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Screening For Potential Exopolysaccharide Producers From *Lactobacillus* spp Isolated From Locally Fermented Milk (*Nono*)

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Abstract

Exopolysaccharides (EPS) are exogenous bacterial sugar polymers with many applications in dairy, pharmaceutical and cosmetic industries, using it as thickeners, stabilizers and gelling agents. The study aimed to screen for potential exopolysaccharide producers from Lactobacillus spp. isolated from locally fermented milk (nono). Twenty-five nono Samples were collected from Wambai market, Kano. Lactic acid bacteria were isolated using de Man Ragosa and Sharpe Agar. Isolates were identified by API 50 CHL kit and web, and screened for EPS production in which the EPS was extracted and quantified using the phenol-sulphuric method. Next, the influence of carbon source (Glucose, Sucrose and Lactose) and concentrations on EPS were evaluated on some of the isolated strains. The functional groups of the EPS were confirmed using FTIR. The isolated Lactobacillus spp. were all Gram positive, catalase and oxidase negative, API identification yielded; Lactobacillus acidophilus 1, Lb. brevis 1, Lb. fermentum, Lb. paracasei ssp paracasei, Lb. acidophilus 3. Ten isolates yielded EPS in the range of 248.33mg/l - 07.83mg/l. The FTIR analysis of extracted EPS produced peaks around 3,300-881cm^{-1}. Hence the study has brought to light the presence of potential EPS producing LAB in nono, which could be further exploited to harness their potential.

Keywords: Exopolysaccharide, Lactic acid Bacteria, Lactobacillus spp, Kano

INTRODUCTION

Lactic Acid Bacteria (LAB) are widely distributed in nature and occur in soil, water, manure, sewage, plant materials, gastrointestinal tract and cereals, to mention a few. Naturally, they occur as indigenous microflora of raw milk, where they are responsible for converting milk into different fermented end products such as yoghurt, cheese, *gariss, rob, chal, katyk, laban,* and *nono* (Khalil and Anwar, 2016). *Nono* is a spontaneously fermented yoghurt-like milk product consumed as a staple food commodity in some parts of northern Nigeria and Sub-Saharan West Africa . In Nigeria, *nono* is produced, hawked and sold by Fulanis', commonly Fulfulde, an ethnic group in northern Nigeria. *Nono* is made from fresh cow milk, mostly collected and processed through traditional methods. The method involves collecting the milk directly by massaging the cows' mammary into containers, usually calabashes, and then some quantity of overnight fermented milk is added to serve as a starter culture. The inoculated fresh milk is left overnight at room temperature for fermentation to occur.

Nono usually has a sharp sour, acidic taste (Okiki *et al*., 2018). Lactic acid bacteria are involved in the fermentation of milk to *nono*. LAB are Gram-positive, catalase-negative, fastidious, non respiring, non sporulating rods, cocci or coccobacilli that produce lactic acid as the major product of fermentative metabolism (Shabana *et al*., 2013). They grow best under anaerobic conditions but can also grow in microaerophilic and aerobic environments (Khalil and Anwar, 2016). Some Lactic Acid Bacteria (LAB) can produce extracellular polysaccharides that occur as cell wall constituents (peptidoglycan). EPS are long-chain polysaccharides consisting of branched repeating units of sugars or sugar derivatives. These sugar units are mainly glucose, galactose, and rhamnose in a different ratio. EPS are secreted into their surrounding environment during growth or are loosely associated with the cell surface via electrostatic interactions, often forming a slime layer; thus, they are not attached permanently to the surface of the microbial cell (Ruas-madiedo and Reyes-Gavilan, 2005; Zeidan *et al*., 2017). EPS produced by LAB can be classified into two groups based on the monomer composition: homoploysaccharide (example; cellulose dextran, mutan, alteran, pulluan, levan and curdlan) and heteropolysaccharides (gellan and xanthan) (Welman and Maddox, 2003).

LAB-produced EPS in fermented food helps improve products' structure, texture, mouth feel and viscosity without impacting their taste. Conversely, some EPS produced by LAB present potential health-beneficial properties such as immune stimulation, antiulcer and cholesterol-lowering activities. *Lactobacillus* spp and their exopolysaccharide have significant economic and therapeutic potential for the development of nutrient-rich functional food products with prolonged human health beneficial effects (Chelule *et al.*, 2010).

Lactobacillus spp that can produce exopolysaccharides might be present in indigenously fermented foods such as *nono,* but little is known locally about them. Likewise, indigenous LAB EPS formation information is meagre and scantily described (Adebayo-tayo and Onilude, 2008). Studying these microorganisms and their EPS will aid in developing and enhancing local strains for application in the local food and other related industries and also help preserve indigenous *Lactobacillus* spp. (Bajpai *et al.*, 2016a).

MATERIALS AND METHODS

Isolation and Identification of *Lactobacillus* spp.

Twenty-five samples of *nono* were collected from Wambai market in Kano, located at 12˚00'64" and longitude 8˚52'62" North-West Nigeria. Twenty-five (25 mL) of *nono* sample was homogenized with 225 mL of sterile peptone water and serially diluted up to 10^{-8}. One mL was transferred in duplicate from the serially diluted test tubes into sterile, appropriately labelled Petri dish. This was followed by the addition of molten de Man Ragosa and Sharpe Agar (MRS;Titan Media,India) fortified with 100 mgL^{-1}of cyclohexamide to prevent fungal growth. The inoculated MRS plates were placed in anaerobic jars containing disposable gas generating pack (AnaeroGen: Thermo scientific) and incubated at 37ºC. Pure discrete colonies were obtained by successive streaking and subculture (Azadnia and Khan, 2009). The pure isolates were subjected to gram staining, catalase and oxidase test. Gram-positive, catalase and oxidase-negative isolates capable of EPS production were identified using API CHL 50 kit (Biomerieux SA, France). The results obtained were analyzed using API web.

Screening of Isolates for Exopolysaccharide Production

Fresh cultures from overnight incubation were inoculated into MRS broth fortified with 1 g/L of sucrose and incubated for 24 hours at 37˚C. After incubation, 1.5 mL of the culture was centrifuged at 5000g for 10 mins. One (1) ml from the supernatant was collected and dispensed into a fresh test tube, adding 1ml of 95% ethanol. The presence of an opaque line at the boundary of the two mixtures indicates a positive result (Akabanda *et al.*, 2014).

Extraction and Quantification of Exopolysaccharide

The method of Bajpai *et al.* (2016b), for extraction, isolation and purification of exopolysaccharides from Lactic acid bacteria was adopted. Pure isolates of the test organism were cultured in MRS broth supplemented with 10 grams of glucose per litre at 37˚C for 24hours. After incubation, the culture was centrifuged at 8,000g for 20mins at 4˚C. The supernatant was collected and mixed with 14% trichloroacetic acid to denature the protein content. The pre-mentioned mixture was allowed to homogenize on a shaker for 30 min at 90rpm. This was followed by centrifugation for 20mins at 8,000g at 4˚C. Two-fold volumes of absolute cold ethanol were added to the collected supernatant. This was kept at 4˚C for 24 hours in order to precipitate the exopolysaccharide (EPS). The EPS precipitate was recovered by centrifugation at 4˚C at 8,000g for 20mins. The collected precipitate was dissolved in sterile deionized water and purified in a dialysis membrane (Solarbio Biotech. Ltd, China; molecular weight cut-off, 8-14kDa) for 2 days. The EPS yield was determined by the phenol-sulphuric acid method according to Dubois *et al.* (1956); Chun-lei *et al.*, (2014).

Effect of Different Carbon source and Concentrations on EPS Production

The three high yielding exopolysaccharide producing isolates; *Lb. acidophilus 1, Lb. brevis 1* and *Lb. fermentum 1* were cultured in MRS broths fortified with different carbon sources. These were Glucose, Lactose and Sucrose. The concentrations of these sugars were varied; 5%, 10%, 15%, and 20%. The pH of the growth medium was adjusted and fixed at pH 6.5±0.2. This was shared into two unequal portions of 10mL and 90mL. A single colony of the isolate was inoculated into the 10ml broth and incubated for 24h at 40ºC.

Following inoculation, the 10ml inocula were completely transferred into the 90ml broth; then incubated at 40ºC for 36h. After which EPS was extracted and quantified as earlier described, according to the method of Chun-lei *et al.*, (2014).

Confirmation of EPS Functional Group Using Fourier Transform Infrared Spectroscopy (FTIR)

The Major functional groups of the purified EPS were detected using Fourier transform infrared spectroscopy. The extracted EPS were recorded on an Agilent Technologies FTIR machine at the Department of Biochemistry, Bayero University Kano. The FTIR spectrum was determined in the region of 4000 – 400cm^{-1} transmission mode, and the number of scans was 32. The infrared spectral resolution was obtained at 8cm^{-1}.

Statistical Analysis

All experiments were performed in triplicate and were reported as means of ± standard deviation.

Significant differences among optimized EPS samples were evaluated using the statistical tool of two factor analysis of variance (ANOVA). The statistical significance of the relationship was analyzed at 95% confidence level.

RESULTS

Lactobacillus spp were distinguished into species based on their ability to ferment different sugars, using API 50CHL system (Table 1). The isolated *Lactobacillus* spp were identified as *Lb. acidophilus 1, Lb. brevis 1, Lb. fermentum* 1, *Lb. paracasei* subsp. *paracasei* and *Lb. acidophilus* 3, *Lb. delbruekii* subsp. *delbruekii*, *Lb. pentosus*, *Lb. helveticus Lb. plantarum*1, *Lb. plantarum* 2 respectively.

Results presented in Table 2 shows that ten (10) isolates out of 136 *Lactobacillus* spp screened were capable of EPS production. The isolated *Lactobacillus* spp produced EPS in the range 248.33 – 07.83 mg/L in MRS broth. Table 3 gives the different Exopolysaccharide yields obtained from isolates using different carbon sources (Glucose, Lactose and Sucrose) at concentrations of 5% - 20%. Statistically, there is a significant difference ($P>0.05$) with regards to the EPS yield in relation to the interaction between carbon source and concentration.

Fourier Transform Infrared Spectroscopy (FTIR) was employed to characterise the extracted polysaccharide. The mid-infrared region (4000 – 400cm^{-1}) spectra were used to determine the bands of the functional groups of the samples. The spectra obtained showed different bands between 400cm^{-1} to 3,500cm^{-1}, a characteristic of exopolysaccharides. The fingerprint region of polysaccharides is indicated by the presence of peaks between 1,500 - 950cm^{-1} region (Figures 1 - 3).

Table 1: Sugar Fermentation Profile (API) of Isolates

S/NO	Carbohydrates	A	J	S	L	N	H	D	Y	R	B
1.	Control	-	-	-	-	-	-	-	-	-	-
2.	Glycerol	-	-	-	-	-	-	+	-	-	-
3.	Erythirol	-	-	-	-	-	-	-	-	-	-
4.	D - arabinose	-	-	-	-	-	-	-	-	-	-
5.	L - arabinose	-	+	-	-	-	-	+	-	-	-
6.	Ribose	-	+	+	+	-	+	+	-	+	+
7.	D - xylose	-	+	-	-	-	-	+	-	-	-
8.	L - xylose	-	-	-	-	-	-	-	-	-	-
9.	Adonitol	-	-	-	+	-	-	-	-	-	-
10.	ß-metil - D - xyloside	-	-	-	+	-	-	-	-	-	-
11.	Galactose	+	+	+	+	-	-	+	+	-	+
12.	D - glucose	+	+	+	+	+	-	+	+	+	+
13.	D - fructose	+	+	+	+	+	+	+	+	+	+
14.	D - mannose	+	+	-	+	+	+	+	+	+	+
15.	L - sorbose	-	-	-	-	+	+	-	-	+	-
16.	Rhamnose	-	-	-	-	-	-	-	-	-	-
17.	Dulcitol	-	-	-	-	-	-	-	-	-	-
18.	Inositol	-	-	-	-	-	-	-	-	-	-
19.	Manitol	-	+	-	+	-	-	+	-	-	+
20.	Sorbitol	-	-	-	+	-	-	+	-	+	-
21.	α-methyl-D-mannosidse	-	-	-	-	-	-	-	-	+	-
22.	α-methyl-D-glucoside	-	-	-	+	-	-	+	-	-	-
23.	N-acetyl-glucosamine	-	+	-	+	+	-	+	+	-	+
24.	Amigdalin	-	+	-	+	-	-	+	-	-	+
25.	Arbutin	-	+	-	+	-	-	+	-	-	-
26.	Esculin	+	+	-	+	+	-	+	-	-	+
27.	Salicin	+	+	-	+	-	-	+	-	-	-
28.	Cellobiose	+	+	-	+	-	-	+	-	-	+
29.	Maltose	+	+	+	+	-	+	+	-	-	+
30.	Lactose	+	+	+	-	+	-	+	-	-	+
31.	Melibiose	+	+	+	-	+	-	+	-	-	-
32.	Saccharose	+	+	+	+	+	+	+	-	-	-
33.	Trehalose	+	+	-	+	+	-	+	-	-	-
34.	Inulin	-	-	-	+	-	-	-	-	-	-
35.	Melezitose	-	-	-	+	-	-	-	-	+	-
36.	D-raffinose	-	+	+	-	-	-	+	-	+	-
37.	Amidon	-	-	-	-	+	-	-	-	-	-
38.	Glycogen	-	-	-	-	+	-	-	-	-	-
39.	Xylitol	-	-	-	-	-	-	-	-	-	-
40.	ß-gentiobiose	+	+	-	+	-	-	+	-	+	+
41.	D -turanose	-	-	-	+	-	-	+	-	+	-
42.	D - lyxose	-	-	-	-	-	-	-	-	-	-
43.	D - tagarose	-	-	-	+	-	-	-	-	-	-
44.	D - fuccose	-	-	-	-	-	-	-	-	-	-
45.	L - fuccose	-	+	-	-	-	-	-	-	-	-
46.	D - arabitol	-	-	-	-	-	-	-	-	-	-
47.	L - arabitol	-	-	-	-	-	-	-	-	-	-
48.	Gluconate	-	+	-	+	-	-	+	-	+	-
49.	2 - keto - gluconate	-	-	-	-	-	-	-	-	-	-
50.	5 - keto - gluconate	-	-	-	-	-	-	-	-	-	-

KEY: A = *Lb.acidophilus 1, J = Lb. brevis 1, S = Lb. fermentum* 1, *L = Lb. paracasei subsp.paracasei* and N = *Lb. acidophilus* 3, H = *Lb. delbruekii subsp. delbruekii*, D = *Lb. pentosus*, Y= *Lb. helveticus* R= *Lb. plantarum*1 B=*Lb. plantarum* 2

Table 2: Exopolysaccharide (EPS) Production Capacity of the Isolates

No of isolates	EPS producers	Non EPS producers	Cultural characteristics	Morphology
20	02	18	Transverse Cream colour and shiny surface	Coccobacilli
84	05	79	Raised circular cream coloured and wet surface	Rods (*Bacilli)* of short to medium height
32	03	29	Cream coloured and shiny surface	Long rods (*Bacilli*)
136	10	126	Total	

KEY: A = *Lb. acidophilus 1, J = Lb. brevis 1, S = Lb. fermentum 1, L = Lb. paracasei* subsp. *paracasei* and N = *Lb. acidophilus* 3, *H= Lb. delbruekii* subsp. *delbruekii, D= Lb. pentosus, Y= Lb. helveticus, R= Lb. plantarum 1, B= Lb. plantarum 2*

Table 3: Quantity of Exopolysaccharide (EPS) Produced by the Isolated Strains

Isolates	EPS (mg/l) ±SD
A	248.33±0.31
J	219.40±0.20
S	200.57±0.25
L	147.60±0.30
N	201.63±0.35
H	111.80±0.30
D	31.00±1.00
Y	37.20±0.60
R	68.23±0.22
B	07.83±0.15

Table 4: Quantity of Optimised EPS (mg/L) Produced by Isolated Local Strains

		CARBON CONCENTRATION (%)			
Isolates	**Carbon Source**	**5**	**10**	**15**	**20**
A	**GLU**	252.0 ± 0.06^{h}	331.2 ± 0.05^{fg}	363.6 ± 0.04^{def}	401.4 ± 0.02^{bc}
	LAC	322.2 ± 0.04^{g}	361.8 ± 0.06^{def}	378.0 ± 0.04^{cd}	414.0 ± 0.03^{ab}
	SUC	343.8 ± 0.05^{efg}	379.8 ± 0.07^{cde}	394.2 ± 0.05^{bcd}	439.2 ± 0.03^{a}
J	**GLU**	225.0 ± 0.05^{h}	246.6 ± 0.03^{g}	280.8 ± 0.02^{e}	324.0 ± 0.05^{c}
	LAC	252.0 ± 0.08^{fg}	298.8 ± 0.07^{d}	320.4 ± 0.03^{c}	369.0 ± 0.03^{a}
	SUC	264.6 ± 0.03^{f}	304.2 ± 0.05^{d}	347.4 ± 0.03^{b}	374.4 ± 0.02^{a}
S	**GLU**	203.4 ± 0.04^{cde}	212.4 ± 0.03^{cd}	221.4 ± 0.06^{c}	253.8 ± 0.02^{a}
	LAC	185.4 ± 0.04^{fg}	192.6 ± 0.02^{ef}	216.0 ± 0.03^{c}	237.6 ± 0.02^{b}
	SUC	178.2 ± 0.04^{g}	201.6 ± 0.02^{de}	210.6 ± 0.05^{cd}	221.4 ± 0.03^{c}

Key: A = *Lb. acidophilus 1, J = Lb. brevis 1, S = Lb. fermentum 1;* GLU = Glucose, LAC = Lactose, and SUC = Sucrose

*Means along columns with different superscript are statistically different at 95% confidence level.

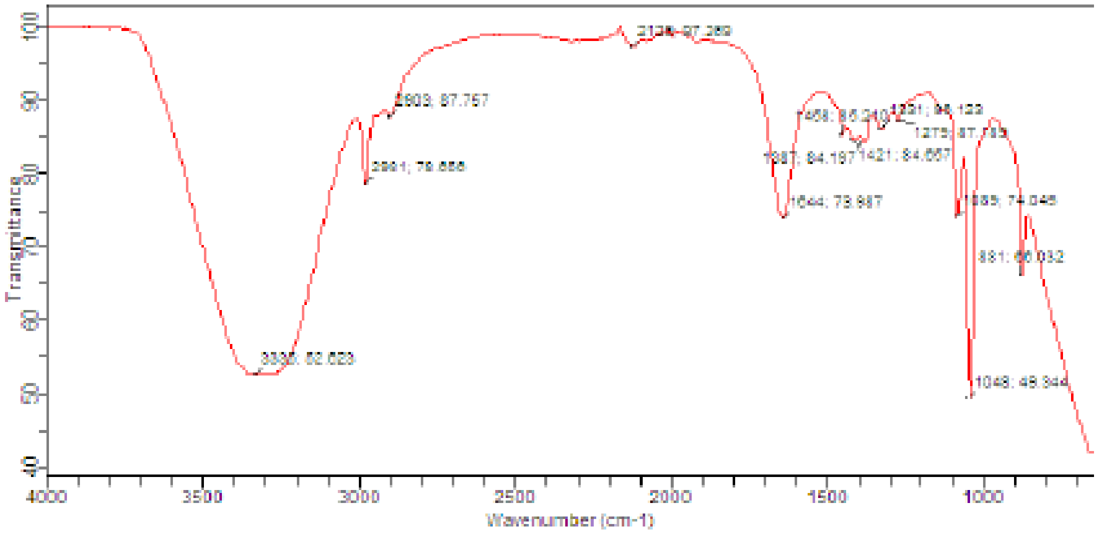

Figure 1: FTIR Spectra of EPS Produced by *Lb. acidophilus 1*

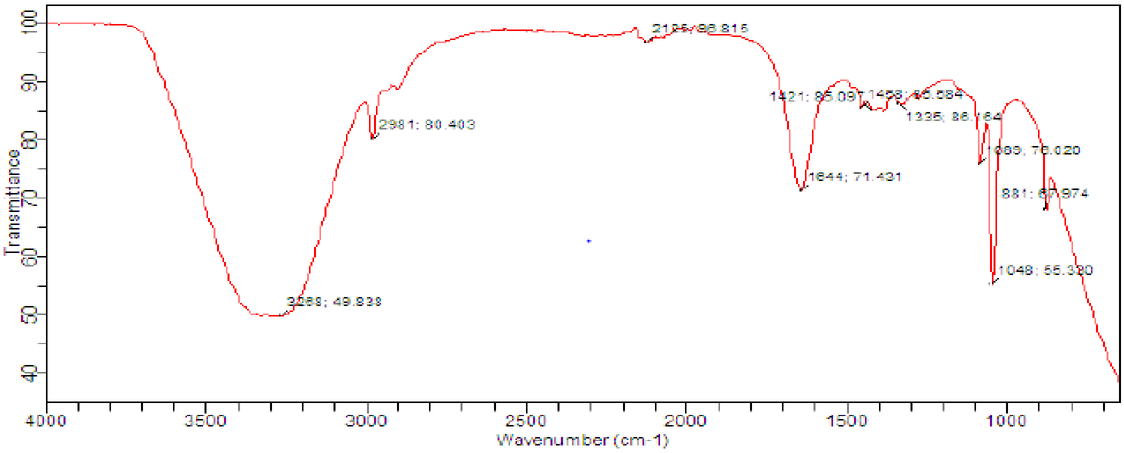

Figure 2: FTIR Spectra of EPS Produced by *Lb. brevis 1*,

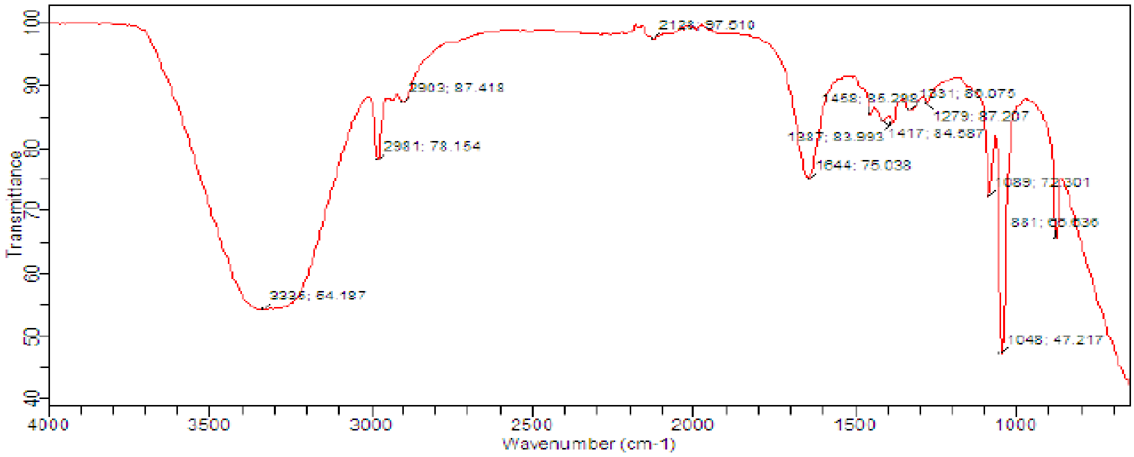

Figure 3: FTIR Spectra of EPS Produced by *Lb. fermentum*

Plate 1: Pure Isolates of *Lactobacillus* spp on MRS Agar

Plate 2: Analytical Profile Index (API) Reaction

Plate 3: EPS Quantification

DISCUSSION

The results obtained in the present study on the twenty-five *nono* samples collected from Wambai Market Kano, has indicated that these products contain *Lactobacillus* spp. The analytical profile identification (API) kit gave the sugar fermentation profile of the strains, which identified isolates(A- B) respectively as; *Lb. acidophilus 1, Lb. brevis 1, Lb. fermentum 1, Lb. paracasei* subsp. *paracasei, Lb. acidophilus* 3, *Lb. delbruekii* subsp. *delbruekii, Lb. pentosus, Lb. helveticus, Lb. plantarum 1,and Lb. plantarum 2* respectively. The presence of these *Lactobacillus* spp in locally fermented milk is of great significance; this has brought to light the type of *Lactobacillus* spp present in the locally fermented milk. *Lactobacillus* spp have been identified as the dominant LAB flora in hot tropical climates (Savadogo *et al*., 2004). Mozzi (2016), reported some of the isolates to have probiotic properties such as *Lb. acidophilus* (antidiarrheal), *Lb. plantarum* (prevent gut infection), *Lb. paracasei* (anticarcinogenic effect*)*, *Lb. helveticus* (improvement of gut immune barrier). Bintsis (2018a) reported *Lb. fermentum* to have immune-enhancing, anti-inflammatory and antioxidant activity.

The strains reported in this study are similar to the findings of Adebayo and Onilude (2008), who isolated *Lb. brevis* and *Lb. fermentum*, *Lb. plantarum*, *Lb. helveticus* from some Nigerian fermented foods. Also Fagbemigun *et al*. (2021), reported the presence of *Lb. fermentum, Lb. helveticus and Lb. delbrueckii,* from *nono* sourced from Jigawa, Bauchi, Katsina and Kano states of Nigeria.

Exopolysaccharide (EPS) production potential was observed in ten isolates (7.94%). Lactic acid bacteria can produce EPS because of the specific enzymes glycosyltransferase and fructosyltransferase, which are responsible for assembling the sugar moieties of the EPS polymer (Malaka *et al*., 2020; Angelin and Kavitha 2020). This study's findings agree with Bachtarzi *et al*. (2019), where only 10 (1.71%) isolates were EPS producers out of 584 Lactic acid bacteria screened for EPS production from traditional Algerian dairy products. These results do not correspond with Savadogo *et al*. (2004) and Abdellah *et al*. (2014). The isolated strains produced varying quantities of exopolysaccharide; the least quantity of EPS (7.83mg/l) was produced by isolate B, while isolate A (248.33mg/l) produced the highest quantity of EPS. Exopolysaccharide is reported to protect the bacterium from adverse environmental conditions such as desiccation, phagocytosis and antibiotics to mention a few. LAB-produced EPS benefits human health due to their immunomodulatory, antitumor properties and cholesterol-lowering ability. These polymers help to improve gut health because they serve as a substrate for beneficial gut bacteria; Gerwig, (2019): Angelin and Kavitha, (2020). The findings of this study are somewhat similar to the findings of Adebayo and Onilude (2008).

Effect of Carbon Source and Concentration on EPS Yield

The isolates produced varying quantities of exopolysaccharide, the influence of different carbon sources (glucose, lactose and sucrose) and concentrations on exopolysaccharide yield. The tested strains generally showed a statistically significant (P>0.05) increase in EPS yield as the carbon concentration was increased. However, the strains each had a preferred carbon source, resulting in significantly better yields. This preference could be attributed to a difference in the regulation of EPS biosynthetic pathways influenced by the different carbon sources. This implies that the most efficient carbon source for EPS production for *Lb. acidophilus* and *Lb. brevis* is sucrose; while *Lb. fermentum* preferred glucose. Gayathiri *et al*., (2017) reported that EPS yields by LAB depend on the efficiency of carbon source use and its concentration. Similar findings were reported by Shi *et al*. (2013) for *Lb. fermentum* where glucose was the preferred carbon source, and the results of Yuksekdag and Aslim, (2008) in which glucose was the more preferred carbon source for two different strains of *Lactobacillus delbrueckii*. Sucrose has also been reported to be the preferred carbon source as reported by Kuntiya *et al*., (2010); Oleksy-sobczak, (2020) reported sucrose to be the preferred carbon source for three different strains of *Lb. rahmnosus* yielding up to 987.84 ml/L

Confirmation of EPS Functional Groups by FTIR Spectra

The spectrum of purified EPS studied in the region of 400 – 4000cm^{-1} showed numerous peaks, from 3335 -881cm^{-1}. The spectra of the analyzed EPS for the three local EPS producing isolates; A, J and S (*Lb. acidophilus, Lb. brevis and Lb. fermentum)* were similar. The broad absorbance peak observed at 3335-3333cm^{-1} indicates the presence of intensive hydroxyl group (OH) stretching frequency, confirming that the compound is a polysaccharide. The stretching vibration signals between 2981cm^{-1}, and 2903cm^{-1} is because of carbon-hydrogen (C-H) bonds. Minor peaks and vibrations around 2125cm^{-1} could be attributed to OH bond groups which could be due to their carbohydrate nature. Peak around 1644cm^{-1} are assigned to C=O stretching of carboxylic group. Bands in the region of 1458 - 1421cm^{-1}are consigned to C-O-C vibrations of glycosidic linkage of glucose and O-H deformation (Li *et al*., 2013b; Zaidi *et al*., 2018). The vibrations peaks between 900 - 1,200cm^{-1} indicate the pyranose ring's presence, similar EPS spectra and bands have been reported by, Ismail and Nampoothiri (2010) obtained from *Lactobacillus plantarum* EPS which had FTIR peaks between 3304.06 – 1056.99cm^{-1}, 1200cm^{-1} and between 1030-944cm^{-1}.Also Zaidi *et al*., (2018) observed EPS from *Lactococcus lactis* SLT10, *Lactobacillus plantarum* C7, and *Leuconostoc mesenteroides* having peaks within range of; 3443 -534 cm^{-1} at specific regions of 3434-3420cm^{-1}, 2928-2850cm^{-1}, 2366cm^{1},1634cm^{1},1404 cm^{-1} and 1000 cm^{-1}. Emnace (2020) had similar peaks from *Lb. rhamosus* EPS.

CONCLUSION AND RECOMMENDATION

The study revealed the presence of different *Lactobacillus* spp., such as *Lb. acidophilus, Lb. brevis, Lb. fermentum, Lb. paracasei* from *nono*. The study revealed that 10 (7.94%)

Lactobacillus spp produced EPS out of the 136 screened. The three highest yielding EPS produces were *Lb. acidophilus, Lb. brevis, Lb. fermentum*. EPS production and yield were influenced by the type of carbon and its concentration, EPS yield increased with increase in sugar concentration. However, each isolate had a preferred carbon for EPS synthesis. Further molecular studies should be conducted on the isolated EPS producing *Lactobacillus* spp., so that the EPS genes can be isolated, characterized and studied. Exopolysaccharide production should be further optimized under different cultural (carbon and nitrogen sources) and environmental conditions (pH, temperature). Extracted EPS should be characterized by nuclear magnetic resonance (NMR) to ascertain its type (either homopolysaccharide or heteropolysaccharide) and its constituent monosaccharide (glucose, fructose or rhamnose).

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