with morphological results, the target strain was further identified as *Gluconacetobacter*.

The 16S rDNA sequence of the strain ZHCJ618 (GenBank accession no. MG451840) had a length of 1390 bp. As is shown in Fig. 2, the phylogenetic tree was constructed using the 16S rDNA gene sequences of the isolates ZHCJ618, suggesting that ZHCJ618 had the maximum homology with species of *Gluconacetobacter xylinus* NCIB 11664 (99%), when compared with the 16 s rDNA sequences of other microorganisms available in the databases.

To sum up, a BC synthesizing strain named *G. xylinus* ZHCJ618 was isolated from kombucha and selected as the species with the highest potential for commercial applications owing to its high phenotypic stability after five consecutive batches, in addition to its sustainable production capacity of 7.56 ± 0.57 g/L BC under static cultivation conditions. This value was significantly higher than the production capacity of *G. xylinus* K3 (0.217 ± 0.027 g/L) reported by Nguyen et al. [17], *G. hansenii* PJK (0.35 g/L) reported by Park et al. [18], and *G. hansenii* P2A (1.89 g/L) reported by Aydin et al. [19]. Yet, it is possible to enhance production capacity by manipulation of medium composition [20, 21] or cultivation conditions [22, 23]. The morphological, physiological and biochemical characteristics of this strain, as well as 16S rDNA sequence alignment results showed that the isolated strain can be identified as *G. xylinus*.

Identification of BC

The IR results of the fermentation product are shown in Fig. 3. The IR Spectra of product synthesized either through static culturing (BC-ST) or shaking culturing (BC-SK) were almost the same. Absorption peaks were found at 3344, 1163, 1111 and 1060 cm^{-1}, suggesting the presence of a large number of –OH; absorption peaks at 2916, 1420, 1336, 667 and 623 cm^{-1}, indicated the presence of $–CH_2–$, > CH– and CH; absorption peaks at 1280 and 877 cm^{-1}, demonstrated the presence of C–O–C ring; absorption

Table 1 Physiological–biochemical characteristics of strain ZHCJ618

Tests	Results
> 60 °C	No growth
Growth under anaerobic condition	Negative reaction
ethanol as the sole carbon source	Positive reaction
Ethanol oxidation test	Positive reaction
Catalase test	Positive reaction
Cytochrome oxidase test	Negative reaction
Nitrate reduction	Negative reaction
Indole test	Negative reaction
2-Keto gluconate	Positive reaction
5-Ketone gluconate	Positive reaction
Hydrogen sulfide	Negative reaction
Catalase test	Positive reaction
Gelatin liquefaction	Negative reaction
Lactic acid oxidation test	Negative reaction
Glucose zymolysis test	Positive reaction
Methyl red test	Positive reaction
Glycerin ketogenic	Positive reaction
Acetic acid oxidation test	Negative reaction

peaks at 1235 and 1035 cm^{-1}, revealed the presence of a linear C–O–C. These results were characteristic for the absorption spectra of BC glucans, indicating the component of products was BC [16, 24]. Product hydrolysate was subjected to TLC analysis, with analytically pure glucose as control. Compared with the glucose, the hydrolyzed sample had a same R_f value with glucose, indicating that the product could be hydrolyzed into glucose, suggesting that the product was a polymer of glucose. HPLC detection results showed that when glucose and the hydrolysates were analyzed by HPLC under the same chromatographic conditions, the characteristic peak of the standard glucose

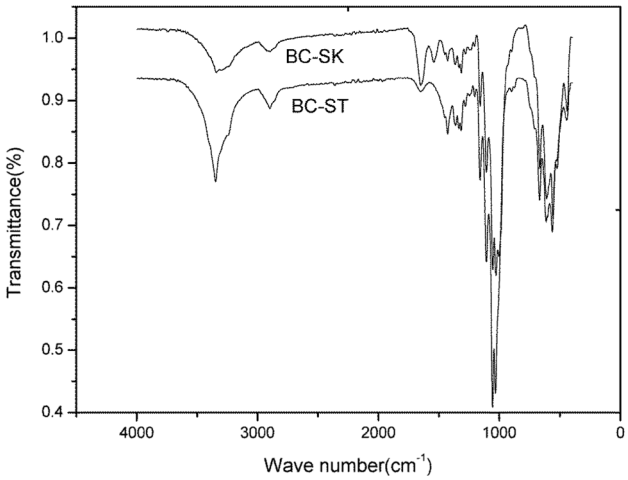

Fig. 3 FT-IR spectrumcolonial of products under different culturing condition (BC-ST: BC synthesized by strain *G. Xylinus* ZHCJ618 under static condition; BC-SK: BC synthesized by strain *G. Xylinus* ZHCJ618 under shaking condition)

appeared at 16 min, and in the HPLC chromatogram of the hydrolysates, a chromatographic peak appeared at the same time, suggesting the presence of glucose. It was most probably produced during the BC hydrolytic process, further indicating that the fermentation product was a polymer of glucose. Apart from glucose peaks, other peaks were found in the HPLC chromatogram of products' hydrolysates, suggesting that there was some incomplete hydrolysis of cellulose. Combined with infrared detection results, the product of strain *G. xylinus* ZHCJ618 was proved to be BC.

BC properties

The properties of cellulose largely depend on the specific assembling and supramolecular order controlled by the

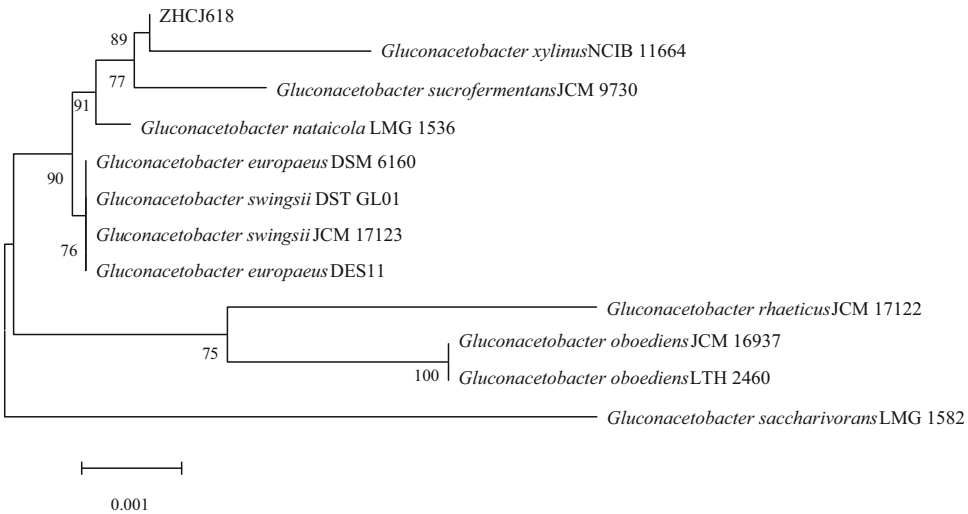

Fig. 2 Phylogenetic tree for strain ZHCJ618 based on the 16S rDNA sequence

origin and treatment of cellulose [7]. So cultivation conditions would affect structures and properties of BC synthesized by *G. xylinus* ZHCJ618.

Cultured under static conditions, a thick film was found on the gas–liquid interface on the following day. With the extension of culturing time, BC film became thicker, and filled all the nutrition solution at last. As a result, hard and dense BC film was formed. *G. xylinus* is aerobic, so when cultured under static conditions, there was not enough dissolved oxygen in internal nutrition solution, As a result, only cells in the gas–liquid contact area could grow and synthesize BC with oxygen in the air. However, under shaking conditions, plenty of dissolved oxygen was available in internal nutrition solution for cells to breathe, so BC could be synthesized during the experiment, lump BC was formed in internal nutrition solution on the following day of the culturing period. With the extension of culturing time, BC lump became bigger and bigger, and BC product was formed quite different from BC product under static conditions.

Morphological structure was studied in detail using SEM images as is shown in Fig. 4. The scanning electron micrographs of BC synthesized under static culturing conditions (BC-ST) and shaking culturing conditions (BC-ST) showed that microfibrils of BC was synthesized when the cellulose molecules were secreted outside the cell surface through pores during cell growth [Fig. 4(A), (B)], then forming fibre bundle [Fig. 4(C)]. The cellulose molecules were interconnected by hydrogen bonds, forming nanoscale crimped winding microfibrils, with a large number of micro-fibrils connected by hydrogen bonds, forming a network structure [Fig. 4(D), (E), (G), (H)]. These structures were then connected to each other with hydrogen bonds in a multi-layered network, giving the formation of a unique layered three-dimensional network structure [Fig. 4(F), (I)] [2].

However, culture conditions affected morphological and structural properties of BC. As shown in Fig. 4, the fibrils in BC synthesized under static culturing lined up as a criss-cross dense network [Fig. 4(D), (E)]. The cross-sectional view [Fig. 4(F)] showed that the mat was composed of ordered interconnected layers, and the fibers between layers served as connection and could form a large amount of longitudinal holes which ensured good behavior of BC in water-holding and permeability. Together with the vertical hole existing in each layer. Meanwhile, because of its highly ordered arrangement of fibers, BC-ST tended to exhibit a hard and dense BC film during its formation. On the other hand, BC synthesized under shaking culturing showed that the irregular fibrils were much looser than those of BC-ST [Fig. 4(G), (H)]. The cross-sectional view [Fig. 4(I)] showed that BC-SK also had a multi-layered three-dimensional network structure, which was determined by the way of microfibrils' assembling. However, compared with the structure of BC-ST, the layered structure of BC-SK was much irregular and few longitudinal holes were found between layers. During the BC formation, the cells are in a dynamic environment, the secreting and assembling of BC microfibrils would be damaged, so it is difficult for BC to form ordered layer structure and hard and dense network. The difference in aggregation behavior of cellulose fibrils induced by cultivation conditions led to other property changes such as mechanical property, crystallinity and water holdup rate as shown below.

The XRD results of BC by strain *G. xylinus* ZHCJ618 showed that the X- ray pattern of both BC-ST and BC-SK had three diffraction peaks near 14°, 17° and 22°, corresponding to the crystal plane <101>, $<\overline{101}>$ and <002> of crystalline cellulose, indicating that BC synthesized by strain *G. xylinus* ZHCJ618 was type I cellulose [3]. However, as the physical properties of BC showed, the crystallinity of BC-ST was 55.78%, which was significantly higher than that of BC-SK (25.61%). The results above showed that, the crystal type of cellulose synthesized by strain *G. xylinus* ZHCJ618 would not be influenced by the shaking conditions but their crystallinity would decrease. This is probably because of the dynamic environment during the shaking culturing, which damaged the regularity of the bacterial cellulose chain, leading to the irregularity of the crystalline structure.

The tensile strength of BC-ST and BC-SK by strain *G. xylinus* ZHCJ618 were tested. As for BC-ST, the tear strength was 138.77 MPa, the break elongation rate was 6.31%, Young's modulus was 3.55 GPa. For BC-SK, the tear strength was 111.01 MPa, the break elongation rate was 5.05%, and Young's modulus was 3.82 GPa. The tear strength and the break elongation rate of BC-SK decreased, while the Young's modulus increased, indicating that the strength of the material decreased, which was attributed to the decrease of the regularity of crystalline structure and of the crystallinity of BC. As the SEM result showed, there were fewer holes within the BC-SK, which led to the lower porosity and break elongation rate. All these were consistent with the results above.

Thermogravimetric analysis (TGA) is a continuous process, involving the measurement of sample weight in accordance with increasing temperature in the form of programmed heating. Generally, TGA curves of samples produced under static and shaking conditions were similar (Fig. 5), and both of them had three stages of weight loss that could be used to derive the chemical composition. The first phase: from room temperature to 220 °C, the percentile weight loss for BC-ST and BC-SK were 2.8, 3.2% respectively, which was mainly from the loss of moisture

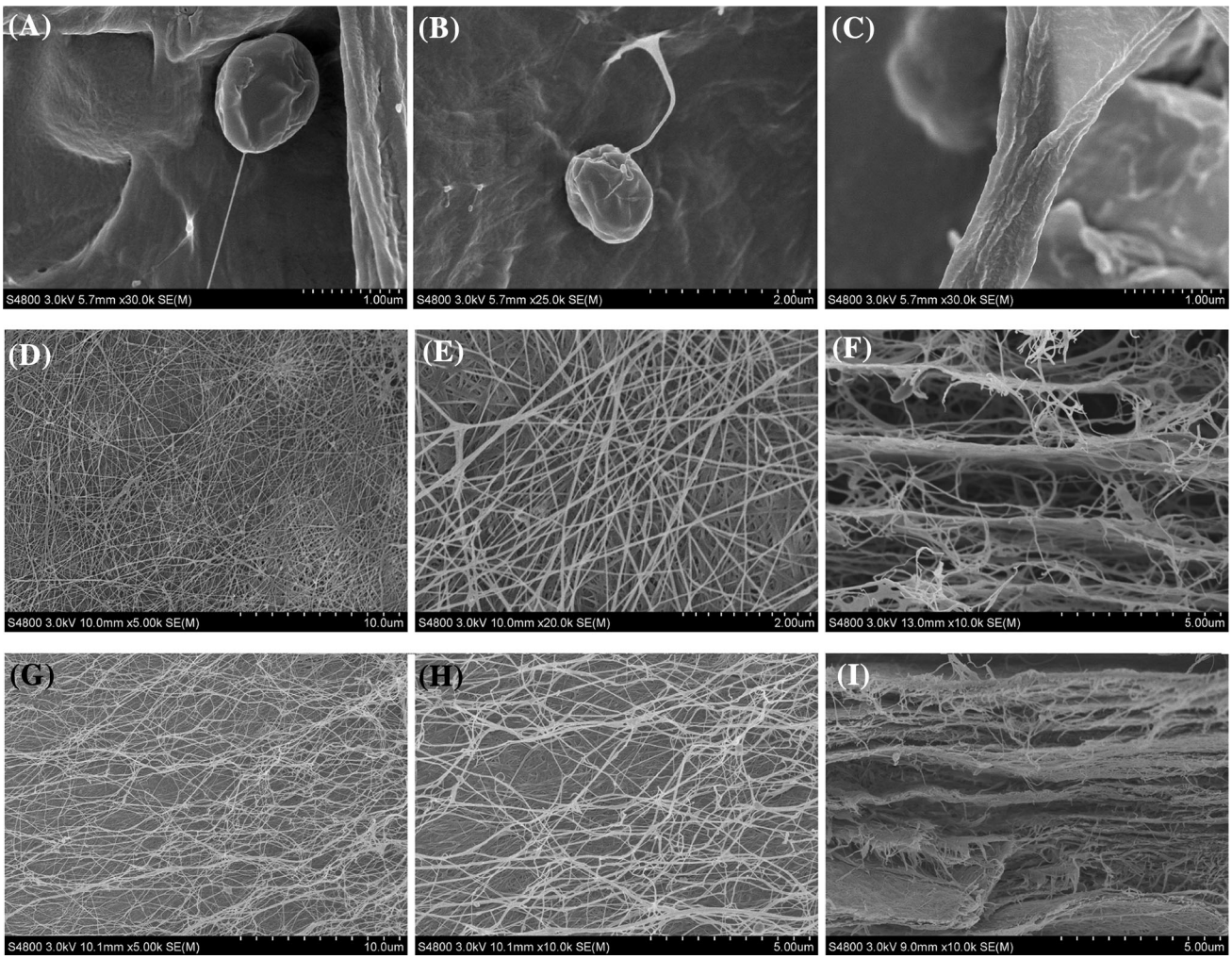

Fig. 4 SEM of BC synthesized by strain *G. Xylinus* ZHCJ618. (**A**), (**B**): The cellulose are secreted outside the cell surface. (**C**) Microfibril curly form fiber bundle. (**D**), (**E**), (**F**) BC synthesized under static condition, of which, (**D**), (**E**) show the vertical view of BC, and (**F**) shows the cross-sectional view of BC-ST. (**G**)–(**I**) BC synthesized under shaking condition, of which, (**G**), (**H**) show the vertical view of BC, and I shows the cross-sectional view of BC-SK

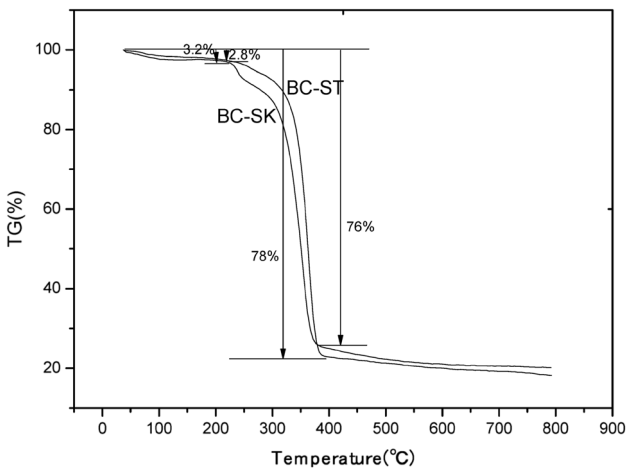

Fig. 5 TG curves of BC synthesized by strain *G. Xylinus* ZHCJ618 under different culturing condition (BC-ST: BC synthesized under static condition; BC-SK: BC synthesized under shaking condition)

existing in BC, revealing that BC-ST and BC-SK were stable under 220 °C. The second phase: from 220 to 400 °C, the percentile weight loss for BC-ST and BC-SK were 78, 76% respectively, which was mainly caused by BC pyrolysis. In the third phase, the percentile weight loss for BC-ST and BC-SK were not noticeable from 400 to 780 °C, indicating there were probably some residues such as cells, lipoprotein etc. in BC. TGA result showed that BC synthesized by strain *G. xylinus* ZHCJ618 had good thermostability, and the culturing conditions of shaking culturing had little influence on thermal performance of BC.

Then the physical properties of BC-ST and BC-SK in this work were summarized. BC yield of strain *G. xylinus* ZHCJ618 through static culturing and shaking culturing was 7.56 and 8.31 g/L respectively. The higher yield of BC-SK probably related to the plenty of oxygen caused by shaking conditions. During its synthesizing, cells could

grow rapidly and synthesize BC efficiently. As mentioned above, both BC-ST and BC-SK formed unique layered three-dimensional network structures, but because secreting and assembling of BC microfibrils was damaged by shaking conditions the fibrils became much looser, the crystalline structure was in disorder and fewer longitudinal holes appeared. The highly ordered arrangement of fibers made BC-ST exhibiting stronger tear strength and higher crystallinity than BC-SK. Because of the layered three-dimensional network structures and more longitudinal holes, BC-ST had higher waterhold rate of 96.67% and rehydration of 74.10%, while BC-SK had a waterhold rate of 85.45% and rehydration of 64.28%. Also, with higher porosity, BC-ST showed higher elongation at the break point.

In summary, BC synthesized by *G. xylinus* ZHCJ618 under shaking conditions (BC-SK) exhibited different properties compared with BC under static conditions (BC-ST). BC-ST exhibited stronger tear strength and higher crystallinity than BC-SK. BC-ST had higher waterhold rate and rehydration. However, when cultured under shaking conditions, BC productivity could be upgraded to 8.31 ± 079 g/L in contrast to previous literature [25], suggesting the importance of efficient aeration [19]. So different culturing conditions could be chosen according to the application field of BC. BC-ST could be applied in fields which need material having superior mechanical strength such as tissue scaffolds, acoustic diaphragms, biosensors and so on. BC-SK could be used as dietary fiber in food. Due to the significant production capacity and the different properties of the product under different culturing conditions, *G. xylinus* ZHCJ618 would be a candidate microorganism for commercial production of BC. And further studies would be planned for the development of an optimized medium and determination of optimal culture conditions such as pH and temperature aiming for enhancement of the yield and production capacity.

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References

1. Barud HS, Rodrigo TR, Marques FC, Lustri WR, Messaddeq Y, Ribeiro SJL. Antimicrobial Bacterial Cellulose-Silver Nanoparticles Composite Membranes. J. Nanomater. 8: 1–8 (2011)
2. Cai ZJ, Yang G. Bacterial cellulose/collagen composite: Characterization and first evaluation of cytocompatibility. J. Appl. Polym. Sci. 120: 2938–2944 (2011)
3. Saska S, Teixeir LN, Oliveira PTD, Messaddeq Y. Bacterial cellulose-collagen nanocomposite for bone tissue engineering. J. Mater. Chem. 22: 22102–22112 (2012)
4. Klemm D, Kramer F, Moritz S, Lindstrom T, Ankerfors M, Gray D, Dorris A. Nanocelluloses: a new family of nature-based materials. Angew. Chem. Int. Ed. 50: 5438–5466 (2011)
5. Gao C, Yan T, Dai K, Wan Y. Immobilization of gelatin onto natural nanofibers for tissue engineering scaffold applications without utilization of any crosslinking agent. Cellulose. 19: 761–768 (2012)
6. Liu B, Zhang Z, Huang K. Cellulose sulfuric acid as a bio-supported and recyclable solid acid catalyst for the synthesis of 5-hydroxymethylfurfural and 5-ethoxymethylfurfural from fructose. Cellulose. 20: 2081–2089 (2013)
7. Eichhorn SJ, Dufresne A, Aranguren M, Marcovich NE, Capadona JR, Rowan SJ, Weder C, Thielemans W, Roman M, Renneckar S. Review: Current international research into cellulose nanofibres and nanocomposites. J. Mater. Sci. 45: 1–33 (2010)
8. Bungay HR, Serafica. Production of microbial cellulose. U.S. Patent, 6,071,727 (1997)
9. Xie JJ, Hong F. Recent Progress in fermentation feedstocks of bacterial cellulose. Journal of Cellulose Science and Technology. 19: 68–77 (2011) **(in Chinese)**
10. Stapleton PC, Dobson ADW. Carbon repression of cellobiose dehydrogenase production in the white rot fungus trametes versicolor is mediated at the level of gene transcription. FEMS Microbiol. Lett. 221: 167–172 (2003)
11. Serafica G, Mormino R, Bungay H. Inclusion of solid particles in bacterial cellulose. Appl. Microbiol. Biotechnol. 58: 756–760 (2002)
12. Mormino R. Incorporation of common cellulose into bacterial cellulose. Ph. Thesis, Rensselaer Polytechnic Institute, Troy, N. Y.
13. Shen XK. Review of kombucha research progress. Ind. Sci. Tribune. 11: 104–105 (2012) **(in Chinese)**
14. Zhang W, Qi XJ. Studies on the method of collecting the cells Producing Bacterial Cellulose. Food Ind. Sci. Technol. 27: 57–58 (2006) **(in Chinese)**
15. Dong XZ, Cai MY. Manual of Commonly Determinative Bacteriology. Science Press, Beijing, China, pp. 364–390 (2001) **(in Chinese)**
16. Zhang LP, Lu HM, Peng XP, Dai R. Study on structure and properties of bacterial cellulose produced by trickling fermentation. Food Ind. Sci. Technol. 33: 197–201 (2012) **(in Chinese)**
17. Nguyen VT, Flanagan B, Mikkelsen D, Ramirez S, Rivas L, Gidley MJ, Dykes GA. Spontaneous mutation result in lower cellulose production by a *Gluconacetobacter xylinus* strain from Kombucha. Carbohydr. Polym. 80: 337–343 (2010)
18. Park JK, Park YH, Jung JY. Production of bacterial cellulose by *Gluconacetobacter hansenii* PJK isolated from a rotten apple. Biotechnol. Bioproc. Eng. 8: 83–88 (2003b)
19. Aydin YN, Aksoy ND. Isolation and characterization of an efficient bacterial cellulose producer strain in agitated culture: *Gluconacetobacter hansenii P2A*. Appl. Microbiol. Biotechnol. 98: 1065–1075. (2014)
20. Oikawa T, Morino T, Ameyama M. Production of cellulose from D-arabitol by *Acetobacter xylinum* KU-1. Biosci. Biotechnol. Biochem. 59:1564–1565 (1995)
21. Son HJ, Kim HG, Kim KK, Kim HS, Kim YG, Lee SJ. Increased production of bacterial cellulose by *Acetobacter sp.* V6 in synthetic media under shaking culture conditions. Bioresour. Technol. 86: 215–219 (2003)
22. Park K, Jung JY, Park YH. Cellulose production by *Gluconacetobacter hansenii* in a medium containing ethanol. Biotechnol. Lett. 25: 2055–2059 (2003a)

23. Czaja W, Romanovicz D, Brown RMJ. Structural investigations of microbial cellulose produced in stationery and agitated culture. Cellulose.11: 403–411 (2004)
24. Rajalaxmi D, Marcus F, Arthur JR. Improving the mechanical and thermal properties of gelatin hydrogels cross-linked by cellulose nanowhiskers. Carbohydr. Polym. 91: 638–645 (2013)
25. Shoda M, Sugano Y. Recent advances in bacterial cellulose production. Biotechnol. Bioproc. Eng. 10: 1–8 (2005)