Environment and Ecology 40 (3D) : 1865—1872, July–September 2022
ISSN 0970-0420

Optimum Temperature for Bacterial Cellulose Production from Cassava Peel by Acetic Acid Bacteria Isolated from Palmwine in Abakaliki, Nigeria

Elom E. E. , Okeh C.O., Uzoh CV, Igwe P. C., Yusuf I., Aroh K. E., Isirue A. M. C., Ugwuocha C. S., Obimma C.A.

Received 6 Aujust 2022, Accepted 2 September 2022, Published 17 September 2022

ABSTRACT

Bacteria cellulose production from cassava peel by acetic acid bacteria isolated from palm wine was carried out in Abakaliki, Ebonyi State. 100 palm wine samples were collected at Nkwagu market from different palm wine vendors while cassava peel was collected from processes cassava vendors at Nkaliki village respectively. Standard microbiological procedures were followed to isolate and identify the isolates. After the substrates were subjected to acid hydrolysis and heat treatment, static culture method was used in the production of the bacterial cellulose (BC). Nine (9) species of *Gluconacetobacter* and 3 species of *Komagataeibacter* were isolated. Bacterial cellulose production from the standard Hestrin-Schramm (HS) broth and the modified HS broth with cassava peel as the carbon source using the acetic acid bacteria species revealed that *Gluconacetobacter* and *Komagataeibacter* species produced varying quantities of bacteria cellulose from the standard and modified HS medium. *Gluconacetobacter* species GS1 produced 0.51±0.01 grams of bacteria cellulose per 250ml of standard HS medium with percentage yield of 10.2 and 0.20±0.01 grams of bacteria cellulose, while *Gluconacetobacter* species GS4 produced 0.42 ± 0.01, 0.36±0.01 and 0.18 grams of bacteria cellulose from the standard HS and cassava peel modified HS medium with percentage yield of 8.4, 7.2 and 3.6 respectively. The optimum temperature for growth and production of bacteria cellulose by the *Gluconacetobacter* and *Komagataeibacter* species was 30^{o}C. There was no growth at 45^{o}C for all tested species. This study demonstrated that cassava peel can serve as an alternative, cost effective carbon sources for producing bacteria cellulose. Utilizing these wastes helps in reduction of environmental pollution.

Keywords Cassava peels, Bacterial cellulose, Rice husk, Fermentation, Acetic acid bacteria.

Elom E. E.[1]*, Igwe P. C.[2]
[1,2]Lecturer, Department of Applied Microbiology, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria

Okeh C.O.[3], Uzoh CV[4], Yusuf I.[5], Aroh K.E.[6], Isirue A.M.C.[7], Ugwuocha C.S.[8],
[3]Senior Lecturer, [4] Lecturer, [5,6,7]Assistant Lecturer, [8]Graduate Assistant,
Department of Microbiology, Alex-Ekwueme Federal University Ndufu-Alike Ikwo P.M.B 1010, Abakaliki, Ebonyi State, Nigeria, Obimma C.A.
Email: optchuks@yahoo.com
*Corresponding author

INTRODUCTION

Microbial cellulose exists as a basic structure known as microfibrils, which are composed of glucan chains interlocked by hydrogen bonds so that a crystalline domain is produced. This microfibrillar structure of bacteria cellulose was first described by Muhlethaler (Cheng *et al.* 2017). Electron microscopic observations showed that the cellulose produced by *Acetobacter xylinum* occurs in the form of fibers. The bacteria first secreted a structurally homogeneous slimy substance within which, after a short time, the cellulose fibers were formed (Yu and Attala 1996). Cassava (*Manihot esculenta*) is a short lived erect perennial shrub, planted vegetatively from hard wood stem cutting. Cassava peels derived from garri processing are normally discarded as wastes and allowed to rot in the open, thus resulting in health hazard. Cassava peels contain high level of hydrogen cyanide and this cyanide from cassava waste peels significantly reduces soil microbial population, increases soil acidity and heavy metal content of soils around processing cites (Asien and Ikenebomhe 2017). This huge waste can be converted to useful feedstock for bacteria cellulose production. Nigeria is one of the world's largest producers of cassava, with a yearly output of about 50 million tonnes. Cassava production is projected to increase to up to 150 million tons by 2020. Nearly half of the quantity of cassava produced is processed into *garri, elubo* and other cassava-based staple foods. For every ton of cassava processed, 10%-15%~125kg/tons, are lost in form of wet peels, which are poorly utilized, dumped as waste or burnt. Hundreds of thousand women spend up to ten hours a day in various cassava processing centers, peeling cassava and earning less than $2.5 dollars a day (Akpan *et a*l. 2017). Cassava peel are abundant and practically of no economic value in many developing countries like Nigeria (Ogbo and Odo 2011). This research aims at developing a low cost feedstock for bacterial cellulose production from cassava peel. By this process, cassava peel hydrolysates could be developed.

MATERIALS AND METHODS

Study area

The study was carried out in Abakaliki local government area in Ebonyi State, Nigeria. Ebonyi State is located in the south-eastern part of Nigeria which lies approximately within longitude $7^{o}30^{1}$ and 7^{o}E and, latitude $5^{o}40^{1}$ and $6^{o}45^{1}$ N. It has a population of 149,683 and a land mass of about 5,935 square kilometers.

Sample collection

A total of 100 palm wine samples (25ml each) were sourced from palm wine tappers at Nkwagu market which is within the study location. The palm wine was taken in a sterile sample bottle and immediately transported to the Microbiology Laboratory of Ebonyi State University, Abakaliki for processing.

Isolation of test organism

The isolation of acetic acid bacteria species that was used for the fermentation was done according to the method described by Amoa-Awua *et al.* (2007). The bacteria were isolated from overnight palm wine (*Elais guineensis*).

Sample processing

The palm wine sample in the bijou bottle was shaken by hand and 1.0 ml pipetted into 9 ml sterile salt peptone solution containing 0.1% peptone (Oxoid LTD. UK), 0.85% NaCl with pH adjusted to 7.2 as the 1:10 dilution. After serial dilution, 0.1ml of the 10^{-4} and 10^{-5} were cultured by spread plate method on GYC and YPM agar at 30^{o}C for two weeks. To both GYC and YPM 10 mg/l of cycloheximide made up in 70 ml/l of ethanol and 20 ml/l of procaine penicillin was added to inhibit the growth of yeast and lactic acid bacteria respectively (Andelib and Nuran 2009).

Characterization of the isolates

The observed colonies on GYC and YPM with clear zones around them were subcultured repeatedly by streaking on fresh same agar until pure cultures were obtained. The suspected acetic acid bacteria isolates were subjected to morphological, biochemical and physiological tests following the method of Andelib and Nuran (2009).

Morphological characterization

The colonial appearance of the isolates on culture media, color and shape was observed and recorded accordingly. Gram staining and other biochemical tests were carried out in accordance with Cheesbrough (2006).

Preliminary assessment of the isolates for cellulose formation and detection

This was done according to the method described by Andelib and Nuran (2009). Seed broth was prepared by inoculation of 25ml test tubes containing 10ml Hestrin-Schramm broth. The tubes were incubated statically at 30^{o}C for ten days. Inoculations were carried out by the addition of 1% (v/v) seed broth to the culture medium. The acetic acid bacteria isolates that were able to produce cellulose from the standard Hestrin-Schramm broth and either of the modified Hestrin Schramm (cassava peel) broth during the preliminary assessment were used for the scale up (Hong *et al*. 2011).

Production of bacteria cellulose from the standard Hestrin-Schramm broth using the acetic acid bacteria isolates

The prepared 250 ml Standard Hestrin Schramm medium was taken in 500 ml (cross sectional area 56.75 cm^{2}) beaker and sealed with aluminium foil to control the volume of air. The culture was carried out by the addition of 1.25ml HS seed broth equal to McFarland no 1 turbidity or about 3.0 x 10^{8} CFU/ml. The medium was incubated at 30^{o}C in a static incubator for 10 days (Cheng *et al*. 2017).

Determination of the optimum growth temperature of the isolated acetic acid bacteria for the production of bacteria cellulose

The ability of the isolates to grow at temperatures of 20, 25, 30, 35 and 45^{o}C was investigated using the standard and modified Hestrin-Schramm broth (HS-broth). The Hestrin-Schramm broth was prepared and 5 ml of it taken into each of the bijou bottle. A 0.1 ml of 0.5 McFarland turbidity standard of the acetic acid bacteria broth was poured into the bijou bottles and incubated differently at the temperatures of 20, 25, 30, 35, 40 and 45^{o}C respectively for 92 hours (4 days). The turbidity (microbial cell numbers) was estimated using the spectrophotometric method at 600nm. The spectrophotometer measures turbidity directly (Firdaus *et al*. 2012, Jia *et al.* 2017).

Bacteria cellulose synthesis from rice husk and cassava peel

Sample collection

The cassava (*Manihot esculenta* Crantz) peel was collected from a farm in Nkaliki village, Abakaliki Local Government Area, Ebonyi State. It was collected into the cellophane bag using the fork. The above mentioned types of rice and cassava are popularly cultivated in Abakaliki (Martin and Ejike 2018).

Sample processing

Acid pre-treatment of cassava peel: The acid pre-treatment was done using the method described by Ang *et al*. (2013). Acid pre-treatment was conducted with 0.5% w/v sulphuric acid for 30 minutes. A total of 5g of the cassava peel and 50 ml of the acid solution (10% load) was used. The reaction was conducted in a borosilicate glass vessel resistant to pressure and temperature using an autoclave at 121^{o}C with a pressure of 1 atmosphere.

Preparation of cassava peel for microbial utilization: The cassava peel was cut and rinsed severally before soaking it in distilled water for four days. This was followed by steaming to further reduce the hydrogen cyanide. The material was dried in an oven at 65^{o}C until constant weight was achieved. A mortar was washed with distilled water and allowed to dry. Next, the dry cassava peel was ground in the mortar to get powder. The powder (5g) was soaked in 12.5 ml of 0.5% sodium hypochlorite (NaOCl) solution at 30^{o}C for one hour to remove lignin and colored materials

Harvest and weighing of bacteria cellulose : Bacterial cellulose membranes were collected and impregnated with 0.5M NaOH at 80^{o}C for two hours, followed by repeated soaking and washing in deionized water until the washing liquid attained neutral pH (Cheng

Table 1. Morphological, microscopic and biochemical characteristics of the *Gluconacetobacter* species isolated from palm wine samples collected from Abakaliki, Ebonyi State.

	Morphological characteristics		Micro CTX			Biochemical characteristics										Carbohydrate utilization									
Species	Color	Shape	GGM	GMA	BPP	M	GS	OT	CT	IT	UT	CUT	OA	OL	GHT	Gl	S	Rh	Ma	F	X	MA	M	I	Probable Organisms
GS1	C-B	Rods	-	+	+	-	-	-	+	-	-	+	+	+	-	+	+	+	-	-	+	-	+	-	*Gluconacetobacter*
GS2	C-B	Rods	-	+	+	-	-	-	+	-	-	-	+	+	-	+	+	+	+	+	+	+	+	-	*Gluconacetobacter*
GS3	C-B	Rods	-	+	+	+	-	-	+	-	-	+	+	+	-	+	+	+	-	-	+	+	+	-	*Gluconacetobacter*
GS4	C-B	Rods	-	+	+	-	-	-	+	-	-	-	+	+	-	+	+	+	-	-	+	+	+	-	*Gluconacetobacter*
GS5	C-B	Rods	-	+	+	-	-	-	+	-	-	+	+	+	-	+	+	+	-	-	+	+	+	-	*Gluconacetobacter*
GS6	C-B	Rods	-	+	+	-	-	-	+	-	-	-	+	+	-	+	+	+	-	-	+	+	+	-	*Gluconacetobacter*
GS7	C-B	Rods	-	+	+	-	-	-	+	-	-	+	+	+	-	+	+	+	-	-	+	+	+	-	*Gluconacetobacter*
GS8	C-B	Rods	-	+	+	+	-	-	+	-	-	+	+	+	-	+	+	+	-	-	+	-	+	-	*Gluconacetobacter*
GS9	C-B	Rods	-	+	+	-	-	-	+	-	-	-	+	+	-	+	+	+	-	+	+	+	+	-	*Gluconacetobacter*

KEY:CTX = Characteristics, Micro = Microscopic, C-B = Cream to beige, M-W = Milky White, GGM = Growth on glutamate agar, GMA = Growth on Mannitol agar, GHT = Gelatine hydrolysis test, Gl = Glucose, S = Sucrose, Rh = Rhamnose, Ma = Maltose, F = Fructose, X = Xylose, MA = Mannose, M = Mannitol, I = Inositol, BPP = Brown Pigment Production, M = motility, GS = Gram stain, OT = Oxidase test, CT = Catalase test, IT = Indole test, UT = Urease test, CUT = Citrate utilization test, OA = Oxidation of acetate, OL = Oxidation of lactate, GS = *Gluconacetobacter* specie, Positive (+), Negative (-).

et al. 2017). The bacterial cellulose was filtered and dried to constant weight at 105°C. The mass of the bacteria cellulose were determined and the bacteria cellulose yield was calculated (Cheng *et al.* (2017). The percentage yield of bacteria cellulose dry film was calculated by following the equation:

$$\text{Percent yield} = \frac{\text{Dry weight of BC film}}{\text{Weight of carbon source used in production medium}} \times 100$$

Statistical analysis : The raw data obtained from this study on bacteria cellulose production were presented as mean ± standard deviation in tables and bar charts while relevant data were interpreted using simple descriptive statistics. One way analysis of variance (ANOVA) with the aid of IBM Statistical Package for Social Sciences (SPSS) version 22 and Microsoft Excel 2013 software were used.

RESULTS

Morphological, microscopic and biochemical Characteristics of acetic acid bacteria isolated from palm wine samples collected from Abakaliki, Ebonyi State

The results of the morphological, microscopic and biochemical characteristics of the acetic acid bacteria isolated from palm wine samples collected from Abakaliki, Ebonyi State are presented in Tables 1and 2. The result showed that the colonies of 9 isolates were cream to beige in color on Hestrin Schramm

Table 2. Morphological, microscopic and biochemical characteristics of the *Komagataeibacte*r species isolated from palm wine samples collected from Abakaliki, Ebonyi State.

	Morphological					Micro CTX			Biochemical characteristics							Carbohydrate utilization									
Species	Color	Shape	GGM	GMA	BPP	M	GS	OT	CT	IT	UT	CUT	OA	OL	GHT	Gl	S	Rh	Ma	F	X	MA	M	I	Organisms
KS1	M-W	Rods	-	+	-	-	-	-	+	-	-	+	+	+	-	+	+	+	-	-	+	-	+	-	*Komagataeibacter*
KS2	M-W	Rods	-	+	-	-	-	-	+	-	-	+	+	+	-	+	+	+	-	-	+	+	+	-	*Komagataeibacter*
KS3	M-W	Rods	-	+	-	-	-	-	+	-	-	+	+	+	-	+	+	+	+	-	-	+	+	-	*Komagataeibacter*

KEY:CTX = Characteristics, Micro = Microscopic, C-B = Cream to beige, M-W = Milky White, GGM = Growth on glutamate agar, GMA = Growth on Mannitol agar, BPP = Brown Pigment Production, OA = Oxidation of acetate, OL = Oxidation of lactate, GHT = Gelatine hydrolysis test, Gl = Glucose, S = Sucrose, Rh = Rhamnose, Ma = Maltose, F = Fructose, X = Xylose, MA = Mannose M = Motility, GS = Gram stain, OT = Oxidase test, CT = Catalase test, IT = Indole test, UT = Urease test, CUT = Citrate utilization test, , M = Mannitol, I = Inositol, KS = *Komagataeibacter* specie, Positive (+), Negative (-).

Table 3. Mass of bacteria cellulose pellicles produced from the standard HS medium by *Gluconacetobacter* species (Weight in grams).

Species	Preliminary assessment for BCP	Quantity of BC obtained from 250 ml HS broth			Mean ± SD of BC obtained	(%)Yield of BC
		1st trial	2nd trial	3rd trial		
GS1	+	0.51	0.52	0.50	0.51±0.01	10.2
GS2	+	0.43	0.41	0.41	0.42±0.01	8.4
GS3	+	0.25	0.26	0.24	0.25±0.01	5.0
GS4	+	0.37	0.35	0.37	0.36±0.01	7.2
GS5	+	0.31	0.30	0.32	0.31±0.01	6.2
GS6	+	0.45	0.42	0.44	0.44±0.02	8.8
GS7	+	0.34	0.35	0.36	0.35±0.01	7.0
GS8	+	0.38	0.39	0.37	0.38±0.01	7.6
GS9	+	0.20	0.21	0.22	0.21±0.01	4.2

Key: GS = *Gluconacetobacter* specie, BC = Bacteria cellulose, + = Positive.

(HS) agar while three isolates appeared milky white.

Bacteria cellulose produced from the standard Hestrin-Schramm broth using the isolated *Gluconacetobacter* and *Komagataeibacter* species

The result of bacteria cellulose production from the standard Hestrin-Schramm broth using *Gluconacetobacter* and *Komagataeibacter* species are presented in Tables 3 and 4. The results showed that *Gluconacetobacter* species GS1 produced an average of 0.51±001 grams of bacteria cellulose per 250ml HS medium with percentage yield of 10.2, while *Gluconacetobacter* species GS2 produced an average of 0.42 ± 0.01grams of bacteria cellulose with percentage yield of 8.4

Growth of *Gluconacetobacter* and *Komagataeibacter* species at different temperatures of 20, 25, 30, 35, 40 and 45°C

The results of the growth of *Gluconacetobacter* and *Komagataeibacter* species at temperatures of 20, 25,

Table 4. Mass of bacteria cellulose pellicles produced from the standard HS medium by species of *Komagataeibacter s*pecies (Weight in grams).

Species	Preliminary assessment for BCP	Quantity of BC obtained from 250 ml HS broth			Mean ± SD of BC obtained	(%)Yield of BC
		1st trial	2nd trial	3rd trial		
KS1	+	0.24	0.23	0.24	0.24±0.01	4.8
KS2	+	0.29	0.29	0.28	0.29±0.01	5.8
KS3	+	0.32	0.34	0.33	0.33±0.01	6.6

Key: *Komagataeibacter* specie, ± = Positive. Key: GS = *Gluconacetobacter s*pecie, BC = Bacteria cellulose, + = Positive.

Table 5. Growth of *Gluconacetobacter* species at different temperatures.

Species	Temperature (°C)					
	20	25	30	35	40	45
GS1	1.2×10^7	8.39×10^7	5.99×10^8	1.05×10^8	9.68×10^7	NG
GS2	7.19×10^7	1.52×10^8	5.9×10^8	1.07×10^8	9.92×10^7	NG
GS3	7.39×10^7	1.54×10^8	2.11×10^8	1.6×10^8	1.52×10^7	NG
GS4	3.44×10^7	9.12×10^7	1.14×10^8	1.11×10^8	8.09×10^7	NG
GS5	6.75×10^7	1.47×10^8	6.17×10^8	4.97×10^8	4.1×10^7	NG
GS6	2.2×10^7	1.02×10^8	6.85×10^8	1.14×10^8	1.05×10^7	NG
GS7	7.37×10^7	1.53×10^8	2.41×10^8	1.54×10^8	1.50×10^7	NG
GS8	6.68×10^7	1.6×10^8	7.6×10^8	1.25×10^8	1.12×10^7	NG
GS9	3.54×10^7	9.22×10^7	1.34×10^8	1.13×10^8	8.07×10^7	NG

Key: NG = No growth, GS = *Gluconacetobacte*r specie.

Table 6. Growth of *Komagataeibacter* species at Different Temperatures.

Species	Temperature (°C) 20	25	30	35	40	45
KS1	7.38×10^{7}	1.44×10^{8}	7.26×10^{8}	6.19×10^{8}	3.79×10^{7}	NG
KS2	2.85×10^{7}	1.08×10^{8}	4.74×10^{8}	9.94×10^{7}	8.98×10^{7}	NG
KS3	6.98×10^{7}	1.5×10^{8}	7.7×10^{8}	1.26×10^{8}	1.17×10^{8}	NG

Key: NG = No growth, KS = *Komagataeibacter* specie.

30, 35, 40 and 45°C are presented in Tables 5 and 6. The results revealed that at the temperatures of 20, 25, 30, 35 and 40°C, *Gluconacetobacter* specie GS1 recorded 1.2×10^{7}, 8.39×10^{7}, 5.99×10^{8}, 1.05×10^{8}, 9.68×10^{7} cells per ml respectively.

Bacteria cellulose produced by *Gluconacetobacter* species at different Temperatures of 20, 25, 30, 35, 40 and 45°C from the standard HS medium

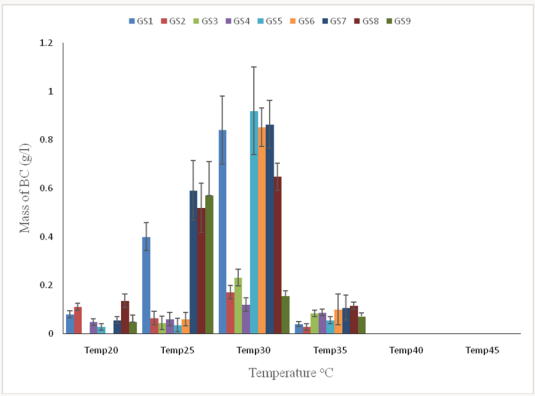
The result of bacteria cellulose produced by *Gluconacetobacter* species at different temperatures from the standard HS medium is presented in Fig. 1. The result showed that at the temperatures of 20, 25, 30 and 35°C, *Gluconacetobacter* specie GS1 produced 0.08, 0.40, 0.84 and 0.04 grams of bacteria cellulose per liter of HS medium. Bacteria cellulose production was not recorded at the temperature of 40 and 45°C for all tested species. At the temperature of 20, 25, 30 and 35°C.

Bacteria cellulose produced by *Gluconacetobacter* species at different temperatures of 20, 25, 30, 35, 40 and 45°C from the rice husk modified HS medium

The result of bacteria cellulose produced by ***Gluconacetobacter*** species at different temperatures is presented in Fig. 3. It revealed that at the temperature of 20, 25, 30 and 35°C, *Gluconacetobacter* species GS1 produced 0.13, 0.51, 0.80, and 0.09 g/l of bacteria cellulose respectively. Bacteria cellulose was not produced at 40 and 45°C for all tested species.

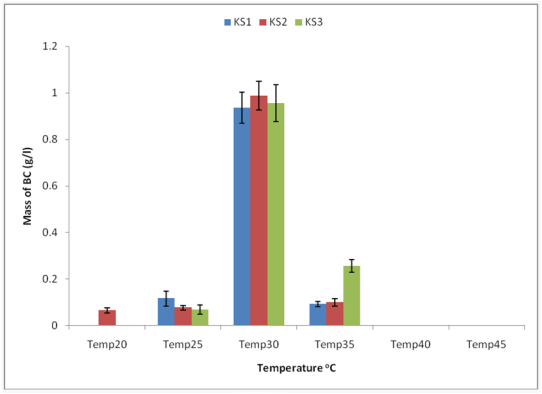
Bacteria cellulose produced by *Gluconacetobacter* species at different temperatures of 20, 25, 30, 35, 40 and 45°C from the cassava peel modified HS medium

The result of the quantity of bacteria cellulose produced by *Gluconacetobacter* species at different



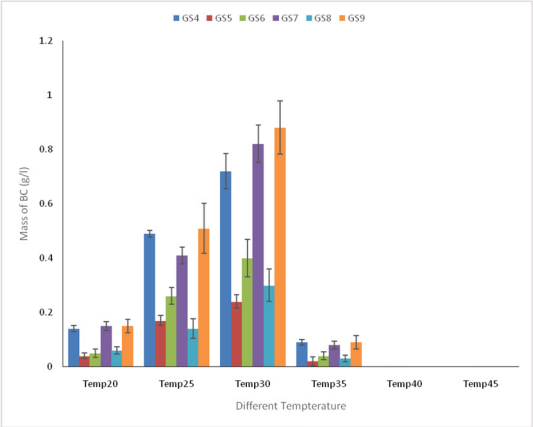
Key: GS= *Gluconacetobacter* species, g/l= gram per liter of medium.

Fig. 1. Mass of bacteria cellulose produced by *Gluconacetobacter* species at different temperatures from the standard HS medium (weight in grams).



Key:KS=Komagataeibacter species.

Fig. 2. Mass of bacteria cellulose produced by *Komagataeibacter* species at different temperatures from the standard HS medium (weight in grams).



Key: GS= *Gluconacetobacter* species, temp = temperature, CMHS = cassava peel modified HS medium.

Fig. 3. Mass of bacteria cellulose produced by *Gluconacetobacter* species at different temperatures of 20, 25, 30, 35 and 45°C from the cassava peel modified Hestrin-Schramm medium.

temperatures from the cassava peel modified medium is presented in Fig. 3.

DISCUSSION

Bacteria cellulose producing acetic acid bacteria isolated from palm wine

The bacteria cellulose producing acetic acid bacteria obtained from the palm wine were *Gluconacetobacter* and the *Komagataeibacter* genera (Tables 1 and 2). This is in line with the findings of Amoa-Awua (2007) which reported that the overnight palm wine (from *Elaisguineensis*) were repository of some acetic acid bacteria with cellulose producing capabilities. All the isolates showed negative for gelatine hydrolysis test. The result of the sugar fermentation test revealed that all the isolates were positive for glucose, sucrose, rhamnose and Mannitol, but varied with other carbohydrates. According to the different test carried out, nine (9) of the isolates belong to the *Gluconacetobacter* genera while three isolates belong to the genera of *Komagataeibacter (*Tables 1 and 2). The screening of the isolated *Gluconacetobacter* and *Komagataeibacter* species for bacteria cellulose production using the standard Hestrin Schramm medium as contained in Tables 3 and 4 showed that maximum yield of bacteria cellulose (0.51 g/250 ml) was obtained from *Gluconacetobacte*r species GS1 while *Komagataeibacter* specie KS3 produced an average of 0.33 g/250 ml of BC. There was no significant difference ($P > 0.05$) in the quantity of cellulose produced by the isolated *Gluconacetobacter* and *Komagataeibacter* species. Keshak and Shamshina (2006) reported maximum cellulose production of 0.2g/250 ml from standard HS medium by *Gluconacetobacter xylinus* ATCC 10245 when cultivated on HS medium under static condition for 7 days. Suwanposri *et al.* (2013) also reported the maximum bacteria cellulose production (0.29g/250 ml) from new *Gluconacetobacter* strain isolated from tropical fruit of Thailand and cultivated in HS medium for 10 days. Jia *et al.* (2017) reported that *Komagataeibacter hansenii* JR-02 produced 2.09g /250 ml from the standard HS medium under static condition which is higher compared to our report of 0.33g / 250ml by *Komagataeibacter* species KS3 and the difference may be attributed to improved culture condition. It was observed that the *Gluconacetobacter* and *Komagataeibacter* species showed highest microbial cell numbers and cellulose production at the temperature of 30°C. There was a significant difference ($P < 0.05$) in cell numbers and bacteria cellulose production at different temperatures investigated. Firdaus *et al.* (2012), reported that *Gluconacetobacter* sp. F6 had maximum cell growth and cellulose production (0.9 g/l) at the temperature of 30°C when grown in standard HS medium. The growth and cellulose production was found to decrease when the temperature was increased or decreased from 30°C. Esin and Halil (2011) reported that the highest yield of bacteria cellulose (0.040 g/l) was obtained at 30°C by *Acetobacter pastorianus* cultivated in HS medium. Kizitalis *et al.* (2015) reported that maximum production of BC (0.15 g/l) by G. xylinum occurred at 28°C using a medium formulated with a residual material originating from pulp mills and lignocellulose biorefineries. Basavaraj *et al*. (2013) reported that maximum temperature for growth and cellulose production by *Gluconacetobacter persimmonis* (GH-2) was 30°C in standared HS medium. The growth and production of cellulose by bacteria cellulose producers at 30°C or slightly around it might be due to the fact that various enzymes and complexes which takes part in the carbon metabolism of bacteria cellulose synthesis has their optimum temperature at 30°C and any deviation in their optimum temperature

will slow down the cellulose production (Rodrigo *et al*. 2018).

CONCLUSION

In conclusion, the results obtained from this study will help design a better strategy for the production of bacteria cellulose. It is feasible to produce bacterial cellulose from cassava peel as an alternative, low cost carbon substrate. A total of nine (9) *Gluconacetobacter* and three (3) Komagataeibacter species that were able to produce BC were isolated from palm wine. Temperature for optimum growth and production of bacteria cellulose by *Gluconacetobacter* and *Komagataeibacter* species were observed to be 30^{o}C and that was the same optimum temperature for the growth of cellulose producing *Gluconacetobacter* and *Komagataeibacter* species. This study will assist immensely in the development of sustainable and economic industrial strategies for modern waste utilization.

REFERENCES

Akpan JF, Eyong MO, Isong IA (2017) The impact of long-term cassava mill effluent discharge on soil pH and microbial characteristics in cross river state. *Asian J Soil Sci Pl Nutrition* 2(1): 1-9.

Amoa-Awua WK, Sampson E, Tano-Debrah K (2007) Growth of veasts, lactic and acetic bacteria in palm wine during tapping and fermentation from felled oil palm (*Elaeis guineensis*) in ghana. *J Appl Microbiol* 102: 599- 606.

Andelib YA, Nuran DA (2009) Isolation of cellulose producing bacteria from wastes of vinegar fermentation. Proc *World Congress Eng Computer Sci* 1: 1- 4.

Ang TN, Ngoh GC, Chua AS (2013) Comparative study of various pretreatment reagents on rice husk and structural changes assessment of the optimized pretreated rice husk. *BioresTechnol* 135: 116-119.

Asien ET, Ikenebomhe MJ (2017) Enhancing the biodegradation process of cassava (*Manihot esculenta* Crantz) peels in dump site. *Nigerian J Biotechnol* 33: 97- 105.

Basavaraj H, Prabhu S, Shetty C, Acharya S, Prabhu V (2013) Production of bacterial cellulose from *Gluconacetobacter persimmonis* GH-2 using dual and cheaper carbon sources. *J Microb Biochem Techno*l 5: 031- 033.

Cheesbrough M (2006) District Laboratory Practices in Tropical Countries. Part 1, 2nd ed. Cambridge University press, South Africa. pp 355 –361.

Cheng Z, Yang R, Liu X (2017) Production of bacteria cellulose by *Acetobacter xylinum* through utilizing acetic acid hydrolysate of bagasse as low cost carbon source. *Biol Re*s 12(1): 1190-1200.

Esin PC, Halil B (2011) Evaluation of different pH for bacteria cellulose production in HS (Hestrin-Schramm) medium and beet molasses medium. *Afr J Microbiol Res* 5 (9): 1037-1045.

Firdaus J, Vinod K, Garma R, Saxana RK (2012) Production of microbial cellulose by a bacterium isolated from fruit. appl *Biochem Biotechnol* 167: 1157-1171

Hong F, Zhu XY, Yang G, Yang XX (2011) Wheat straw acid hydrolysate as a potential cost-effective feedstock for production of bacteria cellulose. *J ChemTechnol Biotechnol* 86(5): 675- 680.

Jia B, Chun BH, Cho GY, Kim KH, Moon JY, Yeo SH (2017) Complete genome sequences of two acetic acid-producing *acetobacter pasteurianus* strains (subsp ascendens LMG 1590^{T} and subsp. *paradoxus* LMG 1591^{T}). *Front Bioeng Biotechnol* 5: 33.

Keshek SM, Sameshima K (2006) Evaluation of Different Carbon sources for bacteria cellulose production. *Afr J Biotechnol* 4: 478 – 482.

Kizitalis EE, Kizitalis A, Gardner DJ (2015) Synthesis of bacteria cellulose using hot water extracted wood sugars. *Carbohydrate Polymers* 124: 131-138.

Ogbo FC, Odo MO (2011) Potential of rice husk and cassava peel as carriers for biofertilizer production. *Nigerian J Biotechnol* 23:1- 4.

Rodrigo JG, Maria FB, Morsylede FR, Wilma AP (2018) Acetic acid bacteria in food industry: Systematics, characteristics and applications. *Food Technol Biotechnol* 56 (2) : 139-151.

Yu X, Atalla RH (1996) Production of cellulose II by *Acetobacter xylinum* in the presence of 2, 6 dichlorobenzonitrile. *Int J Biol Macromol* 19:145 –146.

Martin AN, Ejike EI (2018) Ecophysiology and Production Prin ciples of Cassava (*Manihot* species) in South Eastern Nigeria. Intechopen, pp 1-15.

Suwanposri A, Yukphan P, Yamada Y, Ochaikul D (2013) Identification and biocellulose production of Gluconacetobacter strains isolated from tropical fruits in Thailand *Maejo Int J Sci Technol* 7 : 70 – 82.