



Komagataeibacter saccharivorans strain BC-G1: an alternative strain for production of bacterial cellulose

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Abstract

The current study represents the isolation and characterization of cellulose producing bacteria from rotten grape fruit. The best Bacterial Cellulose producer isolated from rotten grape fruit was identified as *Komagataeibacter saccharivorans* BC-G1 upon biochemical and molecular characterization. Bacterial Cellulose production was done in Hestrin and Schramm (HS) medium under static condition. Studies showed that BC was produced as a thick layer on the surface of medium and yield of BC was 14 g/L after 96 h of fermentation period. BC films were characterized by Scanning Electron Microscopy, Fourier Transform Infrared Spectroscopy and X-ray Diffraction. SEM analysis revealed the presence of highly extended interwoven cellulose fibrils. Characteristics of FTIR spectrum depicted the presence of characteristic absorption bands and less crystalline behavior of BC. XRD patterns demonstrated three major characteristic diffraction peaks corresponding to (110), (100) and (002) indicating presence of less crystalline behavior of BC produced in this study. Results confirmed the produced biopolymer by *K. saccharivorans* BC-G1 is BC.

Keywords Bacterial cellulose · Biopolymer · *Komagataeibacter saccharivorans* · FTIR · SEM · XRD

Abbreviations

BC	Bacterial Cellulose
HMP	Hexose monophosphate pathway
TCA	Tricarboxylic acid
UDPG	Uridine diphosphate glucose
SEM	Scanning electron microscope
FTIR	Fourier-transform infrared spectroscopy
XRD	X-ray diffraction
HS	Hestrin-Schramm
BLAST	Basic Local Alignment Search Tool
MEGA	Molecular Evolutionary Genetic Analysis
CR	Crystallinity Ratio
RPM	Rotation per minute

Introduction

Bacterial Cellulose (BC) is a homo-biopolymer produced by different bacterial strain in a sugar rich medium. Although the molecular formula of BC resembles to that of plant cellulose but the former has more unique and refined three-dimensional porous net like structures. Acetic acid bacteria belong to the family *Acetobacteraceae* and are known to produce acetic acid by oxidizing ethanol (Jose et al. 2020; Saichana et al. 2015). Most common genera which belongs to classification of the AAB (acetic acid producing bacteria) includes *Acetobacter*, *Acidomonas*, *Asaia*, *Gluconacetobacter*, *Kozakia*, *Swaminathania* and *Saccharibacter*. Some species like *Komagataeibacter*, *Aerobacter*, *Agrobacterium*, *Acetobacter*, *Rhizobium*, *Sarine*, *Achromobacter*, *Alcaligenes*, *Azotobacter* and many other bacterial strains are known for producing cellulose but the major reported producer of cellulose is *Komagataeibacter xylinus* (Yamada 2016).

The *Komagataeibacter* genus typically relates to the family *Acetobacteraceae*. Based on analysis of their gene sequences, members of this genus were divided into two groups: the *Komagataeibacter xylinus* group (includes *K. xylinus*, *K. sucrofermentans*, *K. hansenii*, *K. europaeus*,

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K. entanii, *K. saccharivorans*, *K. swingsii*, *K. rhaeticus*, *K. nataicola*, *K. oboediens*, and *K. intermedius*) and *Komagataeibacter liquefaciens* group (includes *K. liquefaciens*, *K. sacchari*, *K. diazotrophicus*, *K. johanna*, and *K. azotocaptans*) (Revin et al. 2020; Brandão et al. 2022 reported several types of strains form an independent genomic group from those of other *Komagataeibacter*, reclassified as *K. hansenii*, *K. cocois*, *K. maltaceti* and *K. pomaceti* as novel members of the genus *Novacetimonas*. The *Novacetimonas hansenii* species are the proposed representative of the novel genus (Brandão et al. 2022).

The production of BC involves the conversion of cellulose monomers into the cellulose chain using biosynthetic pathways like HMP (Hexose monophosphate pathway) and TCA (Tricarboxylic acid) with the network of several important enzymes (Ross et al. 1991). Some of the key enzymes are glucokinases, UDPG- pyrophosphorylase and cellulose synthases, which are involved in phosphorylation of glucose units, isomerization and polymerization of monomers to cellulose chains. The glucose monomers formed from biosynthetic pathways are phosphorylated to UDPG and isomerized by UDPG-pyrophosphorylase. The isomerized forms are then further polymerized to precursor of cellulose by cellulose synthase enzyme (Römling and Galperin 2015).

Various natural sources such as agro-waste, rotten vegetables and fruits, soil as well as waste water are natural reservoirs of these cellulose producing bacteria. With the complex chemical structure, BC evinces many physico-chemical properties such as high extent of polymerization (up to 7000), high crystallinity level (of 70–80%) as well as capacity of water holding/ water retention capacity (up to 95%), advance mechanical stability, great elasticity, ultra-fine nano-structure, which distinguishes it from plant cellulose (Revin et al. 2020; Costa et al. 2017).

Microorganisms as well as the production method are important parameters which regulate the enhanced yield of cellulose. Commonly, BC is produced either static fermentation condition or by agitation. Though agitation enhances the oxygen diffusion rate, it causes the formation of cellulose less mutants, thus lowering the yield most of the time (Srivastava and Mathur 2021; Rastogi et al. 2018).

Recently, several researcher studies are focused on isolating the new strain for high-end production of BC. BC producers which able to synthesize thick cellulosic mat in the air liquid region mainly are Gram negative (-) with rod shape, non- disease causing aerobic bacteria (Ross et al. 1991). This biopolymer is known to exist in free form without any presence of polysaccharides like hemicellulose and lignin. The improved physicochemical properties have gathered great interest from both researchers as well as industrialists, making it an excellent choice of material in

food industry, tissue engineering, biosensors, pharmaceutical and textile industries, packaging, protective coatings and flexible electronics (Molina-Ramírez et al. 2018). Extensive research reports are available on BC production from *Acetobacter* and *Gluconacetobacter* strains. Till date, there are limited studies available on production of BC from *Komagataeibacter saccharivorans* (Gopu and Govindan 2018). Hence the objective of this study was to screen and isolate bacteria cellulose producer with high yield from rotten fruits. The isolated BC producer *K. saccharivorans* BC-G1 was investigated for BC production under static culture condition. Physicochemical characterization of BC was done using SEM, FTIR and XRD. To the best of our knowledge, this is one of the few reports representing the characterization and production of BC from *Komagataeibacter saccharivorans* under static conditions.

Materials and methods

Screening and isolation of cellulose producing bacteria

Isolation of BC producing bacteria was done using different samples of rotten fruits namely- apple, grapes, banana and coconut were collected. A one grams of fruit samples was added to 100 mL modified Hestrin-Schramm (HS) medium containing 2.0% D-glucose (carbon source) (w/v), 0.5% peptone (nitrogen source) (w/v), 0.5% yeast extract (w/v), 0.27% Na₂HPO₄ (w/v), 0.12% citric acid (w/v), 0.2% acetic acid (v/v), 0.5% alcohol (ethanol) (v/v), pH 6.5 (Hestrin and Schramm 1954). The inoculated media was incubated at 30 °C for 5–7 days under static conditions to obtain stable BC layer on the top. The produced BC layer was obtained in the form of white mat on the surface of culture media which was further serially diluted to 1×10^{-6} times with 0.9% NaCl (saline water) (Powers and Latt et al. 1977). Serially diluted samples were further spread on acetic acid bacteria medium (Sharafi et al. 2010) (2.0% D-glucose (w/v), 1.0% yeast extract (w/v), 5% ethanol (w/v), 0.3% CaCO₃ (w/v) (mix CaCO₃ by shaking the medium before pouring) and 2% agar with 0.2mL of inoculum size. The plates were incubated further at 30 °C for 48 h to 72 h to obtain clear colonies of bacteria. The bacterial growth was accompanied with the development of clear zones due to CO₂ production. The eleven bacterial isolates were selected for the further screening and production of cellulose.

Identification of cellulose producing bacterial isolates

Identification of selected BC producer was done using biochemical tests, carbohydrate fermentation test, morphology and gram staining analysis (Singh et al. 2017; Rangaswamy et al. 2015). Further, the isolate bacterial strain (BC- G1) from rotten grape fruit was evaluated for gene analysis using 16 S rRNA sequencing at NCIM, CSIR-NCL, India. The sequencing analysis was performed by following Sanger method using ABI 3500xL genetic analyzer (Life Technologies, USA). Two 16 S rRNA gene primer sequence used for sequencing (forward primer 704F_GTAGCGGT-GAAATGCGTAGA and reverse primer- 907R_CCGT-CAATTCTTTGAGTTT) were used. Once the sequencing has been done, the files obtained were edited using CHROMASLITE (version 1.5) (<https://technelysium.com.au>). The obtained nucleotide sequences were compared with available sequences on the National Center for Biotechnology Information GenBank (NCBI GenBank) database using BLASTn through Basic Local Alignment Search Tool (BLAST) (Zhang et al. 2000b). Moreover, the phylogenetic tree was constructed to obtain the evolutionary relationship of identified strain with other closely related species. Phylogenetic tree was constructed by Neighbor- joining method using MEGA 10.2.6 (Molecular Evolutionary Genetic Analysis) (Kumar et al. 2018) with bootstrap value of 1000 (Thongwai et al. 2022) and CLUSTAL W to align gene sequences for finding gene similarities (Thompson et al. 1994). The obtained nucleotide sequence deposited to NCBI database using GenBank submission portal (<https://submit.ncbi.nlm.nih.gov/subs/genbank/>).

Cellulose production from prepared fermentation media using BC-G1 isolate

To examine the capability of producing cellulose from isolated BC-G1 isolate, the fermentation media (HS medium) was prepared using glucose (20 g/L), yeast extract (5 g/L), peptone (5 g/L), citric acid (2.7 g/L), disodium hydrogen phosphate (Na_2HPO_4) (1.17 g/L). This media (100mL) was additionally supplemented with acetic acid, which was used to maintain final pH up to 6.5. The prepared fermentative media was further autoclaved at 121 °C for 20 min, followed by inoculation using 1% inoculum of isolated BC-G1.

Growth kinetics specific growth rate

The sampling from the culture flask was done at regular intervals to determine cell growth. Growth kinetics was estimated by measuring the optical density at 600 nm (A_{600}) after cellulase treatment of culture broth and bacterial

growth was estimated as specific growth rate (h^{-1}) using Eq. 1 (Kovárová-Kovar and Egli 1998).

$$N_t = N_0 e^{\mu(t_2 - t_1)} \quad (1)$$

Where, N_t and N_0 represent the number of bacterial cells/mL at time t_2 and t_1 , respectively during the exponential growth phase whereas μ represents the specific growth rate of bacterial cells. Doubling time of bacteria was calculated by Eq. 2.

$$t_d = \frac{0.693}{\mu} \quad (2)$$

Production and purification of cellulose from BC-G1 isolate

The produced white mat like layer on the surface of fermentative media was collected and further purified by treating with 1% NaOH (w/v). The harvested cellulose layer was treated for 90 min (thrice) in order to remove the existing impurities from cellulose matrix, present in the form of dead cells. In addition to this, the purified cellulose was neutralized by sterile water (Vasconcelos et al. 2020). The recovered BC sheets were dried using oven drying method at 40 °C for around 1–2 h until its weight was constant (Bodea et al. 2021; Gopu and Govindan 2018).

Effect of carbon sources in BC production

Different carbon sources namely Glucose, Glycerol, Sucrose and Fructose were chosen to evaluate the effect on cellulose production using the isolated BC-G1 strain. The selected carbon sources were added into the growth media (HS media) at 2% (w/v) concentration (Mikkelsen et al. 2009; Molina-Ramírez et al. 2017).

Physicochemical characterization

Fourier transform infrared spectroscopy (FTIR)

The produced and purified BC was characterized in comparison with commercial cellulose (Himedia) using FTIR spectroscopy using Spectrum BX-II Spectrophotometer at material characterization laboratory, Jaypee Institute of Information Technology, India. For FTIR analysis, the transmission mode was selected to collect the spectra over the range of 4000–400 cm^{-1} with the resolution of 4 cm^{-1} . In addition to this, the crystallinity was calculated by obtaining

ratio using the absorption ratio method. In this method, the transmission absorbance from 1372 cm^{-1} (A_{1372}) and 2900 cm^{-1} (A_{2900}) was used to obtain Cr. R1 values whereas transmission absorbance ratio from 1430 cm^{-1} (A_{1430}) and 893 cm^{-1} (A_{893}) was used to obtain Cr. R2 value. The values obtained in terms of Cr. R1 and Cr. R2 were further used to calculate crystallinity ratio (Ciolacu et al. 2011).

X-ray diffraction (XRD)

The obtained BC was also characterized along with commercial cellulose using X-ray diffraction technique using X-ray diffractometer (Shimadzu 6000 XRD, Japan) at material characterization laboratory, Department of Physics and Materials Science and Engineering, Jaypee Institute of Information Technology, India. This technique is used to examine the crystallinity of samples using patterns formed by Cu-K α radiations. During the analysis, patterns were collected in scan range of $2\theta=5-70^\circ$ with speed of $2^\circ/\text{min}$ in order to distinguish several changes within the crystal structures of the cellulose samples. By XRD analysis, the crystallinity index (Cr. I) and crystallinity (Cr%) calculated using Segal's equation (Eq. 3) (Segal et al. 1991; Ioelovich and Veveris 1987).

$$Cr.I(\%) = \frac{I_{002} - I_{am}}{I_{002}} \times 100 \quad (3)$$

Where I_{am} is the minimum intensity which represents the amorphous material. $I_{(002)}$ is the maximum intensity of major crystalline corresponding to the (002) plane at 2θ angle.

Scanning electron microscopy (SEM)

To investigate the structural properties of the cellulose produced by BC-G1 isolate, an electron microscope Zeiss EVO®40 (Germany) at an accelerating voltage of 5 kV was used. The morphology of the samples was studied from the surface as well as the cross-sections. Samples were sputter coated with gold before examination and were photographed at Advanced Instrumentation Research Facility (AIRF) (JNU, India).

Results and discussion

Isolation and screening of cellulose producing BC-G1 bacterial isolate

Rotten fruits were used for isolation of BC producing bacteria in modified HS media. Flask inoculated with rotten grapes was reported positive for BC production as formation of creamy, white mat like structure on the surface of modified HS media after 7 days of incubation. The isolates were then purified using serial dilution method and were tested for BC production on GEY agar plates containing 0.3% (w/v) CaCO_3 . Since all strains of *Komagataeibacter* sp. are known an acid-producers, the development of clear zone around the bacterial colonies is due to dissolution of CaCO_3 in GEY agar plates supplemented with 0.3% (w/v) CaCO_3 . The similar findings have also been reported by Mangayil et al. 2021. The isolate was named as BC-G1 strain, which was further identified using morphological, biochemical and molecular characterization methods (Buchanan and Gibbons 1975; Rangaswamy et al. 2015). Physiological and biochemical test characteristics further demonstrated that the isolated strain belongs to the family of *Acetobacter* sp. as showed in Table 1 (Vos et al. 2011).

16 S rRNA gene serves as a genetic marker and used in study of bacterial taxonomy and phylogenetic analysis. The gene sequence data was aligned using CLUSTAL for finding gene similarities. The sequence analysis studies confirmed isolated BC-G1 strain as *Komagataeibacter saccharivorans* (Accession No. ON514605.1) (<http://www.ncbi.nlm.nih.gov/genbank/>).

Phylogenetic tree provides information related to evolutionary relationship of new strain to their ancestors. 16 S rRNA partial sequence analysis of the isolated strain BC-G1 revealed that our strain is closely related to *Komagataeibacter saccharivorans* (LMG 1582) with 100% identity score (Fig. 1) (Zhang et al. 2000b). The tree was constructed using Neighbor Joining method using MEGA 10.2.6 (Fig. 1) (Kumar et al. 2018).

Production of cellulose using BC-G1 isolate

The strain BC-G1 was examined for BC production under static condition at 30°C for 6 days. The BC was recovered as the floating films on the liquid medium surface after 4th day of incubation. The maximum amount of BC was obtained on day 4 of incubation (14 g/L), after which the amount of BC started decreasing (Fig. 2). Studies have shown that maximum BC from different strains of genus *Komagataeibacter* has been reported during 4-5th days of incubation under static condition after which the BC production was decreased subsequently (Vigentini et al. 2019). Increase in

Table 1 Biochemical Characteristics of the BC-G1 isolate and *Acetobacter aceti* MTCC 2623* (*as reference culture)

Biochemical Test	BC-G1 isolate	<i>Acetobacter aceti</i> MTCC 2623
Gram Staining Analysis	-	-
Methyl Red (MR) Test	+	+
Indole Test	-	-
Citrate Utilization	-	-
V-P test	+	+
Catalase Test	+	+
Oxidase Test	+	+
Fermentation Test		
D-glucose	+	+
D-fructose	+	+
Lactose	+	+
Maltose	+	+
Sucrose	+	+
Starch	+	+
Cellulose	+	+
pH		
2	-	-
3	-	-
4	+	+
5	+	+
6	+	+
7	+	+
8	+	+
Temperature (°C)		
20	+	+
25	+	+
30	+	+
35	+	+
Morphological Characteristics of the BC-G1 isolate		
Characteristics	Observation	
Color	Creamy	
Shape	whitish	
Elevation	Circular	
Surface	Convex	
Shape of cell	Smooth	
Growth on Glucose yeast CaCO ₃ -ethanol medium	Rod shaped Clear zone formation that became cloudy in the center	

incubation time results in formation of disrupted and irregular BC membrane and slight acidity of media, as previously reported (Bodea et al. 2021; Lahiri et al. 2021).

BC yield obtained from *Komagataeibacter saccharivorans* BC-G1 in this study is in well agreement with the reported literature (Gopu and Govindan 2018; Mohite and Patil 2014), supporting the fact that cellulose production is dependent on number of incubation days, choice of bacteria, growth medium as well as the culture conditions. To study the effect of agitation on production of cellulose,

BC-G1 strain was inoculated in HS media and incubated at 30° C, 120 rpm for 5 days (Aydin and Aksoy 2014). Results showed that upon agitation, the production of cellulose was observed to be 0.3 g/ 100 mL, dry weight. Shaking/ agitation is known to produce several non- cellulose producing mutants which negatively affect the production of desirable cellulose in culture media, hence producing less cellulose in agitation conditions than static conditions (Kadier et al. 2021).

Growth studies of BC-G1 isolate in HS medium

The isolated BC-G1 strain was further used for evaluating the cell growth. The bacteria were inoculated in HS medium and incubated for 120 h under static and agitated conditions-Sharma et al. 2016a, b; Singh et al. 2016).

The specific growth rate and doubling time variations were observed in both culture conditions using Eqs. 1 and 2 (Table 2) (Fig. 3) (Sharma et al. 2016a, b; Singh et al. 2016).

Results demonstrated that the specific growth rate of BC-G1 in HS media under static condition (0.49 h⁻¹) was lower than that of agitation condition (0.56 h⁻¹) (Table 2). Since under agitation condition, the bacteria receive more oxygen due to aeration hence the specific growth rate of bacteria is more when compared to static condition (Mathur et al. 2015). Agitation is also known to cause shear stress to the bacterial cell and may result in reduction of BC productivity. (Wang et al. 2019).

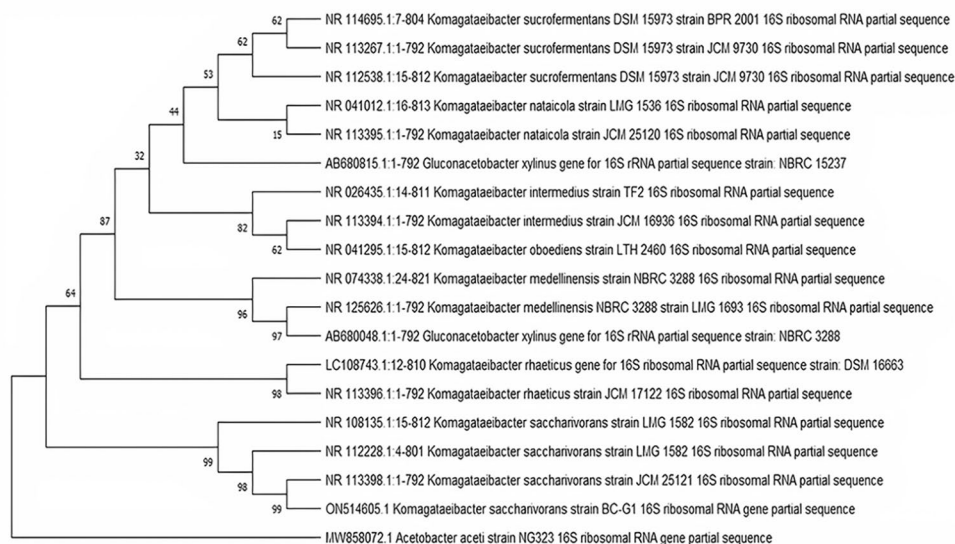
Table 2 Effect of culture conditions on specific growth rate (μ) and doubling time of *Komagataeibacter saccharivorans* BC-G1 isolate in HS medium.

Effect of different carbon sources on BC production

The dynamics of different carbon sources on BC production was evaluated and it was observed that the production of BC is greatly affected by the type of carbon source used. Our results demonstrate that the maximum BC production (13.65 g/L) was observed when the media was supplemented with Glucose. In addition to this, BC production was observed to be 11.9, 8.45 and 11.75 g/L for Glycerol, Sucrose and Fructose respectively (Fig. 4) (Molina-Ramírez et al. 2017).

Maximum BC production was observed till 96 h under static condition with all carbon sources as a floating gelatinous mat-like structure which became irregular and distorted upon further incubation due to depletion of growth factors in the media. When the pellicle growth slows down and all the bacteria are entrapped, the synthesis of BC reaches its threshold and bacteria starts consuming the already formed BC films for survival apart from growth media (Molina-Ramírez et al. 2017). In addition to this, the change in pH

Fig. 1 16 S rRNA partial gene sequence and Phylogenetic tree of isolate BC-G1



>907RC_Seq19_BCG-1

GCTCAGAGCGAACGCTGGCGGCATGCTTAACACATGCAAGTCGCACGAACCTTTCGGGGTT
 AGTGGCGGACGGGTGAGTAACGCGTAGGGATCTGTCCATGGGTGGGGGATAACTTTGGGAA
 ACTGAAGCTAATACCGCATGACACCTGAGGGTCAAAGGCGCAAGTCGCCTGTGGAGGAACC
 TGCGTTCGATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGATGATCGATAGCTGGTC
 TGAGAGGATGATCAGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCA
 GTGGGGAATATTGGACAATGGGCGCAAGCCTGATCCAGCAATGCCGCGTGTGTGAAGAAGG
 TTTTCGGATTGTAAAGCACTTTCAGCGGGGACGATGATGACGGTACCCGCAGAAGAAGCCC
 CGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGGGCAAGCGTTGCTCGGAATGAC
 TGGGCGTAAAGGGCGCTAGGCGGTTTAAACAGTCAGATGTGAAATTCCTGGGCTTAACCT
 GGGGGCTGCATTTGATACGTTGAGACTAGAGTGTGAGAGAGGGTTGTGGAATTCAGTGT
 AGAGGTGAAATTCGTAGATATTGGGAAGAACACCGGTGGCGAAGGCGGCAACCTGGCTCAT
 TACTGACGCTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCCTGGTAGTCCAC
 GCTGTAAACGATGTGTGCTGGATGTTGGGTGACTTTGTTCATTTCAGTGTCTAGTTAACGCG
 ATAAG

was also observed which states that the decrease of pH during cellulose production is directly linked to decrease in the synthesis of the polymer. Our results are also in compliance with the studies of Molina and others, 2017 (Molina-Ramirez et al. 2017) who have indicated the BC production using three different carbon sources namely glucose, sucrose and fructose. The results from their study also showed the maximum production using glucose as carbon source. It is considered that the glucose being the source of ultimate energy as well as the precursor of cellulose polymerization, resulted in maximum yield of BC as compared to other carbon sources.

FTIR spectra of commercial cellulose and BC

The FTIR spectra analysis comprises two regions namely functional group region as well as the fingerprint region. It is known that functional group region contains the absorption peaks having wave numbers exceeding the value of 1450 cm^{-1} providing the information regarding functional groups present in the sample whereas the fingerprints region contains the absorption peaks lying in range lesser than 1450 cm^{-1} , providing the information regarding vibrational characteristics in the sample (Fig. 5) (Ciolacu et al. 2011). Our results showed that the first spectra region of produced BC was observed at wave number 3284 cm^{-1} , 3033 cm^{-1} , 2971 cm^{-1} , hence indicating the presence of functional groups having hydrogen as the main element. The common vibrational pattern was observed to be C-H,

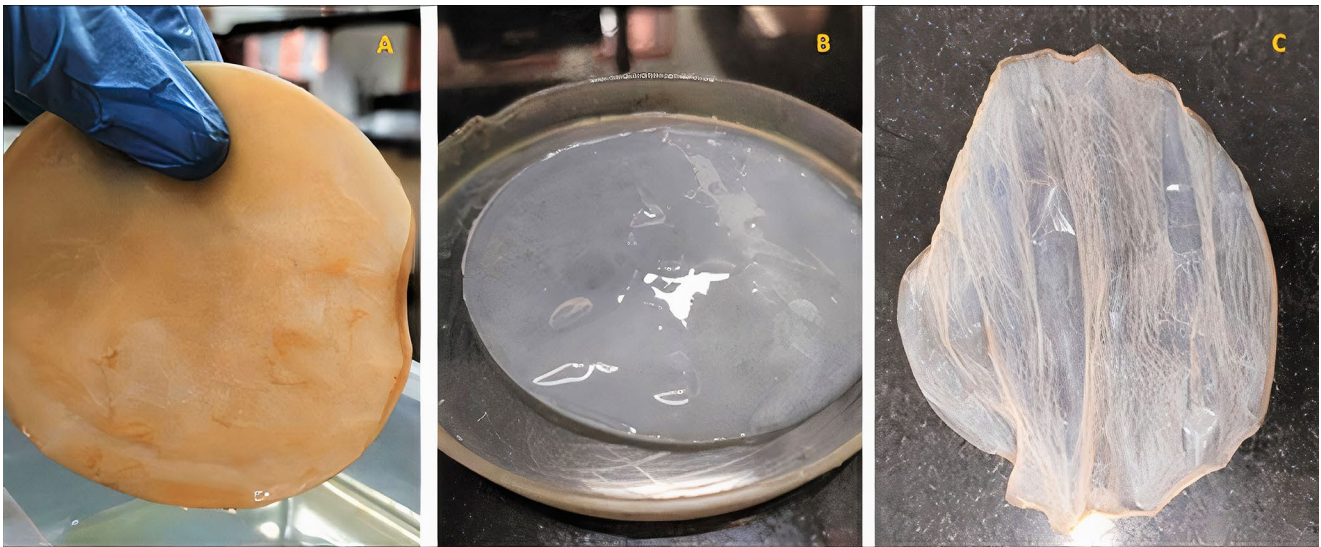


Fig. 2 Produced BC from (A) *Komagataeibacter saccharivorans* BC-G1; (B) Purified BC; (C) BC film in dried form

Table 2 Effect of culture condition on specific growth rate (μ) and doubling time of *Komagataeibacter saccharivorans* BC-G1 isolate in HS medium

Culture Condition	Specific growth rate (h^{-1})	Doubling time (h)
Agitation condition	0.56	1.40
Static condition	0.49	1.22

N-H, and O-H with stretching vibrational patterns present in the samples. In addition to this, the second spectra region was observed from wave number 2000 cm^{-1} to 2500 cm^{-1} indicating the presence of functional groups with two major triple bonds vibrational stretching patterns as $C \equiv C$ and $C \equiv N$, hence showing very less peak shift in this second spectral region. The third region in our FTIR spectra was observed from wavenumber 1350 cm^{-1} to 2000 cm^{-1} having absorption peaks at 1367 cm^{-1} , 1541 cm^{-1} , 1646 cm^{-1} , 173

Fig. 3 Growth studies of *Komagataeibacter saccharivorans* BC-G1 in HS medium in static and agitation conditions

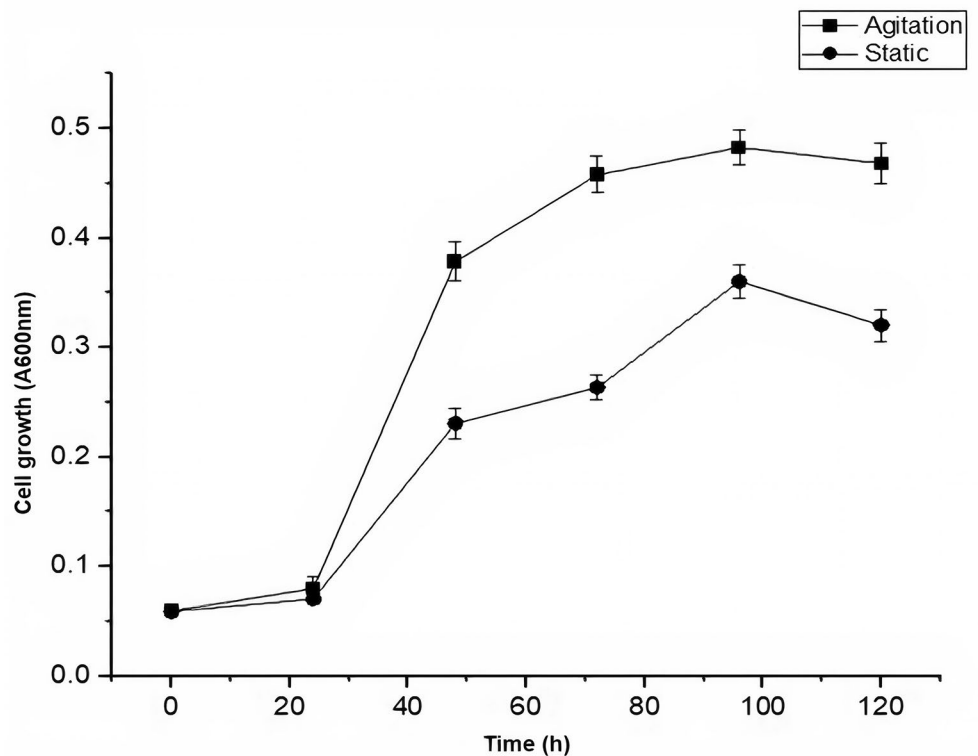
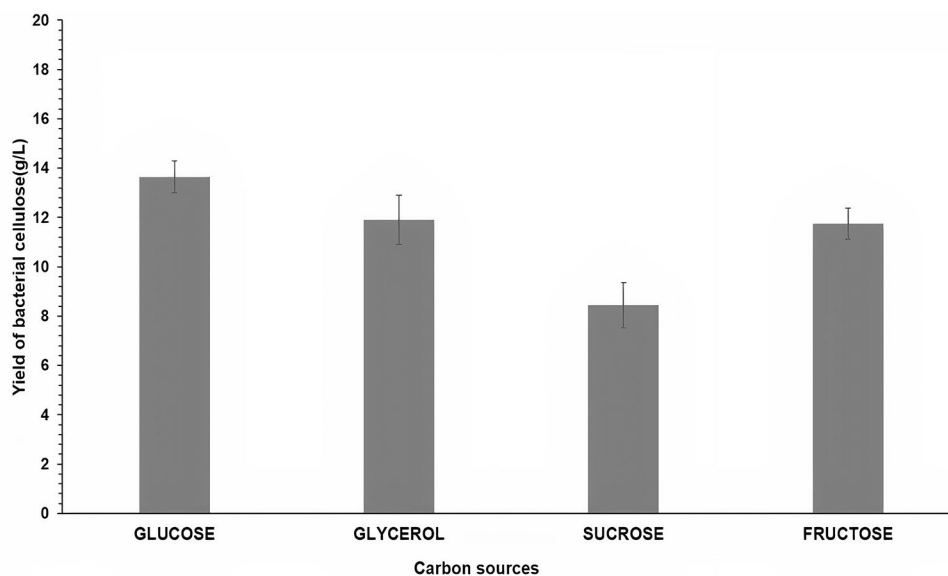


Fig. 4 Yield of BC with different carbon sources supplemented in HS medium



5 cm^{-1} exhibiting the general double bond stretching with functional groups like C=N, C=O and C=C. The presence of CH functional groups with absorption peaks included in alkanes (2800–3000 cm^{-1}), alkenes (3000–3100 cm^{-1}), alkynes (3300 cm^{-1}) as well as other aromatic compounds was noted in spectra region above 2500 cm^{-1} . Moreover, it was observed that aldehyde composed of two distinct absorption peaks at 2700–2900 cm^{-1} due to O=C-H stretching vibrations and O-H stretching vibrations and carboxylic acids (2400–3300 cm^{-1}) (Fig. 5) (Ciolacu et al. 2011). Figure 5 shows the presence of carbonyl stretching due to aldehyde group (1720–1740 cm^{-1}), ketones group (1705–1725 cm^{-1}), carboxylic acids (1700–1725 cm^{-1}) (Yao et al. 2015). The double bond stretching vibration region contains the absorption peaks produced by C=C stretching vibrations (1640–1670 cm^{-1}), aromatic ring C-C absorption peaks (1450–1600 cm^{-1}) (Oh et al. 2005). In addition, the FTIR absorption at 1450 cm^{-1} of CH₂ bending vibration refers to crystallinity band, indicating that a decrease in its intensity reflects reduction in the degree of crystallinity of the samples (Ioelovich 2021). The FTIR absorption at 893 cm^{-1} of C–O–C stretching at beta glycoside linkages, is designed as an amorphous absorption band, an increase in its intensity occurring in the amorphous samples (Ciolacu et al. 2011; Nelson and O'Connor et al. 1964). The ratios of crystallinity (Cr.R) of commercial cellulose using Eq. 3 (Cr. R1 (A_{1372}/A_{2900})=2.33, Cr. R2 (A_{1430}/A_{893})=0.60) and BC produced by BC-G1 (Cr. R1 (A_{1372}/A_{2900})=0.96, Cr. R2 (A_{1430}/A_{893})=0.92) obtained using FTIR spectra.

X-ray analysis of commercial cellulose and BC

The X-ray diffraction analysis of produced cellulose from BC-G1 and commercial cellulose was conducted. The X

ray diffractograms depicted that the peaks were observed in case of cellulose produced from BC-G1 at 14.08°, 16.72° and 22.48° (Fig. 6). These peaks are known to correspond to defined crystallographic planes of (100), (010) and (002) respectively, hence revealing the presence of amorphous nature of the BC samples. In compliance to this result, the commercial cellulose was also observed to have less amorphous nature when compared to BC sample (Nelson and O'Connor et al. 1964). Less crystalline cellulose is characterized by increased hydrophilicity, reactivity, and enzymatic digestibility. Due to its amorphous structure, it can be used as a promising substrate for enzymatic hydrolysis to produce glucose, which can be applied in biotechnology for growing various microorganisms (Ioelovich, 2021).

The crystallinity index (Cr. I) is calculated as %Cr using Segal's equation (Eq. 3) (peak intensity method) (Table 3 (Poletto et al. 2014; Segal et al. 1991).

SEM micrographs of produced BC

Figure 7 shows the morphological structure of BC produced by *Komagataeibacter saccharivorans* BC-G1 at 5kx and 2kx magnifications. The micrographs obtained from SEM analysis revealed that the BC retained its network structure and twisting ribbons without any specific orientation, the fine microfibril structure in an interconnected network form, arranged in crossed random orientation with superimposed layers which ensured good behavior of BC in water-holding and permeability (Zhang et al. 2018a). 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 led to have property such as mechanical property, crystallinity and water holdup rate. These

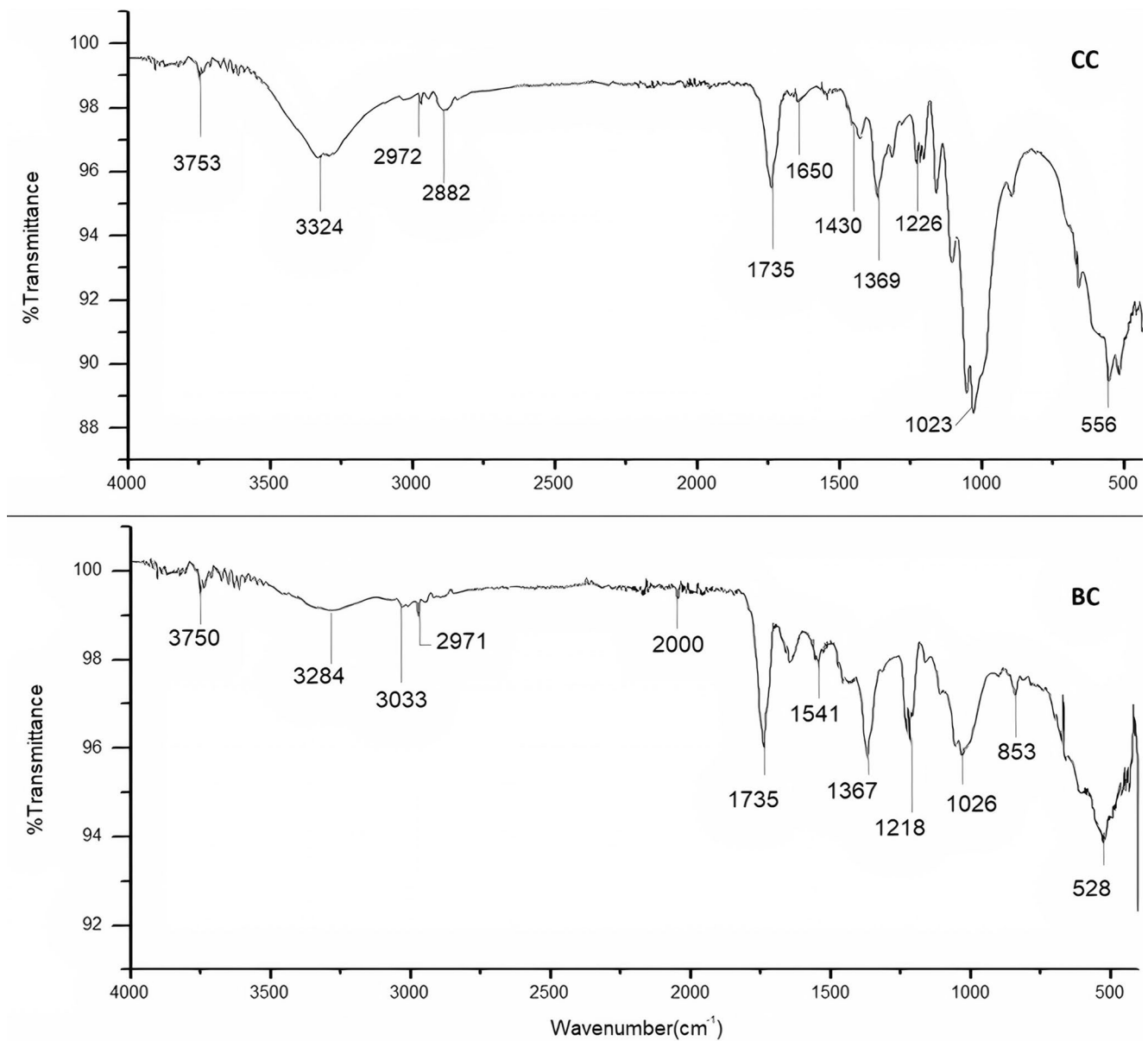


Fig. 5 FTIR of Bacterial Cellulose (BC) produced by *Komagataeibacter saccharivorans* BC-G1 isolate on HS medium in static condition and Commercial Cellulose (CC)

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 (Poletto et al. 2014).

Conclusion

From this study, we have concluded that BC producing bacterial strain has been isolated from rotten grape fruit and was identified as *Komagataeibacter saccharivorans*. The culture was explored for cellulose production under static culture condition in HS medium. BC yield was high compared to

other strains reported so far. Physicochemical and structural characterization of BC allowed the detailed analysis of produced biopolymer for various applications. The FTIR spectra confirmed the purity of BC and less crystalline behavior of BC-G1. X-ray diffraction pattern revealed three characteristic diffraction peaks indicating the presence of less crystalline behavior of the cellulose produced by BC-G1 isolate. SEM studies showed the reticulated fibrillar structure of BC produced by BC-G1 isolate. The study can be further extended to utilize agro-industrial raw materials as an alternative cheap and cost-effective substrate for BC production. *Komagataeibacter saccharivorans* BC-G1 reported in this study may be a potential candidate for large scale, cost

Fig. 6 X-Ray diffraction pattern of CC (Commercial Cellulose) and Bacterial Cellulose (BC) produced by *Komagataeibacter saccharivorans* BC-G1 isolate on HS medium in static condition

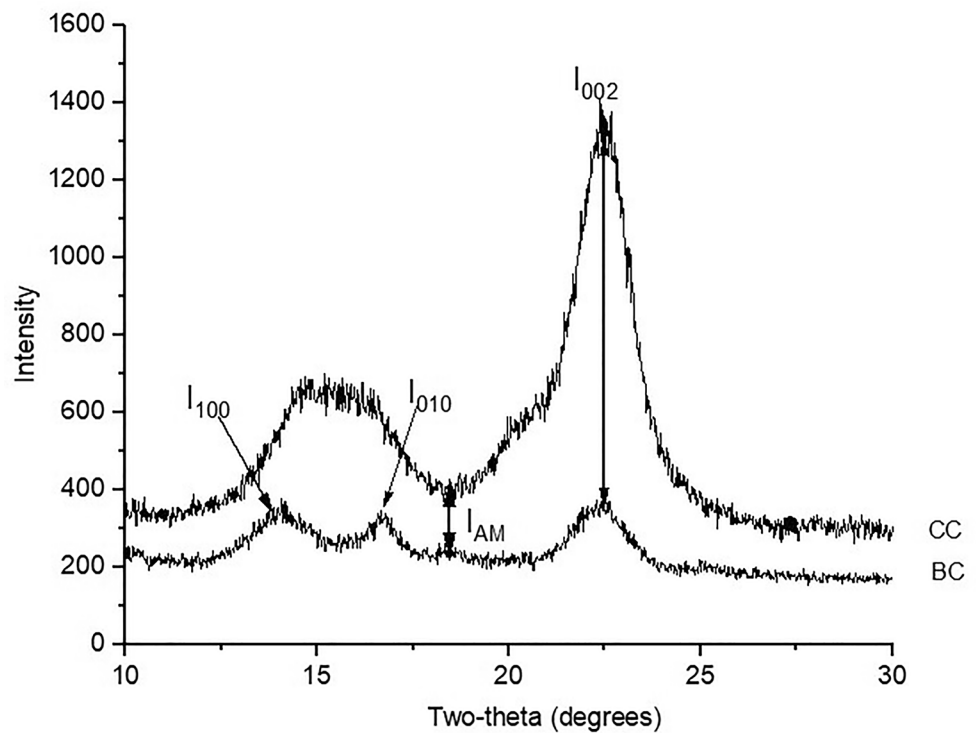


Table 3 Crystallinity index calculation using peak intensity method

Samples	Cr. I (Crystallinity index)
Commercial Cellulose (CC)	71.5%
Bacterial Cellulose (BC)	39.7%

effective production of BC with improved physicochemical properties for widespread applications.

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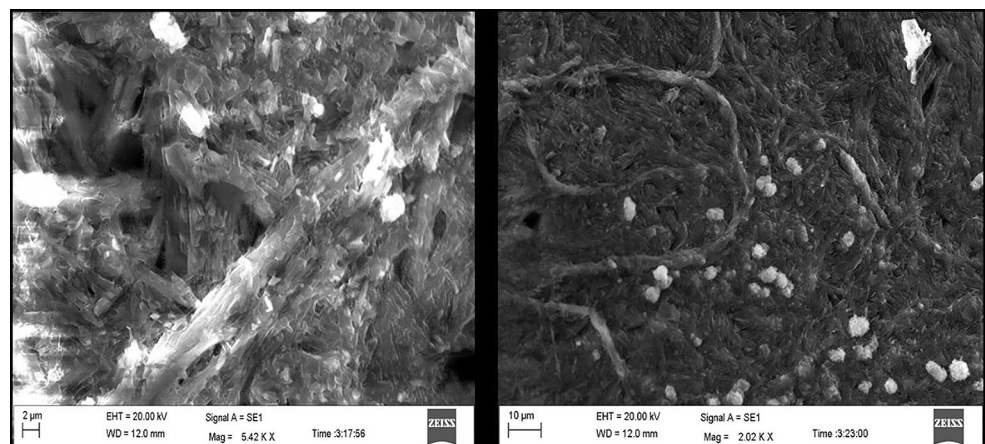
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Declarations

Conflict of interest The authors declare that they have no conflict of interest in the publication.

Competing interests The authors have no financial and nonfinancial competing interests to declare that are relevant to the content of this article.

Fig. 7 SEM micrographs of BC produced by *Komagataeibacter saccharivorans* BC-G1 at 5kx and 2kx magnifications



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