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Production, characterization and biological evaluation of bacterial cellulose from *Bacillus species* BC1

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Abstract

In this study, a bacterial cellulose (BC) producing strain BC1 was isolated from open air environment by exposing fresh wine. Based on 16S rRNA sequence analysis, the strain was identified as *Bacillus sp.* BC1. BC production was carried out in liquid static culture using both Hestrin-Schramm (HS) broth and Tea Extract (TE) broth. Enhanced quantity of BC was produced in TE broth when compared to the HS broth. The cellulose nature of the pellicle produced by *Bacillus sp.* BC1 was confirmed through SEM, FT-IR, and XRD analysis. Characterization studies revealed good thermal stability, excellent tensile strength and superior water-holding and retention capacities whereas the zeta potential proved the colloidal stability and surface charge of BC. Molecular mass of BC was confirmed through LC-MS, MALDI-TOF MS analysis. In antimicrobial study, the povidone -iodine -coated BC showed 25-29 mm inhibition whereas the Squalene-coated-BC showed 10-19 mm inhibition against the tested pathogens. In antioxidant radical scavenging study, the squalene coated BC was showed maximum of 93% radical scavenging activity at 350µg/ml concentration. The results revealed that the BC produced in this study is considered a safe material for wound healing applications with promising antimicrobial and antioxidant properties. Further whole genome sequence analysis of *Bacillus sp.* is in progress to reveal the genetic and biochemical pathways behind the cellulose production.

Keywords: Bacterial cellulose, *Bacillus sp.* biopolymer, antimicrobial, antioxidant.

Introduction

Cellulose is a naturally occurring, renewable linear homopolysaccharides composed by β-D-glucopyranose units linked by β-1,4 glycosidic bonds. This polymer is the structural material for most of the plants but it can also be produced by seaweeds, fungi, some bacteria and marine organisms (Girard VD et al., 2024). Due to its diverse applications in sectors such as medical, environment, energy, agriculture and aquaculture (Aziz T et al., 2022), Seddiqi H et al., 2021), there is a steady increase in the market demand for cellulose. Wood is the most commonly exploited natural source of cellulose due to its high and ease availability. However, the plant cellulose is considered as impure due to the co-existence of other polymers such as hemicellulose and lignin. Removal of these impurities is essential for utilizing cellulose in various fields such as paper production, food and food packaging materials, biosensors, cosmetics, and textile garments. Cellulose purification from plant is commonly carried out through harsh, complex and cost intensive physical, chemical and enzymatic methods (UI-Islam et al., 2019). Hence there will be a dire need for exploring alternate and viable source for cellulose production with high purity and low downstream cost.

In recent years, bacterial genera such as *Acetobacter, Komagataeibacter, Achromobacter, Enterobacter, analysis., Agrobacterium, Clostridium, Pseudomonas, Alcaligenes* and *Pseudomonas* (Gullo M, et al., 2018) are reported to synthesize cellulose and is called as bacterial cellulose (BC) or bacterial nanocellulose (BNC). Production of cellulose can also be achieved through the enzymes from certain bacterium like *Rhodobacter sphaeroides* and yeast, *Pichia pastoris* (Wang SS. et al., 2017). The bacterial cellulose is produced at the maximum purity and quantity when compared to cellulose from other sources (Naomi R, et al., 2020). Among all the cellulose types BC is naturally synthesized by certain bacterial genera in the form of 3D nanofibrillar network with high purity, biocompatibility and non-toxicity which makes them as promising candidate for drug delivery, dressing material for wound healing, antimicrobials, food packing materials, emulsifiers and also as additives in cosmetics (Thorat MN et al., 2018), (Joseph A et al., 2025), (Wang Z, et al., 2025).

BC can be produced by static culture, in general, using HS medium and several other optimized media using different agricultural wastes as substrate (Yilmaz M 2024), (Sathianathan N et al., 2025). Members of the bacterial genus *Gluconacetobacter* are well studied in terms of bioprocess and biological evaluation of cellulose production (Li G, Li W, et al., 2021). However, reports on cellulose production by Gram positive bacteria very limited which is restricted to certain species of *Bacillus* like *B. velezensis* (Abouelkheir SS, 2020) and *B. amyloliquefaciens* (Bektas I, et al., 2025). Abouelkheir et al., (Abouelkheir SS, et al., 2020) reported that the Gram-positive bacterium, *Bacillus*

velezensis isolated from sea water sample was found to produce twice the quantity of BC when compared to the well investigated BC producing Gram negative bacterium *Gluconoacetobacter xylinus* ATCC 10245. Hence, the present study is initiated to study the production, characterization and biological evaluation of cellulose from gram positive bacterium *Bacillus sp.* BC1 isolated from contaminated wine sample in open environment.

Materials and Methods

Isolation and screening of cellulose producing bacteria

In this study, for the isolation of cellulose producing bacteria, about 50 ml of fresh wine obtained from local shop at Chennai, Tamil Nadu was exposed to open air in a sterile beaker for 4 days. Then hundred microliters of air-exposed wine sample were plated on nutrient agar plate for 2 days at 27℃. Morphologically distinct bacterial colonies were isolated from the HS agar plates marked as BC1 and preserved on nutrient agar slants at 4ºC as well as 30% glycerol broth at -80ºC. These strains were inoculated into HS agar (g/L) glucose 20.0, yeast extract 5.0, peptone 5.0, disodium hydrogen phosphate 6.8, citric acid 1.15, agar 18, pH 6 and incubated for four days at 28°C. Inoculum of all the bacterial cultures was prepared by growing on each 10 ml of nutrient broth for 24 h. Then 5 ml inoculum of all the cultures were transferred in to each 50 ml of HS broth prepared in 250 ml conical flask and incubated at static condition for 10 d at 28ºC. Cellulose production was assessed based on the formation of white cellulose pellicles on the air-liquid interface of the culture medium (AI-Deresawi TS, et al., 2023). The presence of cellulose was qualitatively confirmed further by the Schultz's test (Saranya RJ, et al., 2022). Briefly, a small piece of cellulose pellicle was taken in a glass petri plate and few drops of Shultz reagent was added into it. After 10 min, the cellulose pellicle was observed for purple color which indicates the cellulose nature of the pellicle.

Selection and characterization of cellulose producing bacteria

Among the four bacterial cultures tested, only strain BC1 was found to produce cellulose on HS broth. Bacterial strain BC1 was further stored as stored as glycerol stock as well as in nutrient ager slants at -80ºC and 4ºC, respectively (Chen J, et al., 2024). The molecular characterization of the strain BC1 was done by performing 16s rRNA gene sequence analysis at Bioserve Biotechnologies Ltd, Hyderabad. The genomic DNA of potential strain was isolated, amplified and sequenced by adopting the method described by (Vaishnavi M, et al., 2022). The 16s rRNA gene sequence of strain BC1 was compared with the gene sequences available in the GenBank database by BLAST analysis. The phylogenetic tree was constructed using neighbour joining algorithm with MEGA 11 software (Saitou

N, Nei M 1987). The partial 16s rRNA nucleotide sequence of the strain BC1 was deposited at Gene Bank data base by using Bankit tool (http://www.ncbi.nlm.nih.gov/WebSub/?tool=genbank).

Production and characterization of cellulose from *Bacillus sp.* BC1

Freshly grown culture of strain BC1 was inoculated into 50 ml of sterile nutrient broth and kept in rotary shaker at 160 rpm for 24-48 ^{0}C. Initially, BC production was carried out as static flask culture using each 100 ml of HS medium and tea extract medium. Tea extracts medium was prepared by adding 1 gm of green tea leaves and 1 gm of sugar into 100 ml of distilled water in 250 ml conical flask and sterilized by autoclaving at 121^{o}C for 15 min. Each one ml fresh inoculum of strain BC1 was transferred into both HS broth and tea extract broth and kept static condition at 26℃ - 27℃ for 15 d. After incubation, the surface BC layer was collected and kept in a beaker with 0.1 M NaOH at 70˚C and stirred for 1 h. Then the BC was washed with 90% ethanol for 10 min followed by washing with distilled water until it reached the neutral pH value. Then the wet weight and dry weight of the BC was calculated. Further, BC production was carried out in a clean plastic tray sterilized by UV treatment using each 1000 ml of HS medium and Tea extract medium. Each 10 ml fresh inoculum of strain BC1 was added in to both the medium and closed with sterile muslin cloth. The production trays were incubated at 26℃-27℃ under static condition for 25 days. After incubation, the BC layer was collected, processed and quantified by adopting the same procedure as described above for flask experiment (Costa AFS, et al., 2017).

All the spectral, microscopic and physical properties of BC were studied at the Research Facilities available at Sathyabama Institute of Science and Technology, Chennai, India. The FT-IR spectrum of BC was determined using the instrument FT-IR-6600 type A model in the region of 500 to 4500 at ambient temperature and the signal obtained at 1 cm^{-1} resolution. The surface structure of BC was studied by SEM analysis using FESEM (Supra55-Carl Zeiss, Germany). The X-ray diffraction (XRD) analysis of BC sample crystallinity index was carried out with a wavelength of 1.540560 Å at the 2θ˚ of 22.80 observation using XRD Smart Lab-9kW-RIKAGU, Japan (Wang SS et al., 2017). TGA analysis was performed with a LINSEIS STA PT6000 (Germany) Thermal analyser (Potivara K et al., 2019). To determine thermal decomposition property, cellulose samples were dried at 50 °C for 48 h. Water content was calculated by the following equation where $W0$ and Wt represent the weight of dried and wet NCs, respectively. The tensile strength of the BC sample was restrained using a 15 mm width, 4 mm thickness, and instrument model M-50 with serial No.2022/81. The PSA, zeta potential with instrument model HORIBA SZ-100 WINDOWS [Z Type]

ver2.40 were done to know the dimensions of nano fibers and the surface charge properties of BC. BC was dissolved in DMSO solution, homogenized at a high speed, and kept for 10 min in an ultrasonic bath at room temperature.

To determine the water absorption property, the dried BC with 3X3 mm size was taken and measured its weight. The BC material was put into 100 ml of sterile water and kept at 25^{o}C for 24 h. Then the BC layer war removed, and its wet weight was measured (Wang SS et al., 2017). The percentage of water absorption was calculated by adopting the following formula.

Water absorption (%) = [(wet weight of BC-dry weight of BC)/ dry weight of BC] X100

The water retention capacity of BC was determined by dried bacterial cellulose made into 3X3 mm size, and the dried weight was measured, the BC was soaked into 100 ml of distilled water for 24 h incubation. Then the BC layer was taken out and excess water was removed; the percentage of water retention was calculated by using the formula (Abouelkheir SS et al., 2020).

Water retention (%) = [(wet weight - dry weight)/dry weight] X100

Mass Spectrometric analysis of bacterial cellulose

The LC-MS analysis of BC was done with instrument Agilent 1260 infinity II preparative pump mode. 2mg of BC was dissolved in 2mL methanol in volumetric flask. The solution was using SRP-4 membrane 0.45 mm filter. The LC-MS spectrum was carried out with the flow rate 0.5- 50 mL/min and the 1µL injection volume was used (Razmavar S et al., 2014).

The BC sample was subjected to matrix- assisted laser desorption/ionization with time-of-flight (MALDI-TOF) with 2,5-dihydroxy benzoic acid (DHB) as matrix (≥99.5%) to determine unknown oligomers. Instrument model used UTG-10132 type-18 the mass spectra was obtained by ultra-flex MALDI-TOF mass spectrophotometer it equipped with 337 nm nitrogen laser. To dissolve the sample TA30 (30% acetonitrile/70% TFA) solution mixed with 20 mg/mL of DHB (1:1). Then 2µL of sample was placed in steel plate to dry and the mass spectra were used analysed linear positive mode (Lasch P et al., 2025).

Optimization of cellulose production from the Bacillus sp. BC1

Effect BC production of BC1 was carried out by using different types of sugars such as (g/L) of sucrose, fructose, mannitol, xylose, dextrose in HS media by replacing instead of glucose as alternative carbon source. All the reaction

flasks were kept at 25℃ - 27ºC under static condition. Similarly, nitrogen source such as (g/L) yeast extract, peptone, and tryptone and different pH levels such as 5, 5.5, 6.0 and 6.5 was also tested for their effect of BC production (Almihyawi R.A et al., 2024), (Velmurugan P et al., 2015).

Evaluation of BC from *Bacillus sp.* BC1 for biological activities

Antimicrobial activity

The antimicrobial activity of BC impregnated with bioactive compounds was tested against selected microbial pathogens such as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Candida albicans.* All the test pathogens were grown in 10 ml of Muller Hinton broth for 12 h at 37ºC. Then the freshly grown cultures were inoculated on Muller Hinton Agar (MHA) plates as a lawn using sterile cotton swab. The sterile BC membrane was coated with povidone iodine concentration of 5% w/w, squalene concentration of 10% and Gentamicin antibiotic disc (10µg) was placed over MHA plates inoculated with test pathogens. All the plates were incubated at 37ºC for 24 h and then observed for zone of inhibition (Jeong JS et al., 2012).

Antioxidant activity

The '2,2-diphenyl-1-picrylhydrazyl (DPPH)' method used to determine the antioxidant activity of BC coated with 50µl of squalene 40% w/w concentration. The reaction between DPPH and squalene impregnated BC sample was measured with UV-visible spectroscopy OD at 515 nm. The 'methanol solution' was used as blank with different concentrations (50, 20, 40, 50, 60, 80 and 100) µg of the sample, and 30 min incubation in the dark at room temperature. The stock solution was newly made by dissolving 80 µg of DPPH in 98% methanol sonication for fifteen min and stored at dark room temperature. Then 250 µl of sample was pipetted in a tube containing 1750 µl DPPH solution. The blank with 150 µl of DPPH and 250 µl of methanol (UI-Islam M et al., 2012). The antioxidant activity was articulated in mM Trolox equivalents (TE)/100g DW using the calibration curve. The percentage of radical scavenging activity 1% was calculated by using the following formula.

Radical scavenging activity (%) = [(A blank - A sample) / A blank] X 100

Statistical analysis

Origin pro software was used for the statistical analysis of data. The experiments were performed in the triplicate form and the data are expressed as mean value ± SD. The two-way Anova method was used for the analysis of the statistical data differences between the significant of *=$p \leq 0.05$ it was considered as statistically significant.

Results

Isolation and characterization of cellulose producing bacteria

In this study, four distinct bacterial colonies (BC1 – BC4) were observed on the nutrient agar plates seeded with air exposed wine sample. Upon cultivating on HS broth, only the strain BC1 has produced the white pellicle like structure on the surface of the HS medium (Fig.1A). This is a presumptive indication for cellulose production. The white pellicle produced by the strain BC 1 has showed positive results for Schultz's test which further confirm the cellulose production (Fig. 1B).

Strain BC 1 showed good, rough, opaque and fuzzy while colonies on nutrient agar. It showed purple-coloured rod-shaped cells under bright field microscopic observation which confirm it as Gram positive bacterium (Fig. 2A-B). The morphological and physiological characteristics revealed that the potential strain BC 1 belongs to *Bacillus sp*. Further, the 16s rRNA gene amplification yielded a gene sequence of 908bp. Based on the Tamura – Nei model, the phylogenetic tree for the potential strain BC 1 along with its closest relatives of genus *Bacillus* is shown in (Fig. 3). It is accurately depicted that then strain BC 1 was closely clade with *Bacillus sp.* SUB14723091 BC1 PQ312690. 1 (99.89%). Based on the results, the strain BC 1 was identified as *Bacillus sp*. The gene sequence of the potential strain *Bacillus sp.* BC 1 was submitted to NCBI GenBank with the accession number SUB14723091 BC1 PQ312690.

Production and characterization of BC

In static flask and tray method, *Bacillus sp.* BC1 has formed a thick, while pellicle on the surface of both the HS medium and tea extract medium. The yield of BC obtained in both HS medium and tea extract in both static flask and tray method was calculated and given as wet and dry weights in table 1. The results showed that the tea extract medium enhanced the bacterial cellulose production from *Bacillus sp.* BC1 when compared to HS medium (Fig. 4).

The Scanning Electron Microscopic (SEM) observation has revealed the dense porous nano fibrillar network characteristic of bacterial cellulose, with porous sizes varying across 50–200 nm (Fig. 5). FT-IR spectral analysis confirmed the presence of a broad O–H stretching band at 3333.36 cm^{-1}, a C–H stretching peak at 2905.24 cm^{-1}, and strong absorptions at 1368.25 cm^{-1} and 1156.12 cm^{-1} correspond to CH_2 groups and C–O stretching, respectively (Fig. 6). The XRD pattern of bacterial cellulose produced by strain BC1 showed the main peaks at 2θ = 17.133 and 22.803 in the primary direction of the cellulose pellicles (Fig. 7). The small upsurge in the 'relative peak intensity' of

the diffraction peaks relative to the treated bacterial cellulose membrane slightly changes the location of the BC micro fibril crystalline structure in the X-ray diffraction analysis.

The TGA curve (Fig. 8A) demonstrated that the BC produced from the *Bacillus sp.* BC1 has maintained thermal stability up to 395°C, with weight loss observed at three degradation phases (14.02%, 14.8%, and 31.9%). Tensile testing (Fig. 8B) revealed a yield stress of 0.6 N/mm² and a tensile strength of 1.0 N/mm², with an elongation at break reaching 9.546 mm. (Fig. 9 A, B) shows the particle size peak at 65 nm represent the excellent nanofiber dimension high colloidal nature of BC and zeta potential analysis value -52 mv has high surface charge rich colloidal stability of BC. indicated a mean diameter of 65 nm, supporting the nanocellulose classification of the material. As shown in (Fig. 10A-B), the BC produced by *Bacillus sp.* BC1 exhibited an initial water absorption of 82% and a water retention capacity of 80.93% on day 1. Both values gradually decreased over time, consistent with typical moisture loss through evaporation and material relaxation. (Fig. 13) shows the LC-MS analysis of BC revealed many peaks at different retention between 0.074 min to 9.54 min from that retention time peak 55 indicating the presence of cellulose molecular mass 162.90 at 9.540 min and this peak shows the high intense in the chromatogram for cellulose. This data significantly exposes the chemical composition of BC (Bimmer, M et AL., 2023). The (Fig. 14) MALDI-TOF spectrum strongly indicates the presence of cellulose oligomers with various degree of polymerization DP. One DP=162.05Da It shows the distinct peaks between m/z 500 to 2500 with sodium ions [Na^+] in very regular spacing 507.294 DP3 cello-triose, 585.513 DP3, 833.566 DP6 cellopentose, 1017-1019 DP6 cellohexose, 1173.694 DP7 cellohetaose, 1329.829 DP8 celloctaose, 1508.910 DP9 cellononaose. These peaks are perfectly matched with cellulose oligosaccharide (Song J.E et al., 2018). (Fig. 15) shows the optimal carbon, nitrogen source of BC which are glucose and nitrogen optimal pH with 15 days of incubation period.

Evaluation of BC for biological activities

Antimicrobial activity

Antimicrobial properties (and) The BC produced from *Bacillus sp.* BC1 did not exhibit antimicrobial activity as such and it can act as carrier material because of its rich porosity Instead, when coated with bioactive agents such as povidone iodine and squalene, the BC membranes showed considerable zones of inhibition against *Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus,* and *Candida* spp. (Fig. 11 Table 2)

Antioxidant assay DPPH:

The DPPH assay demonstrated that BC extracts possess moderate antioxidant capacity, increasing from 40% to 93.28% as the concentration rose from 50 to 350 µg/ml (Fig. 12).

Discussion

Bacteria are the real renewable resources for cellulose when compared to plants. Cellulose producing bacteria are widely distributed in nature such as soil, decaying organic matter, waste water, fruits and vegetables. Mostly they are isolated from rotten fruits and vegetables (throat MN et al., 2018) followed by other sources like soil and marine sediments (Bharathi S et al., 2024). In the present study, cellulose producing bacterial strain BC1 was isolated from the open-air by exposing wine sample. Schultz's test is the preliminary biochemical test used to qualitatively confirm cellulose which turns cellulose purple (Gohel HR et al., 2014).

According to the literature, most of the BC producers are comes under the Gram-negative bacterial genera such as *Achromobacter, Agrobacterium, Acetobacter, Rhizobium, Azotobacter,* and *Alcaligenes* (Muhammad H, et al., 2024), (Montenegro-Silva P 2024)**.** There are limited studies reported the BC production by the Gram-positive bacteria which include *Bacillus* (Bektas I et al., 2025), *Leifsonia sp., Lactobacillus sp*., and *Rhodococcus sp.* (Gracia EL, et al., 2022). Some previously identified BC producing *Bacillus* species include *Bacillus amyloliquefaciens* B1, *Bacillus subtilis* B2, *Bacillus velezensis* B3, *Bacillus siamensis* B4, and *B. amyloliquefaciens* B5 (Bektas I et al., 2025). Further, the morphology, structure, properties, and applications of bacterial cellulose (BC) are significantly dependent on bacterium type (Liu X et al., 2023). *Bacillus sp.* is a biotechnologically significant bacterium with diverse biological activities such as the production of enzymes, antifungal metabolites (Yu Q, He P et al., 2024). plant growth promotion. In addition, the whole genome sequencing of *Bacillus sp.* by (Wang B et al., 2021) has revealed the presence of several genes for the production of plant defence related enzymes and several biosynthetic gene clusters for the production of secondary metabolites. However, to the best of our knowledge, there is no report on cellulose production from *Bacillus sp.* hence, the BC1 reported in this study is a newly added source for bacterial cellulose production.

The cost of substrates is the major challenge and limiting factor in any fermentation process. To reduce the production cost and increase the productivity, different wastes from food, vegetables and agriculture sectors are widely used in fermentation industries (Guzel M et al., 2020). There are several studies reported the cost-effective production of BC from different bacterial species using different agriculture and fruit wastes (Abol-Fotouh D et al., 2020), (Rodrigues DM et al., 2024) including black tea extracts (Hamed DA et al., 2023). In this study, *Bacillus sp.* BC1 produced around 17.29 grams of wet BC when using green tea extract medium. This is 1.5 times higher than the BC produced

in standard HS medium under same conditions which revealed that tea extract medium is found suitable medium for the enhanced production of BC from *Bacillus sp.* BC1.

The dense porous with fibrous morphology under SEM supports previous reports of BC structures produced by other Gram-positive and Gram-negative bacteria (Machado RTA et al., 2018). These patterns align with cellulose I as previously reported (Zhong C 2020). The TGA pattern of BC1 has similar to the multi-phase thermal breakdown patterns of bacterial cellulose reported by Castro Cet et al., 2011). The tensile strength analysis revealed that the dimensions enhance the surface area-to-volume ratio, making BC suitable for functional coatings and nano-enabled drug delivery systems (Costa AFS et al., 2017). These values reflect the flexibility and mechanical robustness of BC and highlights BC's mechanical suitability for biomedical scaffolds (Girard VD et al., 2024). The hydration properties are critical for biomedical applications, particularly in wound dressings where moisture regulation enhances healing (Oliveira Barud HG, et al., 2015). The ability to retain water also implies potential utility in drug delivery matrices, where sustained hydration is required to support release kinetics.

Bacterial cellulose, in general, is a versatile biopolymer. It can control wound exudate and provide moist environment which facilitate wound healing but it lacks inherent antimicrobial activity. However, BC based composites can be prepared by incorporating various antibiotics, natural molecules or nanoparticles for wound healing (Zheng L et al., 2020). Preparation of BC-based composite enhance the bioactivity of those respective biomolecules (Echeverria C et al., 2024), (Bodea IM et al., 2022). Povidone iodine has a broad-spectrum antimicrobial activity used as common antiseptics (Bigliardi PL et al., 2017). Similarly, squalene, a saturated form of squalene, was also reported to exhibit promising antioxidant and anticancer activities than antimicrobial activities (Huang ZR et al., 2009). In the antimicrobial study, the biomolecules coated BC showed enhanced activity against the test pathogens. This is likely due to the physical disruption of microbial membranes, suggesting that BC serves as an excellent delivery matrix for antimicrobial compounds, echoing findings by (Oliveira Barud et al., 2015) and (Tsouko E et al., 2015) While the direct antioxidant activity may be limited, the data suggest potential for BC to serve as a controlled-release platform for antioxidants, especially when combined with molecules like squalene. This aligns with the emerging concept of dual-function BC scaffolds for oxidative stress-related therapies (Keshk SM 2014).

Findings of the present study revealed that the *Bacillus sp.* BC1 isolated from the open-air sample is a newly added source for cellulose production. Further Whole genome sequencing of Bacillus sp. BC1 is in progress to decipher the molecular mechanism behind the cellulose production.

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Author Contribution

All authors contributed to the conception and design of the study. Supervision, validation, visualization, reviewing and editing were performed by Radhakrishnan Manikkam. Material preparation, data collection and analysis, manuscript writing were performed by Akila Kesavan and Kishore Kumar Annamalai. All authors read and approved the final manuscript.

Data Availability

The data used to support the findings of this study are available from the corresponding authors upon request.

Ethics Approval

Not applicable.

Consent to Participate

Not applicable

Consent to Publish

Not applicable

Conflict of Interest

The authors declare no conflict of interest.

References

1. Girard VD, Chaussé J, Vermette P (2024) Bacterial cellulose: A comprehensive review. J Appl Polym Sci 141:e55163.
2. Aziz T, Farid A, Haq F, Kiran M, Ullah A, Zhang K, Li C, Ghazanfar S, Sun H, Ullah R, et al. (2022) Review on the modification of cellulose and its applications. Polymers 14:3206.

3. Seddiqi H, Oliaei E, Honarkar H, et al. (2021) Cellulose and its derivatives: towards biomedical applications. Cellulose 28:1893–1931.

4. Ul-Islam M, Khan S, Ullah MW, Park JK (2019) Comparative study of plant and bacterial cellulose pellicles regenerated from dissolved states. Int J Biol Macromol 137:247–252.

5. Gullo M, La China S, Falcone PM, Giudici P (2018) Biotechnological production of cellulose by acetic acid bacteria: Current state and perspectives. Appl Microbiol Biotechnol 102:6885–6898.

6. Wang SS, Han YH, Ye YX, Shi XX, Xiang P, Chen DL, Li M (2017) Physicochemical characterization of high-quality bacterial cellulose produced by Komagataeibacter sp. strain W1 and identification of the associated genes in bacterial cellulose production. RSC Adv 7:45145–45155.

7. Naomi R, Bt Hj Idrus R, Fauzi MB (2020) Plant- vs. bacterial-derived cellulose for wound healing: A review. Int J Environ Res Public Health 17:6803.

8. Thorat MN, Dastager SG (2018) High yield production of cellulose by a Komagataeibacter rhaeticus PG2 strain isolated from pomegranate as a new host. RSC Adv 8:29797.

9. Joseph A, Umamaheswari S, Vassou MC (2025) Bacterial cellulose: A versatile biomaterial for biomedical application. Carbohydr Res 552:109350.

10. Wang Z, Li S, Zhao X, Liu Z, Shi R, Hao M (2025) Applications of bacterial cellulose in the food industry and its health-promoting potential. Food Chem 464:141763.

11. Yilmaz M, Goksungur Y (2024) Optimization of bacterial cellulose production from waste figs by Komagataeibacter xylinus. Fermentation 10:466.

12. Sathianathan N, Karri RR, Gunavijayan G, Raj A, Mubarak NM, Latha V, Mukundan AV, Krishnankutty RE, Chellappanpillai S (2025) Optimisation of bacterial cellulose production by Novacetimonas hansenii isolated from pomegranate fruit wastes. J Mol Liq 422:126912.

13. Li G, Li W, Yu D, Qufu W (2021) Research progress of the biosynthetic strains and pathways of bacterial cellulose. J Ind Microbiol Biotechnol 49:kuab071.

14. Abouelkheir SS, Kamara MS, Atia SM, Amer SA, Youssef MI, Abdelkawy RS, Khattab SN, Sabry SA (2020) Novel research on nanocellulose production by a marine Bacillus velezensis strain SMR: a comparative study. Sci Rep 10:14202.

15. Bektas I, Yildirim NB (2025) Molecular characterization of bacterial cellulose producing Bacillus strains isolated from soil. J Basic Microbiol 65:e70026.

16. Al-Deresawi TS, Mohammed MK, Khudhair SH (2023) Isolation, screening, and identification of local bacterial isolates producing bio-cellulose. J Med Chem Sci 6:622–633.

17. Saranya RJ, Vani C, Gobikrishnan S (2022) Production and characterization of bacterial cellulose scaffold from Acetobacter sp. for tissue engineering. J Appl Biol Biotechnol 10:87–94.

18. Chen J, Zheng FHH, Zhen L, Du B (2024) Using static culture method to increase the production of Acetobacter xylinum bacterial cellulose. J Nat Fibers 21:2288286.

19. Vaishnavi M, Abirami B, Vignesh A, Manigundan K, Gopikrishnan V, Radhakrishnan M (2022) Production and characterization of glycolipid biosurfactant from Streptomyces enissocaesilis HRB1 and its evaluation for biomedical and bioremediation applications. J Surfactants Deterg 4:491–503.

20. Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425.

21. Costa AFS, Almeida FCG, Vinhas GM, Sarubbo LA (2017) Production of bacterial cellulose by Gluconacetobacter hansenii using corn steep liquor as nutrient sources. Front Microbiol 8:2027.

22. Potivara K, Phisalaphong M (2019) Development and characterization of bacterial cellulose reinforced with natural rubber. Materials 12:2323.

23. Jeong JS, Park JY (2012) Water absorption and maintenance of nanofiber cellulose production by Gluconacetobacter rhaeticus TL-2C. Afr J Biotechnol 11:9630–9632.

24. Ul-Islam M, Khan T, Park JK (2012) Water holding and release properties of bacterial cellulose obtained by in situ and ex situ modification. Carbohydr Polym 88:596–603.

25. Bharathi S, Kumaran S, Saravanan P, Suresh G, Reshma S, Sundararajan G, Lakshmipathy R (2024) Harnessing the potential of marine bacteria: Production, kinetics, and characterization of bacterial nanocellulose. Biocatal Agric Biotechnol 58:103156.

26. Gohel HR, Contractor CN, Ghosh SK, Braganza VJ (2014) A comparative study of various staining techniques for determination of extracellular cellulase activity on carboxy methyl cellulose (CMC) agar plates. Int J Curr Microbiol Appl Sci 3:261–266.

27. Muhammad H, Alburae NA, Salam MA, Badshah M, Khan T, Abo-Aba SEM (2024) Identification of cellulose producing bacterial strains: An eco-friendly and cost-effective approach. J Pure Appl Microbiol 18:483–499.

28. Montenegro-Silva P, Ellis T, Dourado F, et al. (2024) Enhanced bacterial cellulose production in Komagataeibacter sucrofermentans: Impact of different PQQ-dependent dehydrogenase knockouts and ethanol supplementation. Biotechnol Biofuels 17:35.

29. Garcia EL, Mojicevic M, Milivojevic D, Aleksic I, Vojnovic S, Stevanovic M, Murray J, Attallah OA, Devine D, Fournet MB (2022) Enhanced antimicrobial activity of biocompatible bacterial cellulose films via dual synergistic action of curcumin and triangular silver nanoplates. Int J Mol Sci 23:12198.

30. Liu X, Cao L, Wang S, et al. (2023) Isolation and characterization of bacterial cellulose produced from soybean whey and soybean hydrolyzate. Sci Rep 13:16024.

31. Yu Q, He P, Qi Y, He P, Ahmed A, Zhang X, Zhang H, Wu Y, Munir S, He Y (2024) Insight into antifungal metabolites from Bacillus stercoris 92p against banana cordana leaf spot caused by Neocordana musae. Biomolecules 14:1495.

32. Wang B, Peng H, Wu W, Yang B, Chen Y, Xu F, Peng Y, Qin Y, Fu P, Lu J (2021) Genomic insights into biocontrol potential of Bacillus stercoris LJBS06. 3 Biotech 11:458.

33. Güzel M, Akpınar O (2020) Preparation and characterization of bacterial cellulose produced from fruit and vegetable peels by Komagataeibacter hansenii GA2016. Int J Biol Macromol 162:1597–1604.

34. Abol-Fotouh D, Hassan MA, Shokry H, et al. (2020) Bacterial nanocellulose from agro-industrial wastes: Low-cost and enhanced production by Komagataeibacter saccharivorans MD1. Sci Rep 10:3491.

35. Rodrigues DM, da Silva MF, Almeida FLC, de Mélo AHF, Forte MBS, da Silva Barud CMH, Baudel HM, Goldbeck R (2024) A green approach to biomass residue valorization: Bacterial nanocellulose production from agro-industrial waste. Biocatal Agric Biotechnol 56:103036.

36. Hamed DA, Maghrawy HH, Abdel Kareem H (2023) Biosynthesis of bacterial cellulose nanofibrils in black tea media by a symbiotic culture of bacteria and yeast isolated from commercial kombucha beverage. World J Microbiol Biotechnol 39:48.

37. Machado RTA, Meneguin AB, Sábio RM, Franco DF, Antonio SG, Gutierrez J, Tercjak A, et al. (2018) Komagataeibacter rhaeticus grown in sugarcane molasses-supplemented culture medium as a strategy for enhancing bacterial cellulose production. Ind Crops Prod 122:637–646.

38. Zhong C (2020) Industrial-scale production and applications of bacterial cellulose. Front Bioeng Biotechnol 8:605374.

39. Castro C, Zuluaga R, Putaux J, Caro G, Mondragon I, Gañán P (2011) Structural characterization of bacterial cellulose produced by Gluconacetobacter swingsii sp. from Colombian agro-industrial wastes. Carbohydr Polym 84:96–102.

40. Oliveira Barud HG, Barud HDS, Cavicchioli M, Do Amaral TS, De Oliveira Junior OB, Santos DM, De Oliveira Almeida Petersen AL, Celes F, Borges VM, De Oliveira CI, et al. (2015) Preparation and characterization of a bacterial cellulose/silk fibroin sponge scaffold for tissue regeneration. Carbohydr Polym 128:41–51.

41. Zheng L, Li S, Luo J, Wang X (2020) Latest advances on bacterial cellulose-based antibacterial materials as wound dressings. Front Bioeng Biotechnol 8:593768.

42. Echeverría C, Arnaldos-Pérez I, Barranco R, et al. (2024) Synthesis and characterization of cellulose derivatives for enhanced antimicrobial activity. Cellulose 31:1729–1740.

43. Bodea IM, Cătunescu GM, Pop CR, Fiț NI, David AP, Dudescu MC, Stănilă A, Rotar AM, Beteg FI (2022) Antimicrobial properties of bacterial cellulose films enriched with bioactive herbal extracts obtained by microwave-assisted extraction. Polymers 14:1435.

44. Bigliardi PL, Alsagoff ABL, El-Kafrawi HY, Pyon JK, Wa CTC, Villa MA (2017) Povidone iodine in wound healing: A review of current concepts and practices. Int J Surg 44:260–268.

45. Huang ZR, Lin YK, Fang JY (2009) Biological and pharmacological activities of squalene and related compounds: Potential uses in cosmetic dermatology. Molecules 14:540–554.

46. Tsouko E, Kourmentza C, Ladakis D, Kopsahelis N, Mandala I, Papanikolaou S, Koutinas A (2015) Bacterial cellulose production from industrial waste and by-product streams. Int J Mol Sci 16:14832–14849.

47. Keshk SM (2014) Vitamin C enhances bacterial cellulose production in Gluconacetobacter xylinus. Carbohydr Polym 99:98–100.

48. Almihyawi, R. A., Musazade, E., Alhussany, N., Zhang, S., & Chen, H. (2024). Production and characterization of bacterial cellulose by Rhizobium sp. isolated from bean root. *Scientific Reports*, *14*(1), 10848.

49. Velmurugan, P., Myung, H., Govarthanan, M., Yi, Y. J., Seo, S. K., Cho, K. M., ... & Oh, B. T. (2015). Production and characterization of bacterial cellulose by Leifsonia sp. CBNU-EW3 isolated from the earthworm, Eisenia fetida. *Biotechnology and bioprocess engineering*, *20*(3), 410-416

50. Razmavar, S., Abdulla, M. A., Ismail, S. B., & Hassandarvish, P. (2014). Antibacterial activity of leaf extracts of Baeckea frutescens against methicillin-resistant Staphylococcus aureus. *BioMed research international*, *2014*(1), 521287.

51. Bimmer, M., Reimer, M., Klingl, A., Ludwig, C., Zollfrank, C., Liebl, W., & Ehrenreich, A. (2023). Analysis of cellulose synthesis in a high-producing acetic acid bacterium Komagataeibacter hansenii. *Applied Microbiology and Biotechnology*, *107*(9), 2947-2967.

52. Lasch, P., Beyer, W., Bosch, A., Borriss, R., Drevinek, M., Dupke, S., ... & Doellinger, J. (2025). A MALDI-ToF mass spectrometry database for identification and classification of highly pathogenic bacteria. *Scientific Data*, *12*(1), 187.

53. Song, J. E., Su, J., Noro, J., Cavaco-Paulo, A., Silva, C., & Kim, H. R. (2018). Bio-coloration of bacterial cellulose assisted by immobilized laccase. *Amb express*, *8*(1), 19.

Fig. 1 A. Bacterial strain BC1 showing pellicle formation on HS broth. **B.** Schultz's test showing the cellulose nature of the pellicle.

Fig. 2 Strain BC1 A Cultural morphology on nutrient agar. B. Strain micromorphology on under bright field magnification (40X)

Fig. 3 Phylogenetic tree of strain BC1 based on maximum Likelihood approach. Tamura Nei was used as best substitution model. Consensus tree is constructed from 1000bootstrap trees. 16S rRNA sequence of *Pontibacillus chunwhensis* was used as an outgroup.

Fig. 4 Production of Bacterial Cellulose by *Bacillus sp.* in **A**. Tea extract medium in conical flask **B** Wet BC from the bottle **C**. Dry BC from the bottle **D**. Tea extract medium in **E** Wet BC from the Tray fermentation **F**. Dry BC from the Tray fermentation

Fig. 5 Surface structure of cellulose produced by *Bacillus sp.* BC1 under scanning electron microscope

Fig. 6 FT IR spectrum of bacterial cellulose produced from *Bacillus sp.* BC1

Fig. 7 X Ray Diffraction (XRD) pattern of bacterial cellulose

Fig. 8 A. TGA and B. Tensile strength pattern of bacterial cellulose

Fig. 9 Particle size analysis of bacterial cellulose

Fig. 10 A. Water absorption and B. Water retention capacity of BC

Fig. 11 Antibacterial activity of bacterial cellulose against five different pathogens compared with control plate a. *Pseudomonas aeruginosa* sp., b. *Klebsiella pneumonia*. c. *Candida* sp. d. *Escherichia coli*, f. *Staphylococcus aureus*.

Fig. 12 Antioxidant activity of bacterial cellulose combined with bioactive compound squalane

Fig. 13 LC-MS analysis of bacterial cellulose

Fig. 14 MALDI-TOF spectrum of bacterial cellulose

Fig. 15 Optimization of bacterial cellulose

Table 1. Quantity of BC obtained in two different fermentation method and fermentation medium

	Bacterial cellulose yield	In gram	
Fermentation type	**Production Medium**	**Wet weight**	**Dry weight**
Static flask method	HS medium	12.36 ±3.541	1.97±0.546
	Tea extract medium	17.29 ±2.818	2.20±0.529
Tray method static	HS medium	87.95± 11.35	7.08±1.24
	Tea extract medium	95.67±21.62	9.45±4.947

Table 2. Antibacterial activity of bacterial cellulose isolated from *B. stercoris* BC1

Test compounds	Zone of inhibition in (milli meter in diameter)				
	S. aureus	***E. coli***	***K. pneumoniae***	***P. aeruginosa***	***Candida*. sp**
BC coated with Povidone iodine	28.0±3.16	14.0±2	14.0±1.58	12.0±2.55	25.0±2.65
BC coated with Squalene	19.0±1	12.0±1	16.0±2.65	12.0±2.55	20.0±2
Gentamicin	6.0±1	8.0±1	9.0±3.61	4.0±1.00	4±1

NT – Not Tested

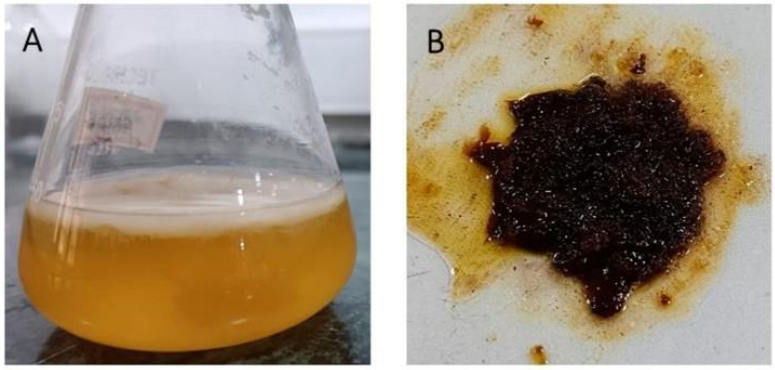


Fig 1. shows the production and conformation of BC A. cellulose B. Schultz's test

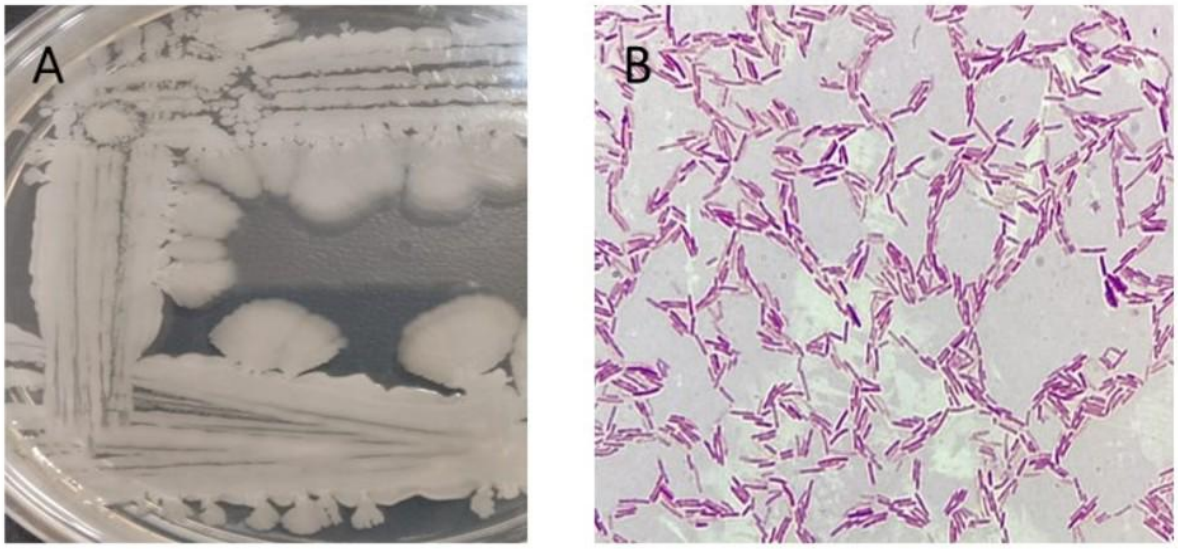


Fig 2. shows the BC1 strains A. morphology B. Grams staining

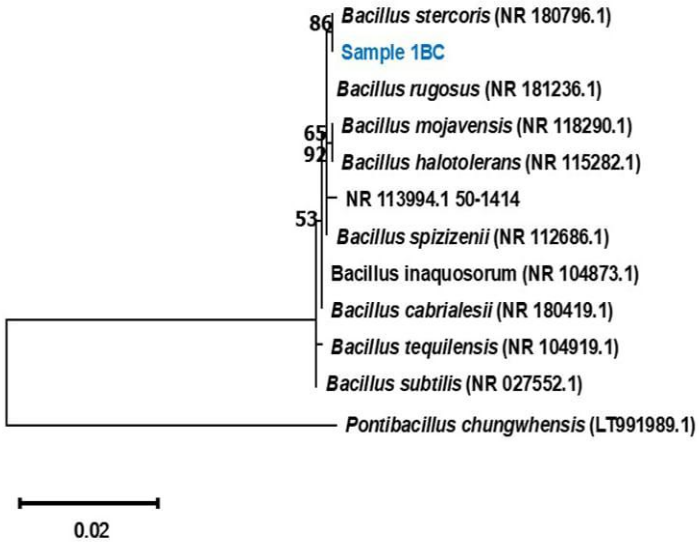


Fig 3. shows the phylogenetic tree 16S DNA sequencing of BC1 strain.

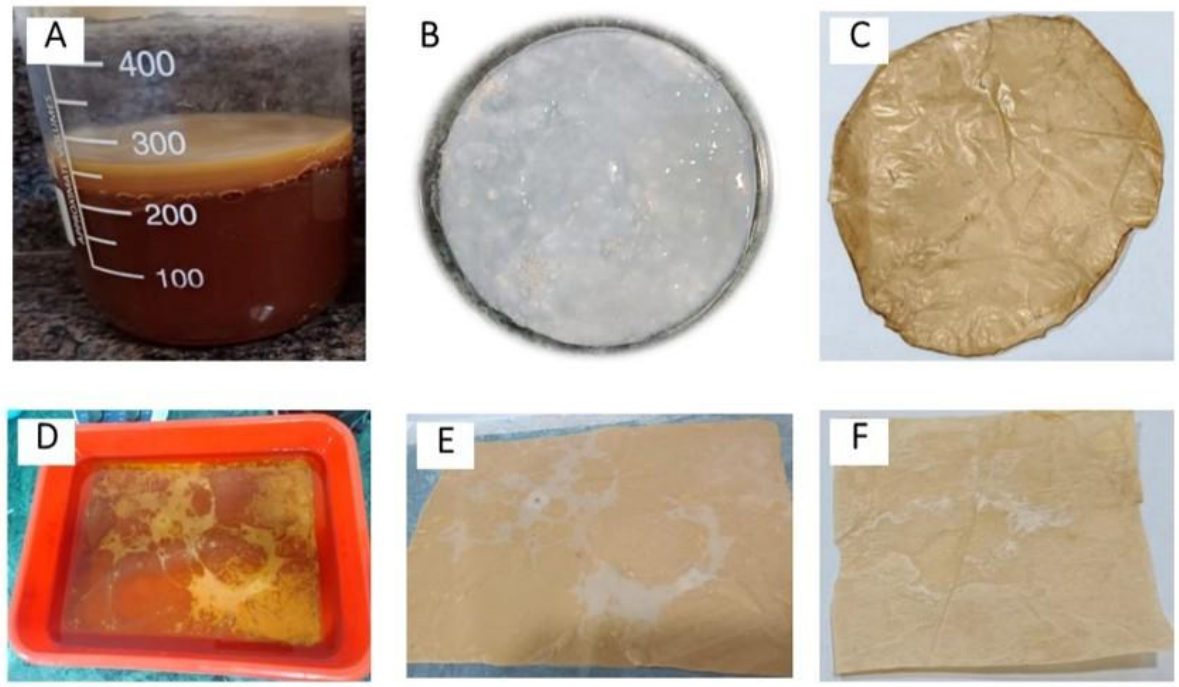
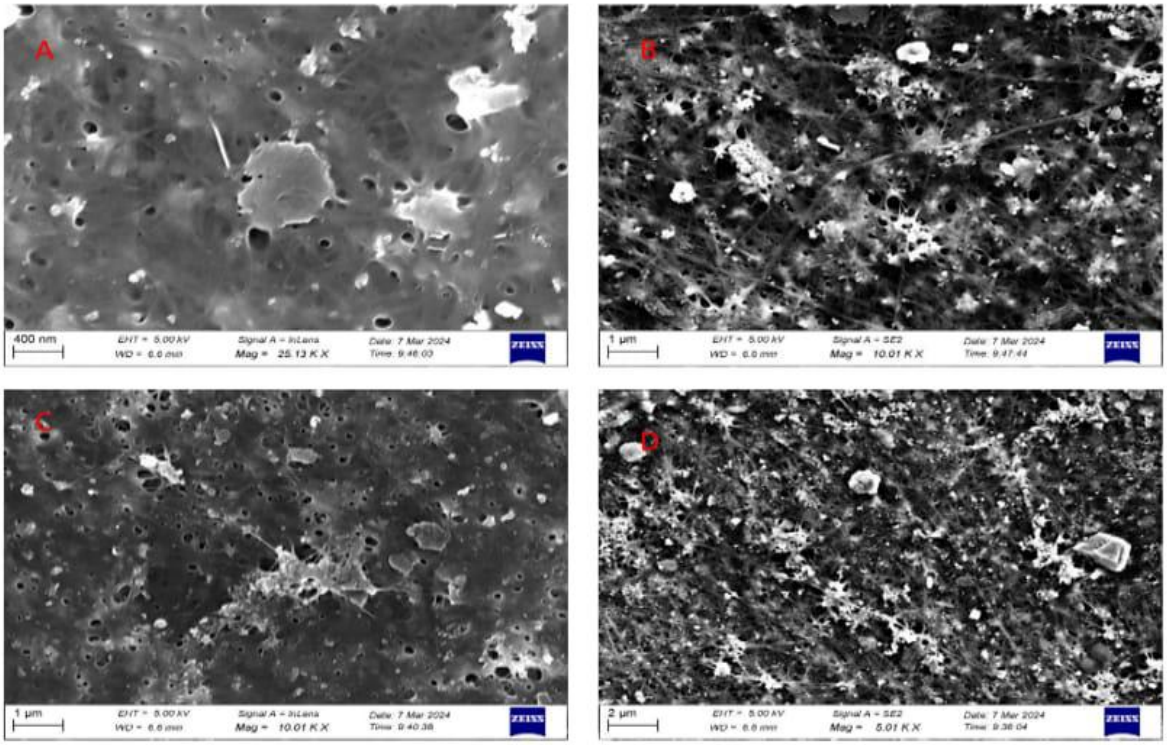


Fig 4. shows the A. production of BC in HS, B. purified BC C. dried BC D. tea extract E. wet BC F. dried BC.



Figu 5. SEM analysis shows the morphological analysis of BC in different nano meters.

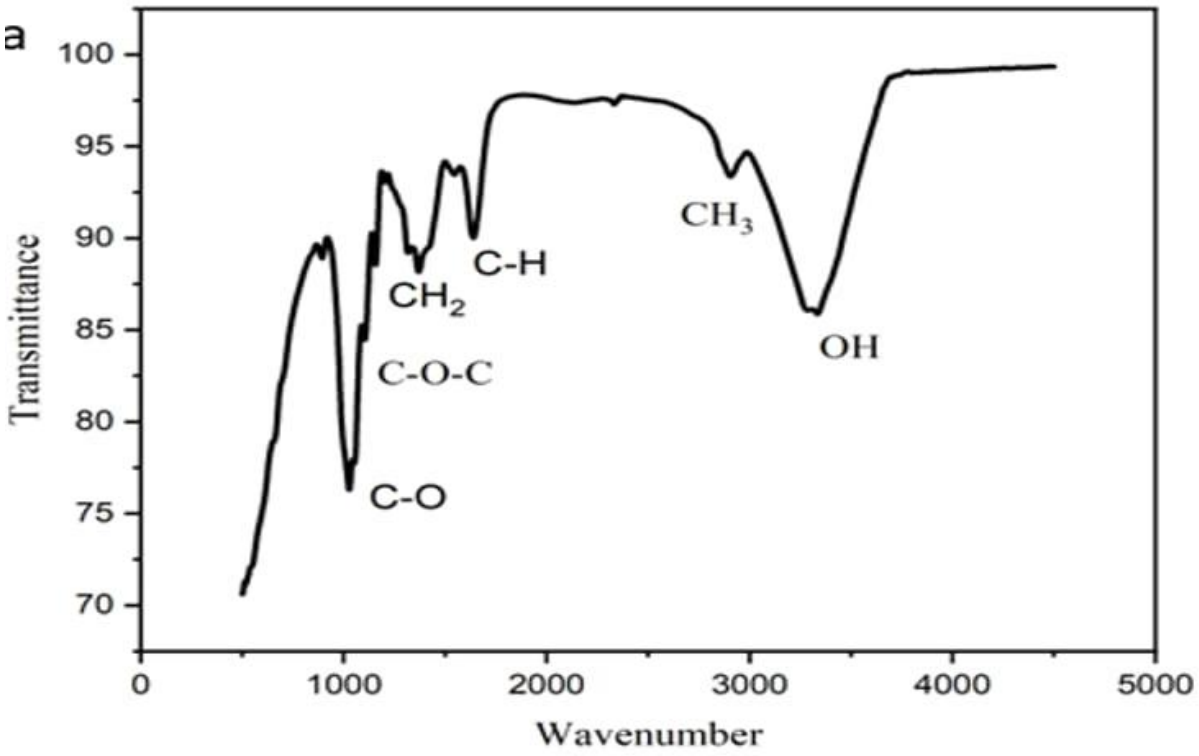


Fig 6. shows the FT-IR spectrum of BC

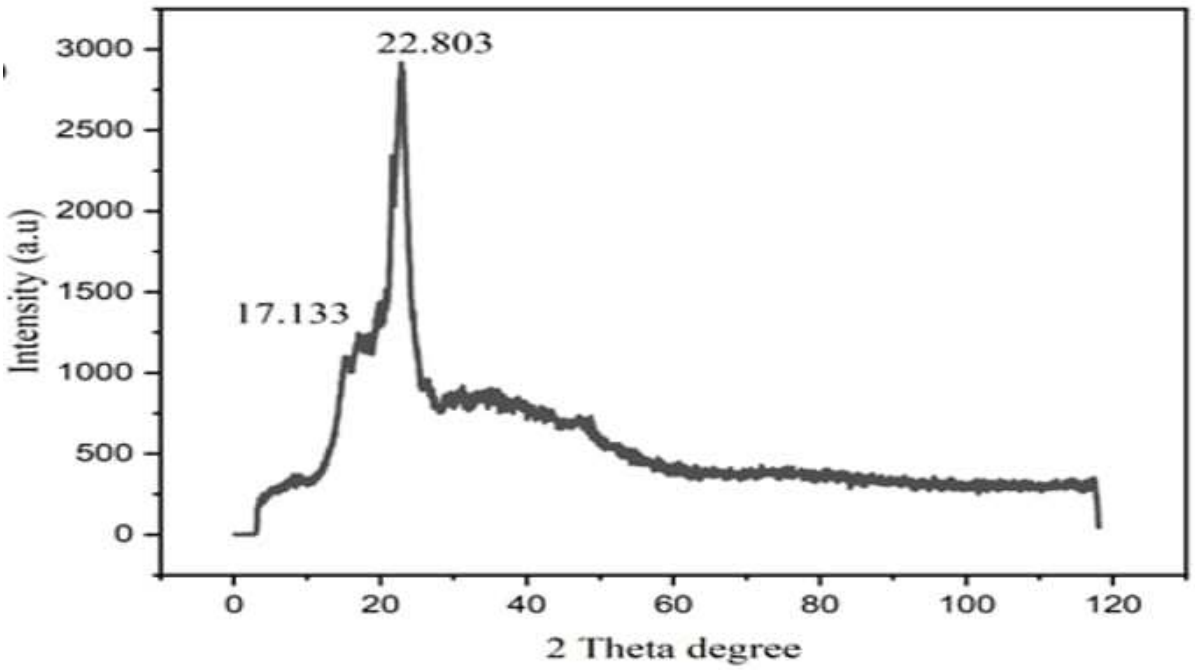


Fig 7 shows the XRD spectrum of BC

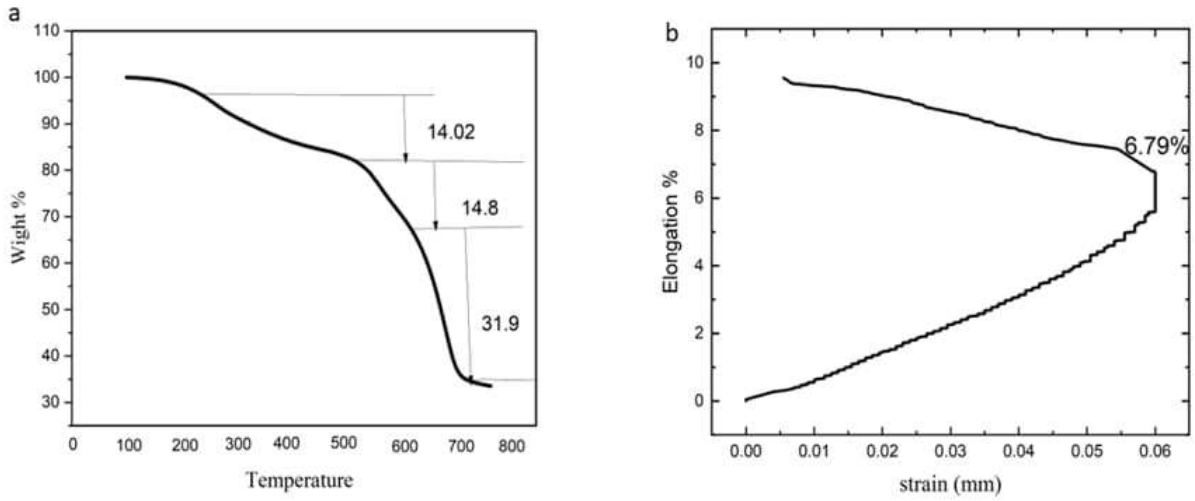


Fig 8. shows the a. thermogravimetric b. tensile analysis of BC

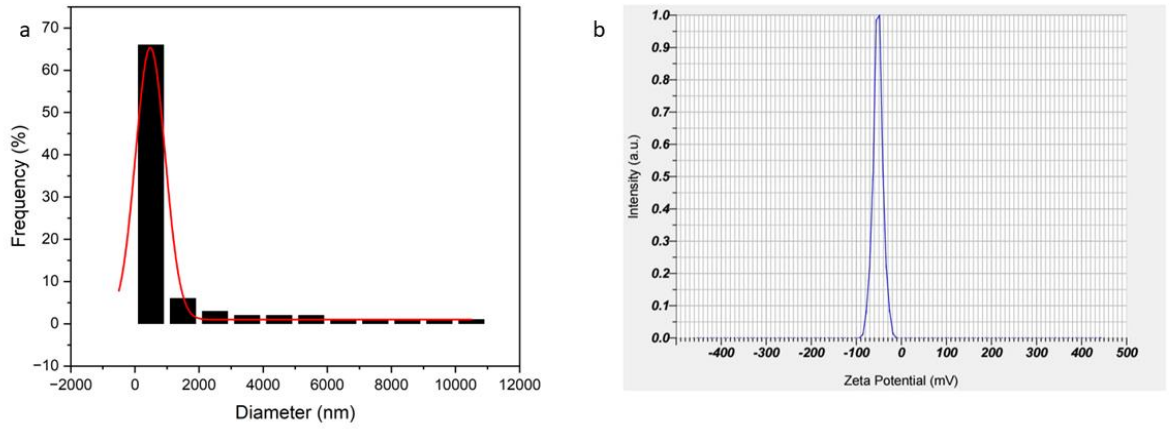


Fig 9. shows the a. particle size analysis of BC b. zeta potential analysis of BC.

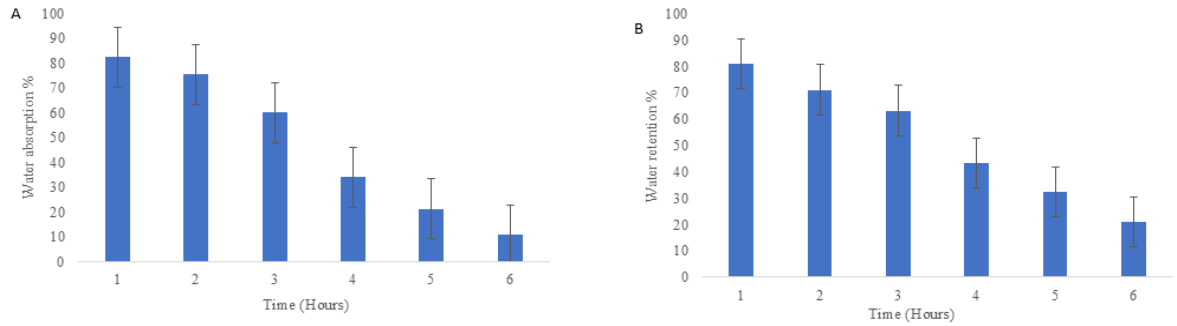


Fig 10. shows the A. water absorption B. water retention of BC

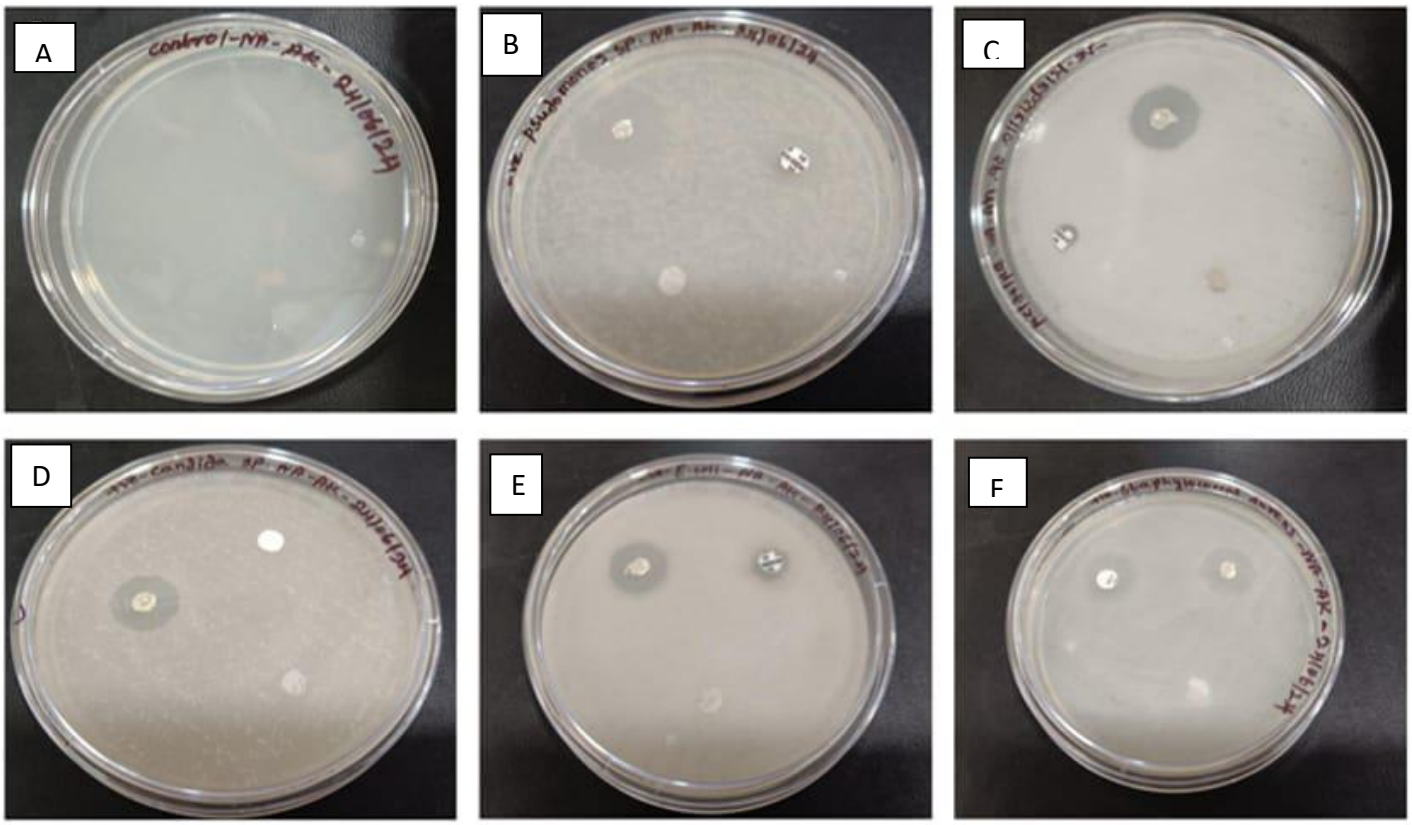


Fig11. shows the antimicrobial activity of BC with A. control B. *pseudomonas sp.* C. *klebsiella pneumoniae* D. *candida sp*. E. *Escherichia coli* F. *streptococcus aureus*

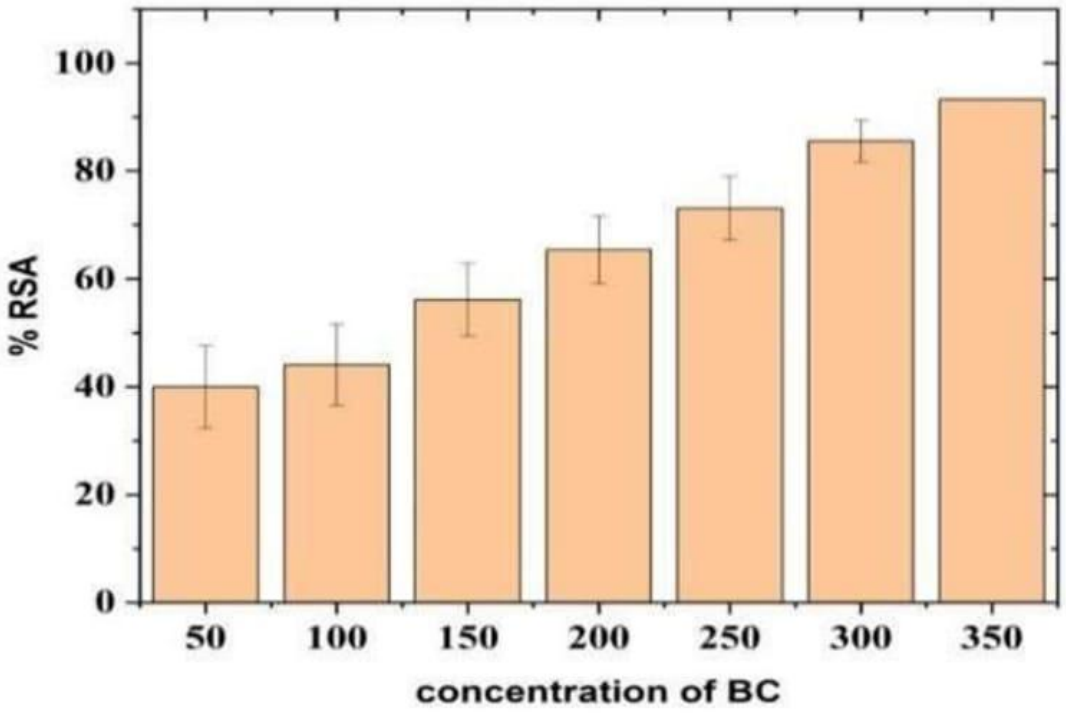


Fig 12. shows the antioxidant - DPPH activity of BC

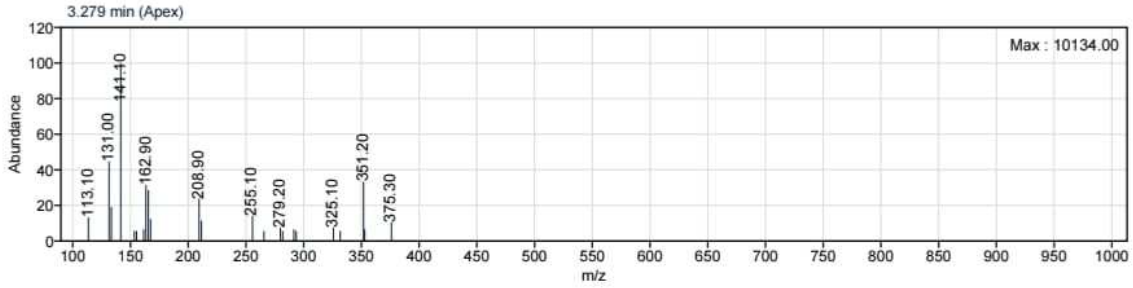


Fig 13. shows the LC-MS analysis of BC

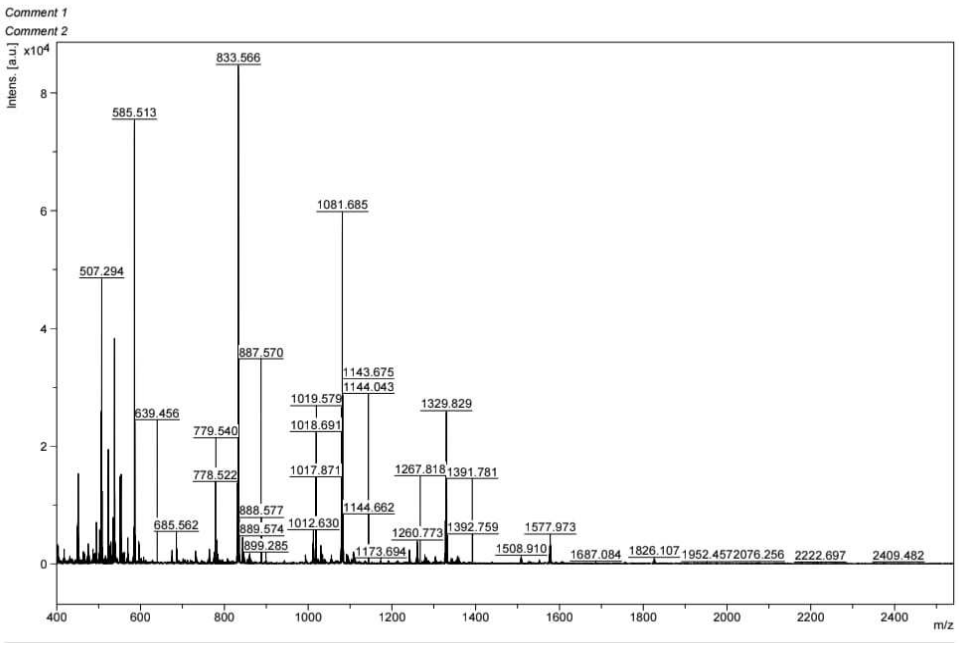


Fig 14. shows the MALDI-TOF spectrum of BC.

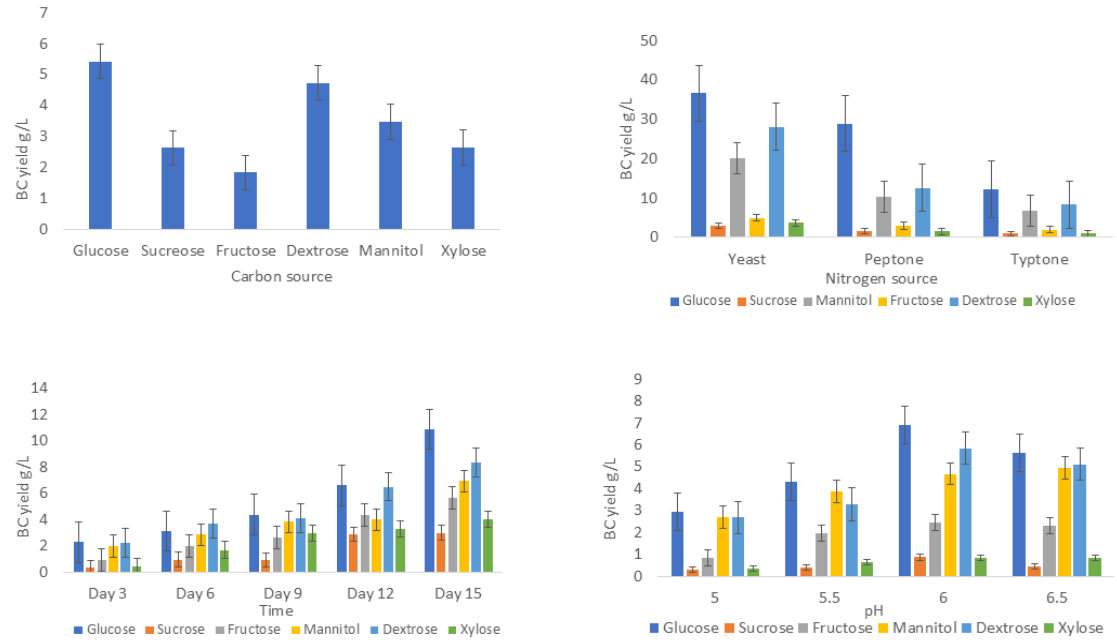


Fig 15. Effect of Carbon sources, nitrogen sources pH on the production of BC by the Bacillus sp. BC1.