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Production and Characterization of Bacterial Cellulose from *Komagataeibacter xylinus*

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Abstract

Eight isolates previously isolated from rhizoplane of sugarcane roots grown in Aswan Governorate, Egypt and biochemically identified as *Gluconacetobacter* spp. were used to evaluate their cellulose production ability. All isolates were subjected to a set of tests that proved their efficiency in producing cellulose i.e. formation of white gelatinous pellicle, absence of precipitate, clear zone formation and yield of bacterial cellulose (BC).The results cleared that the isolate SuGu9 was the potent one that produced 2.7 g/L BC. The isolate was identified using 16S rRNA technique and found to be *Komagataeibacter xylinus* PP431972. This bacterium was known as a modeling microorganism for cellulose production. The morphological, physiochemical and thermal properties of BC produced were also estimated. The BC appeared as a rough surface related to fibrous structure of cellulose by scanning electron microscopy (SEM) as well as SEM pattern appeared as longitudinal fibers. The Fourier transformed infrared spectroscopy (FTIR) and X-ray diffraction (XRD) graphs for the BC were typical for BC of *K. xylinus* with 23% crystallinity. For the thermal stability, thermogravimetric analysis (TGA) curves showed the percentage of weight loss for BC which gradually increased with the increasing of temperature and maximum loss of weight (65.35%) was recorded at 577.7°C. Moreover, derivative thermogravimetry (DTG) curve shows that the maximum rate of this transformation (641.0 µg/min.) occurs at temperature of approximately 297.6°C. While differential scanning colorimetry (DSC) curves indicated that glass transition temperature (*Tg*) of the BC films was found to be 174.9°C.

Keywords: *Komagataeibacter*, *Gluconacetobacter*, bacterial cellulose, physiochemical properties, thermal stability.

Introduction

Cellulose is a biopolymer of glucose connected by β,1-4 glycosidic linkages. It is the most prevalent biomolecule in nature and can be found in a variety of sources, including plants and microorganisms (bacteria, algae, and fungi). Despite its significance and suitability for human use, plant-based cellulose is difficult to separate and does not exist in a pure form **(Patel *et al.*, 2019)**. According to **Sajadi *et al.* (2019)**, microbial-based cellulose, particularly bacterial cellulose (BC), has superior qualities like high purity, surface area, crystallinity, biodegradability, biocompatibility, and high-water retention value. Since BC is regarded as a superior biomaterial for use in a variety of biomedical, pharmaceutical, and packaging applications, there is a growing industrial need for BC, prompting numerous initiatives to raise its productivity. While the chemical make-up of BC derived from various bacterial sources is similar, each source's unique properties are related to the type of bacteria, the method of production, and size of the BC produced **(Jang *et al.*, 2019)**.

Many bacteria produce cellulose include species belonging to the genera *Rhizobium* and *Agrobacterium* **(Moradi *et al.*, 2021)**; *Acetobacter* **(Yamada *et al.*, 2012)**; *Gluconacetobacter* **(Avcioglu, 2022)**; *Klebsiella* and *Sarcina* **(Santoso *et al.*, 2020)**. According to recent reviews **(Gullo *et al.*, 2018; Hur *et al.*, 2020; Rahman *et al.*, 2021)** the genera *Gluconacetobacter* and *Komagataeibacter* produce the most BC among them. The most prolific one was discovered to be *Komagataeibacter xylinus* (originally *Gluconacetobacter xylinus* and *Acetobacter xylinus*). *K. xylinus* is a Gram-negative aerobic bacterium belonging to Acetobacteraceae that produces acetic acid during fermentation **(Liu *et al.*, 2018)**. *Komagataeibacter* wild-type strains were the effectiveness of the selected in producing surface micro-structured bacterial cellulose pouches for making biomedical devices (**Brugnoli *et al.*, 2021**).

It is used as a model organism for research on cellulose production because of its appealing ability to produce massive amounts of cellulose during the formation of biofilms **(Moniri *et al.*, 2017)**. Extracellular cellulose is secreted by *K. xylinus* as a pellicle, which is a very pure and fine network of fibers. The remarkable properties of BC from *K. xylinus*, such as its plasticity, porosity, water absorption capacity, and biodegradability, make it a unique structural composition that can be used in a variety of applications **(Singhania *et al.*, 2022).**

Thus, the purpose of this work was to determine whether eight isolates previously isolated from the sugarcane rhizoplane could produce bacterial cellulose (BC). Subsequently, identified the strongest isolate and assess the physical, chemical, and thermal characteristics of the BC it produces.

Materials and methods

Bacterial isolates and their growth

Eight bacterial isolates which previously isolated from sugarcane rhizoplane and biochemically identified as *Gluconacetobacter* based on the genus level by **Hassan and El-Meihy (2015)** were friendly obtained from Agric. Microbiology Department, Faculty of Agriculture, Benha University. The isolates were grown on glucose, yeast extract and $CaCO_3$ (GYC) medium contained (g/L): 50 glucose, 10 yeast extract, 15 calcium carbonate, pH 6.0 **(Sharafi *et al.*, 2010)** and coded as SuGu2- SuGu9.

Screening for BC production

Primary screening

The isolates were evaluated for their capacity to form white gelatinous pellicle over the surface of Hestrin-Schramm (HS) broth medium, which contained the following concentrations of nutrients (g/L): 20.0 glucose, 5.0 yeast extract, 5.0 peptone, 2.27 disodium hydrogen phosphate, and 1.17 citric acid **(Schramm and Hestrin, 1954)**, One loop of each isolates was added to 100 mL HS medium in 250 mL Erlenmeyer flasks, which were then amended with 0.02% (w/v) cycloheximide. The flasks were then statically incubated at 30°C for seven days at pH 5.0 then observed for formation of white gelatinous pellicle **(Tanskul *et al.*, 2013)**.

The isolates inability to produce white precipitate was also examined using **Ishida *et al.* (2002)'s** methodology. After the same previously growth conditions, the bacterial culture was centrifuged for five minutes at 8,000 rpm, the cell-free supernatant was combined with 90% ethanol at a ratio of 1:3 (v/v), thoroughly shaken, and left to wait under observation. Pure cellulose is indicated by the absence of any precipitate, whereas mixed cellulose and acetan is indicated by the presence of precipitate.

Secondary screening

For knowing if the isolates could produce cellulose, a clear zone formed around their growth when they were cultivated on glucose ethanol yeast extract (GEY) agar medium (pH 7.0) at 37°C for 48 h **(Aydin and Akosy, 2009)**. Lastly, the bacterial cellulose (BC) yield was estimated according to the method outlined by **(Chawla *et al.*, 2009)**. The bacterial isolates were cultured independently for seven days and incubated at 30°C on HS broth medium (pH 5). Then, the interface pellicles that had formed were carefully taken out and cleaned with distilled water. Subsequently, the films were subjected to a 20-minute boiling in 0.1 *M* NaOH at 90°C to eliminate the bacterial cell mass attached to the pellicles. The films were then repeatedly rinsed with deionized water until the pH of the water was neutral. The pellicles that had been purified were dried until the steady weight and the dried BC weight was recorded.

Molecular identification of the highest cellulose-producing isolate

The highest BC producing isolate was identified using 16S rRNA sequencing according to the methods by **Balakrishnan *et al.* (2022)**. The bacterium's whole genomic DNA was extracted and the bacterial 16S rRNA gene was amplified using PCR (MyCycler, Bio-Rad, USA) with primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5´-GGTTACCTTGTTACGACTT-3´). Eventually, a Basic Local Alignment Search Tool (BLAST) was used to determine each sequence's most likely identity. In order to create a phylogenetic tree, the entire 16S rRNA sequence was aligned using MEGA11 software against corresponding sequences of representatives of the relevant bacterial strains that were obtained from the Gene Bank.

Characterization of produced BC

The produced BC was obtained by cultivating 10 mL of the bacterial strain, which was 2 days old, in a 1.0 L Erlenmeyer flask with 200 mL of HS broth medium. The flask was then incubated statically at 30°C for 7 days, with three replicates. At the end of the incubation period, the interface pellicles were carefully removed and cleaned with distilled water. They were then boiled in 0.1 *M* NaOH for 20 minutes at 90°C and rinsed several times in deionized water until the pH of the water reached neutral **(Nie *et al.*, 2022)**. Then, a hot air oven was used to dry the gathered pellicles at 50°C until they reached a constant weight **(Chawla *et al.* 2009)**. Utilizing a variety of analytical techniques, the chemical composition and structural shape of the obtained BC were estimated.

Morphology observation by SEM-EDX

By using scanning electron microscopy (SEM), the dried BC's external surface appearance was examined. Little bits of the dried pellicle were removed. To analyze the surface morphology of the prepared samples, an EDX unit was attached to the Jeol and operated at BED-C 10.0KV, resulting in micrographs with a magnification of 20 kX **(Auta *et al.*, 2017).**

Fourier transform infrared spectroscopy

Using attenuated total reflection Fourier transformed infrared spectroscopy (ATR-FTIR) spectral analyses, the functional groups of the tested BC and the reference were compared for similarity. 24 scans were conducted with a resolution of 4 cm-1 using FT-IR spectroscopy ALPHA II, Bruker, Germany, in the range of 4000 – 400 cm-1 **(Auta *et al.*, 2017)**.

X-ray diffraction

Utilizing an X-ray diffraction (XRD) pattern and a Rigaku MiniFlex 600 diffractometer with Cu Kα radiation, the crystallinity index of the BC produced was ascertained. The Segal method **(Nam *et al.*, 2016)** was used to calculate the crystallinity index of BC, as indicated below:

$$\text{Crystallinity index (Ci)} = \frac{I_{200} - I_{am}}{I_{200}}$$

$$\text{Crystallinity \% (Ci\%)} = 1 - \frac{I_{am}}{I_{200}}\ X\ 100$$

Where: I_{200} represented the highest intensity of the 2θ peak (200) which is around at 22.5°C; I_{AM} represented the intensity of peak situated between (110) and (200) peaks that is at 18°.

Thermogravimetric analyses

An STA200 operator was used to perform thermogravimetric analyses (TGA). In pt pans, a 10.557 mg sample was used for each analysis, and the temperature range for the dynamic nitrogen atmosphere was 10 to 577.7°C. A heating rate of 10°C per minute was used for the experiments. It was possible to obtain the weight loss curve and its derivative weight loss percentage **(Auta *et al.*, 2017)**. A STA200 operator was also used to analyze differential scanning colorimetry (DSC) across a temperature range **(Mohite and Patil, 2014).**

Results and discussion

Screening of *Gluconacetobacter* isolates for their ability to produce BC

The capacity of eight *Gluconacetobacter* isolates to produce bacterial cellulose (BC) was assessed using four testes **(Table 1).** All isolates were able to form white gelatinous pellicle formation over the liquid medium. Three of them produced an intermediate layer, two produced a thick gelatinous layer, and the remaining three produced a thin layer. The findings of multiple studies **(Singhania *et al.*, 2022; Avcioglu, 2022; Rahman *et al.*, 2021)** which indicated that the genera *Gluconacetobacter* and *Komagataeibacter* contain the highest number of species that produce BC, supported this conclusion.

Two isolates were found to be capable of producing cellulose mixed with acetan when tested for their ability to form pure or mixed cellulose. These isolates were the only ones that could form a precipitate when exposed to ethanol. This indicates the purity of the cellulose produced, as the other six isolates failed to form precipitate. According to **Tsalagkas *et al.* (2016)**, this is confirmed by their discovery that while BC does not contain hemicellulose or lignin like plant cellulose, it does contain impurities. For this reason, the removal of these impurities using sodium hydroxide treatment is an essential step in the process. In addition to bacterial nucleic acids and proteins, these contaminants also include organic substances from the culture medium **(Gea *et al.*, 2011).**

After that, six of the eight isolates were then chosen for the second set of testes. Only one isolate was unable to form a zone of clearness, ranging in size from 2.1 to 3.0 cm, whereas five of them did. Similar results were observed by **Tyagi and Suresh (2013)** who recorded distinct zones around the colonies of *Gluconoacetobacter intermedius* on $CaCO_3$-ethanol-agar medium and suggested that this bacterium may be a cellulose producer. Also, **Aydin and Aksoy (2009)** noted that many *Gluconacetobacter* species formed distinct zones on the GEY medium. Finally, the dried BC yield was weighed after seven days of static cultivation in the HS broth at 30°C. Three isolates, despite having tested positive in the previous three tests, did not yield weight because the gel layer they had produced had dissolved after being treated with sodium hydroxide. This could be because, as **Suryanto *et al.* (2019)** noted, they produce any kind of polymer other than cellulose. Furthermore, the high NaOH concentrations might have a major impact on the properties of BC **(McKenna *et al.*, 2009).**

The BC produced by the other three isolates varied from 1.80 to 2.7 g/L. This result was comparable to that of **Nie *et al.* (2022)**, who discovered that the BC yielded by three different acetic acid bacteria ranged from 2.7 to 6.6 g/L. From the previous tests, the isolate SuGu9 was the potent isolate that gave thick white gelatinous pellicle and didn't show any precipitate as well as recoded high clear zone on GEY medium and gave the highest BC yield. Additionally, the SuGu9 isolate produced a comparable amount of BC to the well-known BC producers *G. xylinum* ATCC 10245, *K. europaeus* SGP37 **(Dubey *et al.*, 2017)** and *Gluconacetobacter* sp. and *Komagataeibacter* sp. in the HS medium **(Abdelraof, *et al.*, 2019)**. According to this result, the isolate SuGu9 will be identified and used for the next studies because it is a good BC producer. **Cannazza *et al.*, (2022)** studied that the novel *Komagataeibacter intermedius* strain capable of utilizing glucose, and glycerol sources for biomass and BC synthesis.

Table 1. Screening of *Gluconacetobacter* isolates for their ability to produce BC.

Bacterial isolates	Primary screening		Secondary screening	
	White gelatinous pellicle formation	Precipitation	Zone of Clearness (cm)	Dry weight (g/L)
SuGu2	++	P	--	--
SuGu3	+	P	--	--
SuGu4	++	NP	2.70	2.00
SuGu5	+	NP	2.60	0.00
SuGu6	+	NP	0.00	0.00
SuGu7	++	NP	2.10	1.80
SuGu8	+++	NP	2.30	0.00
SuGu9	+++	NP	3.00	2.70

P: precipitated; **NP:** non- precipitated

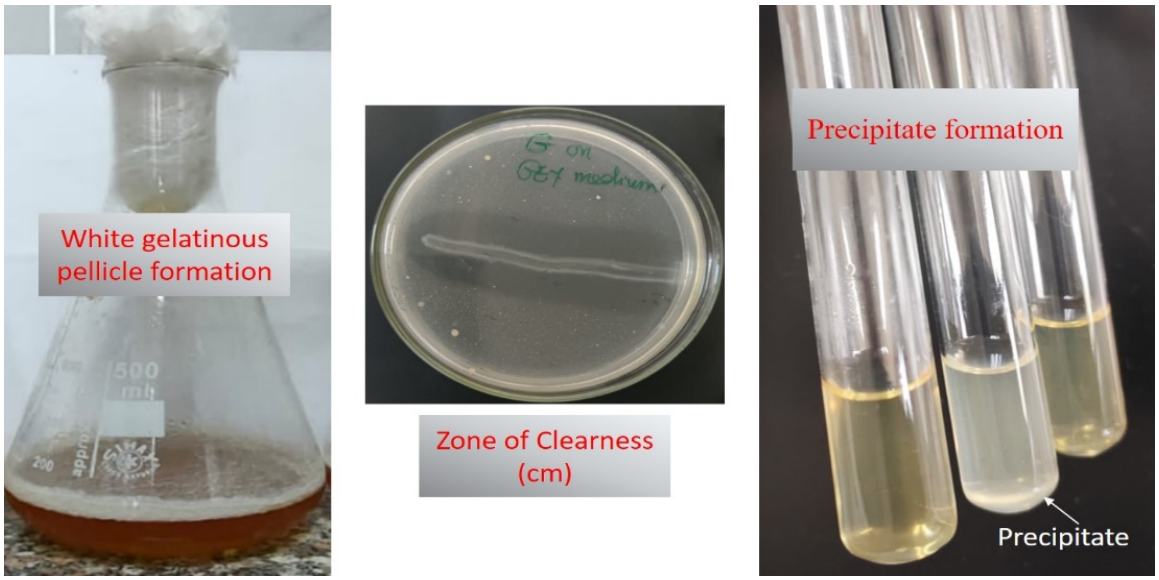


Fig. 1. Screening tests of *Gluconacetobacter* isolates

Identification of the most potent cellulose producer

The sequence of SuGu9 16S rRNA was compared with related species of bacteria to check the similarity of 16S rDNA gene and its phylogenetic lineage. The analysis of the phylogenetic tree further indicated that the SuGu9 isolate was exhibited homology with the species *Komagataeibacter diospyri* 92.37%, *Gluconacetobacter europaeus* 97.08%, *Komagataeibacter europaeus* 94.33%. Its sequence showed a high similarity of the 16S rDNA gene sequence with more than 99.98% with *Komagataeibacter xylinus* strain TJU-D2 **(Fig. 2).** The accession number in the Gene bank: PP431972. Later, *Komagataeibacter xylinus* has gone by a number of different names, most notably *Acetobacter xylinum* and *Gluconacetobacter xylinus.* When the new genus *Komagataeibacter* was formed in 2012, it was given its present name **(Römling and Galperin, 2015)**.

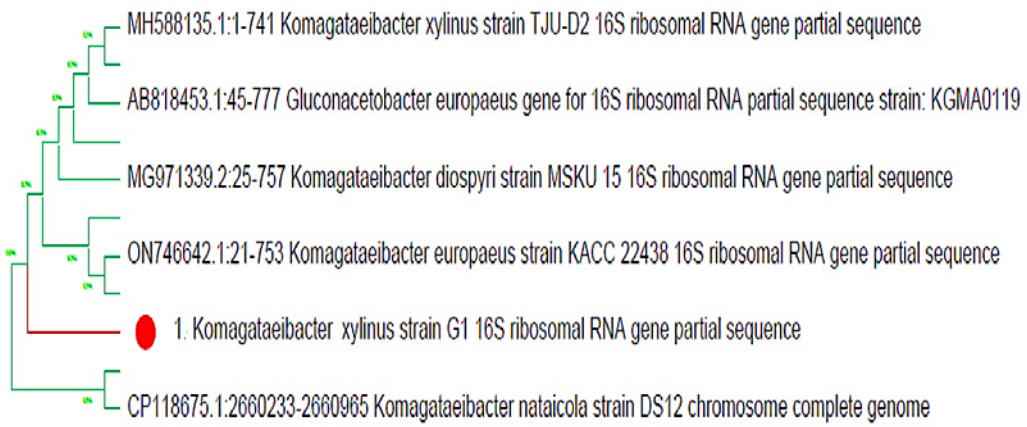


Fig. 2. Analysis of the phylogenetic tree of SuGu9 isolate using 16S rRNA sequencing.

Characterization of BC produced by *K. xylinus* PP431972

Morphology of surface

The SEM pictures that were captured from BC's surface under ideal circumstances are displayed in **(Fig. 3).** According to the SEM results, BC had a rough surface that was associated with the cellulose's fibrous structure. Moreover, longitudinal fibers that indicated low crystallinity were visible in the EDX pattern. The cross-section of BC layers generated by *K. xylinus* at various ascorbic acid concentrations revealed that the highest amount of ascorbic acid in its culture medium showed the least cohesiveness in its structure, despite the fact that the acidity of the culture medium encourages the crystallization of the produced BC **(Raiszadeh-Jahromi *et al.*, 2020)**. This could be because of the synthesis of additional compounds at higher ascorbic acid concentrations, which can cause the BC product's homogenous structure to be disrupted by increasing its porosity and causing cracks as a result of other metabolites being incorporated as impurities within the BC matrix. As byproducts of BC production, acetic acid and glucuronic acid, for instance, can be added to the BC network created, increasing its porosity. According to an EDX analysis, the majority of the cellulose produced is made up of carbon and oxygen atoms combined, at rates of 25.65 and 62.30%, respectively. While calcium and phosphorus were closely related at rates of 3.00 and 0.85%, sodium makes up around 8.20%.

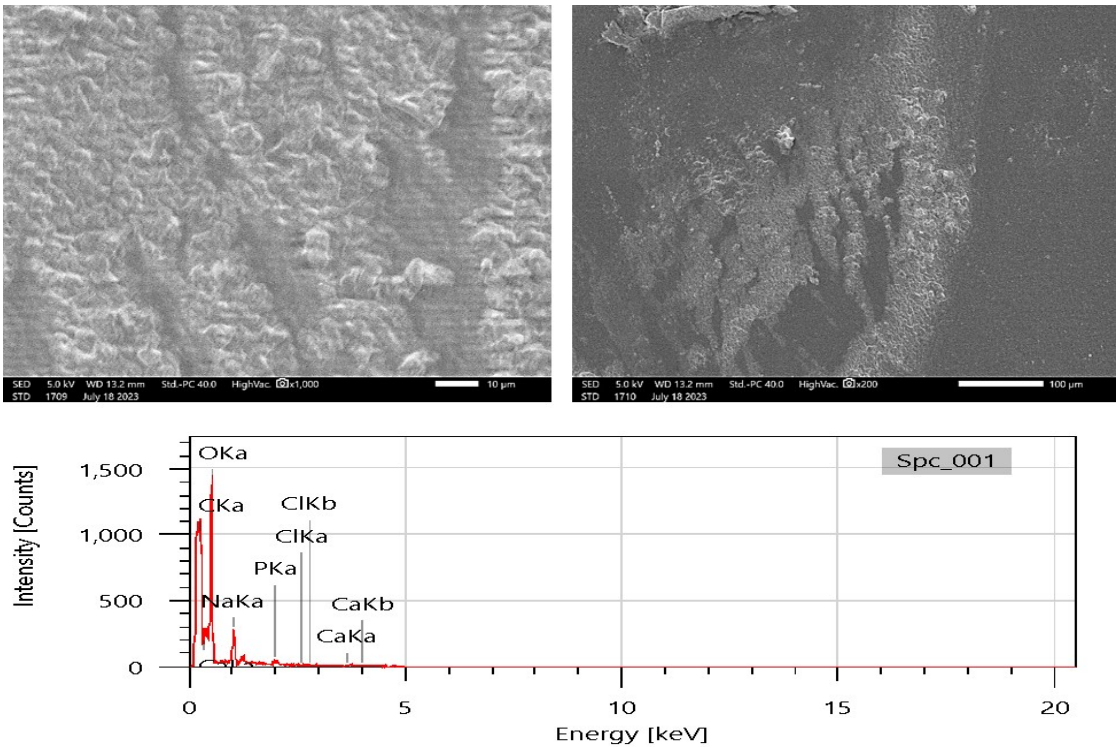


Fig. 3. SEM-EDX images of BC produced by *Komagataeibacter xylinus* PP431972

Structure and composition

The graph of FTIR analysis **(Fig. 4)** shows the formed peaks at different wavelengths ranging from 400-4000 cm^{-1}. The peak at 3322 cm^{-1} corresponding to the stretching frequency of –OH, while peaks at 2928, 2462 and 2458 cm^{-1} are attributed to the tensile vibrations of -CH. These peaks were typically to those in pure BC produced by *K. xylinus* as reported by **(Raiszadeh-Jahromi *et al.*, 2020; Sukhtezari *et al.*, 2017; Stoica-Guzun *et al.*, 2012).** Moreover, peak at 1711 cm^{-1} refer to H-O-H bending while two -CH_2 stretching peaks were recorded at 1586 and 1481 cm^{-1}. Both peaks at 1399 and 1346 cm^{-1} refer to -CH_3 and –CO bending, respectively. While peak at 1198 cm^{-1} refer to C–O–C stretching. In addition, peak at 1001 cm^{-1} was refer to the presence of C-C, OH, C-H ring. The observed peaks with low intensity might be referred to hydrogen bond strength in cellulose fibers **(Hasanin *et al.*, 2023)** and/or the presence of a large number of –CO and C–O–C groups **(Nie *et al.*, 2022)**.

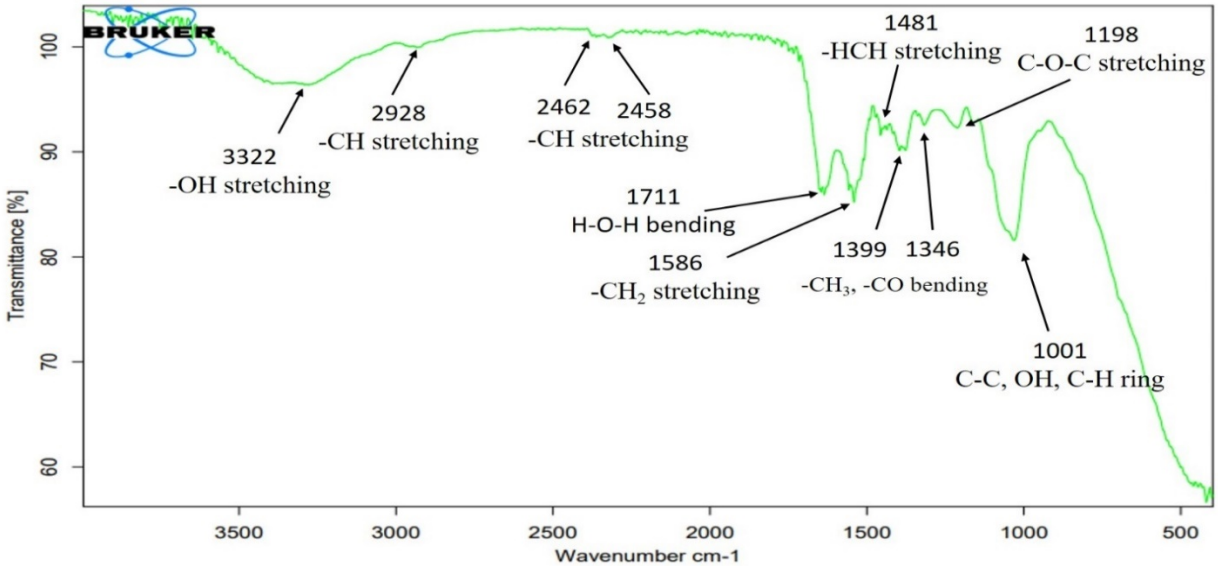


Fig. 4. ART-FTIR profile of BC produced by *Komagataeibacter xylinus* PP431972

The XRD patterns of produced BC exhibited main peaks between 18°, 25°Θ and the sharpest peak at 22.5°Θ **(Fig. 5)** which typically to XRD pattern of pure cellulose **(Jebel and Almasi 2016; Przygrodzka *et al.*, 2022)**. Also, these results can likely be attributed to those by **Gomes *et al.* (2013)** who reported similar XRD pattern for BC produced by *G. xylinus*. The degree of crystallinity of BC produced by *K. xylinus* PP431972 was calculated as 23 %. The acidity of the culture medium had an enhancing effect on the structural orientation by forming compact cellulose domains with regular arrangement of the BC chains produced and this caused an increase in crystallinity **(Raiszadeh-Jahromi *et al.*, 2020).**

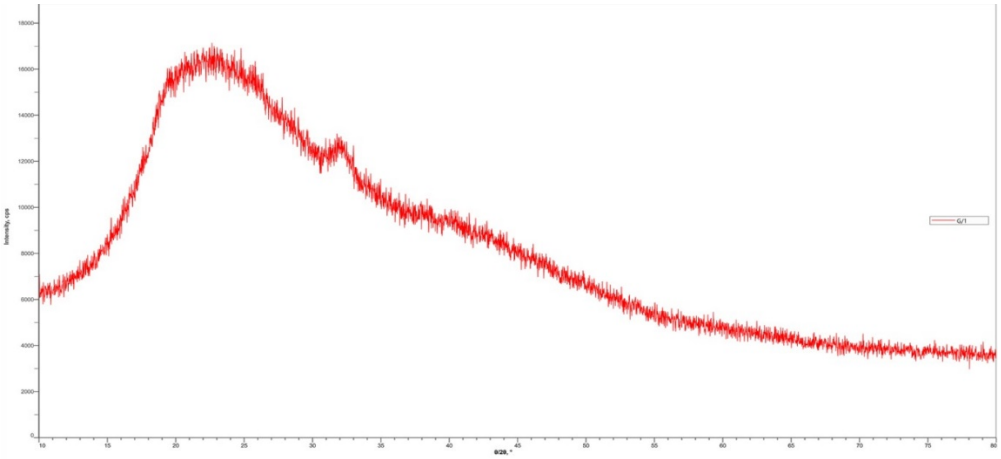


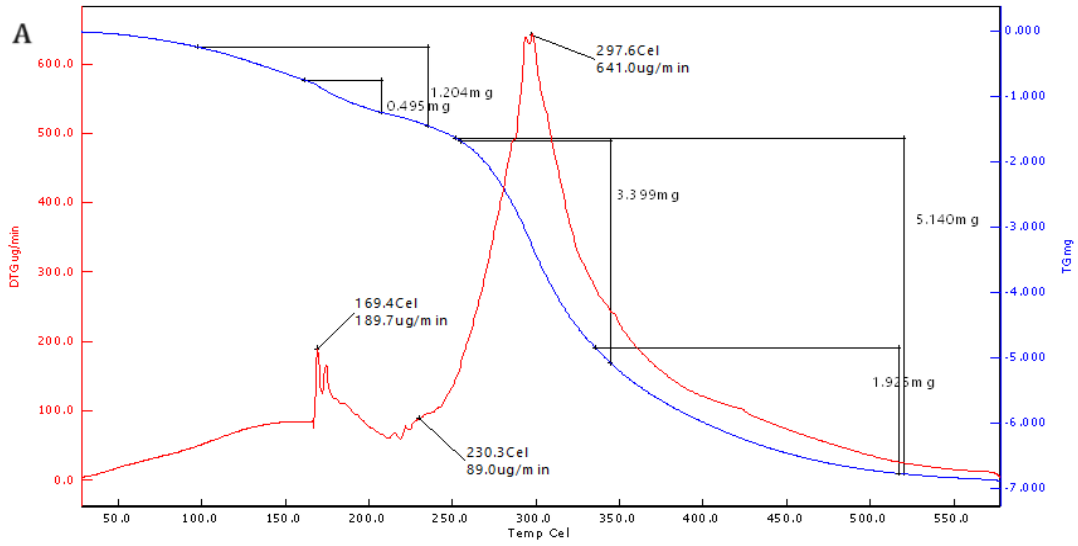
Fig. 5. XRD profile of BC produced by *Komagataeibacter xylinus* PP431972

Thermal stability

A series of analyses, including thermogravimetric analysis (TGA), differential thermogravimetric analysis (DTG), differential scanning calorimetry (DSC), and differential thermal analysis (DTA), were used to assess the thermal stability of the BC obtained by *K. xylinus* PP431972, as shown in **Figs. 6 A, B, and C.** TGA is the process of first subjecting the BC sample (10.56 mg) to a range of temperatures and then monitoring the rate of weight loss in relation to the temperature increase through programmed heating that was gradually increased from 28 to 600°C. At 150°C, the sample was found to have lost 0.649 mg of weight; however, as the temperature rose, the sample continued to lose weight, reaching a total of 1.610 mg at 250°C. Furthermore, it was noted that the significant weight loss (6.898 mg) occurred at 577.7°C **(Fig. 6A)**. Both moisture vaporization and the loss of absorbed water in cellulose fibers are caused by this temperature increase. Also, data in **Fig. 6 (A)** illustrates the thermal breakdown of BC, which is referred to as the TG derivative. The term "DTG curve" describes the BC sample's abrupt change in temperature between 169.4°C and 297.6°C. The change was marginal at the higher temperature. The BC samples' maximum degradation temperature was measured at 577.7°C. These findings could be explained by the BC structure and crystallinity, as well as the high concentration of minerals (EDX data) **(Raiszadeh-Jahromi *et al.*, 2020)**. Similar findings showed that the maximum degradation temperature for BC produced by *Komagataeibacter xylinus* PTCC 1734 was 265°C **(Raiszadeh-Jahromi *et al.*, 2020)**. It was discovered that a high degree of crystallinity improves the thermal stability of BC microfibers by making them more compact.

By measuring the amounts of heat absorbed and emitted during its exposure to a particular and controlled temperature range and calculating the amount of heat exchange with the surrounding environment, DSC aids in the determination of BC's properties.

The obtained results show that the glass transition temperature (*Tg*) of BC films is 174.9°C **(Fig. 6B)**, which is significantly lower than that of **Wang *et al.* (2008)**, who reported that *Tg* of BC was at 280°C, on contrary, **Mohite and Patil (2014)** found *Tg* of BC films to be 44.28°C, which is lower than the results obtained in the current study. In DTA, the temperature differential between a sample and a reference is measured after they are subjected to the same thermal cycles. The DTA curve is useful for assessing BC's thermal behavior **(Dollimore and Hoath, 1981)**. Regarding this, the DTA graph **(Fig. 6C)** clarifies the bacterial cellulose's thermal behavior in the 600°C range. The curve displays a range of thermal event-indicating peaks and inflection points. At 214.5°C, the first peak suggests an endothermic process. The loss of absorbed water from the BC structure may be the cause of this. With a large transformation and a second highest significant peak seen at 308.0°C, this peak most likely represents the primary thermal degradation of bacterial cellulose. A significant endothermic process is indicated by this discernible increase in the DTA signal. This endothermic peak might be an indication of additional cellulose or sample component degradation. The second stage or continuation of the decomposition process is indicated by the steadily increasing DTA signal. The wider temperature range and high intensity suggest significant substantial mass loss and structural collapse.



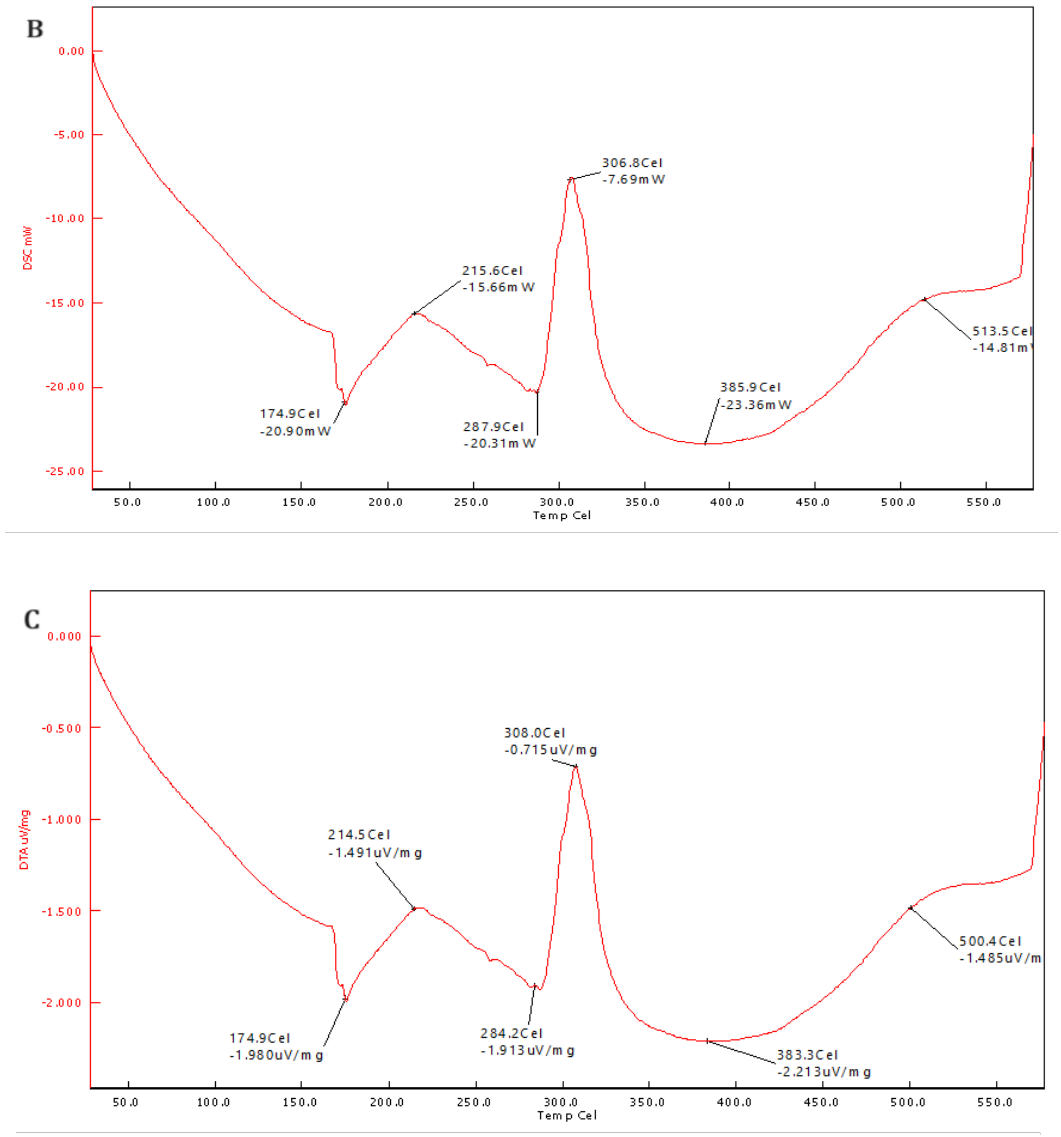


Fig. 6. Thermal analyses of the produced BC by *Komagataeibacter xylinus* PP431972, **a)** TGA and derivative of TG curve (DTG); **b)** DSC; **c)** DTA.

Conclusion

Among eight isolates of the genus *Gluconacetobacter* previously isolated from the rhizoplane of sugarcane, one isolate (SuGu9) was selected as a highly cellulose producer based on the formation of gelatinous pellicle, water soluble polymer and clear zone as well as the dry weight of BC. Later, it was identified as *Komagataeibacter xylinus* PP431972 using 16s rRNA. The produced cellulose was subjected to chemical analysis using (FTIR, XRD and SEM-EDX) as well as the thermal stability properties through (TGA, DTG, DSC and DTA). The resulting data clearly show that BC had a low crystallinity and was comparable to analyses of pure BC. This approach not only enhances bio cellulose yield, but also contributes to the utilization of agricultural by-products, promoting an ecofriendly and economically viable production process.

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إنتاج وتوصيف السليلوز البكتيري بواسطة *Komagataeibacter xylinus*

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أجريت هذه الدراسة باستخدام ثماني عزلات سبق عزلهم من منطقة الريزوسفير الخاصة بمحصول قصب السكر من محافظة أسوان، وكذلك تعريفهم على أساس الاختبارات الكيميائية الحيوية والمورفولوجية وكانوا تابعين لجنس *Gluconacetobacter* وفي هذه الدراسة تم توجيه هذه العزلات على أسس علمية لإنتاج السليلوز البكتيري، وذلك بإجراء عدة اختبارات للتأكد من إمكانية إنتاجهم للسليلوز البكتيري، واشتملت هذه الاختبارات تنمية الميكروبات الثماني على بيئة Hestrin–Schramm (HS) لملاحظة تكون طبقة جيلاتينية بيضاء على السطح، وكذلك اختبار تكون الراسب من عدمه، كما تم تنمية الميكروبات على بيئة Glucose ethanol yeast extract (GEY) agar لملاحظة تكون هالة شفافة، وأخيرا الانتخاب على أساس كمية السليلوز المنتجة بعد استخلاصها وتجفيفها. وتم اختيار العزلة الأعلى إنتاجية وتعريفها على أسس وراثية باستخدام 16S rRNA وتبين أنها *Komagataeibacter xylinus* والتي كانت سابقاً تُسمى *Gluconacetobacter xylinus*. كما تم إجراء بعض الخصائص الفيزيائية والمورفولوجية للسيليلوز المُنتج ومنها FTIR, XRD, SEM وتبين طبقا للمجاميع الوظيفية والشكل المورفولوجي أنه سليلوز غير متبلور له نسبة تبلور 23%، وطبقا للثبات الحراري فإنه ثابت حرارياً حيث فقد جزء كبير من وزنه بتعريضه لدرجات حرارة حتى 600 درجة مئوية.