Trop J Nat Prod Res, March 2023; 7(3):2580-2585 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Purification and Characterization of Bacterial Nanocellulose Produced by *Gluconobacter* 5AC Isolate from Apple Vinegar

Entissar F. Ahmed[1]*, Wala'a Sh. Ali[1], Nadhem H. Heider[2]

[1]*Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq*
[2]*Department of Biotechnology, College of Science, University of Baghdad, Baghdad, Iraq*

ARTICLE INFO

Article history:
Received 15 January 2023
Revised 01 March 2023
Accepted 03 March 2023
Published online 01 April 2023

ABSTRACT

Specific microorganisms can produce bacterial nanocellulose (BNC), with acetic acid bacteria (AAB) being the most active producer. The family Acetobacteraceae includes the obligate aerobic, motile acetic acid bacteria. The BNC has attracted a lot of interest across a wide range of industries, including pharmaceuticals, due to its flexible characteristics, properties, and advantages. The present study was conducted to purify and characterize BNC produced from AAB isolated from apple vinegar. Bacterial nanocellulose was synthesized using a natural date palm liquid medium at pH 6 at 30°C for 8–10 days. The bacterial cellulose produced was then purified using a technique involving 0.1 M sodium hydroxide. To ascertain the surface morphology, size, and form of the BNC membrane, three techniques were used for characterization: X-ray diffraction (XRD), atomic force microscopy (AFM), and transmission electron microscopy (TEM). The results of the XRD analysis confirmed that the BNC particle size ranged between approximately 17.10 and 70.33 nm, while the AFM analysis revealed that the mean diameter of these nanofibers was 26.58 nm. The TEM images clearly showed that the diameters of the BNC fibers ranged between approximately 26-66 nm. The findings of this study reveal that the characterization of the purified BNC using the XRD, AFM, and TEM analyses showed the presence of fibers with varying nanoscale diameters.

***Keywords*:** Atomic force microscopy, Acetic acid bacteria, Bacterial nanocellulose, Characterization, Transmission electron microscopy, X-ray diffraction analysis, *Gluconobacter*.

Introduction

Cellulose $(C_6H_{10}O_5)_n$ is composed of D-glucose units. It contains the C_4-OH group, which serves as the non-reducing end, while the terminating group is C_1-OH.[1] The primary component of plant cell walls is cellulose, which is typically used in manufacturing as a crude composite for paper and rayon fibers. As a result, it is important to look for substitute sources of cellulose with higher purity. Bacterial cellulose is an extracellular polysaccharide produced by several genera of acetic acid bacteria (AAB), including *Acetobacter*, *Agrobacterium*, *Gluconacetobacter*, and other bacteria like *Rhizobium*, *Achromobacter*, *Alcaligenes*, and *Aerobacter*. It can be used as a substitute for plant cellulose in a variety of industries, including the food industry, medicine, and many others.[3] The AAB belong to the family Acetobacteraceae, and its optimal pH range for growth is 5-6.5.[4]

Vinegar is a by-product of AAB metabolism. It is an aqueous solution of acetic acid and other ingredients that is used as a food seasoning and preservative. Vinegar is produced through two stages of fermentation: the first stage is anaerobic fermentation, which involves yeasts converting sugars into ethanol, and the second stage is aerobic fermentation, which involves AAB oxidizing ethanol into acetic acid.[5]

*Corresponding author. E mail: entisar.ahmed1102@sc.uobaghdad.edu.iq
Tel: +964 770 622 0546

Citation: Ahmed EF, Ali WS, Heider NH. Purification and Characterization of Bacterial Nanocellulose Produced by *Gluconobacter* 5AC Isolate from Apple Vinegar. Trop J Nat Prod Res. 2023; 7(3):2580-2585 http://www.doi.org/10.26538/tjnpr/v7i3.18

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Even in conventional vinegar that does not contain preservatives, if the AAB are not completely removed in the filtration process before bottling, it can encourage the growth of cellulose-producing aerobic bacteria. This can lead to the formation of a pellicle layer, either on the surface or the medium of the product.[6]

Currently, there is a lot of research being done and attention being paid to the isolation and production of nanocellulose fibers. This is because nanocellulose combines the physical and chemical properties of cellulose, such as hydrophilicity and its capacity to be chemically modified by a wide range of reactions, with the characteristics of nanomaterials, such as high specific surface area and aspect ratio.[7] Cellulose nanocrystals made by the acid hydrolysis of BC are non-toxic, environmentally friendly, edible, degradable, and biocompatible.[8] The positive outcome of the BNC produced by the nanocellulose pellicles or membrane could be observed as the formation of a gel-like mat that floats on the surface of the medium. The BNC produced by the bacterial isolate could be purified by 0.1 M of NaOH.[9]

The structure of BNC, such as the determination of width, length, and cross-linking of the cellulose fibers, could be characterized using several techniques. These include atomic force microscopy (AFM), which produces images of homogeneous films with distinct grain sizes, and transmission electron microscopy (TEM). Also, other important parameters are used to characterize the BNC, such as the X-ray diffraction (XRD) technique, which uses X-ray diffraction to measure the degree of polymerization (DP) and crystallinity. The XRD technique is a great non-destructive method for characterizing materials because it can be used to determine the crystal structure, orientation, and size of the grain.[10-12]

In the present study, AAB were isolated from apple vinegar and used to produce BNC, which was purified and characterized.

Materials and Methods

Sources of bacterial isolates
The AAB were isolated from apple vinegar and date palm vinegar using three culture media; GYC broth medium,[13] Hestrin-Schramm (HS) and glucose yeast (GY) broth media,[14] and glucose yeast calcium carbonate (GYC) agar medium.[15] A total of 25 samples of different types of vinegar (apple and date palm vinegar) were collected under aseptic conditions from the local market in Baghdad city. The best 5 isolates were characterized as AAB from the total samples, and the isolate 5AC was characterized as *Gluconobacter* according to the procedures of Zahoor *et al.* (2006) and Bellankimath *et al.* (2017).[13-16] The identification of the bacterial isolates was confirmed by the real-time polymerase chain reaction (RT-PCR) amplification technique.[17] After screening in HS and GY broth media and observing its ability to produce the BNC, the bacterial isolate, *Gluconobacter* 5AC was chosen as a BNC producer.

Preparation of the production medium
A date palm liquid medium (pH 6) was used as a medium for the production of bacterial nanocellulose. It was prepared by mixing 15 g of date palm (kind: Osta-Omran) with 100 ml of D.W. The date palm waste was blended with a blender. After that, it was sifted through a sieve, and then 1 g of glucose was added to each screwed-on cup of the medium.[18]

Production of bacterial nanocellulose from acetic acid bacteria
A total of one liter of the production medium (date palm liquid medium) was inoculated with overnight activated bacterial isolate 5AC culture broth (inoculum size: 2%) and incubated at 30ºC for 8-10 days. After this period, BNC was produced in the medium. The BNC wet weight and dry weight were then determined.

Purification of bacterial nanocellulose produced by acetic acid bacterial 5AC isolate
The layers or membranes of BNC that formed at the air-liquid interface of the production medium (date palm liquid solution) were removed and washed with water before being immersed in 0.1 M NaOH at 80°C for 30 to 45 minutes. This was done to eliminate any bacteria that may have become attached to the BNC membrane. The membranes were then left at a neutral pH after being repeatedly washed with distilled water several times to ensure the complete removal of the alkali. At room temperature, the purified cellulose was dried until it reached a constant mass.[3,19]

Characterization of bacterial nanocellulose
After purification and drying, the BNC membrane produced by the AAB 5AC isolate was characterized by three techniques.

a) X-ray diffraction (XRD) analysis
The X-ray diffractometer system was used to analyze the crystalline and epitaxial quality of the BNC nanostructure, which was also utilized to take micrographs. The BNC samples were taken in jars and placed in the system for analysis. The nanoparticles were scanned with a wavelength of 1.5406 Å in two angles ranging from about 10° to 70°. This system records the intensity as a function of Bragg's angle. The crystallite diameter of the bacterial membrane was calculated from the broadening of the X-ray line using Scherrer's equation formula as follows:[20-21]

$D = k\lambda / \beta Cos\theta$

where: λ is the wavelength, D is the crystallite size, and β is the full width out half maximum (FWHM) of the XRD peak.[22]

b) Atomic force microscopy analysis
The AFM analysis was used to obtain a 3D image of the sample. The atomic force microscopic system consists of a position-sensitive photo-detector, a tube scanner, and a cantilever with a sharp tip located at the free end.[23] The surface morphology of the BNC was visualized by AFM under normal atmospheric conditions. Purified dried membrane samples were studied on a tiny slide and investigated using the instrument's contact mode.

c) Transmission electron microscope measurement
Transmission electron microscopy characterization was carried out using TEM equipment. The surface morphology, size, and shape of the BNC membrane were all determined by TEM characterization.[24]

Results and Discussion

Identification of bacterial isolates
Twenty-five samples were collected from different vinegar types (apple and date palm vinegar). Of the total samples, five isolates were identified as AAB. The bacterial colony was described as being aerobic, spherical, smooth, elevated, and opaque with a semi-translucent white or off-white color. Microscopic analysis of the isolates revealed that they were all rod-shaped, gram-negative, and non-spore-producing (Figure 1). The results of the biochemical tests showed that all the isolates were negative for oxidase, positive for catalase, motile, and produced cellulose. These five isolates were confirmed as AAB by the RT-PCR amplification method. The AAB are gram-negative, rod-shaped, and can exist as a single cell, in pairs, or in chains. They are motile because they have flagella, which can either be polar or peritrichous. The bacteria do not form endospores, and they have an obligate aerobic metabolism, which uses oxygen as the terminal electron acceptor.[25-26]

Production of bacterial nanocellulose from acetic acid bacteria
The BNC was observed as a white pellicle with a diameter of about 48 mm covering the surface of the liquid medium (Figure 2) after the AAB 5AC isolate that can produce it was incubated for 8–10 days in date palm liquid medium. It was discovered that 1 L of medium gave about 55.41 g wet weight for BNC. The most generally used bacterial species for making bacterial cellulose (BC) is *Komagataeibacter xylinus* (formerly *Acetobacter xylinum*), which produces quite large amounts of BC from a wide range of carbon and nitrogen sources in broth culture media.[27] Agro-industrial wastes, such as rotten fruit, such as pineapple peels, juice, and sugar, are frequently employed as carbon sources for the production of bacterial cellulose.[28] In the large-scale production of BC, the yield of bacterial cellulose synthesis is up to 40%, depending on the starting carbon source.[7] Following washing, the BNC produced on the wastes exhibited morphological features that were similar to the control BNC. The BNC represents the assimilation of sugars within the bacterial cells and the creation of β-1,4 glucan chains to finally construct the microstructure of cellulose.[9] Bacterial celluloses may be produced industrially and are widely used in many different industries. Donini *et al.* (2010),[29] proposed that the fermentation production of bacterial cellulose can achieve a similar production efficacy to the growth of plant cellulose when the yield of bacterial cellulose reaches up to 15 g/L in 50 hours.[30]

Bacterial nanocellulose purification from acetic acid bacterial 5AC isolate
After the production of BNC in 1 L of natural medium (date palm liquid medium), the BNC layers were harvested and purified. It was observed that the wet weight of the BNC was measured after the purification, and it was found to be about 43.11 g, while the dry weight was about 2.2 g (Figure 3).

Figure 1: Acetic acid bacterial cells.
A: Under the microscope (100x); B: Colonies on GY agar medium.

The isolation and purification of BNC are relatively simple and do not need wide chemical or any other types of treatments, in contrast with wood and plant celluloses.[31] The BNC membrane was separated and purified from the treatment medium with distilled water and a weak alkaline treatment of 0.5 M NaOH, where it was possible to dry these membranes and characterize them by various techniques to analyze the membrane composition, crystallinity, and fibril morphology. The results of the purification process were satisfactory. They included a reduction in the biomaterial's water band, an increase in crystallinity, and the removal of impurities in the membrane, such as bacteria. With the purification procedure completed, it will also be possible to conduct further research on the material to use it as a product in the biomedical field and tissue engineering as a biomaterial reinforcement. The BNC purification using 1 M of NaOH solution is effective and considered eco-friendly, with no signs of recalcitrant development as commonly observed in plant cellulose purification steps.[33]

The characteristics of bacterial nanocellulose according to the X-ray diffraction analysis

The BNC membrane that was produced from the AAB 5AC isolate was characterized by different spectroscopic analytical techniques, including XRD. The X-ray diffraction technique was used for the detection of crystal, size, and shape.[34] This analysis confirmed the BNC particle size to be in the range of approximately 17.10 to 70.33 nm. In comparison to the standard reference codes (96-711-1559, 96-152-6435, 00-028-1757, and reference card 00-022-1758), the BNC membrane has three main peaks that have particle sizes (40.83, 25.60, and 41.33 nm) at a diffraction angle (2θ) of 14.19°, 16.94°, and 22.73°, respectively, as presented in Figure 4. This reflects the crystalline plane of 100, 010, and 110, respectively. The results of the XRD analysis agree with Andritsou *et al*. (2018),[35] and Atykyan *et al*. (2020),[36] which show the XRD diffraction of BC, microcrystalline cellulose (MCC), and the orange peel residues after conventional pectin extraction (CAE-CB). The peaks of X-ray diffraction at 2θ = 14.5, 16.6, and 22.6° match the cellulose structure. These peaks are attributed to the planes of 1 0 0, 0 1 0, and 1 1 0 of cellulose I_α or the planes of 1 1 0, 1 1 0, and 2 0 0 of cellulose I_β. Also, the X-ray diffraction test was directed to investigate the crystalline structure of BC, the diffractogram of BNC membrane displayed peaks of 100, 010, and 110 planes at the angles (2θ) 14.25°, 16.42°, and 22.45°, respectively.[37] Although BC is not a fully crystalline substance, large diffraction peaks were observed. The XRD patterns of BC revealed two distinctive peaks at 14.74° and 22.64° and the crystalline planes 110 and 002, respectively.[38]

Bacterial nanocellulose properties as determined by atomic force microscopy

Surface roughness is an essential component for verifying accuracy in many biological and electrical implementations.[39] The surface area morphology of the BNC membranes formed on the date palm liquid media was described using AFM equipment. The tapping mode was used to image the area of the BNC sample. The results indicated the presence of various nanoscale diameters; the average of these nanofibers' diameters was 26.58 nm, while the minimum and highest values were 24.42 and 70.61 nm, respectively (Figures 5 and 6). The bacterium, *G. xylinus* was used to produce BNC. It was activated in cellulose medium at 30°C for 24 hours, and then *G. xylinu* suspension was inoculated at 5% (v/v) to the cellulose production medium, which was supplemented with chitosan. After 10 days, BC pellicles were produced. The morphology of the BNC was observed by the AFM image, and the mean diameter of the BNC fibers was approximately 50 nm.[38]

Bacterial nanocellulose is a network with a high water-holding capacity that *Komagataeibacter xylinus* produces as an extracellular biopolymer at the liquid-air interface. Atomic force microscopy images of the BNC show diameter measurements of the nanofibers (2-4 nm) and microfibril bundles (15-20 nm). The average height of AFM images was found to be about 28.6 nm for this BNC from *K. xylinus*.[40] The AAB, *Gluconacetobacter hansenii* produced BC, which was examined using an AFM apparatus. The AFM images revealed that BC has a reticulate structure, and the diameters of the bundle, microfibril, and ribbon were approximately 37, 17, and 62 nm, respectively. These ribbons and bundles accumulated together to form a porous, permeable structure with a great aspect ratio. The nanoscale diameters of these fibers confirmed that the bacterial cellulose was nanocellulose when compared to cotton fibers, and the morphology of the microstructure was the basis of the competent characterization of the bacterial cellulose.[41]

Transmission electron microscopy characteristics of bacterial nanocellulose

Transmission electron microscopy was used to determine the BNC's diameter and the size and morphology of the fibers.[42] The TEM images demonstrated that the BNC fibers' sizes ranged between 26 and 66 nm (Figure 7), and these results are in agreement with the ones obtained for the AFM analysis. *Gluconacetobacter xylinus* bacteria were used to produce the BNC from banana peel waste media, and the results were examined using a TEM to identify any variations in the BNC's size and shape. The sample was collected on day 10 when the nano-fibers appeared to be more piled up and concentrated. These nano-fibers have a diameter of 30-50 nm, and it was observed that the BNC from banana peel has a 3D nano-fibrous structure with a large surface area.

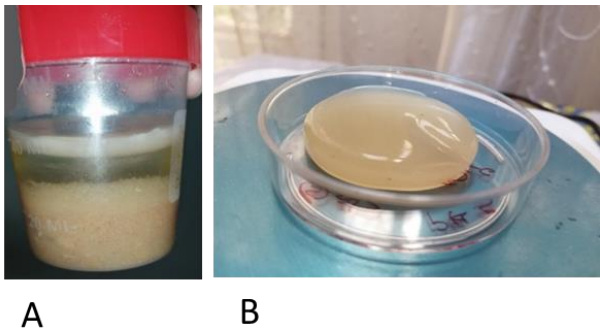


Figure 2: The bacterial nanocellulose membrane.
A: Bacterial nanocellulose at the surface of date palm liquid medium; B: Bacterial nanocellulose (wet membrane).

Figure 3: Bacterial nanocellulose membrane (dry membrane) of acetic acid bacterial 5AC isolate.

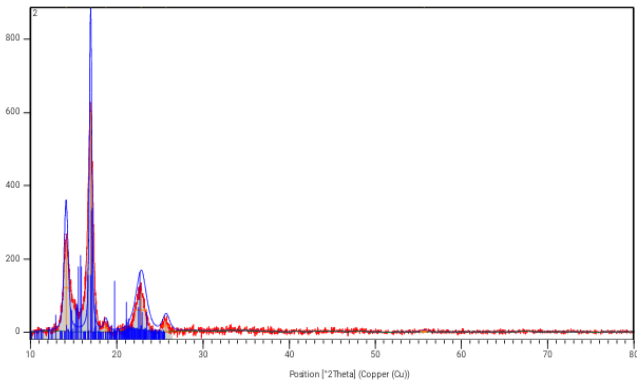


Figure 4: X-ray diffraction pattern of bacterial nanocellulose produced by acetic acid bacterial 5AC isolate.

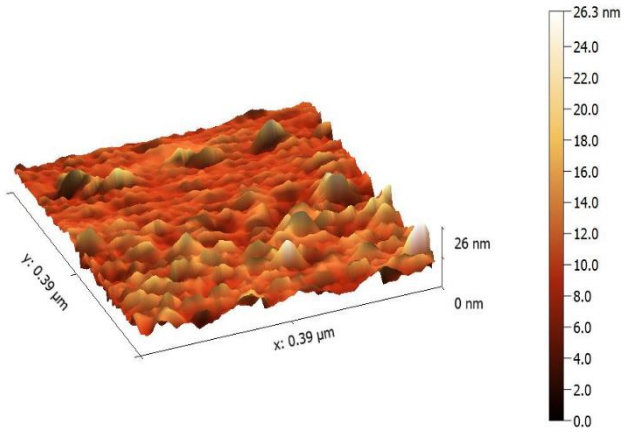
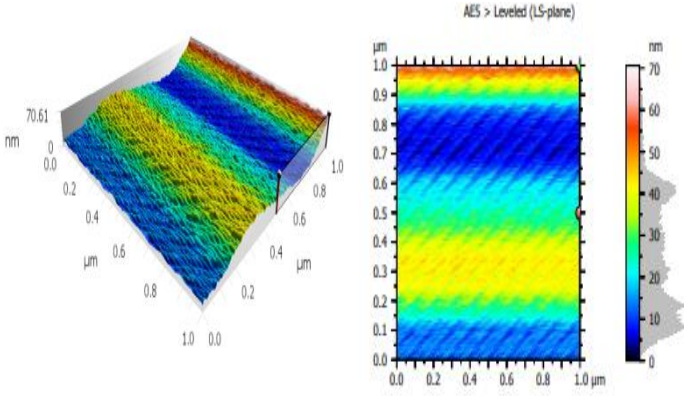


Figure 5: Bacterial nanocellulose picture under atomic force microscopy apparatus.



Statistical summary					
Parameters	Unit	Mean	Std dev	Min	Max
Projected area	µm²	0.007285	0.04539	4.325e-06	0.3499
Z-maximum	nm	26.58	6.373	24.42	70.61

Figure 6: Atomic force microscopy analysis showing the diameters of bacterial nanocellulose nanomaterials.

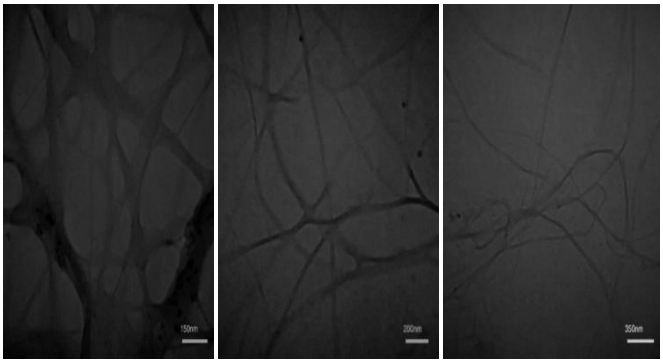


Figure 7: A transmission electron microscopy image of bacterial nanocellulose.

This property is significant in many applications, such as water filter membrane applications, because BNC exhibits many exceptional physical and mechanical properties, such as high porosity, great water holding capacity, good mechanical strength, high purity, and high crystallinity, which reach up to 90%.[24]

These results are in agreement with the findings of Feng *et al*. (2015),[41] who reported that the BNC fiber with a diameter of approximately 30 nm, was certainly categorized as nanoscale cellulose. Bacterial polysaccharides, such as BC, are regarded as promising resources having the potential to be used in a variety of bio-fields as well as other disciplines. By using TEM, the morphology of BNC was found to be around 10–30 nm.[43] The structure of *Acetobacter xylinum* cellulose may be described as an ultrafine net composed of intertwined cellulose ribbons. A TEM micrograph of an *Acetobacter xylinum* revealed the ultrastructure of such thin units of around 3.2 and 33 nm, which is a unique attribute of reticulate BC.[44] Due to importance of the bacterial isolates, it was confirmed by (RT-PCR) amplification technique.[17] the molecular technique applied in different areas related to biology.[45-63]

Conclusion

The findings of this study reveal that BNC synthesized by AAB can be successfully produced under cultural conditions utilizing a date palm liquid production medium. The presence of fibers with various nanoscale diameters was discovered during the characterization of the purified BNC using XRD, AFM, and TEM analyses. As a result of these findings, BNC has gained interest from a variety of fields due to its high capacity for liquid absorption, high tensile strength, and numerous other significant characteristics.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

References

1. Zuppolini S, Salama A, Cruz-Maya I, Guarino V, Borriello A. Cellulose Amphiphilic Materials: Chemistry, Process, and Applications. Pharmaceutics. 2022; 14(2):386-404.
2. Kamona ZK, Alshaikh Daher AA, Al Shamary EI. The use of Vitally Active Cellulose Membranes for the Reduction of Pathogenic Bacterial Count in White Cheese. Ir J Sci. 2021; 62(4):1121-1127.
3. Rangaswamy BE, Vanitha KP, Hungund BS. Microbial cellulose production from bacteria isolated from rotten fruit. Int J Polym Sci. 2015; 5, ID 280784:1-8.
4. Trček J, Barja F. Updates on quick identification of acetic acid bacteria with a focus on the 16S–23S rRNA gene internal transcribed spacer and the analysis of cell proteins by MALDI-TOF mass spectrometry. Int J Food Microbiol. 2015; 196:137–144.
5. Du XJ, Jia SR, Yang Y, Wang S. Genome sequence of Gluconacetobacter sp. strain SXCC-1, isolated from Chinese vinegar fermentation starter. J Bacteriol. 2011; 193(13):3395-3396.
6. Sengun IY. Acetic acid bacteria: Fundamentals and food applications. editor. 2017; Boca Raton, USA: CRC Press.
7. Klemm D, Kramer F, Moritz S, Lindstro¨m T, Ankerfors M, Gray D, Dorris A. Nanocelluloses: a new family of nature-based materials. Angew Chem Int Edit. 2011; 50:5438–5466.
8. Habibi Y, Lucia LA, Rojas OJ. Cellulose nanocrystals: Chemistry, self-assembly, and applications. Chem. Rev. 2010; 110:3479–3500.
9. Abol-Fotouh D, Hassan MA, Shokry H, Roig A, Azab MS, Kashyout AB. Bacterial nanocellulose from agro-industrial wastes: low-cost and enhanced production by Komagataeibacter saccharivorans MD1. Sci. Rep. Natu. Res. 2020; 10 (1):3491-3503.
10. Hasan BA, Ibrahim IM, Shaban SM. Effect the thickness on structural and optical paramteres of PbSe thin films. Ind J App Res. 2011; 3 (3):327-329.

11. Kondo E, Rytczak P, Bielecki S. Chapter 4 - Bacterial NanoCellulose Characterization, Editor(s): Gama, M.; Dourado, F. and Bielecki, S. Bacterial Nanocellulose, From Biotechnol. to Bio-Econ. Elsevier. 2016; P: 59-71.
12. Ail IM, Al-Jenabi MA. Structural and optical properties of In2O3 and Indium Tin Oxide thin films. JUAPS. 2017; 11(1): 9-46.
13. Bellankimath A, Katti A, Hemalata VB, Meti BS. Isolation and characterization of the indigenous acetic acid bacteria from Western Ghats soil samples. Int. J. Curr. Microbiol. App. Sci. 2017; 6(9):1255-1265.
14. Trinh NTN, Masniyom P, Maneesri J. Optimization of culture condition for Acetobacter aceti TISTR 102 in coconut water with supplementary banana juice. Int. Food. Res. J. 2016; 23(3):1300-1307.
15. Klawpiyapamornkun T, Bovonsombut S, Bovonsombut S. Isolation and Characterization of Acetic acid bacteria from Fruits and Fermented fruit juices for Vinegar Production. Food Appl. Biosci. J. 2015; 3 (1): 30–38.
16. Zahoor T, Siddique F, Farooq U. Isolation and characterization of vinegar culture (Acetobacter aceti) from indigenous sources. Br. Food J. 2006; 108(6): 429-439.
17. Longin C, Guilloux-Benatier M, Alexandre H. Design and Performance Testing of a DNA Extraction Assay for Sensitive and Reliable Quantification of Acetic Acid Bacteria Directly in Red Wine Using Real Time PCR. Front. Microbiol. 2016; 7:831.
18. Azeredo HMC, Barud H, Farinas CS, Vasconcellos VM, Claro AM. Bacterial Cellulose as a Raw Material for Food and Food Packaging Applications. Front. Sustain. Food Syst. 2019; 3:7.
19. Costa AFS, Almeida FCG, Vinhas GM, Sarubbo LA. Production of bacterial cellulose by Gluconacetobacter hansenii using corn steep liquor as nutrient sources. Front. microbiol. 2017; 8:2027.
20. Yaaqoob LA. Effect of Using Zinc Oxide and Gold Nanoparticles on Experimentally Induced Diabetic Rats. Ph.D. Thesis. College of Science. University of Baghdad. 2016.
21. Ghafil JA. Synthesis of polyhydroxybutyrate nanopolymer cefotaxime conjugated for treatment of Pseudomonas aeruginosa isolated from burn infections. Ph.D. Thesis. College of Science. University of Baghdad. 2021.
22. Abid NK, Hasan SM. Structural properties of prepared PANI/TiO2 nanocomposite by chemical polymerization. Iraq. J. of Phys. 2022; 20(3):29-39.
23. Ali IM. Investigation on the Effect of Manganese and Cerium on ZnS Nanoparticles Prepared by Microwave Irradiation. Ph.D. Thesis. College of Science. University of Baghdad. 2015.
24. Sijabat EK, Nuruddin A, Aditiawati P, Purwasasmita BS. Optimization on the synthesis of bacterial nanocellulose (BNC) from banana peel waste for water filter membrane applications. Mater. Res. Express. 2020; 7: 055010.
25. Holt JM, Krieg NR, Sneath PHA, Staley JY, Williams ST. Genus Acetobacter and Gluconobaceter. In Bergery's Manual of Determinative Bacteriology.9 th edn., Williams & Ilkens, Maryland, U.S.A. 1994; pp: 71-84.
26. Thongwai N, Futui W, Ladpala N, Sirichai B, Weechan A, Kanklai J, Rungsirivanich P. Characterization of Bacterial Cellulose Produced by Komagataeibacter maltaceti P285 Isolated from Contaminated Honey Wine. Microorg, MDPI. 2022; 10:528.
27. Zhong C, Zhang GC, Liu M, Zheng XT, Han PP, Jia SR. Metabolic flux analysis of Gluconacetobacter xylinus for bacterial cellulose production. Appl Microbiol Biotechnol. 2013; 97:6189–6199.
28. Jozala A, Pe´rtile R, dos Santos C, de Carvalho Santos-Ebinuma V, Seckler M, Gama F, Pessoa AJ. Bacterial cellulose production by Gluconacetobacter xylinus by employing alternative culture media. Appl Microbiol Biot. 2015; 99:1181–1190.
29. Donini Í A N, Salvi DTBD, Fukumoto FK, Lustri WR, Barud HS, Marchetto R, Messaddeq Y, Ribeiro SJL. Biosynthesis and recent advances in production of bacterial cellulose. Eclética Química. 2010; 35: 165–178.
30. Zhong C. Industrial-Scale Production and Applications of Bacterial Cellulose. Front. Bioeng. Biotechnol. 2020; 8:605374.
31. Seddiqi H, Oliaei E, Honarkar H, Jin J, Geonzon CL, Bacabac RG, Klein-Nulend J. Cellulose and its derivatives: towards biomedical applications. Cellu. 2021; 28:1893–1931.
32. Sousa LP, dos S B de, Leite PMSCM, Vieira AA, Faria AC, Vieira L. Effect of water and alkali on purification bacterial cellulose membrane from Kombucha. Res., Soc. Dev. 2021; 10(15): e526101523267.
33. Abba M, Abdullahi M, Md Nor MH, Chong CS, Ibrahim Z. Isolation and characterization of locally isolated Gluconacetobacter xylinus BCZM sp. with nanocellulose producing potentials. IET Nanobiotechnol. 2017; 12(1): 52-56.
34. Khan MR, Ahmad K, Akram R, Asif H, Ahmad B, Ali T, Anjum I, Sami A, Bibi A, Saifullah S. Green Synthesis, Characterization and Antibacterial Potential of Silver Nanoparticles from Onosma bracteatum Extract. Trop. J. Nat. Prod. Res. 2022; 6(2): 202–206.
35. Andritsou V, de Melo EM, Tsouko E, Ladakis D, Maragkoudaki S, Koutinas AA, Matharu AS. Synthesis and Characterization of Bacterial Cellulose from Citrus-Based Sustainable Resources. ACS Omega. 2018; 3(8):10365-10373.
36. Atykyan N, Revin V, Shutova V. Raman and FT-IR Spectroscopy investigation the cellulose structural differences from bacteria Gluconacetobacter sucrofermentans during the different regimes of cultivation on a molasses media. AMB Expr. 2020; 10: 84.
37. Sardjono SA, Suryanto H, Aminnudin M, Muhajir M. Crystallinity and morphology of the bacterial nanocellulose membrane extracted from pineapple peel waste using high pressure homogenize. International Conference on Biology and Applied Science (ICOBAS). AIP Conf. Proc. 2019; 2120, 080015-1–080015-5.
38. Jia Y, Wang X, Huo M, Zhai X, Li F, Zhong C. Preparation and characterization of a novel bacterial cellulose/chitosan bio-hydrogel. Nanomater. Nanotechnol. 2017; 7: 1–8.
39. Choi MC, Kim Y, Ha CS. Polymers for flexible displays: From material selection to device applications. Prog. Polym. Sci. 2008; 33:581–630.
40. Murugarren N, Roig-Sanchez S, Anton-Sales I, Malandain N, Xu K, Solano E, Reparaz J, Laromaine A. Highly aligned bacterial nanocellulose films obtained During Static Biosynthesis in a Reproducible and Straightforward Approach. Adv Sci. 2022 ;9(26):2201947.
41. Feng X, Ullah N, Wang X, Sun X, Li C, Bai Y, Chen L, Li Z. Characterization of Bacterial Cellulose by Gluconacetobacter hansenii CGMCC 3917. J. Food Sci. 2015; 80(10): E2217-E2227.
42. Farid MM, Emam M, Mohammed RS, Hussein SR, Marzouk MM. Green Silver Nanoparticles Based on the Chemical Constituents of Glinus lotoides L.: In Vitro Anticancer and Antiviral Evaluation. Trop. J. Nat. Prod. Res. 2020; 4(10):714–721.
43. Choi SM, Shin E.J. The nanofication and functionalization of bacterial cellulose and its applications. Nanomater. 2020; 10:406.
44. Krystynowicz A, Czaja W, Wiktorowska-Jezierska A, Gonc¸alves-Mis´kiewicz M, Turkiewicz M, Bieleck S. Factors affecting the yield and properties of bacterial cellulose. J. Ind. Microbiol. Biotechnol. 2002; 29:189-195.

45. Hashim ST, Fakhry SS, Rasoul LM, Saleh TH, Alrubaii BAL. Genotyping toxins of Clostridium perfringens strains of rabbit and other animal origins. Trop J Nat Prod Res. 2021; 5(4):613–616.
46. Al-Musawi, AHO, Aziz, HM, Khudair, S, Saleh, TH. Molecular characterization of HBB gene mutations in beta-thalassemia patients of Southern Iraq. Biomedicine. 2022; 42(5):1040-1043.
47. Abdulrazaq, RA., Mahmood, WS., Alwan, B, Saleh, TH, Hashim, ST, Al-Rubaii, BAL. Biological study of protease produced by clinical isolates of Staphylococcus aureus. Res J Pharm Technol.2022; 15(12):5415-5420.
48. Rasoul LM, Allami RH, Alshibib AL, laftaah Al-Rubaii BA, Sale TH. Expression and cytotoxic effect of recombinant Newcastle Disease Virus (rNDV) vector expressing enhanced green fluorescent gene in JHH5 cell line. Biomedicine. 2023; 43(1):205-209.
49. Kadhim AL-Imam MJ, AL-Rubaii BAL. The influence of some amino acids, vitamins and anti-inflammatory drugs on activity of chondroitinase produced by Proteus vulgaris caused urinary tract infection. Iraqi J Sci. 2016; 57(4A):2412-2421.
50. Jawad NK, Numan AT, Ahmed AG, Saleh TH, Al-Rubaii BA. IL-38 gene expression: A new player in Graves' ophthalmopathy patients in Iraq. Biomedicine. 2023;43(1):210-215.
51. Shehab ZH, AL-Rubaii BAL. Effect of D-mannose on gene expression of neuraminidase produced from different clinical isolates of Pseudomonas aeruginosa. Baghdad Sci J. 2019;16(2):291–298.
52. Abdulla L, Ismael MK, Salih TA, Malik SN, Al-Rubaii BAL. Genotyping and evaluation of interleukin-10 and soluble HLA-G in abortion due to toxoplasmosis and HSV- 2 infections. Ann Parasitol. 2022; 68(2):385–390.
53. Jiad AL, Ismael MK, Muhsin SS, Al-Rubaii BAL. ND2 Gene Sequencing of Sub fertile Patients Recovered from COVID-19 in Association with Toxoplasmosis. Bionatura. 2022; 7(3):45. http://dx.doi.org/10.21931/RB/2022.07.03.45.
54. Rasoul LM, Nsaif MM, Al-Tameemi MT, Al-Rubaii BA. Estimation of primer efficiency in multiplex PCR for detecting SARS-Cov-2 variants. Bionatura. 2022; 7(3):48. http://dx.doi.org/10.21931/RB/2022.07.03.49.
55. Al-Rubii, BAL. Cloning LasB gene of *pseudomonas aeruginosa* eiastase 10104-2aI in *E. coli* BL21 and *E.coli* DH5α and investigated their effect on the stripping of vero cells. Pakistan J Biotechnol.2017; 14(4):697-705.
56. Saleh, T.; Hashim, S.; Malik, S.N.; Al-Rubaii, B.A.L. The impact some of nutrients on swarming phenomenon and detection the responsible gene RsbA in clinical isolates of Proteus mirabilis. Int J. Pharm Sci Res. 2020; 1(6):437-444
57. Bresam S, Al-Jumaily RM, Karim GF, Al-Rubaii BA. Polymorphism in SNP rs972283 of the KLF14 gene and genetic disposition to peptic ulcer. Biomedicine. 2023; 43(1):216-220.
58. Shehab ZH, Laftah BA. Correlation of nan1 (Neuraminidase) and production of some type III secretion system in clinical isolates of Pseudomonas aeruginosa. Biomed res. 2018; 15(3):1729-1738.
59. Rasoul, LM., Marhoon AA, Albaayit SFA, Ali RW, Saleh,TH, Al-Rubaii BAL. Cytotoxic effect of cloned EGFP gene on NCI-H727 cell line via genetically engineered gene transfer system. Biomedicine (India). 2022; 42(5):938-942.
60. Ali SM, Laftah BA, Al-Shammary AM, Salih HS. Study the role of bacterial neuraminidase against adenocarcinoma cells in vivo. InAIP Conference Proceedings 2021; 2372, 030009.
61. Hamoode RH, Alkubaisy SA, Sattar DA, Hamzah SS, Saleh TH, Al-Rubaii BAL. Detection of anti-testicular antibodies among infertile males using indirect immunofluorescent technique. Biomedicine (India).2022; 42(5):978-982.
62. Salih HS, Al-Shammari AM, Laftaah BA. Intratumoral co-administration of oncolytic newcastle disease virus and bacterial hyaluronidase enhances virus potency in tumor models. J Glob Pharma Technol. 2018; 10(10):303-310.
63. Abbas MS, Ahmed AG, Ali SQ, AL-Rubaii BA. Immunological inflammatory factors in patients diagnosed with COVID-19. Biomedicine. 2023; 43(1):230-235.