

PRODUCTION AND CHARACTERIZATION OF BACTERIAL CELLULOSE FROM *KOMAGATAEIBACTER XYLINUS* ISOLATED FROM HOME-MADE TURKISH WINE VINEGAR

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In this research, bacterial cellulose (BC) was produced from *Komagataeibacter xylinus* S4 isolated from home-made wine vinegar (Denizli-Çal) and characterized through morphological and biochemical analyses. *K. xylinus* was identified by 16S rDNA sequence analysis. The wet (51.8-52.8 g) and dry (0.43-0.735 g) weights of the produced BC were measured. The morphology of cellulose pellicles was examined by scanning electron microscopy (SEM) and a dense nanofiber network was observed. TGA analysis showed that the weight loss in the dehydration step in the BC samples occurred between 50 °C and 150 °C, while the decomposition step took place between 215 °C and 228 °C. Also, the cytotoxic effect, moisture content, water retention capacity and swelling behavior of BC were evaluated. *In vitro* assays demonstrated that BC had no significant cytotoxic effect. It was found that BC had antibacterial and antibiofilm potential (antibacterial effect > antibiofilm effect). All the results clearly showed that the produced BC can be considered as a safe material for different purposes, such as wound dressings.

Keywords: *Komagataeibacter xylinus*, bacterial cellulose, antibiofilm, cytotoxic activity, SEM, FTIR, TGA

INTRODUCTION

Microorganisms secrete a wide range of polymers, which have potential for use in different areas of the industry. Due to their complex physical and chemical structure, as well as biological properties, these biopolymers have economic and ecological value. Cellulose is one of the bacterial products and the most common biopolymer on earth. Bacterial cellulose is an extracellular polymer, which is the first product of cell metabolism and acts as a preservative. On the other hand, plant cellulose acts as a component of the cell. Plants are the largest source of cellulose in the world, but the world's resources are limited. Therefore, using microorganisms as an alternative to plants for cellulose production is important.

Bacterial cellulose (BC) is an exopolysaccharide produced by various bacteria, such as *Acetobacter*, *Gluconacetobacter*, *Agrobacterium*, *Rhizobium*, *Cyanobacteria* and *Salmonella*.¹⁻⁶ In recent years, there has been an increasing interest in bacterial cellulose as an important alternative to plant cellulose, due to its physical and chemical properties. BC has a high degree of purity and polymerization. In addition, its crystallization index is higher than that of plant cellulose. Moreover, its high tensile strength and high water holding capacity also provide potential for use in the paper and food industries. Moreover, it has potential applications in various industries, such as nano-paper, textiles, medicine, food, tissue engineering and nanotechnology.⁷⁻¹²

In large-scale BC production, the cost effectiveness is mainly dependent on the modification of known strains, finding novel producer strains or improving the BC production conditions. Therefore, the cellulose production conditions of bacteria must be optimized or new bacteria that can produce high amounts of cellulose should be screened. In the literature, many researchers have investigated the effects of novel isolated strains, different media, carbon and nitrogen sources, culture conditions, static and agitated incubation on the production of bacterial cellulose.^{2,13-17}

In the present study, we aimed to determine the ability of BC production of a local isolate *Komagataeibacter xylinus* S4 strain in HS-modified with various carbon sources, such as glucose,

mannitol, sucrose, arabinose and lactose. Also, the obtained BC was characterized by FTIR, SEM and thermal analysis, as well as in terms of its water holding capacity, antimicrobial and cytotoxic activities.

EXPERIMENTAL

Strain isolation of and identification

The strain S4 was isolated from home-made Turkish vinegar (Denizli-Çal, Turkey). First, the BC membrane was washed with saline water containing PBS. After that, for isolation and in all manipulations, Hestrin-Schramm (HS) medium was used (g/L): glucose 20; disodium hydrogen phosphate, 2.7; citric acid, 1.15; peptone 5; yeast extract, 5; agar, 14.¹⁸ The DNA of the isolated S4 strain was isolated by using the GeneJET Genomic DNA Purification Kit (Thermo Scientific) according to the manufacturer's procedure. The 16S rDNA gene was amplified by using the primers 27F (AGAGTTTGATCCTGGCTCAG), 529F (GTGCCAGCMGCCGCGG) and 1491R (ACGGCTACCTTGTTACGACTT) on a Thermalcycler (QLS Optimus 96G). The polymerase chain reaction (PCR) mixtures consisted of 15 µl 2X Amp Master Taq (GeneAll), 2 µl for reverse and forward primers, 5 µl DNA template and 6 µl PCR grade H₂O. The PCR products were checked by using 1% agarose gel electrophoresis. The PCR products were isolated from the gel and sequenced. The bacterial strain was identified by 16S rDNA analysis (Triogen Biotechnology, Istanbul, Turkey).

Biochemical and physiological tests

For the phenotypic and chemotaxonomic characterization of the S4 strain, morphological, physiological and biochemical tests were carried out (Table 1). The acetic acid production of the S4 strain was confirmed by Carr medium (g/L: yeast extract, 30; Bromocresol Purple, 0.022; agar, 20; ethyl alcohol 20 mL/L) and Frateur medium (g/L: glucose, 0.5; peptone, 3; yeast extract, 5; calcium carbonate, 15; agar, 12; ethyl alcohol, 15 mL/L).¹⁹ In order to observe the cellulose production activity of the S4 strain on the solid medium, a fluorescent brightener 28 (FB28, Calcofluor White), with non-specific fluorochrome binding to cellulose, was added at 0.2 g/L in HS medium and the colonies on the surface of the agar medium were examined under UV.²⁰

Bacterial cellulose production, culture conditions and bleaching of cellulose

The S4 strain was incubated at 30 °C for 4-14 days in HS medium. At the end of the incubation period, the BC formed on the surface of the medium was gently taken and kept in 0.1 M NaOH at 80 °C for 1 h. After the BC was washed with dH₂O until the BC reached neutral pH,²¹ it was lyophilized and stored at -20 °C for analyses.

Water absorption

The water absorption capacity of the BC sample was determined by the method described by Lin *et al.*²² with slight modifications. Briefly, lyophilized and dried BC membranes were cut into small pieces (4x4 cm) and were weighed (labelled as W_{dry}). The material was merged into water and incubated at 25 °C for 24 h. Specimens were removed at certain intervals and then weighed (W_{wet}) after removing excess water. The water absorption was determined by calculating the increase in weight percent by the formula:

$$\text{Water absorption (\%)} = [(W_{\text{wet}} - W_{\text{dry}}) / W_{\text{dry}}] \times 100 \quad (1)$$

Similarly, the moisture content of bacterial cellulose was calculated by using the following formula. Two replications were performed for this experiment.

$$\text{Moisture content (\%)} = [(W_{\text{wet}} - W_{\text{dry}}) / W_{\text{wet}}] \times 100 \quad (2)$$

Water retention

The water retention capacity of bacterial cellulose was determined according to the method of Lin *et al.*²² The lyophilized and dried bacterial cellulose membranes were weighed to determine their initial dry weight (W_{dry}). Then, these materials were soaked into deionized water for 24 h. After this duration, the membranes were taken out from water and excess water was removed by using filter paper. The weights of the samples placed on an open plate were measured at certain time periods (W_{wet}). The percent water retention of BC was calculated by using the following formula. Two replications were performed for this experiment.

$$\text{Water retention (\%)} = [(W_{\text{wet}} - W_{\text{dry}}) / W_{\text{dry}}] \times 100 \quad (3)$$

Cell culture and cytotoxicity assay

Human embryonic kidney 293 cells line (HEK293) were obtained from the European Collection of Cell Cultures (ECACC, UK). Cells were cultured in DMEM, including 10% FBS and 1% penicillin/streptomycin mixture, in a humidified atmosphere (95% air with 5% CO₂) at 37 °C. HEK293 cells were grown in 96-well plates at a density of 1x10³ cells/mL culture medium. After 24 h of growth, the medium was removed, and cells were treated with extracted bacterial cellulose. The extraction was performed as described in Lin *et al.*²² with

slight modifications. Briefly, 2x2 cm cut bacterial cellulose was extracted at 37 °C for 24 h in culture media in a shaker. After 24 h, the medium was removed and filtered. An equal amount of medium without extract was added to untreated cells (control). Cellulose treated and control cells were incubated for 48 h. Following incubation, medium containing floating cells were removed, and attached cells were treated with crystal violet [0.5% (w/v) in 10% ethyl alcohol]. The dye absorbed by live cells was solubilized with sodium citrate (0.1 M in 50% ethyl alcohol). Colour intensity was determined at 630 nm. Three replicated wells were used for each experimental condition. Viability was expressed as a percentage of the control.

Antibiofilm activity

The antibiofilm activity of cellulose was determined according Jain *et al.*²³ TSB medium, *S. aureus* ATCC 29213 and cellulose discs (2x2 mm) were added in 96-well microplates. For biofilm production, the plates were incubated at 37 °C for 24 h. At the end of the period, the samples were washed three times with sterile PBS (phosphate buffer saline) and dried. The wells were stained for 15 minutes by 0.1% crystal violet and washed with PBS. Finally, 33% glacial acetic acid was added to each well and the absorbance at 630 nm wavelength was read by a Microplate Reader (Optic Ivymen System 2100-C). The biofilm inhibition of BC was calculated by using the following formula:

$$\text{Biofilm inhibition (\%)} = [(OD_{\text{final}} - OD_{\text{initial}}) / OD_{\text{initial}}] \times 100 \quad (4)$$

Antibacterial activity

S. aureus ATCC 33862, *E. coli* ATCC 25922, *E. coli* O: 157 H: 7, *B. cereus* RSKK 863, *B. pumilis* NRRL-BD-142 and *E. faecalis* ATCC 19433 were used. The bacteria were obtained from Pamukkale University, Department of Biology, Bacteriology Laboratory, Bacterial Culture Collection. Firstly, the cells were incubated at 37 °C for 6-8 h in Muller Hinton Broth (MHB). After that, they were centrifuged at 3000 rpm for 10 min. The pellet was washed 2-3 times with PBS and suspended with PBS according to McFarland 0.5. Sterile cellulose discs (7.5x7.5 mm) were applied in two different ways. First, the cellulose was immersed in PBS with bacteria and removed instantly. Second, the cellulose discs were added in PBS with bacteria and incubated at 37 °C for 24 h. The discs were placed in 1 mL of sterile PBS and vortexed. Finally, 1 mL of this solution was used for serial dilutions and inoculated on solid agar. The Petri dishes were incubated at 37 °C for 24 h. At the end of the period, the colonies were counted as CFU/mL.²⁴

Fourier transform infrared (FTIR) spectroscopy

The FTIR spectra of the samples were recorded using a Bruker Vertex 70 V FTIR spectrometer (Germany), with an ATR (attenuated total reflectance) accessory unit, in the region from 400 to 4000 cm⁻¹ at the ambient temperature.

Thermogravimetric analysis

Thermal decomposition behaviors of the samples were carried out with Perkin-Elmer Diamond thermogravimetric analysis (TG/DTG) device. About 5 mg of a sample was placed in a ceramic pan and heated at 10 °C/min from 50 °C to 600 °C under N₂ atmosphere (200 mL/min). Differential scanning calorimetry (DSC) analyses of the samples were performed using a Perkin-Elmer Pyris 6 DSC instrument. The sample was put in an aluminum pan and heated from 0 °C to 400 °C under N₂ atmosphere (20 mL/min).

Scanning electron microscopy

Electron microscopy analysis was carried out at PAU-ILTAM (Denizli) and Akdeniz University, Medical Faculty (Antalya).

Statistical analysis

Statistical analyses were performed by using the Minitab statistical software package and the SPSS statistical package for Windows. All the results were expressed as means with their Standard Error of Means (SEM). A comparison between the two groups was performed by Student's t-test and p<0.05 was chosen as the level of significance.

RESULTS AND DISCUSSION

Screening and selection of new isolates for efficient production of bacterial cellulose is one of the most important goals in producing large amounts of that material. For this reason, various microorganisms have been isolated from different sources by many researchers and their cellulose production capacity was investigated. Although numerous bacteria, such as *Rhizobium*, *Agrobacterium*, *Acetobacter*, *Salmonella* and *Alcaligenes* can synthesize cellulose, *Komagataeibacter xylinum* are the most used bacteria in cellulose production.²⁵ In other words, it may be a model

cellulose-producer. Therefore, we firstly isolated a local isolate S4 from home-made grape vinegar in the current study. Some biochemical test results of the isolates are given in Table 1. It showed negative rods for gram reaction, aerobic, catalase and cellulose production positive. The isolate was inoculated on Carr and Freuter media for determining acetic acid production. At the end of the incubation, the color of Carr medium turned yellow due to the acid produced by the S4 strain and it was confirmed that it was an acetic acid bacterium (Fig. 1A and 1B). Transparent zones were observed around the colonies on Freuter medium containing CaCO₃ and acetic acid production was confirmed (Fig. 1C). In addition, the S4 strain was cultivated on Calcofluor agar and observed under UV light. The cellulose positive colonies glowed under UV light and separated from those that did not produce cellulose because the Fluorescent brightener 28 in Calcofluor agar binds with cellulose fibers (Fig. 1D). Moreover, the 16S rDNA analysis showed that the strain S4 was 100% identical to *Komagataeibacter xylinus* (GenBank: KX216690.1 and KX216693.1).

Table 1
Identification tests used for *K. xylinus* S4

| <i>K. xylinus</i> S4 | |
|---|---|
| Catalase | + |
| Production of cellulose | + |
| Growth on medium containing CaCO ₃ | + |
| Growth at pH=2 | + |
| Acid formation from glucose | + |
| Acid formation from sucrose | + |
| Acid formation from fructose | + |
| Acid formation from lactose | + |
| Acid formation from maltose | + |
| Oxidase | - |
| Indole production | - |
| Methyl Red | - |
| Voges-Proskauer | - |
| Utilization of sodium citrate | - |
| H ₂ S formation | - |
| Urea utilization | - |
| Gelatin liquefaction | - |

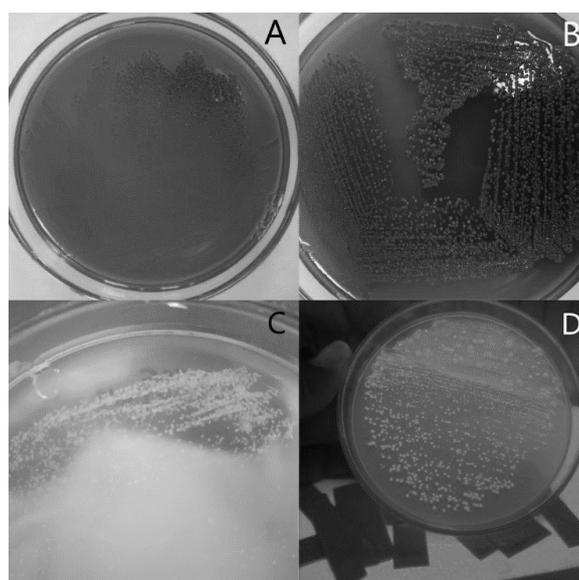


Figure 1: Carr (A and B), Frauter (C) and Calcofluor Agar (D) media used for isolation

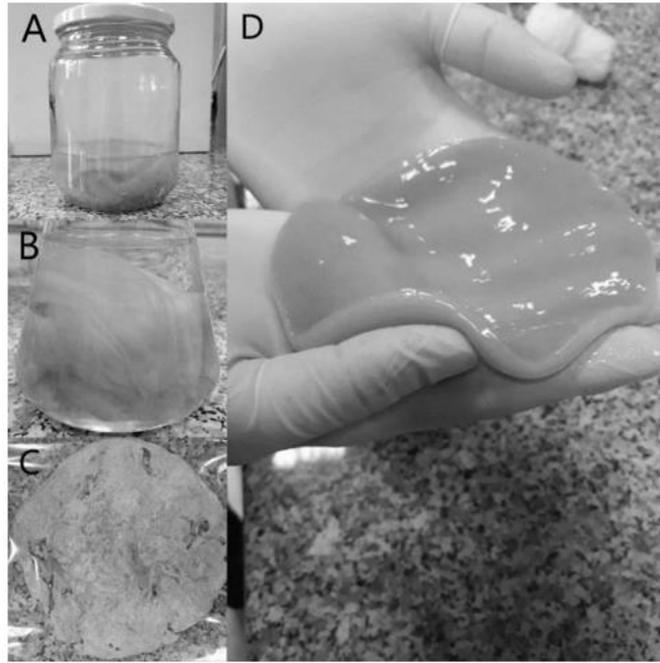


Figure 2: Images of bacterial cellulose production; A: pellicle in main source, B: bleaching of cellulose, C and D: wet and lyophilized cellulose

Table 2
Production of cellulose by *Komagataeibacter xylinus* S4 (g/L)

| Incubation | Dry weight | Wet weight |
|------------|-----------------|------------------|
| 5 days | 0.6320 ± 0.0000 | 51.8000 ± 0.0000 |
| 7 days | 0.5325 ± 0.0205 | 52.0520 ± 1.5000 |
| 10 days | 0.4300 ± 0.0100 | 40.0460 ± 0.9180 |
| 14 days | 0.7350 ± 0.0850 | 52.8035 ± 2.6745 |

The production of BC, a recognized multifunctional biomaterial, depends on the type of culture, oxygen, temperature, pH and culture conditions. The BC from *K. xylinum* S4 was produced in standard HS medium. The BC production and appearance are presented in Figure 2. The weight of wet BC was 51.8-52.8 g, while its dry weight was estimated as 0.43-0.735 g. The only difference was observed regarding the dry weight of BC after 14 days of incubation. There was a slightly higher than 100% increase in dry weight due to incubation (Table 2).

Antibacterial activity

Bacterial cellulose has many uses (*e.g.*, food, cosmetics, medicine, biomedical, *etc.*). The most important reason for having such a wide area of application is undoubtedly its antimicrobial effect.²⁶⁻²⁸ Therefore, the antibacterial effect of BC was investigated in our study. As seen in Table 3, the BC exhibited a strong effect in the presence of both momentary contact and 24 h contact. The antibacterial effect after 24 h contact was more efficient than after momentary contact. Figure 3 shows observations of the antibacterial effect of BC.

Numerous studies have been reported on the antibacterial effect of BC in the literature. In most of these studies, bacterial cellulose was treated with various antimicrobial agents. For example, BC obtained from *A. xylinum* was coated with chitosan (BC-Ch) and the antibacterial effect of pure BC and BC-Ch was tested against *S. aureus* and *E. coli*. While untreated BC and BC-Ch inhibited *S. aureus* growth with 30.4% and 99.9%, respectively; *E. coli* growth was inhibited by 49.2% with untreated BC and 99.9% with BC-Ch, respectively.²² In another study, lysozyme was immobilized onto bacterial cellulose nanofibers (BCNF) and the antimicrobial activity of the lysozyme against *Staphylococcus aureus*, *Escherichia coli*, *Listeria monocytogenes*, *Yersinia enterocolitica*, *Aspergillus niger* and *Saccharomyces cerevisiae* was increased after immobilization.²⁹ According to Adepu and

Khandelwal,³⁰ bacterial cellulose modified by silver (AgBC) exhibited 99.9% antimicrobial activity sustained for 72 h against a mixed culture of both bacteria and fungi.

Table 3
Antibacterial activity results of bacterial cellulose (%)

| Bacteria | Momentary contact | 24 h |
|-------------------------------|-------------------|--------|
| <i>S. aureus</i> ATCC 33862 | 89.08 | 91.56 |
| <i>E. coli</i> ATCC 25922 | 98.86 | 99.99 |
| <i>E. coli</i> O:157 H:7 | 99.79 | 100.00 |
| <i>B. cereus</i> RSKK 863 | 99.57 | 99.99 |
| <i>B. pumilis</i> NRRL-BD-142 | 99.26 | 99.99 |
| <i>E. faecalis</i> ATCC 19433 | 97.87 | 95.83 |

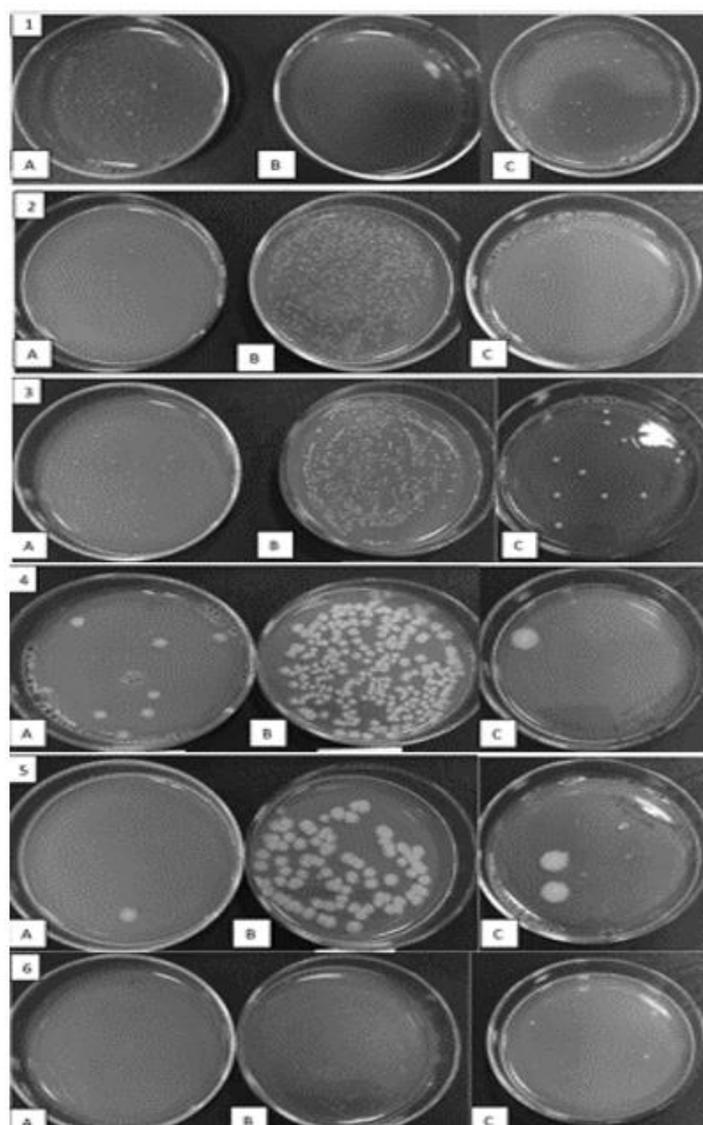


Figure 3: Antibacterial efficacy of bacterial cellulose against *S. aureus* ATCC 33862 (1A, control; 1B, after momentary contact and 1C, 24 h contact with BC), *E. coli* O:157 H:7 (2A and 2C after momentary and 24 h contact with BC, respectively, 2B, control), *E. coli* ATCC 25922 (3B, control, 3A and 3C, after momentary and 24 h contact with BC), *B. cereus* RSKK 863 (4B, control, 4A and 4C, after momentary and 24 h contact with BC), *B. pumilis* NRRL-BD-142 (5B, control, 5A and 5C, after momentary and 24 h contact with BC), *E. faecalis* ATCC 19433 (6B, control, 6A and 6C, after momentary and 24 h contact with BC)

Contrary to these results, BC purified from our local isolate exhibited high antibacterial activity, even without being coated with any substance. In other words, our pure BC was clearly more effective than modified BC used in the literature. According to the antibacterial screening test, it was shown that the activity of BC was good against all the used organisms (Table 3). However, *S. aureus* ATCC 33862 and *E. faecalis* ATCC 19433 were less resistant than *E. coli* ATCC 25922, *E. coli* O:157 H:7, *B. cereus* RSKK 863, *B. pumilis* NRRL-BD 142. This result means more specific interaction of BC with these bacteria. Moreover, the cell walls composition and structure of gram-positive and gram-negative bacteria are also different. That is why, the attachment of BC fibers may be strain specific.

Biofilm inhibition and eradication by BC

Many bacterial infections, for example, chronic wounds, are associated with biofilms. Combating with antibiotic applications of biofilm-associated infections is more difficult because biofilm increases antibiotics resistance of pathogens. Therefore, non-antibiotics strategies are being developed to eradicate biofilm infections. Bacterial cellulose as an antibiofilm composite can be one of these alternatives.^{31,32} In our study, the antibiofilm activity of BC was detected by using the crystal violet method. *S. aureus* ATCC 33862, *S. aureus* ATCC 29213, *E. coli* ATCC 25922, *E. coli* O:157H:7, *B. cereus* RSKK 863, *B. pumilis* NRRL-BD-142 and *E. faecalis* ATCC 19433 were used as indicator pathogens. According to our results tabulated in Table 4, the BC inhibited only biofilms of *S. aureus* ATCC 29213. The antimicrobial and antibiofilm activity of BC modified with various antimicrobial substances were mostly screened. The rates of biofilm inhibition and eradication were of 22.05% and 9.495%, respectively. According to the literature information, there is only one article investigating the antibiofilm effect of cellulose. For example, Zhang *et al.*³² indicated that the BC-modified-tannic acid composites significantly reduced biofilm formation of *S. aureus* and *P. aeruginosa* after 24 h incubation by ~80% and ~87%, respectively. In the study by Krasowski *et al.*,³¹ bacterial cellulose was treated with oral antiseptics and a very high antibiofilm effect was found. Contrary to Zhang *et al.*³² and Krasowski *et al.*,³¹ we tested the antibiofilm efficacy of pure BC and because of this, the antibiofilm effect was lower than the results of Zhang and co-authors, and Krasowski and co-authors. Indeed, most bacterial exopolysaccharides are not biofilm specific. SEM images of bacterial cellulose indicated cellulose fibers and pores on the surface. We considered that the antibacterial and antibiofilm effect of pure cellulose might be due to its fibrils and porous structure that attach to bacteria. This result proved the potential of our BC in biofilm inhibition, but pure BC must be modified to be used in many more applications.

Cytotoxicity of bacterial cellulose

The cytotoxic effect of BC was determined as described in the experimental part by using HEK293 cell line. As shown in Figure 4, the viability of cells decreased slightly because of BC treatment. However, this decrease was not found to be statistically significant. Quite similar results were observed in different studies.^{22,33} Although a 15% decrease was observed in the BC obtained from *Acetobacter xylinum*,²² our isolated BC caused an 8% decrease in cell viability. It is well established that a small decrease in viability can be considered as safe in the application of bacterial cellulose for different purposes.

Table 4
Antibiofilm effect of bacterial cellulose (%)

| | |
|-----------------------------|----------------|
| <i>S. aureus</i> ATCC 29213 | |
| Biofilm inhibition | 22.050 ± 6.675 |
| Biofilm eradication | 9.495 ± 0.808 |

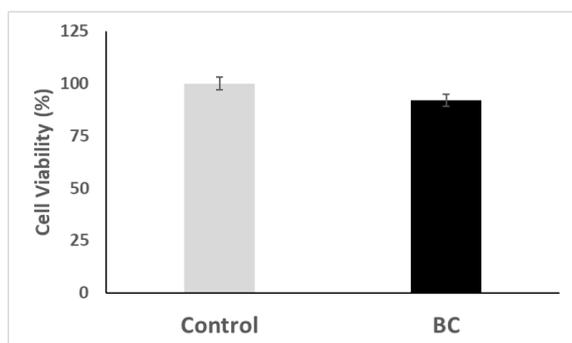


Figure 4: Cytotoxic effect of bacterial cellulose (BC) on HEK293 cells after 48 h incubation. Results are mean \pm SD values for three independent experiments

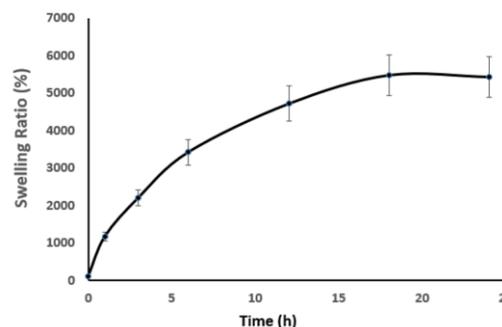


Figure 5: Swelling ratio of bacterial cellulose. Results are mean \pm SD values for three independent experiments

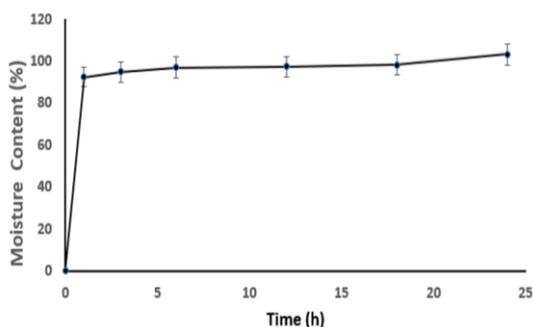


Figure 6: Moisture content of bacterial cellulose. Results are mean \pm SD values for three independent experiments

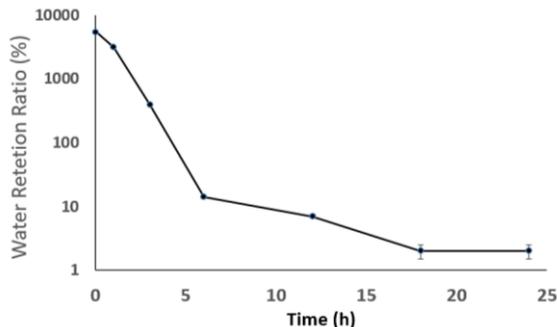


Figure 7: Water retention capacity of bacterial cellulose. Results are mean \pm SD values for three independent experiments

In addition to the cytotoxic effects, the moisture content, water retention capacity and swelling behavior of BC were evaluated in this study. As can be seen in Figure 5, the isolated BC has a 55.3-fold swelling ratio, compared to its dry weight, after 24 h. Similarly, the moisture content was found to be of 98.2% (Fig. 6). Water retention was evaluated for 24 h in this study. Most of the water was removed from BC after 24 h (Fig. 7).

One of the possible applications of bacterial cellulose may be in wound healing. It is well known that many factors can have an influence in wound healing process, including age, sex, moisture, infection.^{34,35} To increase this physiological process, natural and synthetic materials have been examined for their usage potential as wound dressings. One of the materials that can be used for this purpose is cellulose. It is found extensively in plants and produced by bacteria as well. Although their chemical nature is the same, their microstructures are different.³⁶ Our results showed that BC can maintain a moisture environment around the wound and can help the wound healing process. Isolated bacterial celluloses from different sources showed similar results, as described previously.^{22,37} All these results showed that BC may have potential in contributing to the wound healing process. Further experiments, such as *in vivo* animal tests, will be required to clarify the role of BC in wound healing.

FTIR analysis

Fourier transform infrared spectroscopy (FTIR) analysis provides important information about the existence of functional groups in a molecule, polymer, or composite material.³⁸⁻⁴⁰ FTIR analysis was used to detect functional groups and the binding properties of these groups in the cellulose samples (Fig. 8). Stretching vibrations of the hydroxyl groups ($-OH$) of the samples, due to hydrogen bonds formation, appeared as a broad absorption band around $3400-3300\text{ cm}^{-1}$. The absorption bands that appeared around $2950-2850\text{ cm}^{-1}$ are probably related to the $C-H$ asymmetric/symmetric stretching vibrations of methylene (CH_2) and methyl (CH_3) groups. The stretching vibration of the carbonyl ($C=O$) group of proteins and lipids can be easily recognized at 1727 cm^{-1} . The peaks in the range of 1426 and 1315 cm^{-1} possibly belong to the bending vibrations of CH_2 and $C-H$ groups of polysaccharides. The stretching vibration of the $C-O$ group was observed at 1159 cm^{-1} . The absorption bands around $1000-1200\text{ cm}^{-1}$ are attributed to the existence of $C-O$ and $C-O-C$ functional

groups of the pyranose ring. The other vibration peaks observed for the commercial cellulose and main source material used in the study (BC) are assigned to $-C-H$ out-of-plane bending (896 cm^{-1}), and $-OH$ out-of-plane bending ($666-619\text{ cm}^{-1}$). Both absorption bands are typical of the cellulose structure, with a few exceptions in the case of the main source (BC) spectra. The absorption band around 1640 cm^{-1} , which exhibited higher peak intensity in the spectrum of the main source (BC), compared to that of commercial cellulose, is due to absorbed water. Moreover, the peak at 1552 cm^{-1} , which corresponds to protein amide II absorption, disappeared in commercial cellulose.⁴¹ This is thought to be the result of insufficient purification applied to the main source. All the above characteristics of the FTIR spectra provide evidence of BC synthesis.

Thermogravimetric analysis

Thermogravimetric analyses (TGA) of the samples were accomplished in order to detect the thermal decomposition behavior of the samples during the thermochemical transformation. Since TGA results are dependent on many parameters, including geometries of samples, the amount of sample and heating rate, these parameters were kept the same during the analysis. The decrease in the mass of the samples was determined and the thermogravimetric (TGA) and differential thermal analysis (DTG) curves are given in Figures 9 and 10. In addition, some results obtained from the TGA and DTG curves while heating samples to $550\text{ }^{\circ}\text{C}$, such as the initial temperature of decomposition, the end temperature of the decomposition, the maximum decomposition temperature and the carbonaceous residue after pyrolysis, are given in Table 5.

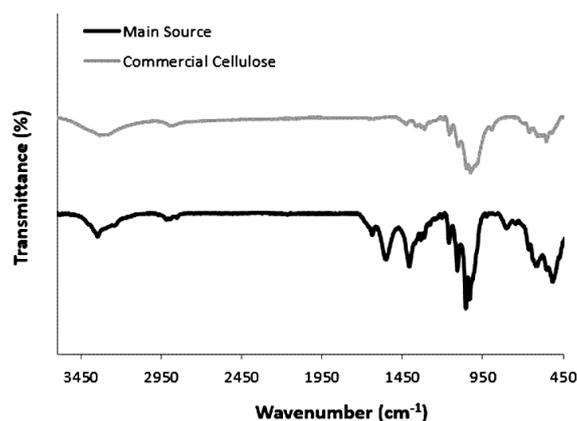


Figure 8: FTIR spectra of main source and synthetic cellulose

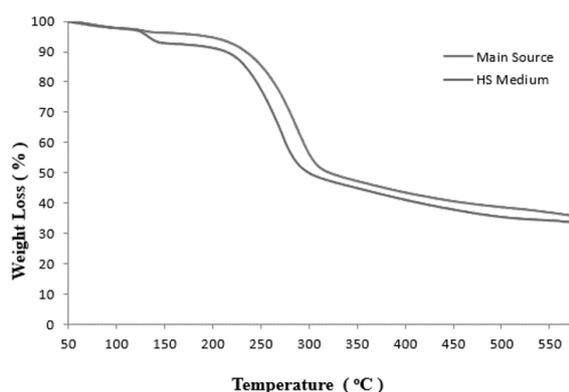


Figure 9: Thermogravimetric analysis curves of BC

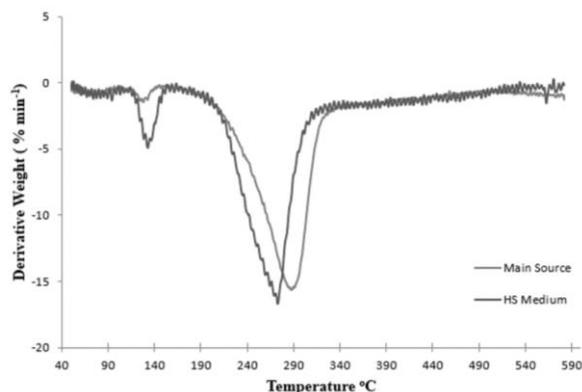


Figure 10: Differential thermal analysis curves of BC and main source

Table 5

Some results read from TGA-DTG curves of BC samples produced in HS medium with different carbon source of *Komagataeibacter xylinus* S4 bacteria

| Carbon source | T _{onset} (°C) | T _{offset} (°C) | T _{max} (°C) | Pyrolysis residue at 550 °C (%) |
|---------------|-------------------------|--------------------------|-----------------------|---------------------------------|
| Main source | 214.49 | 325.84 | 287.46 | 36.8 |
| HS medium | 227.23 | 288.1 | 272.72 | 34.19 |

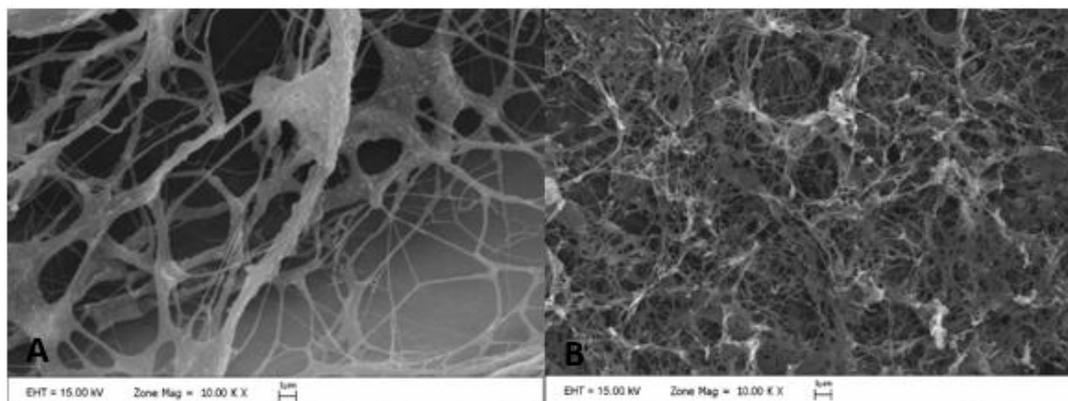


Figure 11: Images of BC from *K. xylinus* S4 (A – the main source from which strain S4 was isolated, and B – BC obtained from HS medium)

The thermal decomposition steps of the samples occur mainly due to the dehydration and depolymerization of the polymeric structures.⁴² As can be seen from the figures, the dehydration step occurs between 50 °C-145 °C, due to the loss of absorbed water in the samples, which is consistent with previous reports.⁴³⁻⁴⁶ BC mainly shows moderate thermal stability under the applied conditions and its decomposition occurs rapidly between 250 °C-350 °C.⁴⁷ The main decomposition step, with a higher percentage mass loss, occurred between 215 and 310 °C in the samples. It is observed that the second degradation step starts at temperatures between 215 and 228 °C, as can be seen from the figures given. In addition, the DTG curves obtained from the thermal analysis of the samples show that the maximum weight loss rate of this degradation is recorded between 272.72-287.46 °C temperatures. The temperature values at which degradation ends in samples obtained with different carbon sources are between 288 °C and 326 °C, as shown in the table. These values are relatively low when compared with the literature results.^{45,46,48-50}

Some structural parameters, such as the orientation, crystallinity and molecular weight of cellulose fibers, affect the thermal degradation behavior.⁴⁶ Besides these structural parameters, reasons such as the cleaning procedure used in the purification of the samples, the method of preparing the samples for thermal analysis, the morphology of the samples, the sample size, *etc.*, can affect the thermal degradation behavior.⁵¹ According to the literature mentioned above, this relative deviation is thought to be mainly due to the method of purification of the samples, as it was observed that the degradation step of the samples obtained by changing the washing procedure applied in cleaning them in another part of the study was more compatible with the results found in the literature. The accuracy of this evaluation is supported by the high residual pyrolysis values recorded after the samples are heated to 550 °C.

SEM analysis

Figure 11 presents the morphology of cellulose pellicles obtained from *Komagataeibacter xylinus* S4. Although the reticular structure of the cellulose pellicles in the main source and pure BC was similar, the fibril thickness was different. The fibrils of pure BC were thinner than those of the pellicles in the main source. Moreover, the main source was more compact in the background, and the fibril distribution was infrequent. The interfacial adhesion was very intense in BC. Jung *et al.*⁵² reported that the BC samples had reticulated structure consisting of ultra-fine cellulose fibrils. *Acetobacter xylinum* cellulosic fibrils were similar to pure microcrystalline cellulose fibrils.⁵³ Thus, the morphological structure of our cellulose was not different from that described in the literature.

CONCLUSION

The antibacterial, antibiofilm and cytotoxic activity, as well as moisture content, water retention capacity and swelling behavior of BC produced by *Komagataeibacter xylinus* were studied for the first time in the present study. Physical properties were determined by FTIR and thermal analysis. SEM results verified the fibril network structure of BC. The antibiofilm effect of BC was remarkable. This effect should be investigated in more detail, especially against various pathogens. In future studies, the antibiofilm effect of BC can be enhanced by modifying cellulose. Moreover, it has no cytotoxic effect. The moisture content, water retention capacity and swelling behavior of the isolated BC showed that it maintained the moisture environment constant, and it may be used for many medical applications. Further experiments will be required to test this hypothesis.

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