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Isolation and characterization of some exopolysaccharide producing bacteria from cassava peel heaps

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ABSTRACT

Eighty (80) bacterial isolates from cassava peel heap samples were tested for their ability to produce biofilm using a modified crystal violet method and the total carbohydrate content of EPSs produced by thirteen (13) strong and medium biofilm formers were determined by the phenol-sulphuric acid method. A significant difference was observed in the carbohydrate values of the EPS produced by the different isolates as compared to the positive control (*Staphylococcus epidermidis*), which produced 0.67 g/L glucose equivalent of EPS; isolate J47 produced the highest (12.76 g/L) EPS, followed by isolates J1 (10.95 g/L), J18 (10.52 g/L) and isolate J2 (10.37 g/L); the lowest EPS quantity was produced by isolate J30 (0.13 g/L). This disparity in the EPS producing ability of these isolate may be dependent on the strains of bacteria in question, as well as the growth environment. Three of the bacterial isolates obtained belong to the genus *Bacillus,* one was identified as *Lactobacillus plantarum* and four of the isolates were *Klebsiella pneumoniae*. Four isolates produced good EPS quantity, with most of the EPS producers being of the *Bacillus* genus.

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Introduction

Cassava (*Manihot esculenta* Crantz) as a staple food possesses many benefits for communities with degraded resource base, unreliable rainfall and feeble market structure. It's being drought tolerant is a characteristic that brands cassava the most apposite food crop for cultivation during drought and famine periods [14]. Nigeria is the world's principal producer of cassava, among other major producers like Indonesia, Thailand, the Democratic republic of Congo and Angola [4].

Processing cassava produces large amounts of waste (both liquid and solid) that, if not appropriately disposed, could produce hygiene and environmental glitches [10]. Ubalua [30] observed that that some cassava processing industries in Ghana reported that almost 28% of the cassava peel produced during '*gari*' production has no usage.

The peel are usually discharged on land or water as wastes and allowed to rot in the open thus resulting in health and environmental hazards. They are either heaped in refuse dump or left lying opening in the municipal. Consequently, soil and vegetation surrounding the heaps of cassava peels are rendered unproductive and distraught, owing to chemical

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and biological reactions going on in the middle of the incessantly fermenting peel heap, the soil, nearby water bodies and vegetation, by this means constituting nuisance to the environment [15].

Cassava mills are one of the main local small scale industries in the southern part of Nigeria. The mills are generally located near where flowing effluent is capable of polluting arable land, fresh water bodies as well as soil around the mill [26]. The increasing supply and demand for cassava in the developing countries has stressed the negative effect of cassava processing and production on environment and biodiversity.

Llamas et al. [24], noted that EPSs of microbial source exist naturally in several habitats and they are vital in biofilm formation, a structure which is engaged in the adherence of cells to surfaces and also in protecting bacteria against harmful environmental influences. Recently, growing consideration is being given these molecules based on their bioactive activity and their broad range of possible uses in pharmaceuticals [2,20], as well as agricultural and numerous other industrial uses [37].Of the numerous EPSs making bacteria, lactic acid bacteria (LAB) have been given special attention [28]. Recently, EPSs produced by LAB received a growing attention due their health benefits to consumers [28]; these advantageous effects include anti-ulcer, antitumor, antioxidant activities, cholesterol lowering activity, as well as immune-stimulating activities [17].

Sourcing and selection of microbes from natural sources is an effective way for finding strains of industrial value [1]. Ayodeji et al. [5] reported abundant bacterial isolates from all *gari* and cassava effluents were Gram positive with some differences in their morphology, catalase and oxidase tests. They reported that 13 of the strains from the effluent matched *Lactobacillus* profile while 4 matched the profile of *Bacillus* sp. with negative catalase and oxidase tests; 16 strains isolated from *gari* showed *Lactobacillus* sp. profile with 2 others showing the profile for *Bacillus* sp. In addition, there were 2 isolates that resembled *Streptococcus* sp. The main objective of this research was to study the bacterial structure of cassava peel heaps and screen the isolates for EPS producing ability.

Methods and materials

Sample collection

Three cassava peel heap samples from cassava processing a factory in Ogbomoso, Nigeria (latitude 8° 6′53.22"N, longitude 4°14′34.50"E); and two samples from the horticultural orchard of Assam Agricultural University, Jorhat, India (latitude 26°43′40.17"N, longitude 94°12′14.54"E) were pooled into two different groups of samples tagged AAG and JHA respectively and used for the present study. Samples were collected at 10–15 cm depth below the surface and transported to the laboratory.

Bacterial isolation

Serial dilution of the samples were carried out after homogenising 10 g of the composite samples in 100 mL sterilized normal saline solution (labelled the stock dilution); with aliquots of 1 mL from stock transferred to 9 mL saline solution and vortexed. 100 µL was spread on plate count agar (PCA) and MRS agar from the fourth and sixth dilution. The plates were incubated for 24 h at 37 °C. Distinct bacterial colonies were picked and streaked continuously on new plates until pure colonies were obtained (Plates 1–3).

Biochemical characters

Catalase test was carried out by adding 2 drops of 3% hydrogen peroxide on the emulsion of each of the isolates made on a clean microscope slide (after 24 h incubation) and examined for the production of oxygen bubbles which indicates the presence of catalase [7]. Citrate, oxidase and motility tests were according to Barrow and Feltham [7] and Murray et al. [21].

Biofilm formation

Eighty bacteria isolates (forty from each sample group) were tested for their ability to produce biofilms using a modified crystal violet method of Shukla and Rao [36]. Isolates were first grown in liquid medium (MRS) overnight and diluted with fresh medium in the microtiter plate at a ratio of 1:10; the plate was then incubated at 37 °C for 48 h. Plates were turned over to dump cells, after this, 125 µL of 0.01% of Crystal violet solution in water was added after washing and the plates were incubated at room temperature for 15 min; after which the plates were rinsed in water, blotted vigorously and turned upside down to dry overnight. Quantification of formed biofilm was done at 492 nm with a HALO MPR-96 Visible Microplate Reader, the buffer readings were deducted from readings as zero blank [19,34] after the addition of 125 µL of 30% acetic acid solution, incubation of the plates at room temperature for 15 min. Isolates were classified into 4 groups: non biofilm

formers, weak biofilm formers, moderate biofilm formers and Strong biofilm formers based on their biofilm forming ability as compared to the negative control (*E. coli* JW0419-1) using the formula of Singh et al. [39]:

i) $OD_{cut} = OD_{avg}$ of negative control + 3 × S.D. of ODs of negative control.
ii) $OD \leq OD_{cut}$ (Non biofilm formers)
iii) $OD_{cut} < OD \leq 2 \times OD_{cut}$ (Weak biofilm formers)
iv) $2 \times OD_{cut} < OD \leq 4 \times OD_{cut}$ (Moderate biofilm formers)
v) $OD > 4 \times OD_{cut}$ (Strong biofilm formers)

Thirteen (13) bacterial isolates classified as strong and moderate biofilm formers were identified and used for the remaining experiments in this research.

$OD_{cut}$was calculated thus:
OD_{avg} of negative control = 0.085
S.D. of ODs of negative control = 0.09
: $0.085 + (3 \times 0.09)$
$= 0.085 + 0.27$
$= 0.355$

Exopolysaccharide production extraction

Qualitative exopolysaccharide production ability of isolates was tested on plate count agar (PCA) supplemented with 0.1% aniline blue [23]. Quantitative EPS production was carried out on thirteen (13) isolates which are strong and moderate biofilm formers, following a modified method of Bajpai et al. [6]. Isolates were cultivated in 500 mL Erlenmeyer flasks with caps, containing 300 mL of MRS broth supplemented with 4% dextrose; with an agitation rate of 130 rpm at 37 °C for 48 h. Each flask was inoculated with approximately 1.12×10^8 cells per 100 mL (v/v) of medium, from overnight grown cultures. Bacterial cells were recovered from the culture medium after given for centrifuge at 8000 rpm for 15 min at 4 °C. EPS was precipitated from the supernatant using a modified method of Sonawdekar and Gupte [29]. 10% tricholoroacetic acid was added to the supernatant and centrifuged at 8000 rpm for 10 min, 4 °C after 30 min incubation in the shaker. Cold absolute methanol was then added at a ratio 2:1 (v/v) and the mixture was left overnight at −20 °C to precipitate EPS. The mixture was centrifuged at 8000 rpm for 20 min at 4 °C and the supernatant was discarded, the debris (EPS) was then air-dried and kept for further analysis. A part of the extracted EPS (for isolates J18 and J47) was purified by dissolving in ultrapure water, and was again precipitated with double volume of chilled ethanol, this step was repeated three times and dialyzed against distilled water for 2 day at 4 °C using 10 kDa dialysis membrane with the changing of the water twice daily and then lyophilized. Freeze-dried EPS was used for LCMS analysis.

Determination of total carbohydrate and protein contents of EPS

The total carbohydrate content of produced EPS was determined by the phenol-sulphuric acid method [12], to 1 mL aliquot drawn from the supernatant after recovery of bacterial cells, was added 1 mL of 6% phenol, 5 mL of conc. H_2SO_4 was added and the mixture vortexed immediately, this was incubated at room temperature for 20 min and absorbance was taken at 480 nm against distilled water as blank in a Spectroquant Prove 300 spectrophotometer (Merck). Glucose standard was prepared and the reagents were added as above. Total protein quality of EPS was resolved following the technique of Bradford (1976) taking the optical density at 280 nm. Protein concentration was resolved using bovine serum albumin (BSA) as a standard.

HPLC analysis of EPS

The exopolysaccharide (0.1 g) was hydrolysed by treating with 1.25 mL of 72% sulphuric acid and was incubated for 60 min at 30 °C. Then 13.5 mL of distilled water was added and placed it in a water bath for 4 h. After 4 h the mixture was cooled and 3.1 mL of 32% sodium hydroxide was added. Then the hydrolysed sample was dissolved in methanol. The EPS were analysed with a high performance liquid chromatography (HPLC) carbohydrate column (Agilent) and eluted with distilled water at a flow rate of 1.0 mL/min at 20 °C. The separated components were monitored by refractive index detector. The EPS after being hydrolysed and derivatives with methanol was analysed for its sugar composition by HPLC (HPLC 1260 Infinity, Agilent Technologies). The column was calibrated with different molecular mass standard and a standard curve was then established.

Genomic DNA isolation and molecular characterization

Genomic DNA isolation was carried out using a modified method of Pearson and Stirling [27]. Molecular characterization was carried out using 16S rRNA gene and a modification of the method of Lemos et al. [22] with U16SF-5≠AGAGTTTGATCMTGGCTCAG-3ánd U16SR-5≠TACGGYTACCTTGTTACGACTT-3ṕrimers. Obtained 16S rRNA gene sequence was

Table 1
Crystal violet biofilm test analysis of bacterial isolates of cassava peel heap samples.

Isolates	Mean	Standard deviation	Isolates	Mean	Standard deviation	Isolates	Mean	Standard deviation
J1	1.80	0.68	J28	1.71	0.21	J55	0.05	0.01
J2	1.06	0.97	J29	0.45	0.13	J56	0.06	0.05
J3	0.24	0.18	J30	1.44	0.25	J57	0.11	0.10
J4	0.30	0.08	J31	0.34	0.22	J58	0.05	0.01
J5	0.23	0.04	J32	0.35	0.11	J59	0.06	0.03
J6	0.34	0.08	J33	0.19	0.07	J60	0.37	0.21
J7	0.18	0.04	J34	0.14	0.05	J61	0.10	0.13
J8	0.16	0.03	J35	0.13	0.02	J62	0.05	0.01
J9	0.16	0.03	J36	0.73	0.07	J63	0.06	0.01
J10	0.27	0.12	J37	0.44	0.03	J64	0.04	0.01
J11	0.16	0.05	J38	0.32	0.05	J65	0.04	0.01
J12	0.24	0.06	J39	0.70	0.06	J66	0.03	0.01
J13	0.21	0.02	J40	1.12	0.13	J67	0.02	0.01
J14	0.44	0.08	J41	1.19	0.07	J68	0.03	0.01
J15	0.95	0.10	J42	1.10	0.16	J69	0.03	0.01
J16	1.86	0.29	J43	0.04	0.01	J70	0.07	0.04
J17	0.49	0.24	J44	0.09	0.02	J71	0.02	0.01
J18	1.20	0.36	J45	0.05	0.02	J72	0.20	0.04
J19	0.43	0.19	J46	0.04	0.02	J73	0.08	0.03
J20	0.17	0.02	J47	1.34	0.75	J74	0.10	0.12
J21	0.22	0.03	J48	0.41	0.15	J75	0.36	0.28
J22	0.36	0.07	J49	0.37	0.08	J76	0.08	0.021
J23	0.22	0.05	J50	0.29	0.06	J77	0.03	0.01
J24	0.44	0.24	J51	0.28	0.11	J78	0.05	0.01
J25	0.15	0.02	J52	0.10	0.01	J79	0.23	0.03
J26	1.06	0.78	J53	0.38	0.04	J80	0.09	0.09
J27	0.43	0.06	J54	0.44	0.12	Control	0.08	0.09

[a]Strong and moderate biofilm producers in red. Isolates J1-J40 were obtained from cassava peel heap samples from Ogbomoso, Nigeria; while isolats J41-J80 were obtained from cassava peel heap samples from Jorhat.

Table 2
Bacterial isolates of cassava peel heaps samples grouped based on biofilm formation.

Groups	Number of isolates	Biofilm yield range	Percentage (%)
Strong biofilm formers	4	>1.42	5.0
Moderate biofilm formers	9	0.72-1.42	11.25
Weak biofilm formers	15	0.356-0.71	18.75
Non-biofilm formers	52	≤ 0.355	65.0

compared with those of other bacteria in GenBank by engaging the BLAST program (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to define the phylogenetic affiliation of the isolates. A neighbour-joining phylogenetic tree based on the16S rRNA gene sequences of the isolates and other related bacterial strains was constructed using MEGA 7.0 software.

Data availability: All data generated or analysed during this study are included in this article.

Results and discussion

Biofilm production ability of the isolate ranged from 0.02 (J67 and J71) to 1.86 (J16) at 492 nm. Using the criteria of Singh et al. [39], only four (4) isolates were classified as strong biofilm formers (J1, J16, J28 and J30) with a production ability of 1.80, 1.86, 1.71 and 1.44 respectively. Nine (9) isolates were classified as moderate biofilm formers (J2, J15, J18, J26, J36, J40, J41, J42 and J47), giving yields of 1.06, 0.95, 1.20, 1.06, 0.73, 1.12, 1.19, 1.10 and 1.34 respectively. Other isolates were grouped into weak biofilm formers (J14, J17, J19, J22, J24, J27, J29, J37, J39, J48, J49, J53, J54, J60 and J75) and non-biofilm formers (Table 1). A very high number of the isolates belong to the non-biofilm formers with 65% of all, while strong biofilm formers had the lowest percentage of all (5%) the isolates (Table 2). Phillips (2016) has earlier reported that biofilm may compose of a single species or mixed species- which is more commonly found in the natural environment. He also stated that biofilms afford a number of bacterial species with a common machinery of persistence, particularly in food processing environments; Costerton et al. [9] posited that the genes which allow for the development of biofilm are activated when enough cells attach to a solid surface, suggesting that attachment itself is major to the synthesis of the extracellular matrix wherein the sessile bacteria are embedded. Zottola and Sasahara [38] opined that biologically viable bacteria attach to solid surface and stick to

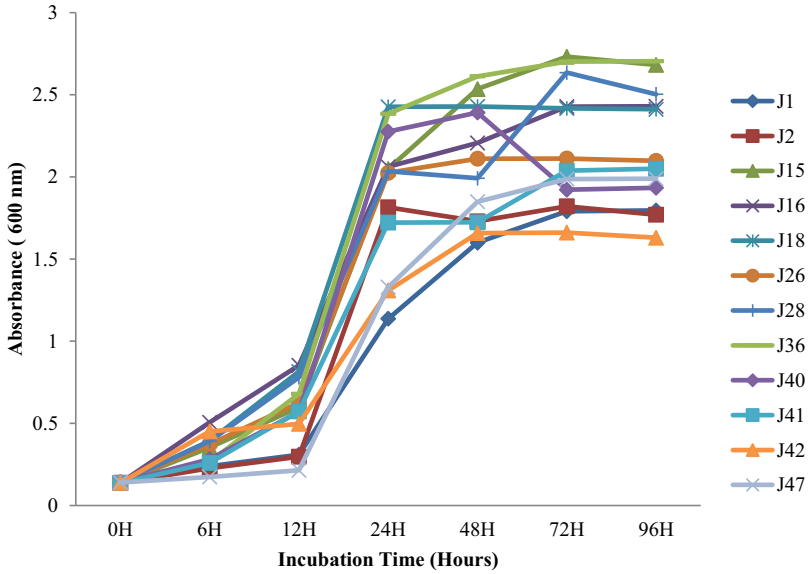

Fig. 1. Growth pattern of EPS producing bacteria isolated from cassava peel heaps at different times of incubation.

it, biofilm formation come to being when these solid surfaces come in contact with different kinds of liquids; these biofilms may be advantageous or harmful to the environment wherein they formed.

A significant difference was observed in the carbohydrate values of the EPS produced by the different isolates when compared with the positive control (*Staphylococcus epidermidis*), which produced 0.67 g/L EPS of glucose equivalent. Isolate J47 produced the highest (12.76 g/L) EPS, followed by isolates J1 (10.95 g/L), J18 (10.52 g/L) and J2 (10.37 g/L) respectively; all these were significantly different from the quantity of EPS produced by the other isolates, J15 (0.55 g/L), J16 (0.51 g/L), J26 (0.82 g/L), J28 (3.37 g/L), J30 (0.13 g/L), J36 (0.82 g/L), J40 (0.82 g/L) and J42 (1.21 g/L); some of these are greater than what Vijayabaskar et al. [32] reported, who recorded total carbohydrate to be 0.91 mg/100 ml and 0.43 mg/100 ml for *Bacillus subtilis* in basal medium and malt medium respectively. This disparity may be dependent on the strains of bacteria in question, as well as the growth environment. Although after J47, J1 had the highest EPS quantity produced, its ability was not significantly different from that of isolates J2 and J18 respectively (Table 4) according to the Duncan's multiple range test at $p \leq 0.05$.

The total protein content of the isolates' EPS is reported is Table 5. Based on the analysis of variance, significant difference was observed in the protein content as matched to the control. Protein content equivalent of bovine serum albumen (mg/mL) for the thirteen bacterial isolates were recorded as 6.95, 4.75, 7.95, 7.31, 7.64, 8.66, 10.55, 9.08, 7.65, 9.04, 8.54, 8.64 and 7.25 respectively; with isolates ***Bifidobacterium animalis* J28** having the highest and isolate ***B. subtilis* J1** having the lowest protein content. Metzger et al. [25] earlier reported that protein content of EPS obtained using the NMR approach was about 30% greater than the one resolved with the Lowry biochemical technique, both for *P. putida* and *A. pullulans*. He also noted that the typical protein carbon content as found out using the NMR approach was roughly 70% of the total carbon content observed.

Some of the compounds detected in the EPS produced by Isolates (Table 6) J18 include flavonoids like Catechin and Epicatechin. Kim et al. [18] reported the examination of secondary metabolites in liquid cultures of a *Hasllibacter halocynthiae*, and was able to isolate two cholic acid derivatives with their structures resolved as 3,3,12-trihydroxy-7-ketocholanic acid and 3,3,12-trihydroxy-7-deoxycholanic acid. Yamagunchi et al. [33] reported the isolation of Trichoderonic acids A and B, a novel terpenoids, as well as (+)-heptelidic acid from a fungus

Results of the growth studies of the isolates revealed some of the isolates (J2, J16, J18, J26, J36, J40 and J41) have their log phase between 12 h of incubation and 24 h of incubation while some delayed until 48 h of incubation (Fig. 1). The highest absorbance (nm) for the isolates was recorded between 24 h and 96 h of incubation with all-time highest shown by J15 at 72 h of incubation (2.732), which was equivalent to 2.19×10^9 cells per mL; this agrees with the result of Dhingra and Chaudhary [11], who observed that *B. thuringiensis* isolate reached maximum growth after 48 h of inoculation. The highest cell density of 2.19×10^9 cells per mL was shown by J15 at 72 h of incubation. However, the growth pattern failed to follow the conventional growth curve; Burdett *et al* [8] had earlier noted a significant deviation from a strictly exponential curve proportional to cell size during his study on growth kinetics of individual *B. subtilis* cells.

Results of the 16S rRNA gene sequencing of the isolates and their accession numbers with NCBI revealed that three of the isolates belonged to the genus *Bacillus* and were identified as *Bacillus* sp. (J1); *B. subtilis* (J2) and *Bacillus amyloliquefaciens*

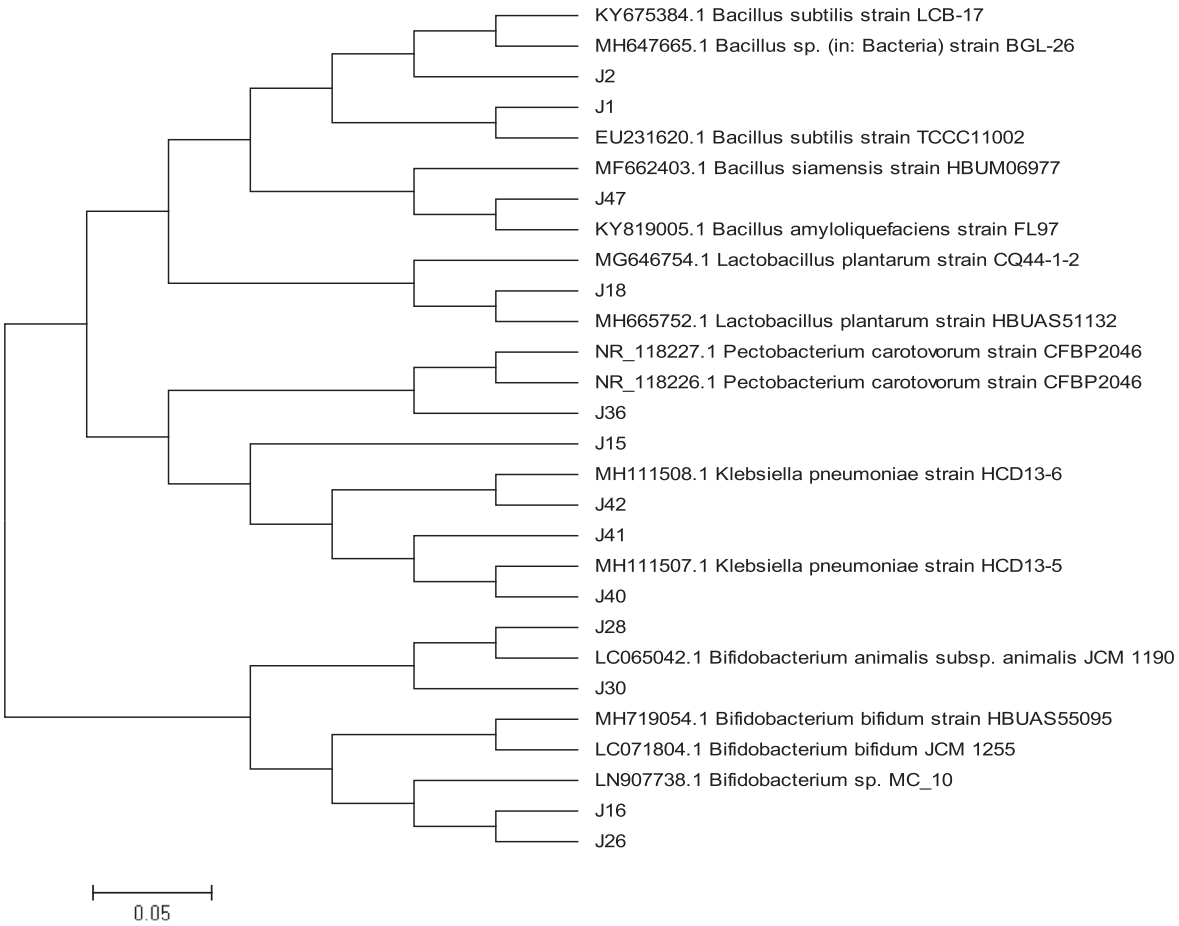

Fig. 2. Molecular phylogenetic analysis of isolates by Maximum Likelihood method.

(J47) with the NCBI blast; four isolates were identified as *Klebsiella pneumoniae* (J15, J40, J41 and J42);; four isolates belonged to genus *Bifidobacterium* (J16, J26, J28 and J30); while one isolate each were identified as *Lactobacillus plantarum* (J18) and *Pectobacterium carotovorum* (J36); Arotupin [3] had earlier reported the isolation of bacteria such as *Aerococcus viridens*, *B. subtilis, Bacillus* sp., *Corynebacterium manihot* and *Lactobacillus acidophilus* from cassava waste water; while Eze and Onyilide [13] isolated *Klebsiella* sp., *Staphylococcus aureus, Escherichia coli*, *Pseudomonas* sp., *Enterobacter* sp., *Bacillus* sp., and *Proteus* sp. from soil receiving cassava effluent (Figs. 2 and 3).

The result of high performance liquid chromatography carried out on the EPS with the best four total sugars (J1, J2, J18 and J47) revealed the presence of glucose as the main sugar component of J1; fructose as the main sugar component of J2; xylose, fructose and ribose as the components of J18, while ribose and sucrose are the components of J47 (Table 8). Hong et al. [16], reported that the major sugar component of biofilm-forming marine bacterium 98TH11317 were glucose and galactose. Sebastião et al. [35] noted that polysaccharides of F. columnare and A. hydrophila bacterial culture filtrates were all hetero-polysaccharides, with different proportions of neutral sugars and uronic acids. Veiga et al. [31] also reported that the major sugar components of EPS produced by *Methanobacterium formicicum* were made up of rhamnose, fucose, mannose, galactose, glucose, glucosamine, galactosamine, and mannosamine sugar components, with trace amounts of ribose (Tables 3 and 7).

Conclusion

As revealed by our study, thirteen bacterial isolates were good biofilm formers, out of which four produced good EPS quantity, with most of the EPS producers being of the *Bacillus* genus. The presence of *K. pneumoniae* indicated that the cassava peel heaps harbour some bacteria of public health importance to man.

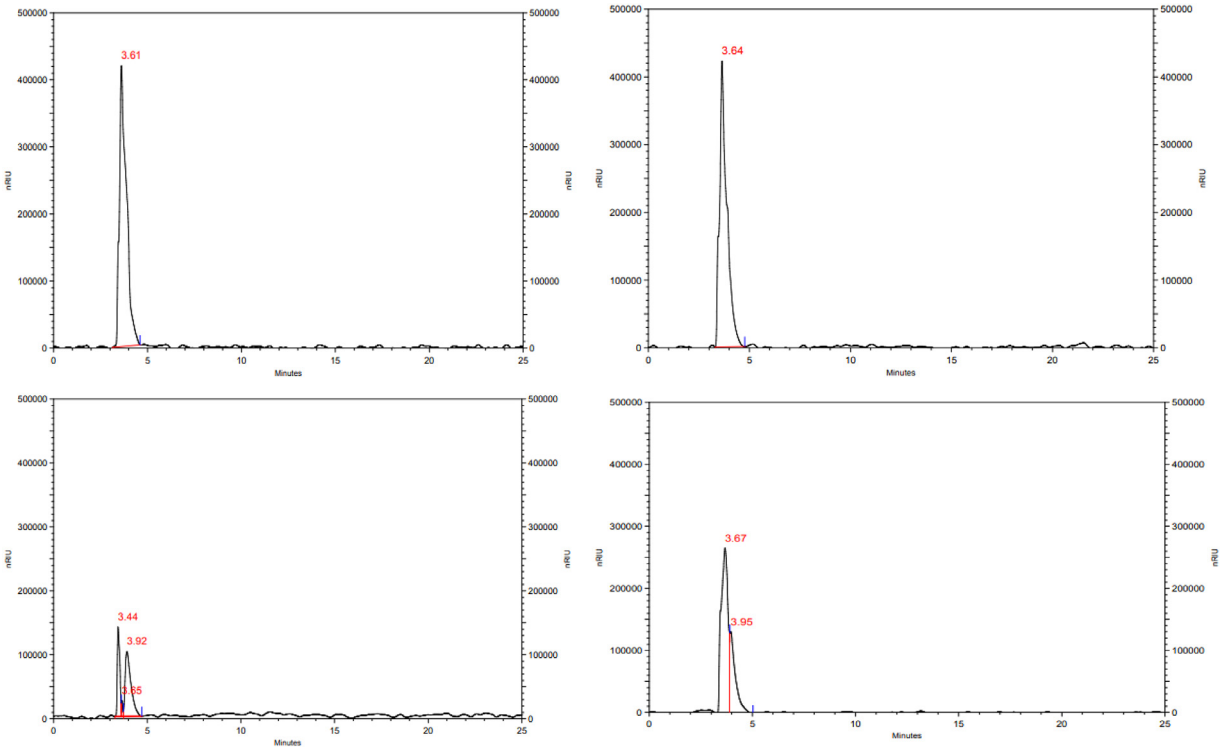

Fig. 3. Chromatograms of EPS produce by isolates J1 (top left), J2 (top right), J18 (bottom left) and J47 (bottom right) respectively.

Plate 1. Photo of a cassava peel heap (Nigeria).

Table 3
Geographic distribution of strong and medium biofilm former.

Isolate	Class of biofilm formation	Isolate origin
J1	Strong biofilm former	Ogbomoso
J2	Medium biofilm former	Ogbomoso
J15	Medium biofilm former	Ogbomoso
J16	Strong biofilm former	Ogbomoso
J18	Medium biofilm former	Ogbomoso
J26	Medium biofilm former	Ogbomoso
J28	Strong biofilm former	Ogbomoso
J30	Strong biofilm former	Ogbomoso
J36	Medium biofilm former	Ogbomoso
J40	Medium biofilm former	Ogbomoso
J41	Medium biofilm former	Ogbomoso
J42	Medium biofilm former	Jorhat
J47	Medium biofilm former	Jorhat

Plate 2. Isolates showing EPS production ability on MRS agar supplemented with aniline blue.

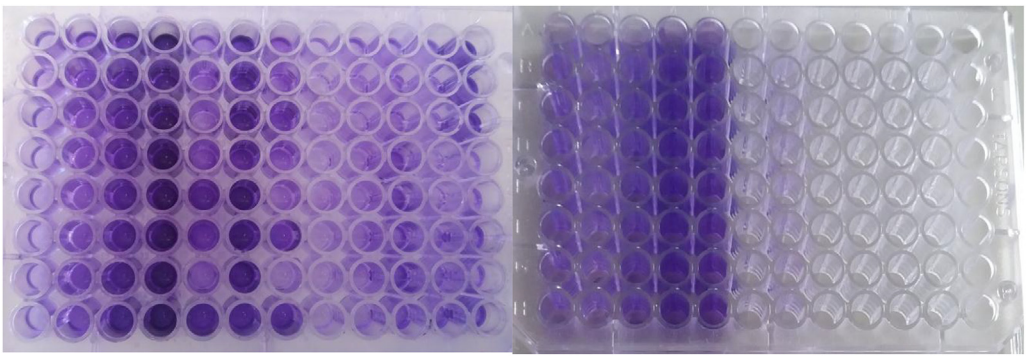

Plate 3. Biofilm production by isolates in microtiter plates.

Table 4
Total carbohydrate content of exopolysaccharides produced by the strong and moderate biofilm formers isolated from cassava peel heaps.

Isolates	Mean (g/L)	Standard deviation	Standard error
J1	10.95[d]	0.37	0.21
J2	10.37[d]	0.56	0.32
J15	0.55[b]	0.09	0.05
J16	0.51[b]	0.06	0.04
J18	10.52[d]	0.42	0.24
J26	0.82[c]	0.03	0.01
J28	3.37[b]	0.35	0.20
J30	0.13[a]	0.03	0.02
J36	0.82[c]	0.06	0.04
J40	0.82[c]	0.02	0.01
J41	0.78[c]	0.01	0.01
J42	1.21[a]	0.07	0.04
J47	12.76[e]	0.03	0.02
Control	0.67[bc]	0.07	0.05

[a] Mean followed by the same superscripts are not significantly different according to Duncan's Multiple Range Test ($p \leq 0.05$).

Table 5
Protein content of produced EPS.

Isolates	Protein content (equivalent of BSA) mg/mL
J1	6.95 ± 0.017[b]
J2	4.75 ± 0.012[a]
J15	7.95 ± 0.010[e]
J16	7.31 ± 0.027[bc]
J18	7.64 ± 0.010[cd]
J26	8.66 ± 0.003[ef]
J28	10.55 ± 0.004[g]
J30	9.08 ± 0.005[h]
J36	7.65 ± 0.002[cd]
J40	9.04 ± 0.006[ef]
J41	8.54 ± 0.007[e]
J42	8.64 ± 0.006[ef]
J47	7.25 ± 0.001[bc]
Control	7.79 ± 0.014[d]

Value = mean of triplicates ± standard error tailed with identical superscripts in the columns remain not significantly dissimilar based on Multiple Range Test of Duncan ($\rho \leq 0.05$).

Table 6
Compounds observed in the LCMS of the EPS with highest total sugars.

J18	J47
(+/−)-Catechin	(+/−)-Catechin
(-)-Epicatechin	Procyanidin B1
(+/−) Salsolinol	-7-Hydroxy-3-(sulfooxy)cholan-24-oic acid
10-Methoxycarbamazepine	S,R)-Noscapine
2′,3′,6-Trimethoxyflavone	3′,4′,5,7-Tetramethylquercetin
2-O-Methylguanosine	Eicosapentaenoic acid
(Z)-3-Hydroxyoctadec-7-enoic acid	4-Methylthiobutyl glucosinolate
11,12-epoxy-5Z,8Z,14Z-eicosatrienoic acid	
2′-Deoxyadenosine-5′-monophosphate	
3-Ketocholanic Acid	
10-Acetyl-trichoderonic-acid	
2′-Hydroxygenistein 8-C-glucoside	
Isorhoifolin	

Table 7
Identification and accession numbers of moderate and strong biofilm producers isolated from cassava peel heaps.

Lab Identity	Isolate	Accession number
J1	*Bacillus* sp.	MH634685
J2	*Bacillus subtilis*	MH656722
J15	*Klebsiella pneumoniae*	MH634686
J16	*Bifidobacterium biavatii*	MH656723
J18	*Lactobacillus plantarum*	MH634689
J26	*Bifidobacterium* sp.	MH634690
J28	*Bifidobacterium* sp.	MH634691
J30	*Bifidobacterium* sp.	MH634692
J36	*Pectobacterium carotovorum*	MH634693
J40	*Klebsiella pneumoniae*	MH634694
J41	*Klebsiella pneumoniae*	MH634695
J42	*Klebsiella pneumoniae*	MH634696
J47	*Bacillus amyloliquefaciens*	MH634697

Table 8
Sugars present in exopolysaccharides produced by moderate and strong biofilm producers isolated from cassava peel heaps.

Sample	Suspected sugar(s)
J1	Glucose
J2	Fructose
J18	Xylose, Fructose, Ribose
J47	Ribose, Sucrose

Conflict of interest

The authors declare that there is no conflict of interest as regarding this research work.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.sciaf.2019.e00093.

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