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Production and Characterization of Bacterial Cellulose Synthesized by *Komagataeibacter* sp. Isolated from Rotten Coconut Pulp

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Bacterial cellulose is a naturally occurring polysaccharide that is produced by acetic acid bacteria. It has become increasingly popular due to its numerous biotechnological applications. Bacterial cellulose is free from contaminants that are often associated with plant cellulose. This study aims at isolation of bacterial cellulose producing microbial strains from rotten fruits. The selected bacterial cellulose producer was identified using biochemical tests, including biochemical, carbohydrate fermentation, morphology, gram staining and 16S rRNA sequence analysis. Furthermore, several different media were evaluated for higher production of cellulose. The produced cellulose sheets were washed, purified and characterized using various analytical techniques, including FTIR, XRD and SEM analysis. A potential high bacterial cellulose yielding microbial strain was isolated from coconut pulp waste and identified as *Komagataeibacter saccharivorans* strain BC-C1 using 16S rRNA sequencing. Effect of media components on bacterial cellulose production was studied. The isolate yielded 0.52 g/100 mL with Hestrin-Schramm (HS) culture media (designated as M1) and 1.10 g/100 mL in M5 media. The physico-chemical characterization demonstrated that produced bacterial cellulose sheets show characteristics IR bands and three-dimensional fibrillar interconnected network structure. The study revealed that isolated *Komagataeibacter saccharivorans* strain BC-C1 can be utilized for large scale production of bacterial cellulose.

Keywords: Bacterial cellulose, Coconut, *Komagataeibacter*, Rotten fruits.

INTRODUCTION

Bacteria derived cellulose represents a crucial biomaterial synthesized by members of the *Acetobacteraceae* family, specifically *K. hansenii*, *K. europaeus*, *K. maltaceti*, *K. melomenus*, *K. rhaeticus*, *K. saccharivorans* and *K. xylinus*. This type of cellulose is considered superior due to its distinctive physico-chemical characteristics, which include high purity, crystallinity and exceptional water retention capacity. Recently, there has been a growing interest in the identification and isolation of cellulose-producing bacteria, driven by the increasing focus on the development of sustainable biomaterials [1]. Bacterial cellulose is a nanofibrillar material composed of glucose molecules linked together in a linear form through β-1,4-glycosidic bonds and these glucose units are organized in a highly structured crystalline pattern. Despite structural similarities, bacterial cellulose exhibit superior physico-chemical properties, including higher levels of purity, polymerization and crystallinity compared to plant cellulose. These unique characteristics are attributed to the stable 3D structure of bacterial cellulose [2,3]. A higher degree of polymerization leads to a more compact and tightly packed structure in bacterial cellulose, making it more resistant to degradation and increasingly stable over time [4].

The structural density of microfibrils in bacterial cellulose is such that they are aligned in parallel, forming ribbon-like structures that are interwoven with one another, ultimately creating a 3D network. The selection of the microbial strain, cultivation conditions, such as temperature, pH and aeration, the age and size of the bacterial inoculum, the composition of carbon and nitrogen sources in the growth medium and the presence of additional nutrients or additives, are all important factors influencing the structural properties of bacterial cellulose. Together, these elements affect the quality of bacterial cellulose pellicles, their thickness and density and the rate of cellulose biosynthesis [5].

Microbial cells have the capability to utilize a variety of carbon sources, including sucrose, glucose, mannitol and others, in order to polymerize and transport glucan chains through specialized pores known as terminal complexes. These transported glucan chains then undergo assembly processes to ultimately form protofibrils, bundles and, eventually, ribbon-shaped microfibrils. The structural characteristics of these glucan chains and their arrangement into microfibrils, bundles, strips or ribbons are controlled by structures similar to terminal complexes [6,7]. Bacterial cellulose can be divided into two different forms based on how the fibrils are arranged: lace-like cellulose type-I and nebulous cellulose type-II. The parallel, unidirectional alignment of the β-1,4-glucose chains in cellulose type-I results in a highly organized, crystalline structure. Contrarily, the placement of the glucose chains in cellulose type-II is random and lacks a defined pattern, resulting in a less structured and amorphous structure. The microfibrillar arrangement of cellulose fibers in these two types significantly determines the tensile strength and crystallinity of bacterial cellulose [7]. The remarkably ultrafine fibrous structure of bacterial cellulose serves as the foundation for its wide-ranging applications. The organization of fibrils and their exceptional purity create a distinct avenue for the production of applications involving bacterial cellulose based composites. The high water retention capacity, nano-porosity, cell adhesion capabilities and flexible properties of bacterial cellulose are directly linked to the structure and arrangement of its cellulose fibrils, which, in turn, enable a broad spectrum of microbial cellulose applications [8,9]. The unique structural composition and distinct properties of bacterial cellulose become a point of attraction for many researchers and industries. Biocompatible and biodegradable feature of bacterial cellulose provides a special place to bacterial cellulose in medical research and bioengineering [10].

Scaling up bacterial cellulose production to an industrial level presents several technological challenges that need to be addressed. These challenges include bacterial cellulose inherent hydrophobicity, limited solubility and its comparatively high production cost. The primary factor contributing to the elevated cost of bacterial cellulose production is the relatively low bacterial cellulose yield obtained with known bacterial strains and the use of expensive culture media. Overcoming these challenges may involve the development of more efficient bacterial cellulose producing strains, optimization of culture conditions to enhance yield and the exploration of cost-effective nutrient sources and cultivation methods. These production process difficulties may be resolved by using the fed-batch operation mode, which encourages bacteria to continue generating cellulose by adding fresh batch of the culture medium. Recently, bacterial cellulose production by *Gluconacetobacter xylinus* using fed batch culture technique in glycerol media resulted enhanced bacterial cellulose yield, substrate consumption and volumetric productivity [11]. Use of genetic modification may be a tool for introducing desired mutations at genetic level for improving bacterial cellulose yield and altering the physicochemical properties of cellulose suitable for a given application sector [12]. Several reports focusing on exploring the utilization of low cost carbon sources for cost effective and large scale bacterial cellulose production have been published [13-15].

Present investigation primarily aimed to isolate efficient cellulose-producing bacteria from the natural habitats of acetic acid bacteria. The isolates were identified at the genus level through biochemical, morphological analysis and 16S rRNA sequencing. Additionally, the impact of different culture media components on bacterial cellulose produced by *K. saccharivorans* BC-C1 as well as the physico-chemical characterization using advanced techniques such as Fourier transform infrared spectroscopy, scanning electron microscopy and X-ray diffraction were also performed.

EXPERIMENTAL

All materials and chemicals utilized in this study were of analytical grade. Glucose, peptone, yeast extract, disodium hydrogen phosphate, citric acid, calcium carbonate, ethanol, acetic acid was purchased from HiMedia, India. Standard cellulose used was procured from HiMedia.

Isolation and identification of bacterial cellulose producing strain: For the isolation of bacterial cellulose producing strains, modified Hestrin-Schramm (HS) medium (% w/v) (D-glucose: 2.0, peptone: 0.5, yeast extract: 0.5, Na_2HPO_4 : 0.27, citric acid: 0.12, acetic acid: 0.2, ethanol: 0.5, pH 6.5) was inoculated with 1 g coconut pulp and incubated at 30 ± 2 ºC in the static cultivation conditions to obtain floating gelatinous bacterial cellulose [16]. Broth was then serially diluted and spread onto acetic acid bacteria medium with composition (% w/v): (D-glucose: 2.0, yeast extract: 1.0, ethanol: 5.0, $CaCO_3$: 0.3 and agar: 2.0) [17] for 2-3 days. After incubation bacterial colonies exhibiting clear zone on agar plate were selected for examination. For identification of bacterial strain, the morphological, biochemical and molecular investigations were also performed. Gram staining, cell morphological characteristics and spore forming ability were determined. The biochemical characteristics of the strain were tested as per Systematic Bacteriology Manual [18]. 16S rDNA sequence analysis performed at NCIM, CSIR-NCL, India, using the Sanger method. The sequencing data was processed using Chromas lite (version 1.5) and compared with existing sequences in the NCBI GenBank database utilizing BLASTn. Phylogenetic tree was built using MEGA 10.2.6 based on the Neighbor-joining method. The gene sequences were analyzed for gene similarities *via* CLUSTALW alignment and a bootstrap value of 1000 was applied. Finally, the nucleotide sequence was submitted to the NCBI.

Culture media and cultivation: Five different fermentation media, which are reported for bacterial cellulose production were selected based on literature review to study cellulose production from selected isolate (Table-1). For primary inoculum preparation, single colony from actively growing pure culture was inoculated into 10 mL of each auto-claved media. Further, cell growth and the bacterial cellulose production were studied in 100 mL different culture media inoculated with 2% (v/v) inoculum [19]. Acetic acid was added to the fermentation media during preparation in order to keep the pH level constant. Cultures were incubated at 30 ± 2 ºC for 5-7 days. Bacterial cellulose

TABLE-1
DIFFERENT CULTURE MEDIA USED IN THE STUDY FOR BACTERIAL CELLULOSE PRODUCTION [20]

HS medium [M1] (%)		Yamanaka medium [M2] (%)		Park medium [M3] (%)		Wei, Yang & Hong medium [M4] (%)		Tang Jia, Jia & Yang medium [M5] (%)	
Glucose	2.0	Sucrose	5.0	Glucose	1.0	Mannitol	2.5	Glucose	2.5
Peptone	0.5	Yeast extract	0.5	Peptone	1.0	Tryptone	0.3	Yeast extract	0.75
Yeast extract	0.5	$(NH_4)_2SO_4$	0.5	Yeast extract	0.7	Yeast extract	0.5	Peptone	1.0
Na_2HPO_4	0.27	KH_2PO_4	0.3	Acetic acid	0.15			Na_2HPO_4	1.0
Citric acid	0.15	$MgSO_4.7H_2O$	0.05	Succinic acid	0.2			Acetic acid	1.0
pH	6.5	pH	6.0	pH	6.5	pH	5.5	pH	6.0

membranes formed on the surface of each medium was harvested as per the standard process [17,19].

Recovery and purification of bacterial cellulose: The bacterial cellulose membranes collected from each flask were washed with distilled water followed by treatment with NaOH solution (1% w/v) for 1 h at 50 ºC to remove any remaining bacteria cells and other impurities. The NaOH treatment was carried out at a high temperature on a magnetic stirrer to ensure that all cells are removed. After NaOH treatment, the bacterial cellulose membranes were rinsed with distilled water until the pH reaches 7. Finally, the bacterial cellulose membranes were stored at 4 ºC for further analysis.

Physico-chemical characterization of bacterial cellulose

FTIR analysis: FTIR analysis is a qualitative tool to investigate the ordering degree of cellulose polymer in signature fingerprint wave number regions [20]. The bacterial cellulose samples were characterized using FTIR spectroscopy (Spectrum BX-II spectrophotometer (Perkin-Elmer, Massachusetts, USA) in transmission mode recorded in the range of 4000-400 cm^{-1}. Commercial cellulose (Himedia, India) was used as a reference sample.

XRD analysis: X-ray data were recorded using an X-ray diffractometer Shimadzu-6000, Japan with Cu*K*α radiation (voltage = 40 kV). Diffraction patterns were collected from 2θ = 5º to 30º, at a speed of 0.5º min^{-1} and a step size of 0.02º. Scherrer's equation (eqn. 1) relates the line broadening of a diffraction peak to the size of the crystallites [21]:

$$d = \frac{K\lambda}{\beta\cos\theta} \quad (1)$$

where d is the mean crystallite size in nanometers (nm); K is the Scherrer constant, typically taken to be around 0.9; λ = wavelength of the X-rays used in nanometers (0.154); β = FWHM; width of the diffraction peak in radians; θ is the diffraction angle in radians, which can be converted from the 2θ angle using the relation θ = π/180 × 2θ. Segal equation (eqn. 2) was used to calculate crystallinity index (Cr. I) and crystallinity (Cr%) of the bacterial cellulose samples [22].

$$\text{Cr. I } (\%) = \frac{I_{(002)} - I_{am}}{I_{(200)}} \times 100 \quad (2)$$

where I_{am} = minimum intensity corresponding to the amorphous region; $I_{(002)}$ = total intensity of (002) plane.

Surface morphological analysis: Scanning electron microscopic technique was used to investigate the morphological characteristics of the cellulose synthesized by *K. saccharivorans* BC-C1. Samples were mounted on the stub, gold coated and then examined under scanning electron microscope (SEM Zeiss EVO®50 series, Germany) with an accelerating voltage of 5 kV.

Statistical analysis: All experiments were carried out in triplicates and data were expressed as mean ± standard deviation (SD).

RESULTS AND DISCUSSION

Isolation of cellulose producing bacteria from coconut pulp: Coconut pulp waste was used for isolation of cellulose producing bacterial strains using HS media, which resulted in the production of four bacterial isolates. All these four bacterial strains were found to produce white creamy mat-like structure over the surface of HS media. Amongst all, it was found that BC-C1 strain exhibited the maximum amount of cellulose production and was considered for further analysis. In addition to this, purified isolates of BC-C1 (purified using serial dilution) exhibited the development of clear zones in GEY agar plates, which was supplemented with $CaCO_3$. The formation of clear zones by bacterial colonies over the desired agar plates containing GYE media further confirms that the isolated bacterial belongs to the genus of *Komagataeibacter* sp. After isolation, the BC-C1 strain was characterized using biochemical and morphological characteristics in accordance with Bergey's Manual [18]. The results demonstrated that the isolated BC-C1 was showing maximum similarity with the known strain *Acetobacter aceti* (MTCC 2623) which belongs to the family of *Acetobacter* sp. (Table-2). All the biochemical tests and the physiological tests exhibited the maximum similarity between both the strains, hence confirming our assumption that the isolated strain belongs to the family of *Acetobacter* sp.

Further, in order to validate the present findings, the 16S rRNA sequence analysis was also performed and the phylogenetic tree was created using MEGA 10.2.6 software through neighbour joining method. 16S rRNA sequence analysis is considered as crucial parameter in precise identification and classification of bacterial species [23]. It is considered that 16S rRNA sequence analysis leads to formation of phylogenetic tree which helps in determining the evolutionary relationships among different bacterial strains (Fig. 1). In this study, the sequence of BC-C1 obtained from 16S rRNA sequence analysis was further aligned using CLUSTAL Mega 10.2.6, in order to determine the similarities between the existing genes. The sequence analysis as well as phylogenetic tree analysis confirms that our isolated strain BC-C1, *K. saccharivorans* (Accession

TABLE-2
BIOCHEMICAL CHARACTERISTICS OF THE BC-C1 ISOLATE (*Acetobacter aceti* MTCC 2623* AS REFERENCE CULTURE)

Biochemical test	BC-C1 isolate	*Acetobacter aceti* MTCC 2623*	Fermentation test	BC-C1 isolate	*Acetobacter aceti* MTCC 2623*	pH	BC-C1 isolate	*Acetobacter aceti* MTCC 2623*
Gram staining analysis	–	–	D-glucose	+	+	2.0	–	–
Methyl red (MR) test	+	+	D-fructose	+	+	3.0	–	–
Indole test	–	–	Lactose	+	+	4.0	+	+
Citrate utilization	–	–	Maltose	+	+	5.0	+	+
V-P test	+	+	Sucrose	+	+	6.0	+	+
Catalase test	+	+	Starch	+	+	7.0	+	+
Oxidase test	+	+	Cellulose	+	+	8.0	+	+

Temp. (°C)	BC-C1 isolate	*Acetobacter aceti* MTCC 2623*
20	+	+
25	+	+
30	+	+
35	+	+

Morphological characteristics of the BC-C1 isolate	
Characteristics	Observation
Colour of the colony	Creamy whitish
Shape of the colony	Circular
Elevation	Convex
Surface appearance	Smooth
Microscopy	Rod shaped
Growth on glucose yeast $CaCO_3$-ethanol medium	Clear zone formation

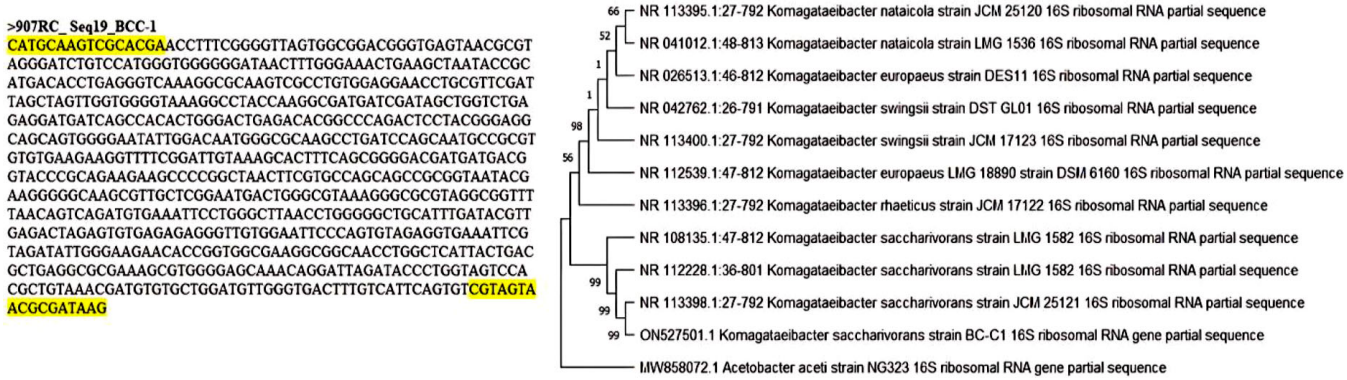

Fig. 1. 16S rRNA sequence and phylogenetic tree analysis for isolated BC-C1 strain

no. ON527501) was found to be identical with *K. saccharivorans* strain JCM 25121, exhibiting 99.74% similarity.

Estimation of bacterial cellulose yield using different culture media: The effect of different culture media was examined upon the production of bacterial cellulose by BC-C1 strain. For analysis, different culture media namely HS medium (M1), Yamanaka medium (M2), Park medium (M3), Wei, Yang & Hong medium (M4) and Tang Jia & Yang medium (M5) were considered. The isolated BC-C1 strain was inoculated in different culture media (Table-1) and incubated for 96 h. After incubation period, the bacterial cellulose yield (on the basis of wet weight) was determined and it was found that M1, M2, M3, M4 and M5 media produced 9.07 g/100 mL,4.00 g/100 mL, 17.90 g/100 mL, 1.80 g/100 mL and 22.2 g/100 mL of bacterial cellulose. On the basis of dry weight (g/100 mL), the bacterial cellulose yield was estimated as 0.52 g/100 mL, 0.12 g/100 mL, 0.9 g/100 mL, 0.26 g/100 mL and 1.10 g/100 mL in M1, M2, M3, M4 and M5 media, respectively. Fig. 2 displays the maximum bacterial cellulose produced in M5 media under favourable conditions. The variation of bacterial cellulose yield was positively correlated with the media composition, thereby indicating that alterations in nutrient content and composition have a noteworthy impact on the production of bacterial cellulose [19,20]. Amongst all five culture media, the maximum bacterial cellulose yield was observed in M5 media, followed by M3 and M1, while M2 media exhibited the lowest bacterial cellulose yield. Glucose and acetic acid are most prominent factors in influencing the production of bacterial cellulose. Glucose serves as a primary carbon source, while acetic acid provides both carbon and energy necessary for bacterial growth and cellulose synthesis [24]. During the cellulose production, the bacteria employ the glycolysis pathway, utilizing glucose as a primary substrate. The carbon derived from glucose is considered as a crucial substrate for the synthesis of cellulose [6]. In addition to being a crucial substrate for bacterial cellulose biosynthesis, glucose is also crucial for the growth and metabolism of the bacterial cells that produce cellulose.

The higher production of bacterial cellulose in M5 media, which contains elevated levels of glucose and acetic acid, followed by M4 media, can be attributed to the significant influence of these components upon bacterial cellulose synthesis. As stated, glucose and acetic are crucial components and hence the increased availability of these essential substrates in M5 and M4 media creates a more favourable environment for bacteria to efficiently produce bacterial cellulose. In addition to this, other carbon sources also influenced the production rate of bacterial cellulose. Present research findings revealed

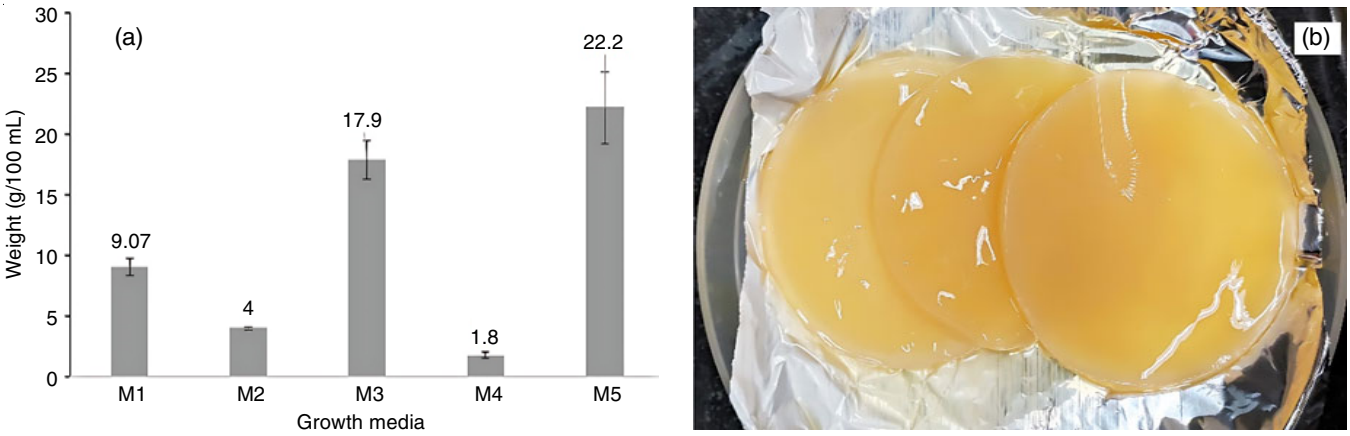

Fig. 2. (a) Yield of bacterial cellulose (wet weight in g/100 mL) produced by BC-C1 strain in different culture media, (b) thick BC films produced by BC-C1 strain in M5 media

that M2 media, which was formulated using sucrose as the primary carbon source, exhibited reduced bacterial cellulose production. This lower bacterial cellulose yield in the presence of sucrose can be attributed to the comparatively slower rate at which sucrose is transported across the cell membrane, in contrast to other sugars like glucose [25]. This reduced transport rate often results in limited availability of sucrose as a substrate required during bacterial growth as well as the synthesis of cellulose [26]. Hence, amongst all the five culture media, M2 media exhibited the lowest bacterial cellulose production.

Besides this, the present study also utilizes mannitol as an alternative carbon source alongside glucose and sucrose in M4 media. The inclusion of mannitol in this medium resulted in the lower production of bacterial cellulose compared to other media containing glucose as carbon source. This lower production of bacterial cellulose can be attributed to the fact that the metabolic pathways associated with mannitol utilization may not be as productive to yield maximum bacterial cellulose as those involving glucose as primary substrate material [27]. In addition to this, some bacteria, especially *Gluconacetobacter xylinus* are found to utilize mannitol as a substrate for energy production and cellulose synthesis, in addition to glucose. Hence, the specific bacterial strain and the components of the culture media can influence the extent of bacterial cellulose production, highlighting the importance of effectively selecting the carbon source to optimize bacterial cellulose yield.

FTIR studies: The FTIR analysis was conducted to determine the presence of functional groups in bacterial cellulose produce by BC-C1 strain in different culture media. Moreover, the functional groups in produced bacterial cellulose were also compared with the standard cellulose. The IR data indicate that a distinctive feature associated with presence of cellulose, namely the peak observed at 3332 cm^{-1}, was consistently present in all bacterial cellulose samples as well as in the standard cellulose. This particular peak can be attributed to the vibrational stretching of O-H bonds, occurring both within the cellulose structure as well as between cellulose molecules, hence indicating the characteristic presence of cellulose in the samples [28]. However, it is significant that the position of this particular peak exhibited slight variations among the bacterial cellulose samples, in case of different culture media. These variations imply differences in the structure of bacterial cellulose, likely arising from variances in the amount of the carbon source and influence of other media components used during the production process. Another region in spectral analysis spanning from 2970 cm^{-1} to 2800 cm^{-1} is significant due to the presence of C-H stretching vibrations. This presence of C-H stretching vibrations indicates the existence of organic compounds within the samples. Within this region, the peaks with reduced transmittance, signifying the absorption of energy at these specific wavelengths. These peaks are indicative of chemical groups that feature C-H bonds and the nature of these groups can vary, encompassing a range of organic molecules like hydrocarbons [28]. It was observed that all the samples exhibit a peak at similar wavenumber, hence indicating the similarity between bacterial cellulose produced by different culture media as well as standard cellulose. Apart from this, a significant peak was also observed at 1650 cm^{-1} which indicated the presence of water content in all the bacterial cellulose samples. The displayed peak shift at this wavenumber could be attributed to structural difference among all the bacterial cellulose samples produced using different culture media. All the bacterial cellulose samples also exhibited the presence of different peaks at 1437 cm^{-1}, 1055 cm^{-1}, 898 cm^{-1} and 810 cm^{-1}, with some variations in their positions indicating the presence of different functional groups. The peak at 810 cm^{-1} was associated with the β-glyosidic linkage between glucose units in cellulose and was present in the bacterial cellulose samples, with slight shifts observed in samples with M1 [29] (Fig. 3).

The peak detected at 1039 cm^{-1} in the spectra indicates the presence of O-CH_3 groups within the bacterial cellulose samples, providing evidence for the existence of these particular polysaccharides. In contrast, the peak at 1107 cm^{-1} may suggest the presence of either C-C bonds in the monomer units of polysaccharides or the bending vibrations of C-O bonds. This observation could potentially be linked to the presence of other polysaccharides, such as starch, alongside bacterial cellulose in the samples. The appearance of a strong peak at 1003 cm^{-1} implies the presence of C_3-O_3 crosslinking structures within the bacterial cellulose [28]. These crosslinks significantly contribute to the mechanical strength of bacterial cellulose materials.

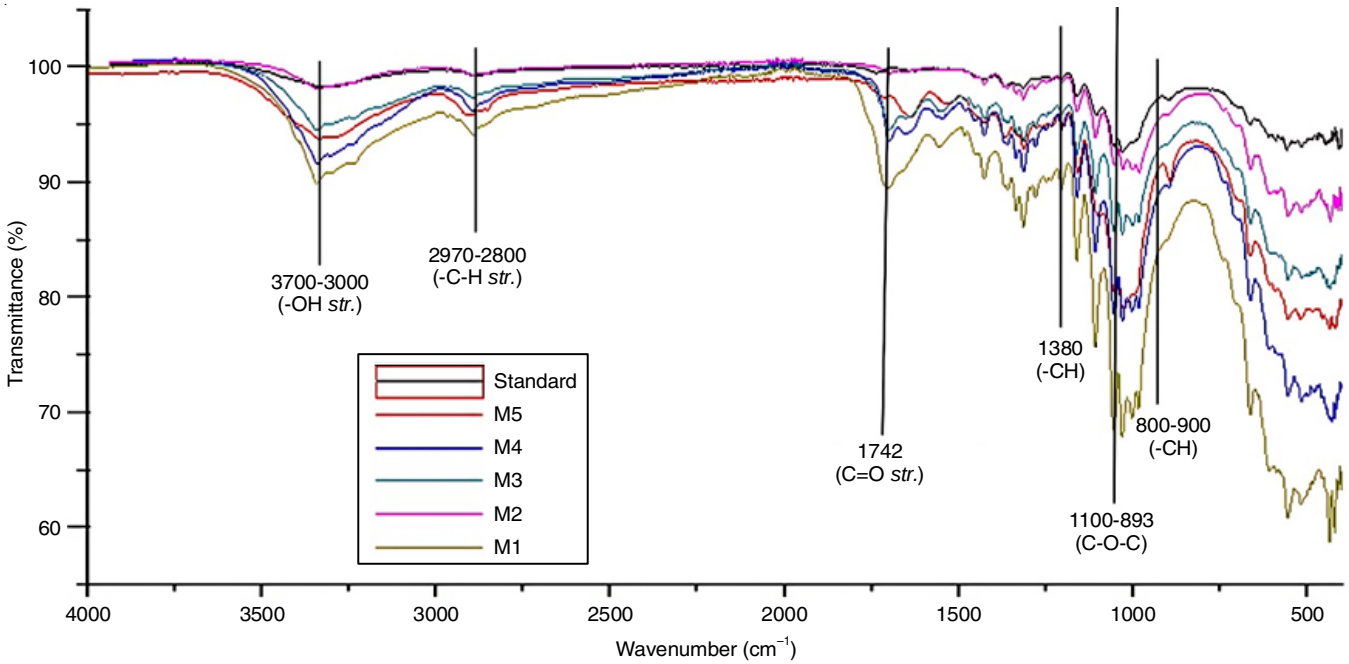

Fig. 3. FTIR spectra of standard cellulose and bacterial cellulose produced from *K. saccharivorans* in different culture media

Furthermore, the peak at 896 cm^{-1} indicates the presence of β-glycoside bond linkages between glucose units, which are indispensable for the formation of cellulose fibers within bacterial cellulose. These distinctive spectral features offer valuable insights into the composition and structural characteristics of bacterial cellulose samples. Thus, the predominant composition of the bacterial cellulose produced by *K. saccharivorans* BC-C1 appears to be pure cellulose I. This is supported by the presence of weak characteristic peaks appeared at 1427 and 898 cm^{-1} of pure cellulose. Furthermore, peaks observed at 3338, 1160 and 900 cm^{-1} are consistent with cellulose 1 peaks. However, the peaks at 1334, 1315, 1278 and 1427 cm^{-1} suggests the coexistence of cellulose II in the bacterial cellulose samples (Fig. 3). These particular peaks may be present in all samples due to the NaOH purification procedure, which has the potential to alter the structural composition of cellulose from type I to type II [30,31].

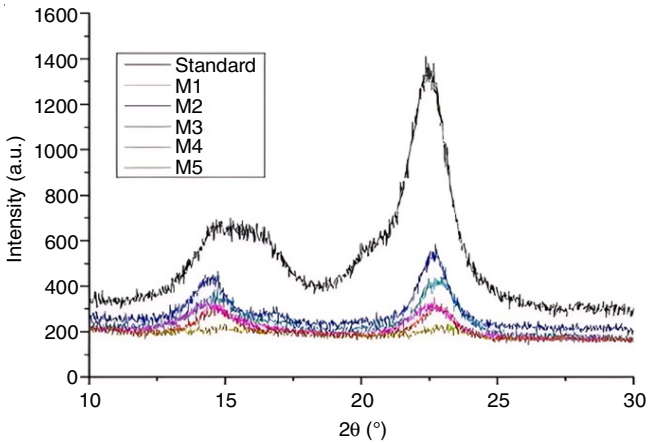

Fig. 4. XRD patterns of bacterial cellulose produced from *K. saccharivorans* in different culture media

XRD studies: The XRD analysis of bacterial cellulose samples produced in different culture media was compared with the standard cellulose. The diffractograms (Fig. 4) depicted the presence of peaks at I_{002} and I_{200} in all the samples as well as standard cellulose. Different samples exhibited peaks at almost similar positions, hence indicating the presence of similar crystal structures. It was observed that all bacterial cellulose samples exhibited the presence of three distinct peaks corresponding to both Iα and Iβ crystal cellulose. Table-3 summarizes crystallinity behaviour and crystallite size data obtained from the XRD analysis. Upon analysis results indicate that M5 and M3 had the highest crystallinity percentages, with values of 40.5% and 42.5%, respectively. On the other hand, bacterial cellulose produced from M1 media exhibited crystallinity

TABLE-3
CRYSTALLINITY AND CRYSTAL SIZE OF PRODUCED BACTERIAL CELLULOSE AND STANDARD CELLULOSE

Media	2θ			Crystallinity (%Cr)	Crystallite size 'd' (nm)	
	I_{002}	I_{am}	I_{200}		I_{002}	I_{200}
Standard	15.4	18.3	22.4	71.5	2.91	5.10
M1	14.7	19.2	22.8	39.7	9.89	6.02
M2	14.5	18.6	22.6	40.5	7.06	7.46
M3	14.5	18.7	22.9	42.5	7.92	6.43
M4	14.4	18.6	22.7	26.2	9.81	8.69
M5	14.8	19.1	23.2	40.5	12.17	15.56

percentage as 39.7% while bacterial cellulose produced using M2 medium exhibited crystallinity percentage as 40.5%. In addition to this, the mean crystallite size was also determined which ranged from 5.1 to 15.5 nm, hence indicating the presence of semi crystalline nature in all bacterial cellulose samples produced using different culture media.

The findings in this study highlighted the significant impact of the chemical composition of culture medium on the crystallinity of bacterial cellulose as presented by X-ray diffraction (XRD) analysis. This phenomenon occurs because the crystallinity and semi-crystalline structure of bacterial cellulose are sensitive to the specific conditions related to its production, including the chemical makeup of the growth medium [32,33]. Consequently, different culture media can yield distinct XRD peak patterns and intensities, providing a means to characterize the resulting bacterial cellulose samples [34]. Bacterial cellulose is recognized for its semi-crystalline nature, indicating the presence of both crystalline and amorphous regions within its structure. The crystalline regions consist of linear chains of glucose molecules (cellulose fibers) arranged in a highly ordered and repeating pattern. These regions were identified by their prominent XRD peaks. In contrast, the amorphous regions exhibit a more disordered cellulose chain arrangement which lacks long-range order, hence resulting in weaker XRD peaks [35,36]. The presence of more crystalline nature in bacterial cellulose samples is also attributed to distinctive mechanical and physical behaviour, including its high tensile strength, water-holding capacity and superior biocompatibility.

SEM studies: Fig. 5 illustrates the surface analysis of bacterial cellulose membranes produced by BC-C1 strain in M3 and M5 medium at 20KX magnification. Upon analysis of these micrographs, it was found that bacterial cellulose produced by utilizing both culture media exhibited well-organized, porous, three-dimensional fibrillar interconnected network, which is consistent with the published reports as well. Moreover, the nanofibers as well as the empty spaces observed in the bacterial cellulose membrane matrix are dispersed at random intervals. Due to this unique network structure, bacterial cellulose membranes are known to exhibit the ability to efficiently absorb and retain water or water-soluble chemicals. The random distribution of nanofibers as well as void spaces allows the membrane to act like a sponge and exhibit higher water holding capacities. This property is particularly advantageous for applications requiring moisture absorption, such as wound dressings or as a scaffold for cell growth in tissue engineering [37,38]. The 3D network structure of bacterial cellulose also offers a substantial surface area and high porosity, which contributes to its special properties like mechanical strength as well as biocompatibility, hence making it a versatile material with a wide range of applications in various industries [38,39].

Conclusion

The present investigations reported the isolation of cellulose producing bacteria from coconut pulp from 5 isolates obtained on GEY media. The isolate was identified as *Komagataeibacter saccharivorans* BC-C1. The composition of the media components greatly affected the bacterial cellulose yield. Under the optimum conditions of growth, *K. saccharivorans* BC-C1 achieved the highest cellulose yield of 1.10 g/100 mL (dry weight). The physico-chemical characterization of the produced bacterial cellulose revealed typical structural characteristics as standard cellulose. Media type resulted in the crystallinity variations amongst different bacterial cellulose samples. Scanning electron micrographs of the bacterial cellulose obtained from this bacterium revealed ultrafine microfibrils. These results have important implications for future bioengineering efforts to create cellulose on a large scale, as well as for the ongoing development of cellulose synthesis.

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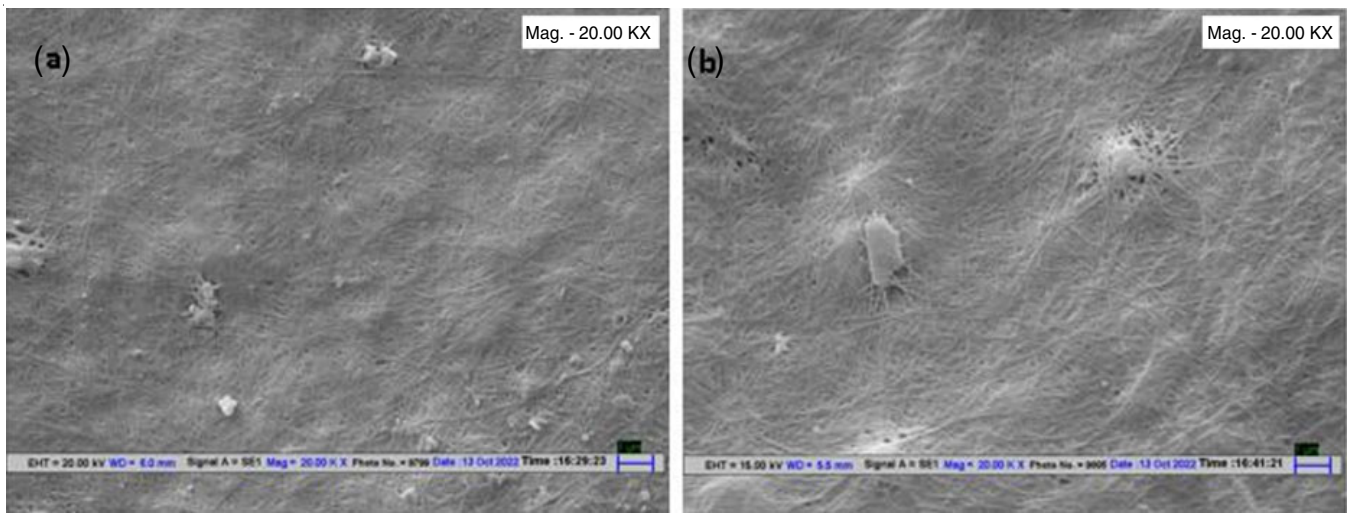

Fig. 5. Scanning electron microscope (SEM) photographs of bacterial cellulose produced in (a) M3 media; (b) M5 media at magnification 20 KX

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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