

# Production of Cellulose and its Characterization by *Bacillus sp.*RS-1

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## Abstract:

Bacterial cellulose is a naturally produced biopolymer synthesized by certain microorganisms and is widely studied because of its unique structural and functional properties. The present study was carried out to produce bacterial cellulose and characterize its important properties for possible industrial and biomedical applications. The isolate RS-1 demonstrated the maximum bacterial cellulose yield (4.80 g/L). RS-1 isolate was identified as *Bacillus sp.* by its morphological, biochemical characteristics and 16S rRNA sequencing. Optimization of carbon source for cellulose production by the isolate was investigated and HS medium supplemented with 2% (w/v) lactose resulted in the highest BC yield (4.45 g/L). Characterization of cellulose were tested by FT-IR test, and other parameters such as weighing dry and wet weight for WRC (water retention capacity), WHC (water holding capacity), WRR (water retention rate), MC (moisture content), swelling ratio and porosity ratio were tested. The produced bacterial cellulose showed good water holding capacity, high purity, flexibility and a strong fibrous structure.

**Keywords:** Bacterial Cellulose, Cellulose Characterization, Biocompatibility, Sustainable Biopolymer, Biodegradable Materials.

## 1. Introduction

Cellulose, the most abundant renewable biopolymer on Earth, serves as a key structural component of plant cell walls. It is sourced from various organisms, including plants and bacteria, and is valued for its non-toxic, stable nature, making it suitable for biomedical applications. Notably, Bacterial Cellulose (BC), produced by certain bacteria, displays superior physicochemical properties such as high crystallinity and excellent water holding capacity, enabling improved cell attachment and quicker wound healing. While plant-derived cellulose has applications in multiple industries, its extraction is intensive. In contrast, BC unique characteristics position it as a potential alternative biodegradable material in medical and other fields, due to its high purity and biodegradability.

Therefore, the study investigates the isolation and screening of bacterial cellulose-producing strains from spoiled fruits, assessing cellulose production potential under various fermentation conditions, and optimizing process parameters with alternative carbon substrates. It includes detailed physicochemical characterization of the cellulose to evaluate its structural properties and applications. The research aims to establish a sustainable bacterial cellulose production system, linking yield and material properties to metabolic and environmental factors. Additionally, cellulose's role in microbial survival and biofilm formation is highlighted, along with the genetic regulation of its biosynthesis through networks involving cellulose synthase operons and cyclic di-GMP signaling.

## 2. Materials and Methodology

**2.1 Isolation & Screening:** Samples of spoiled fruits, spoiled vegetables and soil were used to isolate the bacteria, obtained from the local markets. These samples included Banana, Grapes, Lemon, Orange, Tomato, Potato and soil.

To isolate cellulose-producing microorganisms, samples were mixed with sterilized distilled water, ground aseptically, and filtered to collect juice. Serial dilutions ( $10^{-1}$  to  $10^{-6}$ ) were conducted, and 0.1 ml aliquots from specific dilutions were plated on HS agar. Individual colonies were purified through repeated streaking onto fresh HS plates and preserved on HS agar slants at 4°C.

Screening by pellicle formation: Inoculating 1ml of bacterial isolates in 50ml of sterile HS culture medium at 30°C for 2 weeks led to pellicle formation, suggesting cellulose production. Pellicles were collected via centrifugation, rinsed, and boiled in 0.1 M NaOH to remove cells. After drying at 70°C, the weight of the purified pellicles was measured to assess bacterial cellulose production, with the highest-yield isolate chosen for further study (Hasanin et al., 2023).

$$\text{Yield (g/L)} = \text{Dry weight (g)} \div \text{Volume of medium (L)} \quad (1)$$

## 2.2 Characterization of selected isolates

Biochemical characterization of isolates were carried out using indole production, Methyl red test, Voges Proskauer test, Citrate utilization test, H<sub>2</sub>S production test, Nitrate reduction test, Urea hydrolysis test, Gelatin liquefaction test, Oxidase test, Catalase test and carbohydrate fermentation test.

Molecular identification of the selected isolate involved genomic DNA extraction and unidirectional 16S rRNA sequencing using the BDT v3.1 Cycle sequencing kit on an ABI 3730 genetic analyzer. The bacterial isolates' 16S rRNA sequences were amplified with primers 27F and 1391R. Identification was performed using BLAST against the NCBI gene bank database, focusing on the maximum identity score.

## 2.3 Phylogenetic tree analysis of selected isolate

The phylogenetic analysis involved partial 16S rRNA sequences of a selected isolate. Nucleotide sequences from related organisms were aligned using the ClustalW program, followed by homology calculations. A phylogenetic tree was constructed through the neighbor-joining method with MEGA 11 software. The partial 16S rRNA sequence of R1 was submitted to the NCBI database via the BankIt tool of GeneBank (Tamura K. et al., 2021).

## 2.4 Optimization of carbon source for cellulose production

Optimization of carbon sources was conducted using various sugars, including Glucose, Fructose, Maltose, Sucrose, and Lactose, with a selected isolate. (Idris Bektas and Nazli Betul Yildirim, 2025).

## 2.5 Purification of cellulose

The cellulose was removed from the medium and treated with 0.1 N NaOH at 80° C for 1 hour to eliminate bacterial cells and medium components. The purified cellulose was then washed with distilled water until a pH of 7.0 was achieved, followed by measurement of the dry weight of the produced cellulose (Hasanin et al., 2023).

## 2.6 Characterization of cellulose

### 2.6.1 The water holding capacity (WHC) and water release rate (WRR)

The study measured the water holding capacity (WHC) and water release rate (WRR) of BC composites using a sieve shaking method. Dried BC sheets were soaked in distilled water to swell, then excess water was removed by gentle shaking before weighing. The samples were air-dried and weighed over 45 hours to assess water release rate. Final weights were obtained after drying at 60 °C for 24 hours. WHC was calculated as the mass of water removed during drying divided by the dry weight of the cellulose sample, while WRR was determined by periodic weighing of the samples stored under ambient conditions.

$$\text{WHC} = \text{Mass of water removed during drying (g)} \div \text{Dry weight of cellulose sample (g)} \quad (2)$$

### 2.6.2 Porosity analysis

The porosity of BC samples was measured by immersing them in distilled water for 12 hours at room temperature. The porosity percentage was then calculated using the formula: (Almiyawati et al., 2022).

$$\text{Porosity (\%)} = (\text{wet weight} - \text{dry weight}) \div (\text{wet weight} - \text{weight in water}) \times 100 \quad (3)$$

### 2.6.3 Swelling ratio

The study examined the swelling behavior of BC composites by cutting membranes into  $2 \times 2$  cm pieces and drying them to a constant weight. After drying, the samples were immersed in deionized water at room temperature to facilitate swelling. The swelling ratio (%SR) was calculated using the formula:

$$\text{SR\%} = (\text{Wt} - \text{Wd}) \div \text{Wd} \times 100 \quad (4)$$

where Wt is the swollen weight and Wd is the dry weight. Each swelling experiment was repeated three times, and average values were computed for accuracy (Almiyawati et al., 2022).

### 2.6.4 Determination of the WRC (Water Retention Capacity) and MC (Moisture Content)

The BC samples were dried at 80 °C prior to analyzing water retention capacity (WRC) and moisture content (MC). For WRC, the dry weight was recorded, followed by immersion in 45 mL distilled water at 27 °C for 30 minutes. After removing excess water, the wet weight was measured. WRC and MC were calculated using the formulas:

$$\text{WRC (\%)} = (m_{\text{wet}} - m_{\text{dry}}) \div (m_{\text{dry}}) \times 100 \quad (5)$$

$$\text{MC (\%)} = (m_{\text{wet}} - m_{\text{dry}}) \div (m_{\text{wet}}) \times 100 \quad (6)$$

where  $m_{\text{wet}}$  is the initial weight and  $m_{\text{dry}}$  is the dry weight of the BC sample (Bektas et al., 2025).

### 2.6.5 FTIR Analysis

The characteristics of bacterial cellulose (BC) were analyzed using FTIR. For this, the samples were first finely powdered and then mixed with KBr salt to form small tablets suitable for analysis. The analysis was carried out over a wavelength range of 4000 to 500  $\text{cm}^{-1}$  to identify the important functional groups present in the BC samples (Bektas et al., 2025).

## 3. Results & Discussion

### 3.1 Isolation and screening of cellulose producing microorganisms

Isolation of microorganisms was carried out by serial dilution and spread plate method using Hestrin-Schramm medium. Different dilution ranges including  $10^{-2}$ ,  $10^{-4}$ , and  $10^{-6}$  were prepared for each sample. Screening of bacterial isolates was performed on HS broth medium based on pellicle formation. Pellicle formation is an important indicator of bacterial cellulose production. A total of 20 isolates showed pellicle formation with varying cellulose yield. Among all isolates, RS-1 demonstrated maximum cellulose production with a pellicle dry weight of 0.240 g and cellulose yield of 4.80 g/L.

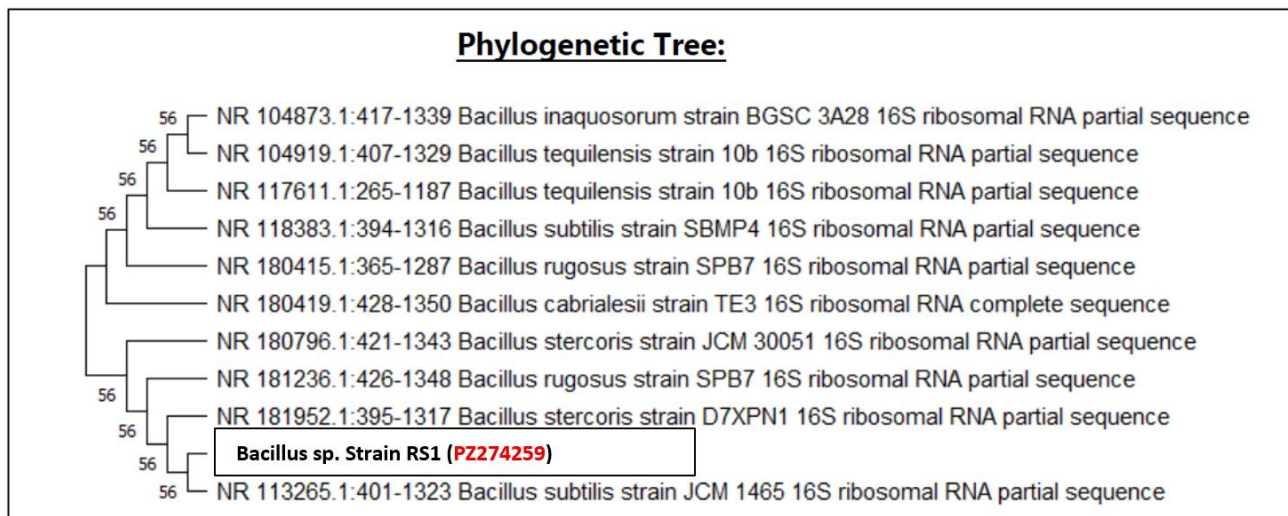


**Figure-1: Growth of isolate RS-1 on HS medium**

Selected isolates RS-1 showing significant cellulose production were characterized morphologically and microscopically. Gram staining results revealed that the RS-1 was gram-positive rods and biochemical characteristics are given in Table-1.

<b>Table 1: Results of Biochemical test of RS-1</b>			
Name of the Biochemical test	Results	Carbohydrate fermentation test	Results
Indole test	-	Nutrient glucose broth	+
MR test	-	Nutrient sucrose broth	+
VP test	+	Nutrient xylose broth	-
Citrate utilization test	+	Nutrient lactose broth	+
H <sub>2</sub> S production test	-	Nutrient mannitol broth	+
Nitrate reduction test	+	Nutrient fructose broth	+
Urea hydrolysis test	+	Nutrient maltose broth	+
Gelatin liquefaction test	+	Triple Sugar Iron Agar	
Oxidase test	+	Slant	Alkaline
Catalase test	+	Butt	Alkaline
		H <sub>2</sub> S Production	-
+ = Positive, - = Negative			

Molecular characterization of isolate RS-1 by 16S rRNA sequencing using Sanger dideoxy sequencing method confirmed that the isolate belonged to the genus *Bacillus*. The obtained partial 16S rRNA sequence consisted of 563 nucleotides. The sequence was submitted to NCBI and assigned accession number PZ274259. Phylogenetic tree analysis demonstrated close evolutionary relationship of the isolate with related *Bacillus* species (Figure-2). Molecular characterization confirmed the biochemical identification results and validated the taxonomic classification of the isolate.



**Figure-2: Phylogenetic tree analysis of isolate no. RS1:** The evolutionary history was inferred using the Neighbor-Joining method (Saitou N. and Nei M., 1987). The bootstrap consensus tree inferred from 500 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches. The evolutionary distances were computed using the Jukes-Cantor method and are in the units of the number of base substitutions per site. This analysis involved 11 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There was a total of 923 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 (Tamura K., Stecher G., and Kumar S., 2021).

### 3.2 Optimization of carbon source for cellulose production

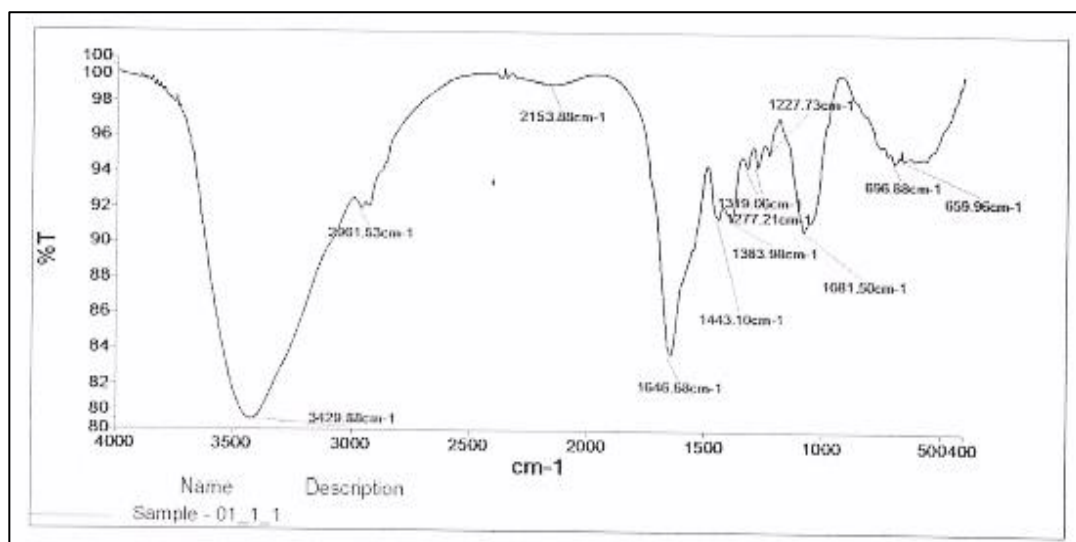
Different carbon sources including lactose, fructose, glucose, maltose, and sucrose were evaluated for cellulose production by isolate RS-. Among all tested sugars, lactose showed maximum cellulose production with a dry weight of 0.89 g and yield of 4.45 g/L. Fructose and glucose also supported good cellulose production with yields of 3.85 g/L and 3.25 g/L respectively. Maltose and sucrose produced comparatively lower cellulose yields. The higher cellulose production observed with lactose may be due to better substrate utilization efficiency and favorable metabolic pathways involved in cellulose biosynthesis.

### 3.3 Characterization of Cellulose

The cellulose produced by isolate RS-1 was characterized based on swelling ratio, moisture content, porosity, water retention capacity, and FT-IR analysis. The wet weight of cellulose before purification was 3.18 g, while purified dry cellulose weight was 0.89 g. The produced cellulose demonstrated excellent swelling ability with a swelling ratio of 930.33%, indicating high water absorption capacity. The moisture content and porosity of cellulose were recorded as 72.01% and 76.3% respectively. Water retention capacity was found to be 257.30%, demonstrating good hydrophilic properties.

FT-IR analysis confirmed the cellulose structure through characteristic absorption peaks. FTIR spectra recorded in transmittance mode over the 4000-400  $\text{cm}^{-1}$  wavenumber range with 2  $\text{cm}^{-1}$  intervals. The typical absorption at around 3400  $\text{cm}^{-1}$  (peak 1) corresponds to stretching vibration of intra and inter O-H in cellulose, While the weak peak at around 2960  $\text{cm}^{-1}$  (peaks 2 and 3) corresponds to C-H stretching of  $\text{CH}_2$  and  $\text{CH}_3$  groups or  $\text{CH}_2$  asymmetric stretching. A peak at around 1650  $\text{cm}^{-1}$  (peak 4) is observed, corresponding to H-O-H bending of absorbed water. Although it is thought that the peak at around 1430

$\text{cm}^{-1}$  (peak 5) may originate from  $\text{CH}_2$  scissoring. The peak at around  $1360 \text{ cm}^{-1}$  corresponds to C–H bending, which also results in a typical absorption at  $1280$  and  $1204 \text{ cm}^{-1}$ . While the peak at  $1319 \text{ cm}^{-1}$  (peak 7) may correspond to C–H deformation or O–H in-plane bending. It has shown that the peaks at  $1000$ – $1100 \text{ cm}^{-1}$  can be assigned to C–O stretching vibration in primary alcohol and C–O–C skeletal vibration (Figure-3).



**Figure-3: FT-IR Spectrum Analysis of Bacterial Cellulose**

## 5. Conclusion

In the present study, bacterial cellulose producing bacteria were isolated from different environmental sources including rotten fruits, vegetables, and soil samples. Out of all the isolates, RS-1 showed the highest bacterial cellulose yield of  $4.80 \text{ g/L}$ , making it the most efficient producer among the screened strains. Based on morphological, biochemical characterization and 16S rRNA sequencing, the RS-1 isolate was identified as *Bacillus sp.* Further optimization studies revealed that HS medium supplemented with 2% (w/v) lactose significantly enhanced bacterial cellulose production, while sucrose resulted in comparatively lower yield. The produced bacterial cellulose was further characterized using FT-IR analysis and evaluated for properties such as water retention capacity, water holding capacity, moisture content, swelling ratio, and porosity. The results confirmed the formation of a highly functional and stable cellulose matrix. Further optimization and large-scale studies may enhance its commercial applicability.

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