



Strain Selection and Characterization of *Komagataeibacter hansenii* for Bacterial Nanocellulose Production from Fruit Waste

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Abstract

Bacterial nanocellulose (BNC) is a microorganism-derived homopolymer with a simpler, less harsh production route than its typical plant-based counterpart, offering unique properties valuable to high-end industries. This study aims to isolate and characterize bacteria that produce BNC from fermented rotten fruit waste, highlighting fermented starfruit as a selective and underexplored source for isolation. Among 80 isolates obtained using the fermented fruit juice method, strain RM-03 produced the highest cellulose concentration (0.092 g/100 mL) and was identified as *Komagataeibacter hansenii* (99.0% similarity). Using HS medium, it produced 0.277 ± 0.007 g/100 mL BNC at Day 16, a production within the range reported for some *Komagataeibacter* strains cultivated under comparable static, unoptimized conditions. Microscopy characterization revealed a dense network of nanofibers with an average fibril width of 35 nm. Spectroscopic and diffraction analyses confirmed cellulose type I with 78.24% crystallinity. The BNC exhibited excellent water-holding capacity (366.24%) and retained this moisture for up to 65 hours. These findings demonstrate that fermented rotten starfruit is a promising source for isolating functional BNC producers and that *K. hansenii* RM-03 shows strong potential for further bioprocess development and industrial applications.

Keywords: Bacterial Nanocellulose, Field Emission Scanning Electron Microscopy, Fourier Transform Infrared, *Komagataeibacter hansenii*, Rotten Starfruit, Transmission Electron Microscopy, X-ray Diffraction.

1. Introduction

Cellulose is a major homopolysaccharide within the extracellular polysaccharides group and is extensively used as a biopolymer worldwide [1]. Although cellulose is generally from plants, certain bacteria can also produce cellulose, known as bacterial nanocellulose (BNC). BNC is a microorganism-based homopolymer of β -(1,4) glucose ($C_6H_{10}O_5$)_n, synthesized primarily by the genera *Komagataeibacter*, *Rhizobium*, *Pseudomonas*, and *Sarcina* [2]. BNC was postulated to protect bacterial cells against harmful ultraviolet rays and furnish bacterial cells with an oxygen-rich environment [3]. Cellulose from plants and bacteria has different production pathways. Plant cellulose extraction requires harsh treatments such as acid hydrolysis to separate lignocellulosic components, which is unsustainable, expensive, and uneconomical due to large acid volumes, high temperatures, and low yield (~30% by weight) [4]. In contrast, BNC biosynthesis involves only fermentation and purification steps without any harsh chemicals or treatments.

BNC is increasingly attractive for various applications owing to its renewability and superior properties. Its dense and well-organized three-dimensional fibril network imparts high crystallinity, thermal stability, high water-

holding capacity, and high mechanical strength compared to plant cellulose [5]. BNC hydrogel also exhibits strong water absorption, biocompatibility, and partial degradability [6]. Its high elasticity, surface area, and porosity add significant value to biomedical, pharmaceutical, food, textiles, and cosmetics industries [7]. Despite these advantages, the number of well-characterized high-productivity BNC-producing strains remains limited, with *Komagataeibacter xylinus* being the most commonly studied producer [8]. Conventional isolation sources such as kombucha, vinegar, and sugarcane juice have been extensively explored and may produce predominantly similar or closely related strains, potentially limiting the discovery of novel biodiversity.

Rotten fruit waste offers a scientifically distinct and underexplored isolation niche while also representing a sustainable and low-cost substrate for biotechnological applications. The decomposition of fruit involves active breakdown of plant polysaccharides by complex microbial communities, creating an environment that naturally enriches for microorganisms with strong polysaccharide-metabolizing capabilities, including cellulose producers [9]. During decomposition, microbes compete for resources and space; cellulose-producing bacteria may gain competitive advantages by forming protective biofilms or floating pellicles that access oxygen [3]. This natural selection pressure could favor strains with robust cellulose production. Using fruit waste not only provides a novel ecological source for BNC-producing bacteria but also aligns with waste valorization strategies, reducing reliance on expensive conventional isolation media. Among various fruit wastes, starfruit represents an underexplored substrate with natural acidity and fermentable sugars that may promote spontaneous fermentation and enrichment of cellulose-producing bacteria, yet its use has received limited attention in BNC isolation studies.

Several studies have demonstrated that fruit wastes and fruit-processing residues can serve as low-cost substrates for bacterial nanocellulose (BNC) production. Agricultural fruit peels such as mango, dragon fruit, banana, and papaya have been reported to support BNC biosynthesis, with concentrations ranging from approximately 0.9 to 3.7 g/L depending on the substrate and isolate, indicating that diverse fruit peels can function effectively as carbon sources for cellulose synthesis [10]. Citrus fruit peel extracts have also been investigated as an alternative medium, where BNC concentrations of 2.06–3.92 g per 100 g peel were obtained using *Komagataeibacter hansenii* GA2016 [11]. In addition, pear juice industry wastewater has been successfully used as a fermentation medium, producing BNC in the range of 0.27–0.31 g/L using *Komagataeibacter* strains, further supporting the feasibility of fruit-related wastes as fermentation substrates [12].

By targeting this niche, the present study addresses the gap in understanding the full diversity of cellulose-producing bacteria beyond conventional sources and aims to uncover strains with potentially different or enhanced characteristics. However, it remains unclear whether rotten fruit waste can serve as a reliable source for isolating bacterial strains capable of consistent bacterial nanocellulose production, with favorable productivity among isolates recovered from this niche, and desirable structural and functional properties. Addressing this gap is important for expanding the diversity of BNC-producing bacteria and identifying strains suitable for further bioprocess and application-oriented studies. This study aims to isolate BNC-producing bacteria from rotten fruit waste, an underexplored ecological niche, and select a strain showing comparatively higher BNC concentration among the isolates recovered under identical static screening conditions, rather than to propose a new bacterial species. Strain selection was based on relative BNC concentration among the isolates recovered in this study under identical static screening conditions. In this context, “comparatively higher” refers only to within-study screening performance and does not imply superiority over all previously reported BNC-producing strains. The best BNC producer was identified through biochemical and molecular approaches. The resulting BNC pellicles were analyzed using Fourier Transform Infrared (FTIR) spectroscopy, X-ray Diffraction (XRD) analysis, Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM) analysis. Functional characterization included water-holding capacity (WHC) and water retention rate (WRR). Furthermore, a preliminary kinetic study was conducted to evaluate the growth, substrate consumption, and BNC production patterns of the selected strain.

2. Materials and methods

2.1 Rotten fruit preparation

Rotten fruits such as apples, oranges, plums, and pineapples were sourced from the Taman Sri Serdang Night Market (3°00'58.9" N 101°42'43.9" E) while felled starfruits were obtained from Taman Sri Serdang, Selangor, Malaysia (3°00'18.9" N 101°42'35.9" E). Fruits discarded due to physical damage or decay were chosen and kept in separate containers to prevent cross-contamination. The collected fruits were ripened beforehand, as they contained high sugar levels and lower pH, favoring BNC producers' growth. After collection, the fruits were cleaned using deionized water to remove dirt, after which 100 g of each fruit was diced (2 × 2 × 2 cm) and stored at 4 °C for less than 24 h before use.

2.2 Isolation and screening of BNC-producer strains

Two media were used: a modified Hestrin-Schramm (HS) medium and a Fermented Fruit Juice (FFJ) medium. The HS and FFJ media were prepared according to Park et al. [13] and Lin et al. [14], respectively, with slight modifications [13–14]. Both media were adjusted to pH 5.5 using acetic acid and autoclaved at 115 °C for 15 min. Sterile 1.5% v/v ethanol and 0.1 g/L cycloheximide were added to HS media and FFJ media, respectively, after autoclaving, using the filter sterilization technique. Screening and isolation of BNC producers followed the methods M1, M2, M3, and M4, according to Park et al. [13] and M5, based on Lin et al. [14]. M1 to M4 approaches used apples, oranges, plums, and pineapples, whereas M5 used starfruits as isolation source (Figure 1). This selection was based on prior reports indicating the suitability of HS-based methods for common fruits, while FFJ medium has been shown to support cellulose-producing bacteria from starfruit effectively [13–14]. A positive result was indicated by BNC pellicle formation at the air-liquid interface (static) or pellet formation in submerged culture (shaking). Pellicles that withstood stirring in 0.1 M NaOH at 80 °C were confirmed as BNC. Then, the positive isolates were streaked onto HS agar for three subculture generations to obtain pure culture, which were then re-inoculated into HS media to reaffirm the pellicle formation and quantify BNC productivity. The procedure should start with M1, repeated four times, and if the result was negative, the following method (M2) would be employed to isolate any BNC producer. This sequence of work would continue until a particular isolation method bore positive results.

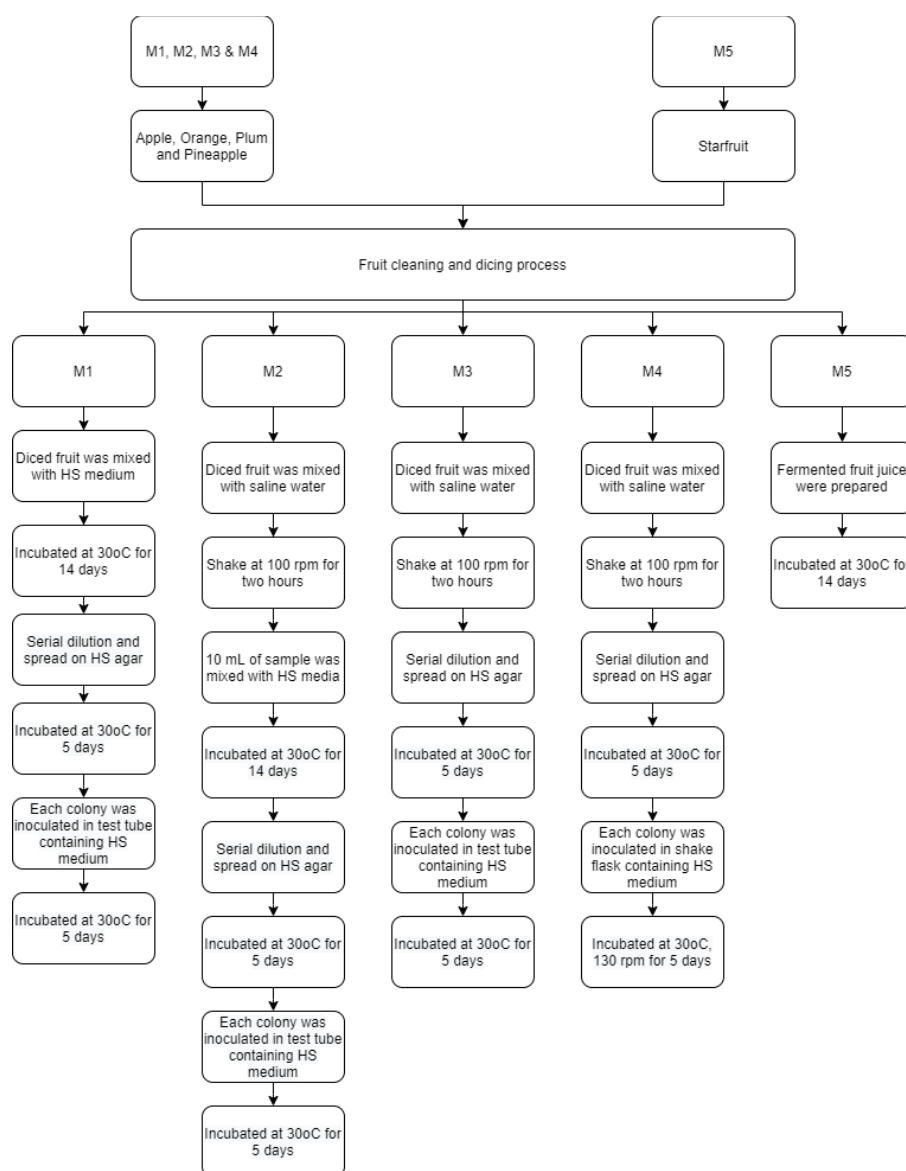


Figure 1 Flowchart of the isolation methods employed in this study.

2.3 Substrate, cell concentration, and BNC quantification

Residual glucose concentration was measured using the 3,5-dinitrosalicylic acid (DNS) method [15]. For cell concentration and BNC quantification, the method described by Lin et al. [14] was followed.

2.4 Identification of Cellulose-Producing Bacteria

2.4.1 Morphological and Biochemical Identification

The morphological and biochemical characterization followed Bergey's Manual of Systematic Bacteriology and identification schemes described by Green [16] and Rosenberg et al. [17]. The tests evaluated included Gram staining, catalase and oxidation tests, growth on selective media (10% (w/v) glucose agar, GYC (Glucose, Yeast, Carbon) agar, mannitol salt agar and 10% (v/v) ethanol in Carr and Passmore medium) and growth at room temperature.

2.4.2 Molecular Identification of Isolates Using 16S rRNA

The genomic DNA extraction followed the method of Poblet et al. [18]. An overnight-grown sample was centrifuged at 10,000 g, and pellets were washed thrice using distilled water. The washed pellet was then mixed with 300 μ L of 3% (w/v) SDS-TE buffer (10 mmol/L Tris-HCl, pH 7.5, and 1 mmol/L EDTA), 200 μ L TE buffer and 500 μ L of phenol-chloroform-isoamyl alcohol (25:24:1, v/v). The mixture was centrifuged for 10 min at 10,000 g and the aqueous phase was mixed with isopropanol and washed with 70% (v/v) ethanol. Finally, the DNA was resuspended in 20 μ L TE buffer and stored at -20 °C before proceeding with amplification. PCR amplification of the 16S rRNA gene was carried out using universal primers 9F and 1510R [19]. The reaction mixture containing 100 ng of DNA, 1.25 U of Ex Taq DNA Polymerase, 200 μ M dNTP Mix, Taq buffer, 10 μ M of each primer, and distilled water was subjected to PCR. The cycling program started with an initial denaturation at 94 °C for 3 min, continued with amplification for 30 cycles, each consisting of 94 °C for 30 seconds, 58 °C for 40 seconds, and 72 °C for 1 minute. Finally, an extension at 72 °C for 5 min was conducted. The PCR product was verified on agarose gel electrophoresis (1.5% w/v agarose and TBE) at 110V for 40 minutes and purified with GFX PCR DNA and Gel Band Purification Kit (Amersham Biosciences, USA). The resulting purified 16S rRNA genes were sequenced with an ABI PRISM Big Dye Terminator v3.1 Cycle Sequencing Ready Reaction Kit on an ABI PRISM model 310 Genetic Analyzer (Applied Biosystems, USA). Primers 9F, 1510R, and 520F were used for sequencing. Sequences were then submitted to GenBank and subjected to BLAST analysis. Next, multiple sequence alignments were constructed using Molecular Evolutionary Genetics Analysis (MEGA) v4.1. Although this version is older, it provides reliable neighbor-joining, maximum parsimony, and maximum likelihood analyses. To ensure the robustness of the phylogenetic results, 1,000 bootstrap replicates were performed, confirming the stability of the tree topology. Phylogenetic trees were constructed using the neighbor-joining, maximum parsimony, and maximum likelihood methods. Pairwise sequence similarities were calculated for the three highest BNC producers (RM-01, RM-02, and RM-03) using DNAMAN 6.0.

2.5 BNC Characterization

The morphology of air-dried BNC pellicles was examined using a FESEM microscope (JSM-7600F, JEOL, Japan) at 5 kV after platinum coating to prevent electron charging. A droplet of homogenized and sonicated BNC suspensions was placed on a carbon-coated copper grid and visualized using a TEM microscope (Hitachi H-7100) at 100 kV. FTIR spectra were recorded using a Perkin Elmer Spectrum 100 spectrometer with ATR accessory at 4 cm^{-1} resolution (16 scans, 4000 to 6000 cm^{-1}). The crystallinity and structural analysis were carried out using a Philips PW 3040/60 MPD X'Pert High Pro PANalytical diffractometer in theta-theta configuration, with a secondary monochromator, $\text{CuK}\alpha$ radiation ($\lambda = 0.154 \text{ nm}$), and a solid-state pixel detector, operated at 40 kV, 40 mA, scanning $2\theta = 5^\circ$ – 70° . The crystallinity index was determined using the method described by Segal et al. (1959) [20]. WHC and WRR of the BNC pellicle were determined as described by Lin et al. [14]. For WHC, homogenized BNC pellicle was centrifuged for 10 min at 10,000 rpm to remove free and loosely bound surface water, ensuring that the measured wet mass represented water retained within the BNC network. The initial wet sediments were weighed. Then, the dried sediment (with water completely removed) was weighed upon drying at 60 °C for 48 h. For WRR test, the dried BNC (1 g) was subsequently immersed in distilled water under shaking conditions (100 rpm) at room temperature for rewetting. After rewetting was completed, the samples were centrifuged for 10 min at 10,000 rpm and analyzed for WRR by continuously measuring their weights at various time intervals at ambient temperature until they were completely dry. The weights of the BNC samples at different time intervals were plotted against time. WHC and WRR measurements were performed in triplicate ($n = 3$), and the results were expressed as mean $\pm$ standard deviation.

3. Results and Discussion

3.1 Screening, isolation, and identification of bacterial nanocellulose producers

Among five isolation methods (M1 - M5) tested (Figure 1), only the M5 method yielded positive results. The M5 method utilized fermented starfruit juice as the isolation source, produced thick and robust pellicles at the medium surface, indicating the presence of BNC producers [21]. The pellicles maintained their structure after 30 min boiling in 0.1M NaOH, confirming BNC production since this technique removes cells and protein agglomerates [22]. Eighty colonies were isolated from the M5 method based on the distinctive morphology of *Komagataeibacter*, characterized by raised, convex to umbonate, smooth to rough, entire to irregular, and butyrous features [15].

Table 1 summarizes the number of isolates obtained from different methods. Among the 80 isolates, RM-01, RM-02, and RM-03 had BNC-producing capability. The observed mean cellulose concentrations were 0.0827 g/100 mL for RM-01, 0.0733 g/100 mL for RM-02, and 0.092 g/100 mL for RM-03 (Figure 2). While the differences are modest and no formal statistical comparison was performed, RM-03 was selected for further study as it consistently showed the highest mean BNC production. This practical selection approach allowed focus on the most promising isolate for detailed characterization. Future studies may include additional replicates and statistical analysis to rigorously compare BNC productivity among isolates. HS medium is a well-established medium for cultivating cellulose-producing bacteria; however, its performance during isolation is strongly influenced by the microbial background of the substrate. In methods M1–M4, apples, oranges, plums, and pineapples introduced diverse and rapidly growing indigenous microorganisms that likely dominated the HS medium, limiting the growth and detection of slow-growing cellulose-producing bacteria despite prolonged incubation. In contrast, method M5 employed clarified fermented starfruit juice supplemented with additional sugars and cycloheximide, which reduced fungal contamination and microbial competition while maintaining an acidic environment favorable to cellulose-producing bacteria. This fermented juice acted as the enrichment and isolation medium, allowing *Komagataeibacter* spp. to dominate and form robust pellicles, resulting in successful recovery of BNC-producing isolates. These findings demonstrate that the selective advantage arises from the combined effects of substrate composition, fermentation-driven enrichment, and inhibitory conditions against competing microbes. A similar study by Singh et al. (2017) obtained a BNC producer from fresh fruit waste [23]. In addition, *Komagataeibacter* sp. SFCB22-18 was isolated from ripened persimmons [24] and *G. xylinus* ZHCJ618 from kombucha [22]. Thus, fruit waste, either fresh or fermented, is a suitable source for isolating BNC producers as their high sugar content and acidic conditions provide favorable conditions for *Komagataeibacter* growth [17].

Table 1 Number of isolates obtained from various sources by different isolation methods.

Method	Isolation Source	Isolation Media	Number of Isolates	Number of positive Isolates
M1	Apple, Orange, Plum, Pineapple	Hestrin-Schramm	235	0
M2	Apple, Orange, Plum, Pineapple	Hestrin-Schramm	254	0
M3	Apple, Orange, Plum, Pineapple	Hestrin-Schramm	267	0
M4	Apple, Orange, Plum, Pineapple	Hestrin-Schramm	319	0
M5	Starfruit	Fermented Fruit Juice	80	RM01, RM02, RM03

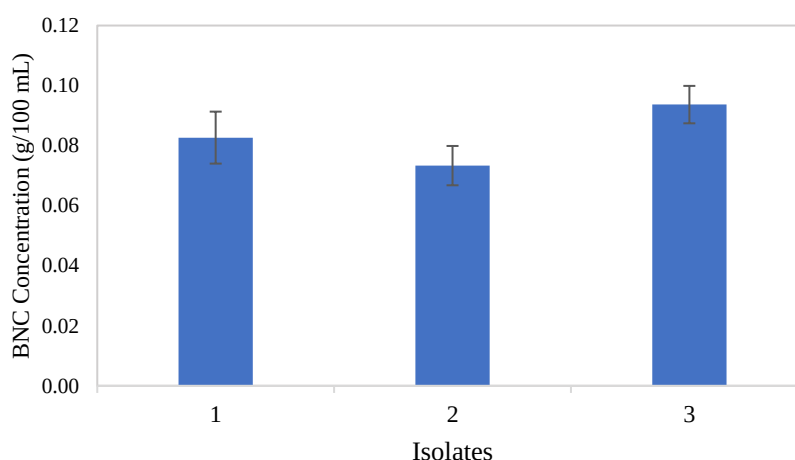


Figure 2 Comparison of BNC production after 5 days for RM01, RM02 and RM03. The error bars represent the standard deviations about the mean (n=3)

Figure 3 displays the growth profile of RM-03 in batch cultivation using HS medium during inoculum development. The growth profile started with a lag phase (0 - 4 h), followed by exponential growth (4 - 32 h) and a stationary phase until 40 h. The glucose consumption profile mirrored the growth pattern, indicating active use as a carbon source by the microbe. Hence, the optimal inoculum incubation time is 32 h, as the highest cell count was observed at this point. During static fermentation, BNC biomass increased considerably until Day 13 before plateauing at Day 16 (Figure 4A). The highest BNC concentration obtained at Day 16 (0.277 ± 0.007 g/100 mL) under static conditions is comparable to values reported for *Komagataeibacter* species cultivated in Hestrin–Schramm medium under similar conditions [25]. Concurrently, glucose consumption was rapid until Day 4, then gradually declined. The cell count increased drastically between Days 1 and 2, followed by a steady decrease until Day 15, and plummeted on Day 16 (Figure 4B). Moreover, a significant decrease in pH was observed from Day 1 (5.13) to Day 4 (4.06), followed by a gradual drop to 3.74 (Day 10) and remained constant until Day 16. The rapid initial glucose consumption supports active cell growth and metabolism during the exponential phase. Part of the glucose was oxidized to gluconic acid, resulting in a sharp drop in pH. This acidification created increasingly unfavorable conditions for cell proliferation, slowing growth and stabilizing cell density. Meanwhile, BNC biosynthesis continued as cells redirect carbon flux toward extracellular cellulose production, particularly under static conditions where oxygen at the air–liquid interface favors pellicle formation. The plateau and slight decline in BNC production after Day 13 likely resulted from a combination of substrate limitation, accumulation of acidic byproducts, and reduced metabolic activity in stationary-phase cells. Similar observations have been reported using *G. xylinus* PTCC 1734, where BNC biosynthesis occurred steadily until Day 14, and declined thereafter [25].

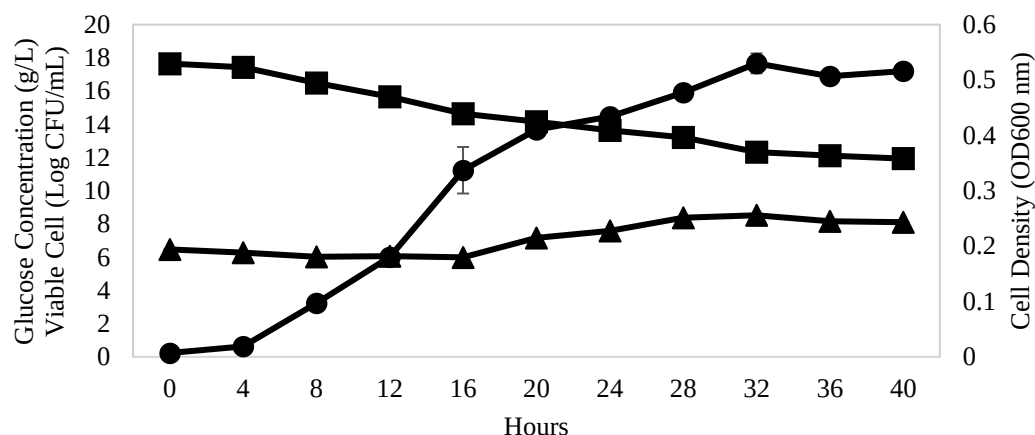


Figure 3 Growth profile of RM03 isolate in batch cultivation using HS medium in shake-flask culture during inoculum development. Symbols represent; ▲ Viable cell (Log₁₀ CFU/mL); ■ Glucose Consumption (g/L); ● Cell Density (OD 600 nm).

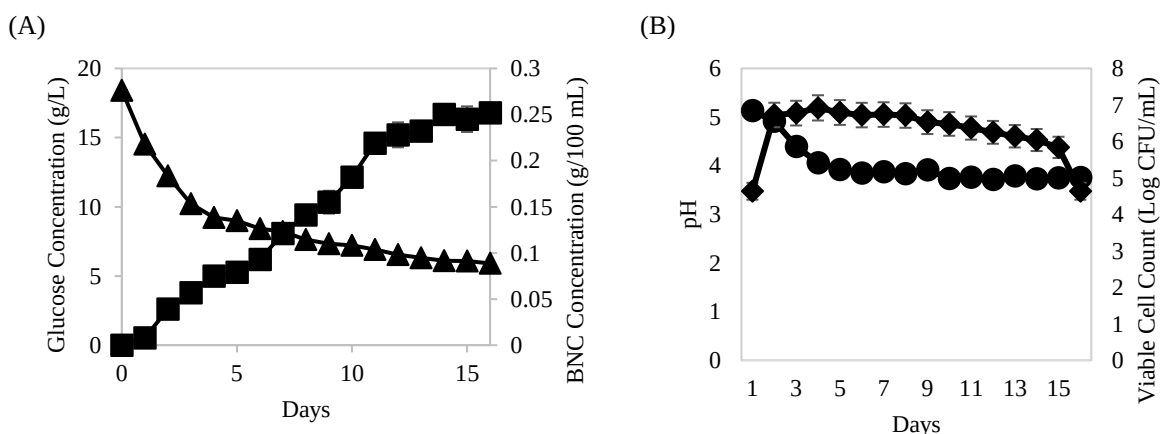


Figure 4 (A) Glucose consumption and BNC dry weight during batch cultivation using HS-medium for BNC production. (B) Growth profile of RM03 isolate in batch cultivation using HS-medium for BNC production. The symbols represent; ■ BNC Dry Weight (g/100 mL); ▲ Glucose Consumption (g/L); ◆ Viable Cell Count (Log₁₀ CFU/mL); ● pH.

The 16S rRNA sequence of RM03 (1356 bp) displayed 99% similarity to *K. hansenii* NBRC 14820 (Figure 5). Hence, the strain was identified as *Komagataeibacter hansenii* RM-03. Table 2 summarizes the biochemical and morphological characteristics of *K. hansenii* RM-03. *K. hansenii* RM-03 formed irregular, cream-to-beige colonies (2-3 mm) with a smooth and shiny texture on HS agar. Gram staining confirmed *K. hansenii* RM-03 as a Gram-negative, rod-shaped bacterium (0.4 – 0.6 x 1.0 – 1.5 μ m). Moreover, *K. hansenii* RM-03 is non-motile, catalase-positive, and oxidase-negative, consistent with other *Komagataeibacter* spp. The strain is also able to use mannitol (forming a yellow color zone around the colony on mannitol salt agar), glucose (clear zone formation around colony on calcium carbonate agar) and ethanol (forming a yellow color zone around the colony on Carr–Passmore agars) [19]. It also tolerated up to 0.35% acetic acid (pH 3.7) and synthesized a thick, robust pellicle on the medium surface.

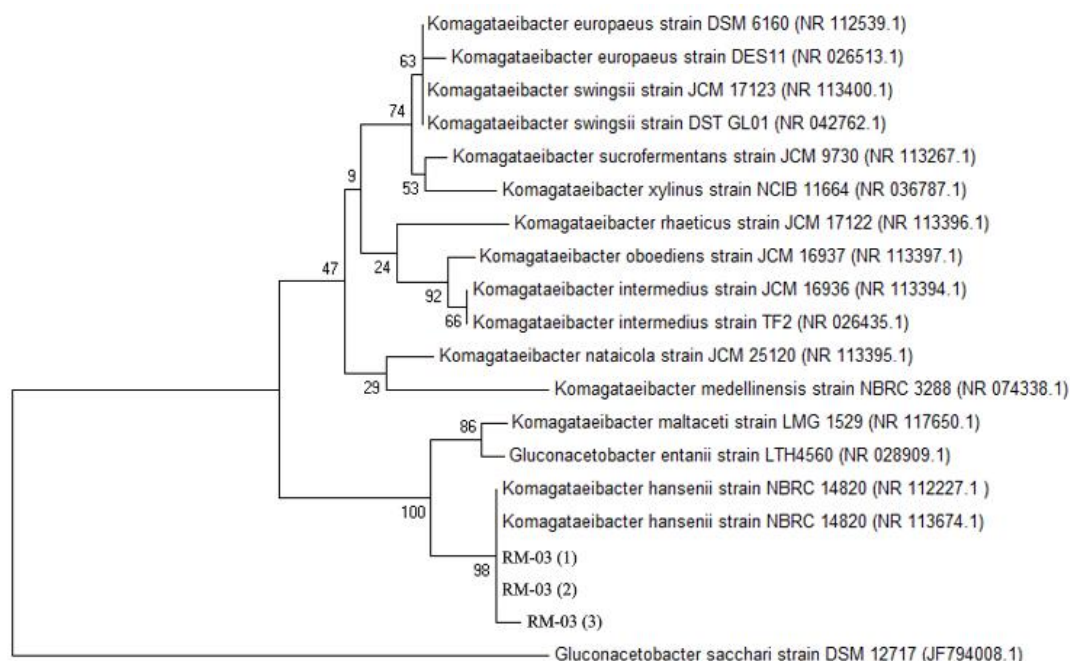


Figure 5 Neighbor-joining tree derived from 16S rRNA sequence data of RM-03 (triplicate) and other closely related species retrieved from NCBI GenBank. Bootstrap values (expressed as percentages of 1000 replications) are shown next to the branches.

Table 2 Biochemical and morphological characteristics of *K. hansenii* RM03.

Characteristic	Value
Shape	Rod
Size	0.4 – 0.6 x 1.0 – 1.5 μ m
Gram stain test	Negative
Catalase test	Positive
Oxidase test	Negative
Growth in the presence of 0.35% acetic acid	Positive
Cellulose production	Positive
Growth on mannitol agar	Positive
Color change on CaCO ₃ agar	Positive
Color change on Carr Passmore agar	Positive
Motility	Non-motile
Brown pigmentation	Negative
Elevation	Convex
Texture	Smooth
Optical property	Opaque
Pigmentation	Non-pigmented (cream)
Form	Irregular

3.2 Characterization of bacterial nanocellulose

To determine the physicochemical properties of BNC, surface, polymer, and functional characterization is essential. Surface characterization generally describes the morphology and structure; polymer characterization explains the chemical and crystalline features while functional characterization evaluates the performance-related properties of BNC. This comprehensive characterization is crucial, particularly when integrated with industrial

applications such as wound dressing, suture materials, scaffolds, and artificial corneas, which relate to attributes such as tensile strength, water-holding capacity, and permeability. Thus, BNC produced in this study underwent surface characterization using FESEM and TEM, polymer characterization using XRD and FTIR, while functional characterization included WHC and WRR.

FESEM images (Figure 6) revealed a dense, fibrous, ribbon-like network of ultrafine cellulose microfibrils forming a mat-like structure, with most fibers averaging 35 nm in diameter (based on 200 samples). Similar nanofibril networks have been reported for BNC by *Komagataeibacter* sp. SFCB22-18 [24]. Thus, BNC by *K. hansenii* RM-03 falls within the range of 1 to 100 nm and is therefore classified as a nanoparticle [26].

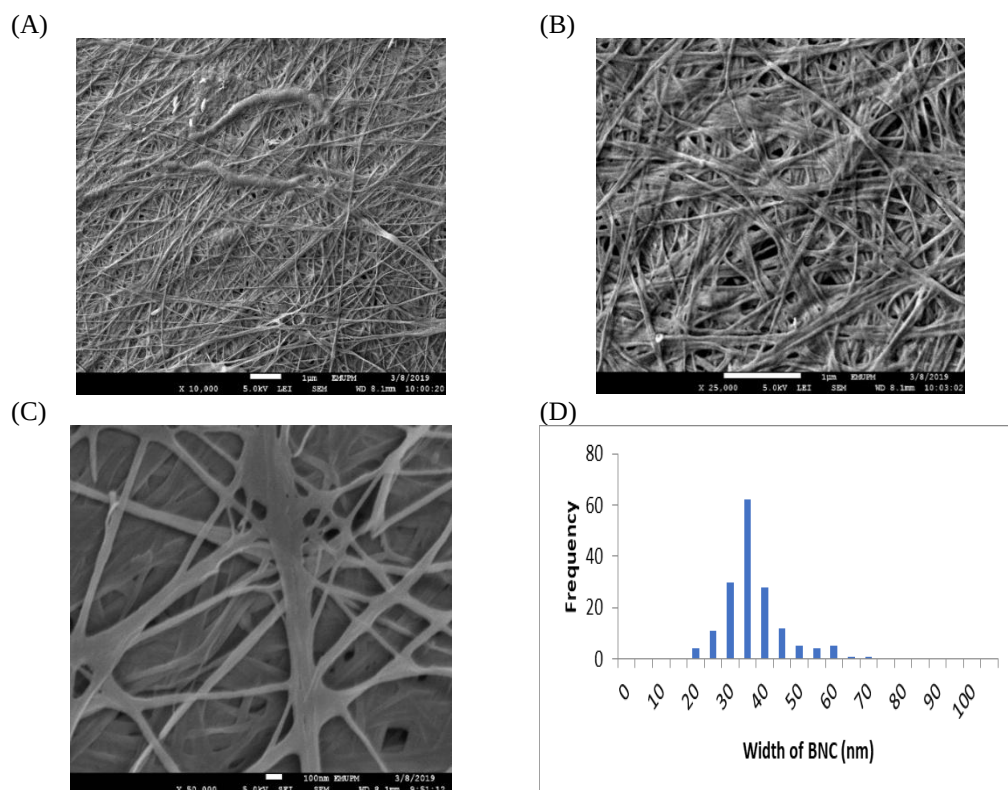


Figure 6 FESEM micrograph of BNC from *K. hansenii* RM-03 taken at 10,000x (A), 25,000x (B) and 50,000x (C) magnification; and the size distribution at the 50,000x magnification (D).

TEM further confirmed the elongated ribbon structure of BNC (Figure 7). At higher magnifications (10000 $\times$ - 25000 $\times$) the BNC strands appeared bundled, forming a highly entangled network with an average width of 35 nm (based on 200 samples). Numerous similar cellulose fiber bundles were observed by Machado et al. (2018) in BNC from *Komagataeibacter rhaeticus* using TEM [27].

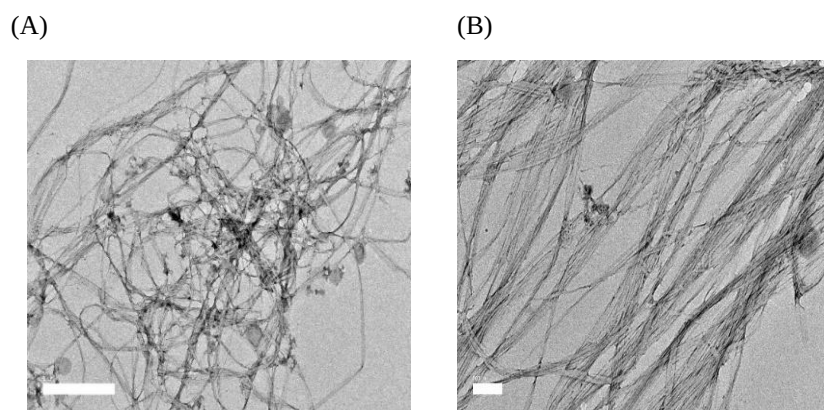


Figure 7 TEM micrograph of BNC from *K. hansenii* RM-03 taken at 5,000x (A), 10,000x (B) and 25,000x (C) magnification; and the size distribution at the 25,000x magnification (D).

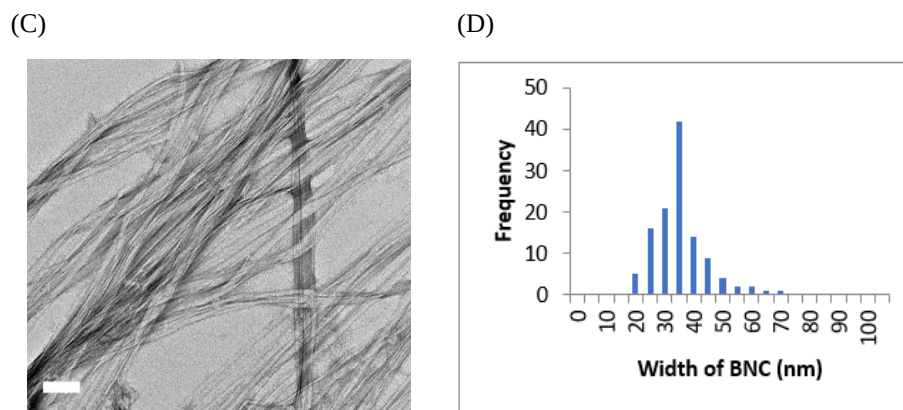


Figure 7 TEM micrograph of BNC from *K. hansenii* RM-03 taken at 5,000x (A), 10,000x (B) and 25,000x (C) magnification; and the size distribution at the 25,000x magnification (D). (Conf.)

The dense and interconnected fibrillar network of BNC contributes to its strong mechanical integrity, making it ideal for lightweight and durable applications [6]. XRD is a commonly used technique to examine the crystallinity of BNC structures, which is closely linked to their mechanical strength and material performance. XRD analysis (Figure 8A) showed three characteristic peaks at 14.4° , 16.9° , and 22.9° , corresponding to the primary diffraction of (100), (010), and (110) planes of cellulose Ia and cellulose Ib [28-29]. The dominant intensity at 14.4° indicates a predominance of cellulose Ia, generally found to be higher in bacterial cellulose. The Z-discriminant function yielded a Z-value of 5.021 (Table 3), confirming high Ia content, consistent with earlier *Komagataeibacter*-produced BNC studies [3], which reported a Z-value of 4.5. The higher intensity at 14.4° also implies that the BNC crystallites have a rectangular cross-section [29]. The crystallinity index (CI), calculated using the Segal method, was 78.24%, comparable to the CI reported for BNC produced by *K. hansenii* GA2016 (79.48%) by Güzel and Akpınar [11].

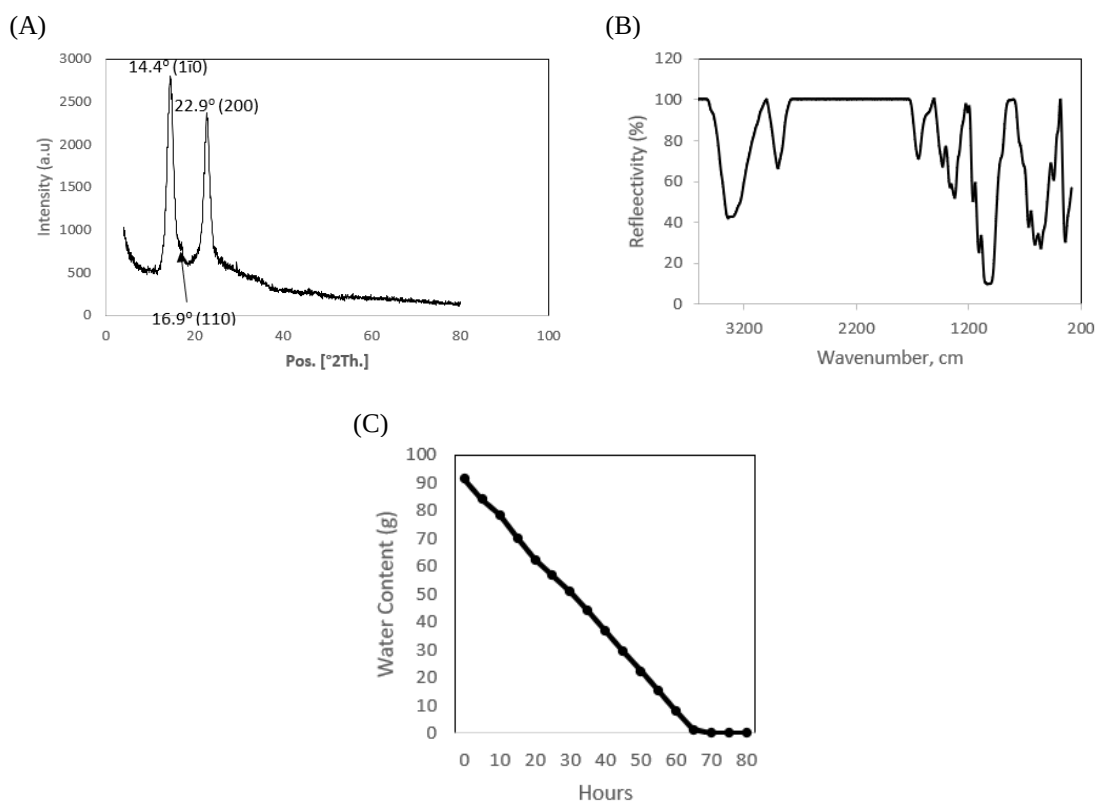


Figure 8 X-ray diffractograms (A), FTIR spectrum (B), and WRR (C) for BNC produced from *K. hansenii* RM-03 using HS medium after 16 day.

Table 3 XRD analysis results of BNC produced by *K. hansenii* RM-03.

Parameter	Value
I ₂₀₀	2761.45
I _{am}	600.78
CI	78.24
d-spacing (Å) (110) (d ₁)	6.12
d-spacing (Å) (200) (d ₂)	5.312
Z-value	5.021

FTIR spectra (Figure 8B) exhibited characteristic cellulose type I bands. A broad absorption at 3301 cm⁻¹ was attributed to OH stretching [30]. Notably, the absence of bands at 3488 cm⁻¹ and 3447 cm⁻¹, confirmed the absence of cellulose type II [31]. The bands at 2889 cm⁻¹ and 2854 cm⁻¹ were related to CH symmetric stretching of CH₂ group and CH₂ asymmetric stretching, respectively [32]. Besides, peaks at 1642 cm⁻¹, 1341 cm⁻¹, 1313 cm⁻¹, 1097 cm⁻¹, 1427 cm⁻¹ and 1128 cm⁻¹ corresponded to absorbed water [33], CH bending and CH₂ wagging [34-35], COC stretching [36], CH₂ symmetric bending [37] and COC asymmetric stretching [38], respectively, typically found in cellulose type I. These results align with previous FTIR findings, for instance, BNC produced by *G. hansenii* NCIM 2529 shows similar bands at 3350 cm⁻¹, 2960 cm⁻¹, 1653 cm⁻¹, as well as a characteristic region between 1500 cm⁻¹ to 1235 cm⁻¹, and 1068 cm⁻¹ [39]. Similarly, BNC produced by *K. saccharivorans* BC1 has peaks at 3300 cm⁻¹, 2930.10 cm⁻¹, 1645.23 cm⁻¹, 1439.39 cm⁻¹, 1167.83 cm⁻¹, 1102.49 cm⁻¹, and 1026.55 cm⁻¹ [3].

While the morphological and structural features observed here (FESEM, TEM, XRD, FTIR) are consistent with typical *Komagataeibacter*-derived BNC, the novelty of this study lies in the isolation of BNC-producing bacteria from a locally fermented starfruit substrate, an agro-waste, rather than in the production process itself. Unlike conventional approaches that rely solely on expensive HS medium for isolation and production, this study demonstrates a practical strategy for selectively enriching cellulose-producing bacteria from underexplored, low-cost fruit-based substrates. However, HS medium was subsequently used for BNC production to ensure high-quality material for detailed characterization. This approach highlights a sustainable and resource-efficient method to expand the diversity of BNC sources.

BNC possesses a remarkable ability to hold a large volume of water, an essential functional property for its numerous applications in pharmaceutical and medical industries. BNC biosynthesis by *K. hansenii* RM-03 showed a high WHC (366.24), where 0.251 g of dry BNC could hold up to 91.9 g of water. This result is comparable to other BNC produced by *G. hansenii* NCIM 2529 (WHC=400) [39], *K. xylinus* 0416 (WHC=316) [40] and *G. hansenii* PJK (WHC=296) [41]. The WRR analysis showed that BNC from *K. hansenii* RM-03 required approximately 65 hours to dry completely, indicating a prolonged moisture retention ability (Figure 8C). This drying duration is within the range reported for other BNC sources, which fully release water at approximately 50–70 hours [42,43]. These functional properties are directly related to the dense, porous, and highly entangled microfibrillar network observed via FESEM, which allows water to be retained through hydrogen bonding and slows its release. This combination of high WHC and slow WRR indicates that BNC from RM-03 performs comparably to other BNC sources and is suitable for moisture-sensitive applications, such as wound dressings.

4. Conclusion

In summary, only rotten starfruit using the M5 fermented fruit juice method yielded BNC-producing isolates, supported by pellicle formation. Among the 80 isolated colonies, only three produced BNC, and the isolate showing the highest mean BNC concentration under the screening conditions (0.092 g/100 mL) was selected for further characterization and identified as *Komagataeibacter hansenii* RM-03 (99.0% 16S rRNA similarity). During static fermentation in HS medium, *K. hansenii* RM-03 produced the highest BNC (0.277 ± 0.007 g/100 mL) on Day 16. FESEM and TEM confirmed the nanocellulosic morphology (~35 nm), while XRD and FTIR analysis indicated cellulose type I, with a CI of 78.24%. The BNC exhibited WHC of 366.24% and retained water for up to 65 hours. Importantly, this study provides a novel insight by demonstrating that low-cost agro-waste substrates, such as fermented starfruit juice, can be effectively used to selectively enrich and isolate BNC-producing bacteria. This practical strategy expands the diversity of potential BNC sources and offers a sustainable, resource-efficient approach for future production.

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6. Authors contributions

Zahimi, MI.: Conceptualization, Methodology, Resources, Data curation, Formal analysis, Investigation, Visualization, Writing - original draft preparation; Nor, NM.: Methodology, Resources, Validation; Rahim, NFC.: Methodology, Resources, Validation; Pannerchelvan, S.: Validation, Writing - review and editing; Tan, JS.: Validation, Writing - review and editing; Mohamad, R.: Conceptualization, Project administration, Methodology, Resources, Validation, Writing - review and editing, Funding acquisition, Supervision; Mohamed, MS.: Methodology, Resources, Validation, Writing - review and editing. All authors have read and agreed to the published version of the manuscript.

7. Conflicts of interest

The authors declare that they have no conflict of interest.

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