Journal of Advances in Biology & Biotechnology

14(3): 1-9, 2017; Article no.JABB.34171
ISSN: 2394-1081

Effect of Different Fruit Juice Media on Bacterial Cellulose Production by *Acinetobacter* sp. BAN1 and *Acetobacter pasteurianus* PW1

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Authors' contributions

This work was carried out in collaboration between all authors. Author BCAT designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors MOA and JFS managed the analyses of the study, managed the literature searches and performed the statistical analysis. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JABB/2017/34171
Editor(s):
(1) Csilla Tothova, Clinic for Ruminants, University of Veterinary Medicine and Pharmacy in Kosice, Slovak Republic.
(2) Andrzej Kowalski, Department of Biochemistry and Genetics, Institute of Biology, Jan Kochanowski University, Kielce, Poland.
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(3) V. Vasanthabharathi, Annamalai University, India.
Complete Peer review History: http://www.sciencedomain.org/review-history/20415

Original Research Article

Received 17th May 2017
Accepted 16th July 2017
Published 8th August 2017

ABSTRACT

Aim: To produce Bacteria Cellulose (BC) using Fruit juice with high sugar contents as the substrate for production.
Study Design: The effect of different fruit juice on BC production by *Acinetobacter* sp. BAN1 and *Acetobacter pasteurianus* PW1 and Characterization of the BC was investigated. This research was carried out in the Department of Microbiology University of Ibadan, Nigeria.
Methodology: Two bacteria *Acinetobacter* sp. BAN1 and *Acetobacter pasteurianus* PW1 were obtained from stock culture. Pineapple, Pawpaw and Watermelon fruit juice media were prepared and used for the production of BC by isolation. The BC produced was characterized.
Results: Two BC producers were selected for BC production using different fruit juice media, Pineapple juice medium (PIJM), Pawpaw juice medium (PAJM) and Watermelon juice medium

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(WMJM). The dry weight of the BC produced by *Acinetobacter* sp. BAN1 and *Acetobacter pasteurianus* PW1 ranged from 0.3 – 6.4 g/l and 0.1 – 7.7 g/l. PAJM supported the highest BC production. FTIR spectrum of the produced BC indicates the presence ofbonds that connect the glucose monomers into a polymer also the presence of carbonyl groups and hydroxyl groups. **Conclusion:** The study has shown that the setected cellulose producing strains can utilize the nutrients and sugars in the fruits for production of BC.

Keywords: Biocellulose; Acinetobacter sp. BAN1; Acetobacter pasteurianus PW1; fruit juice; FTIR.

1. INTRODUCTION

Cellulose is the most abundant macromolecule on earth [1]. Cellulose is a major component of plant cell wall. It can be synthesized by plants, algae and microorganisms [2]. Plants derived cellulose usually contains impurities in form of lignin, hemicellulose and pectin [3,4].

Biocellulose is an extracellular excretion that forms aggregated fibrils, which crystallize into ribbons and assemble into a thick cellulose mat known as pellicle [5]. Biocellulose has unique properties that makes it better than plant cellulose. BC has higher purity, crystallinity, tensile strenght, elasticity and absorbance than plant cellulose [6,2]. Gram negative bacteria like *Acetobacter, Rhizobium, Achromobacter, Aerobacter, Pseudomonas* have the ability to synthesize cellulose [7]. Bacteria of the genus *Acetobacter* are the most studied and efficient cellulose producers with high yields, and have been used as model microorganisms for basic and applied studies on cellulose [8,9].

Monosaccharides and disaccharides are used as substrates for production of BC. Fruits are readily available agricultural products which contain high amount of sugars and have been studied as substrate forproduction of BC. Pineapple and Sugarcane juice [9], Apple, Orange, Grape Juice [10] have been usedin BC production.These fruits have abundant sugars such as glucose, fructose and sucrose that can be bioconverted into bacterial cellulose [11] when used as substrate by the cellulose producing strains.

Bacterial cellulose has application in production of paper products, audio headphone diaphragm, thickner for paints, food additives [2,12-14]. Also, in biomedicals such as wound dressing, tissue engineering [15].

This study is aimed at the production of bacteria cellulose using different fruit juice as substrate.

2. MATERIALS AND METHODS

2.1 Collection of Sample and Microbial Culture

Pineapple, Pawpaw and Watermelon fruits were purchased from Oje market, Ibadan, Oyo State Nigeria. Cultures of *Acetobacter pasteurianus* PW1and *Acinetobacter*sp. BAN1 were obtained from the culture collection of our previous work in the department of Microbiology, University of Ibadan, Nigeria.

2.1.1 Culture maintenance

Cultures obtained from the department of Microbiology, University of Ibadan were maintained on slants of Hestrin-Schramm (HS) medium composed of glucose (2 g), yeast extract (0.5 g), peptone (0.5 g), citric acid (0.12 g), disodium hydrogen phosphate (0.27 g), Agar (1.0 g). The isolates were subcultured unto fresh HS-agar plates.

2.2 Production of Bacterial Cellulose Using Different Fruit Juice as Substrate

BC was produced using different juice samples according to the method of Afreen and Lokeshappa [16] and Kamarudin et al. [17]. The fruits were washed, crushed, squeezed and separated to the juice and shaft.

Seed broth was prepared by inoculating the isolates into 10 ml tubes containing HS broth. The tubes were incubated at 30°C for 3-5 days. After incubation, 5 ml of the seed culture was inoculated into the basal medium containing Na_2HPO_4 (0.34 g), peptone (0.62 g), yeast extract (0.62 g) and citric acid (0.14 g),50 ml of the juice samples watermelon, pineapple and paw paw respectively. The pH of the medium was adjusted to pH 5. The inoculated basal medium was incubated statically at 28-30°C for 15 days.

The growth of the BC producing strains was monitored by reading the optical densitometer (OD) at 620 nm and the pH of the fermenting medium was monitored using a pH meter over a period of 15 days at 5 days interval was studied The production of cellulose was monitored for 15 days and the formation of white pellicles were observed on the surface of the medium. The cellulose produced was quantified and characterized.

2.3 Characterization of BC

2.3.1 Reducing sugar analysis

The BC produced was quanified by determining the reducing sugar in the fermentation broth using the method of Miller [18]. The amount of reducing sugar in the fermenting medium was determined using Di Nitro Salicylic Acid (DNSA) method.

2.3.2 Dry weight measurement

Dry weight of the pellicles produced after fermentation was measuredaccording to the method of Aydin and Akosy [19]. The fermentation medium containing the pellicles was centrifuged at 4000 rpm for 10 mins. 1N NaOH was added to the pellicles and boiled for 15mins at 80ºC to dissolve entrapped cells and cell components within the cellulose pellicles. The cellulose was washed repeatedly with distilled water and dried at 70°C to a constant weight. The weight of the dried cellulose was measured using a weighing balance.

2.3.3 Fourier Transformed Infra-red Spectroscopy (FTIR)

The cellulose samples obtained from the fermentation medium was analysed to study conformational characteristics by FTIR spectrometer using KBr plate method. 1.0 mg of dried bacterial cellulose samples were mixed with KBr powder and pressed into a small tablet. Then FTIR spectrum was measured in the transmittance mode with the resolution of 1.00 cm^{-1} at wavenumbers ranging from 4000 cm^{-1} to 400 cm^{-1} [20].

2.3.4 Scanning Electron Microscope (SEM)

The purified BC pellicles was visualizedusing a scanning electron microscope (ASPEX 3020) to observe the microstructure and surface morphology of the produced [20].

3. RESULTS AND DISCUSSION

Table 1 shows the growth of isolated *Acinetobacter* sp. BAN1 and *Acetobacter pasteurianus* PW1 in PIJM, PAJM and WMJM after incubation for 15 days.

In PIJM, PAJM and WMJM the growth of *Acinetobacter* sp. BAN1 ranged from 1.291^{d} – 2.759^{a}, 1.742^{d} – 2.179^{a} , 0.687^{d} – 1.991^{a}. The highest BC production was recorded on the 5^{th} and 10^{th} day of incubation respectively. At day 0 in the juice medium, the highest growth was supported by PAJM (1.742). On the 5^{th} and 10^{th} day of fermentation, PIJM supported the highest growth (2.759 and 2.273 respectively). On the 15^{th} day of fermentation, the highest growth was supported by PAJM (1.887).

In PIJM, PAJM and WMJM the growth of *Acetobacter pasteurianus* PW1 ranged from1.387^{d} – 2.506^{a}, 0.687^{d} – 1.991^{a}, 0.561^{d} – 1.907^{a}. The highest BC production was recorded on the 10^{th} day of incubation. At day 0 in all the juice media, the highest growth was supported by PAJM (1.648). On the 5^{th} day of fermentation, the highest growth was supported by PIJM (2.325). On the 10^{th} day of fermentation, PIJM supported the highest growth (2.506). On the 15^{th} day of fermentation, the highest growth was supported by PIJM (2.301).

Table 1. Growth of Bacterial cellulose producing strain in fruit juice media at different days of incubation

Incubaion time (Days)	Growth (620 nm)			Growth (620 nm)		
	Acinetobacter sp.BAN 1			*Acetobacter pasteurianus* PW 1		
	PIJM	PAJM	WMJM	PIJM	PAJM	WMJM
0	1.441^{c}	1.742^{d}	0.687^{d}	1.387^{d}	1.648^{d}	0.561^{d}
5	2.759^{a}	2.160^{b}	1.924^{b}	2.325^{b}	2.299^{b}	1.795^{b}
10	2.273^{b}	2.179^{a}	1.991^{a}	2.506^{a}	2.336^{a}	1.907^{a}
15	1.291^{d}	1.887^{c}	1.825^{c}	2.301^{c}	2.218^{c}	1.508^{c}
Standard error	0.1816	0.0557	0.1611	0.1315	0.0841	0.1596

Key: PIJM- Pineapple juice medium, PAJM- Pawpaw juice medium, WMJM- Watermelon juice medium

The ability of the microorganisms to utilize substrates for synthesis of intracellular and extracellular materials can be studied in the growth pattern of the microorganism. The growth pattern of *Acinetobacter* sp. BAN1 in PAJM and WMJM during the incubation period revealed that it grew best at day 10 of incubation. But in PIJM the maximum growth was recorded at day 5 of incubation.

The ability of *Acetobacter pasteurianus* PW1 in PIJM, PAJM and WMJM to have maximum growth at day 10 of incubation is in accordance with the work of Qiang [21] that the growth curve of *Acetobacter pastuerianus* suggested the maximal bacterial growth amount was achieved on the 8th day of incubation. Pawpaw contains glucose, but the sucrose content increases during ripening and reaches up to 80% of the total sugar [22-23]. The fruit also contains minerals and vitamins [24], which may improve the production of BC.

Table 2 shows the pH development during fermentation of fruit juice media for BC production. For *Acinetobacter* sp. BAN1, during fermentation at different time intervals in PIJM, PAJM and WMJM, the pH ranged from 3.5^{d} – 3.8^{a}, 3.9^{c} – 4.2^{a} and 4.0^{b} – 4.3^{a} respectively. The lowest pH was recorded in PIJM. The highest pH was recorded in WMJM. At day 0 – 5, there was significant difference ($P \leq 0.05$) in the pH development during fermentation using the different fruit juice media. At day 10 – 15, there was significant difference in pH development during fermentation except for WMJM.

For *Acetobacter pasteurianus* PW1, during fermentation at different time interval in PIJM, PAJM and WMJM, the pH ranged from 3.6^{c} – 3.8^{a}, 4.0^{c} – 4.2^{a} and 4.1^{c} – 4.3^{a} respectively. The lowest pH was recorded in PIJM. The highest pH was recorded in WMJM. Generally, at day 0 – 15, there was significant difference ($P \leq 0.05$) in the pH development during fermentation, using the different fruit juice media. But there was no significant difference in the pH of PIJM at day 5 – 10, also at day 5 and 15 in PAJM and WMJM.

Accumulation of organic acids may lead to the inhibition of BC production. The lower BC productivity in PIJM may be as a result of the organic acids and spontaneous fermentation in the pineapple juice, which in turn contributes to a decline in the pH [25]. Acidic pH development in the media during fermentation for the production of BC may be due to the release of gluconic acid or acidic by products, this agrees with the findings of Ndoye et al. [26] and Kongruang [27].

Table 3 shows the bacterial cellulose yield (mg/l) after 15 days of fermentation in the different fruit juice media. The BC yield of *Acinetobacter* sp. BAN1 in the juice media ranged from 1.23 – 6.48 mg/l. PAJM supported the highest yield, followed by WMJM (3.36 mg/l) and PIJM supported the lowest yield.

The BC yield of *Acetobacter pasteurianus* PW1 in the juice media ranged from 0.65 – 8.41 mg/l. WMJM supported the highest yield, followed by PAJM (6.74 mg/l) and PIJM supported the lowest yield.

Table 2. pH development during fermentation in different fruit juice media for Bacterial cellulose production

Incubaion time (Days)	pH			pH		
	***Acinetobacter* sp. BAN 1**			***Acetobacter pasteurianus* PW 1**		
	PIJM	**PAJM**	**WMJM**	**PIJM**	**PAJM**	**WMJM**
0	3.7^{b}	4.2^{a}	4.3^{a}	3.6^{c}	4.2^{a}	4.3^{a}
5	3.5^{d}	3.9^{c}	4.0^{b}	3.8^{a}	4.1^{b}	4.2^{b}
10	3.8^{a}	3.9^{c}	4.0^{b}	3.8^{a}	4.0^{c}	4.1^{c}
15	3.6^{c}	4.0^{b}	4.0^{b}	3.7^{b}	4.1^{b}	4.2^{b}
Standard error	0.0337	0.0369	0.0392	0.0250	0.0213	0.0213

Key: PIJM- Pineapple juice medium, PAJM- Pawpaw juice medium, WMJM- Watermelon juice medium

Table 3. Bacterial cellulose yield (mg/l) after 15days of incubation

Isolate	BC yield (mg/l) reducing sugar		
	PIJM	**PAJM**	**WMJM**
Acinetobacter sp. BAN 1	1.23	6.48	3.36
Acetobacter pasteurianus PW 1	0.65	6.74	8.41

Key: PIJM- Pineapple juice medium,PAJM- Pawpaw juice medium, WMJM- Watermelon juice medium

Fig. 1 shows the dry weight of Bacterial cellulose produced by *Acinetobacter* sp. BAN1 and *Acetobacter pasteurianus* PW1 in the juice media.

The dry weight of the BC produced by *Acinetobacter* sp. BAN1 in the juice media ranged from 0.3 – 6.4 g/l. PAJM supported the highest BC, followed by WMJM (0.7 g/l) and PIJM supported the lowest BC.

The dry weight of BC produced by *Acetobacter pasteurianus* PW1 in the juice media ranged from 0.1 – 7.7 g/l. PAJM supported the highest BC, followed by WMJM (0.4 g/l) and PIJM supported the lowest BC.

The FTIR spectrum of BC produced by *Acinetobacter* sp. BAN1 in PIJM, in PAJM and in WMJM is shown in Fig. 2 (a-c). The distinguishing peaks at 3408.33 cm^{-1} in PIJM, at 3417.96 cm^{-1} in PAJM and at 3417.98 cm^{-1} in WMJM indicates O – H stretching. Peaks at 2850.88 cm^{-1} - 2922.25 cm^{-1} in PIJM, 2852.81 cm^{-1} – 2922.25 cm^{-1} in WMJM indicates C – H stretching. Peaks at 1651.12 cm^{-1} in PIJM, at 1687.77 cm^{-1} in PAJM and at 1653.05 cm^{-1} in WMJM indicates presence of carbonyl group (C = O). Peaks at 1041.6 cm^{-1} – 1066.67 cm^{-1} in PIJM, at 1031.95 cm^{-1} – 1064.74 cm^{-1} in PAJM and at 1043.52 cm^{-1} in WMJM indicates C – O stretching. Peaks at 1404.22 cm^{-1} in PIJM, at 1467.88 cm^{-1} in PAJM and at 1410.01 cm^{-1} in WMJM indicates CH_2 bending. Peaks at 1317.43 cm^{-1} in PIJM and at 1336.71 cm^{-1} in PAJM indicates C – H. Peaks at 896.93 $cm^{-1}$and 1155.4 cm^{-1} in PIJM, at 1161.19 cm^{-1} in PAJM and at 1155.4 cm^{-1} in WMJM indicates C – O – C stretching. and peak at 715.61 cm^{-1} indicates out-of-plane bending of C – O - H .

The FTIR spectrum of BC produced by *Acetobacter pasteurianus* PW1 in PIJM, PAJM and WMJM is shown in Fig. 2 (d-f). The distinguishing peaks at 3402.54 cm^{-1} in PIJM, at 3470.06 cm^{-1} in PAJM and at 3396.76 cm^{-1} in WMJM indicates O – H stretching. Peaks at 2850.88 cm^{-1} - 2922.25 cm^{-1} in PIJM, and at 2852.81 cm^{-1} - 2922.25 cm^{-1} in WMJM indicates C – H stretching. Peaks at 1647.26 cm^{-1} in PIJM, at 1693.56 cm^{-1} in PAJM and at 1647.26 cm^{-1} in WMJM indicates presence of carbonyl group (C = O). Peaks at 1039.67 cm^{-1} – 1058.96 cm^{-1} in PIJM, at 1028.09 cm^{-1} – 1066.67 cm^{-1} in PAJM and at 1043.52 cm^{-1} in WMJM indicates C – O stretching. Peaks at 1408.08 cm^{-1} in PIJM, at 1467.88 cm^{-1} in PAJM and at 1417.73 cm^{-1} in WMJM indicates CH_2 bending. Peaks at 1319.35 cm^{-1} in PIJM and at 1319.35 cm^{-1} in WMJM indicates C – H bending. Peaks at 1155.40 cm^{-1} in PIJM, at 1163.11 cm^{-1} in PAJM and at 1155.4 cm^{-1} in WMJM indicates C – O – C stretching and peak at 719.47 cm^{-1} in PIJM indicates out-of-plane bending of C – O - H .

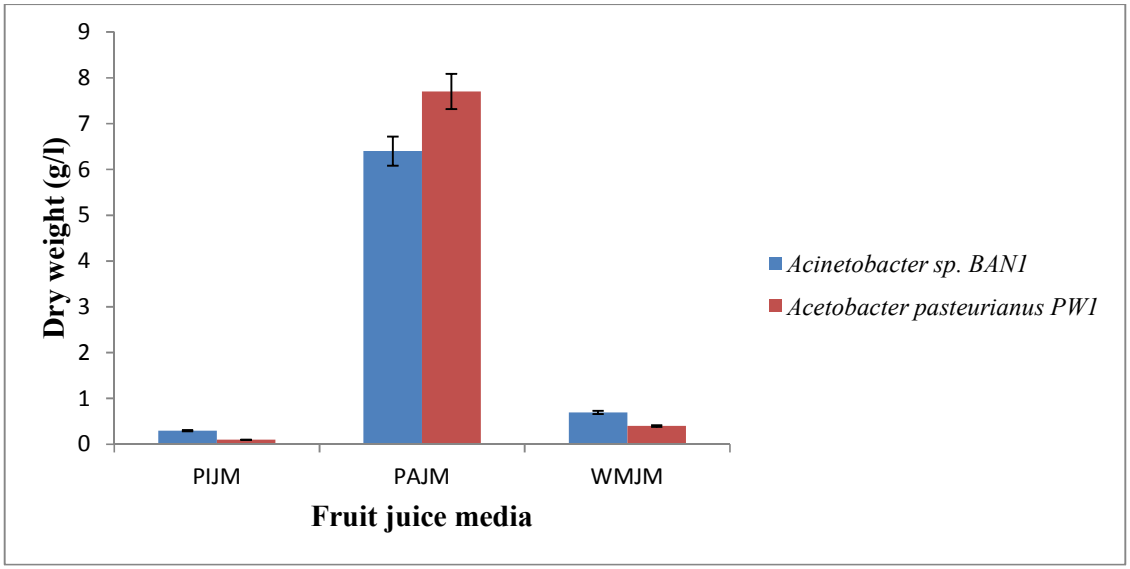

Fig. 1. Dry weight of Bacterial cellulose produced by *Acinetobacter* sp. BAN1 and *Acetobacter pasteurianus* PW1 using different fruit juice media

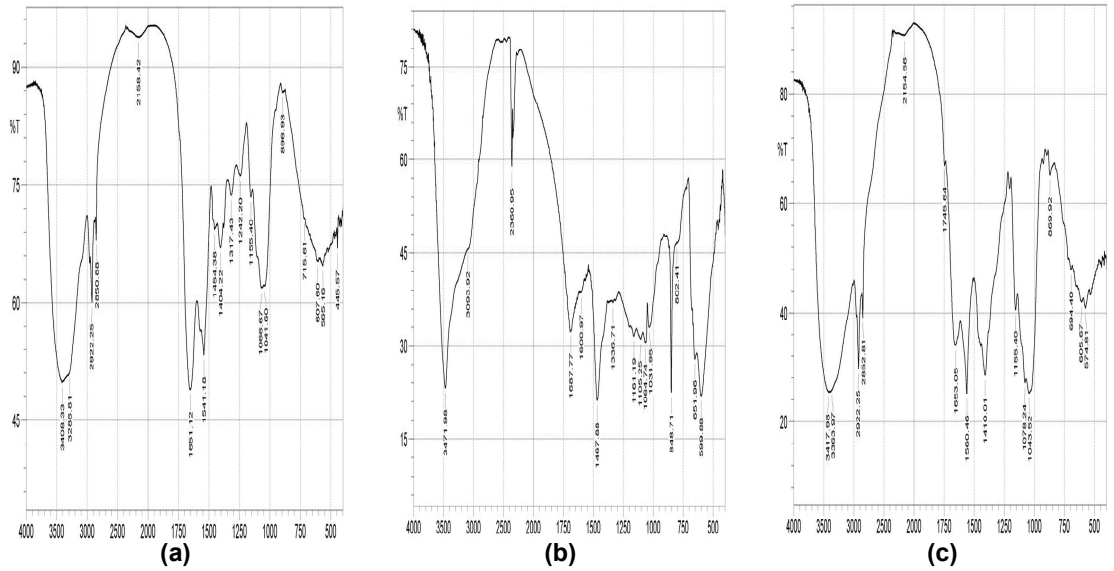

Fig. 2(a–c). FTIR spectrum of Bacterial cellulose produced by *Acinetobacter* sp. BAN1 using (a) PIJM, (b) PAJM and (c) WMJM

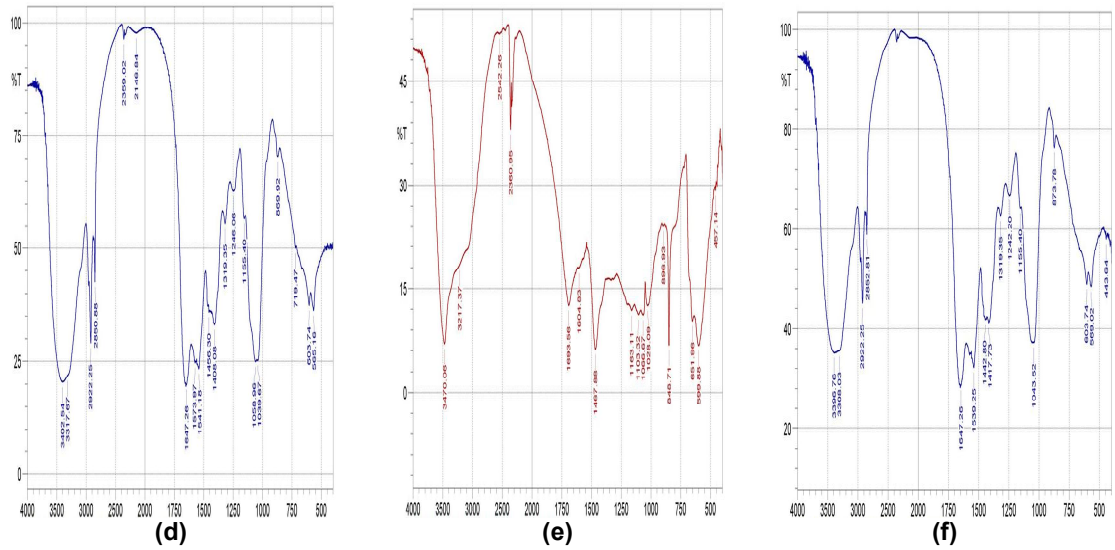

Fig. 2(d – f). FTIR spectrum of Bacterial cellulose produced by *Acetobacter pasteurianus* PW1 using (d) PIJM, (e) PAJM and (f) WMJM

Marchessault and Sundararajan, [28] stated that pure cellulose spectrum had distinguish peaks of 3350 cm^{-1} which shoulders around 3400 cm^{-1} to 3500 cm^{-1} and it indicates O-H stretching, 2800 cm^{-1} 1 to 2900 cm^{-1} indicates C-H stretching, 1160 cm^{-1} indicates C-O-C stretching and 1035 cm^{-1} to 1060 cm^{-1} indicates C-O stretching. Other fingerprint regions for cellulose are peaks around 1300 cm^{-1} indicating C-H bending and around 1400 cm^{-1} indicating CH_2 bending. The spectra of BC produced from *Acinetobacter* sp. BAN1 and *Acetobacter pasteurianus* PW1 in PIJM, PAJM, WMJM indicates that there is a similarity between the BC produced and pure cellulose.

The FTIR spectra of BC produced by *Acinetobacter* sp. and *Acenotobacter* pasteurianus using agrowaste as substrates has been reported by Adebayo-Tayo et al. [29].

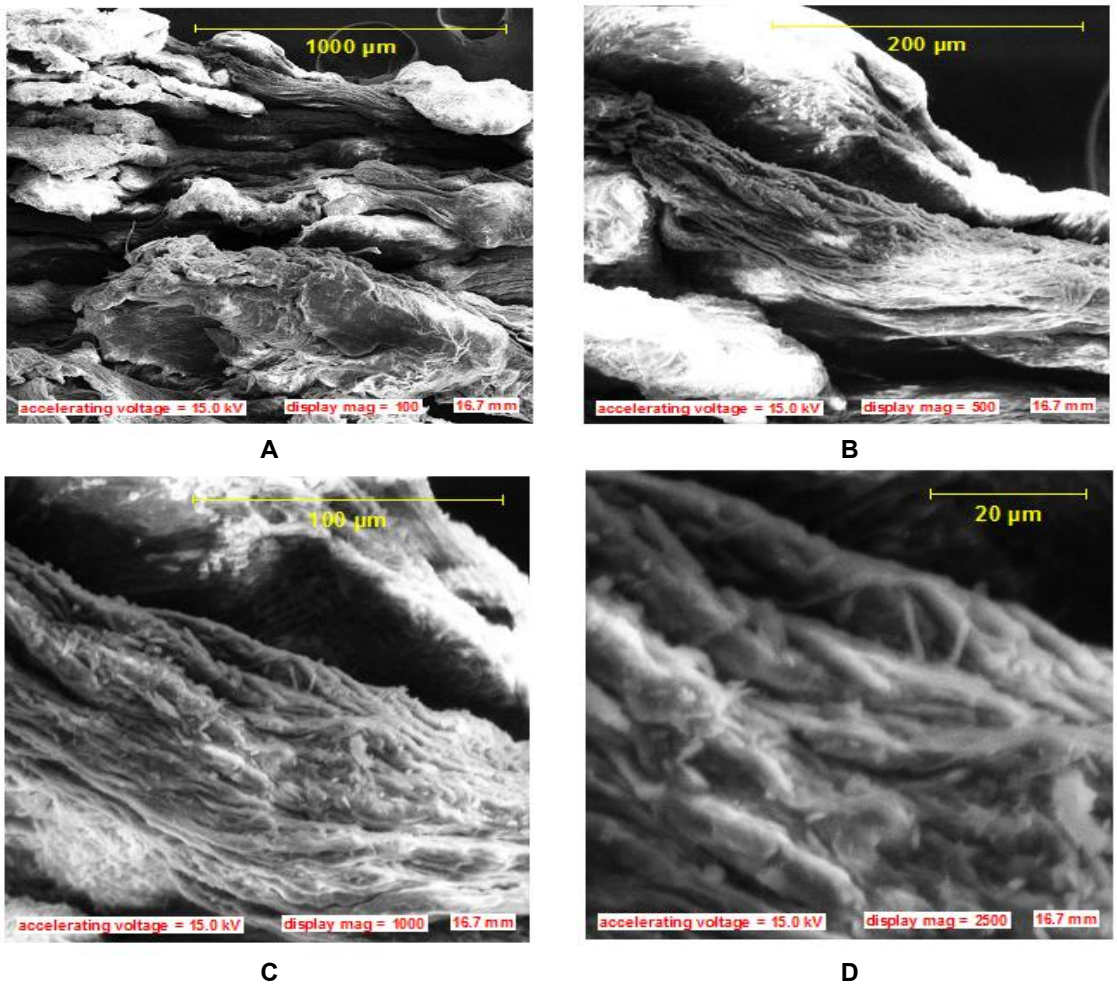

Fig. 3. SEM image of Bacterial cellulose produced by *Acetobacter pasteurianus* PW1 using PAJM at magnifications; (A) 100X, (B) 500X, (C) 1000X and (D) 2500X

4. CONCLUSION

In conclusion, the organisms utilized the nutrients and sugars in the fruits as carbon source for growth and proliferation. The fruit also served as a substrate for BC production.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/20415