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# Probiotic and technological properties of exopolysaccharide producing lactic acid bacteria isolated from cereal-based nigerian fermented food products

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ABSTRACT

The present study aims to evaluate the probiotic potential and technological properties of exopolysaccharide (EPS) producing lactic acid bacteria (LAB) isolated from Nigerian fermented cereal-based foods. Sixty-three autochthonous LAB isolated from cereal-based fermented foods were screened for EPS production, five isolates produced an appreciable amount of EPS on sucrose modified media and were identified by sequencing the 16S rRNA gene as *L.plantarum* YO175, *L.plantarum* OF101, *P. pentosaceus* OF31, *W. confusa* OF126 and *W. confusa* WS90. These five isolates were assessed for their probiotic and technological properties viz., tolerance to low pH, bile salt resistance, bile salt hydrolysis, tolerance to simulated gastric transit, cell surface hydrophobicity, antimicrobial, amylolytic and acidifying activity. The LAB isolates showed good survival at pH 2.0 and 2.5 and were resistant to 0.3% bile salt after 4 h. All the isolates tolerate gastric juice condition, with no reduction in viability except *W. confusa* WS90 that lost viability over 180 min incubation time. *L. plantarum* OF101 showed the highest hydrophobicity values for n-hexadecane and xylene (43.6%, 46.2%). They all showed different antimicrobial activities against five food-borne pathogens. *P. pentosaceus* OF31 possessed the highest ability to inhibit pathogens and also demonstrated better and rapid acid production capability. Albeit the properties tested are strain-dependent, *L. plantarum* and *P. pentosaceus* strains were found to possess interesting functional and probiotic characteristics to a greater extent compared to *W. cibaria* strains. The safety investigations indicate their suitability as good candidates for cereal-based probiotic products/starter culture for the improvement of traditional cereal fermentation process and also the development of functional cereal foods.



## 1. Introduction

Cereals are edible grains which form part of the human diet since ancient times, they remained important food crop with an estimated global production of 2500 million tonnes in 2015 (FAO, 2016). They serve as an important source of energy, carbohydrate, protein and fiber, micronutrients (vitamin E, vitamin B), sodium, magnesium, and zinc (Waters, Mauch, Coffey, Arendt, & Zannini, 2015). Cereals are staple food crops as they often make up the bulk of the diet since they are relatively cheap to produce. In sub-Saharan Africa, cereals such as maize, sorghum, and millets are consumed by people with varying food preferences and socio-economic background, and serves as a source of food security and economic wellbeing (Adesulu & Awojobi, 2014).

Fermented foods contribute enormously to the human diet. Many African foods are fermented before consumption. In Nigeria, larger percentages of cereals grown yearly are subjected to the spontaneous and traditional fermentation process to produce beverages, dough and gruels, both at the household level and industrial set-up. Fermentation provides a simple and economical way to improve the nutritional value and sensory attributes of cereal grains (Ferri, Serrazanetti, Tassoni, Baldissarri, & Gianotti, 2016; Nout, 2009). The fermenting microorganisms involved in many of these cereal-based fermented products are mainly lactic acid bacteria (LAB) and few yeast species (Odunfa & Adeyele, 1985; Sanni & Adesulu, 2013).

Lactic acid bacteria constitute a significant group of food-grade microorganisms. They are most important bacteria in desirable

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food fermentations and have reported being involved during the fermentation of various Nigerian indigenous fermented foods (Adesulu-Dahunsi, Sanni, & Jeyaram, 2017a; Banwo, Sanni, & Tan, 2013; Sanni, Morlon-Guyot, & Guyot, 2002). Some of the traditional fermented cereal products of Africa includes; *ogi, burukutu, kunu-zaki, togwa, fura*, *gowê*, *koko, kenkey*, *ben-saalga*, *boza*, *poto-poto,* and *doklu.* Researchers have reported that careful selection and use of autochthonous microbial strains with desirable characteristics for controlled fermentation, industrial application, probiotics, or as potential starter culture can results to improved quality attributes of fermented cereals which will have effect on the stability, safety and overall quality of the products (Manini et al., 2016; Ogunremi, Banwo, & Sanni, 2017; Oguntoyinbo & Narbad, 2015). Thus, for enhanced quality and production of indigenous fermented foods with beneficial health effects, LAB strains with interesting functional characteristics and enhanced technological and probiotic properties are suitable, since LAB are generally regarded as safe (GRAS) (Adesulu-Dahunsi, Sanni, Jeyaram, & Banwo, 2017b).

Probiotic is defined as a live microorganism which confers a valuable health benefit on the host when an adequate amount is ingested (FAO/WHO, 2002). Many LAB isolated from different fermented foods product across the globe have been documented for their health effect and probiotic potentials (Ducrotté, Sawant, & Jayanthi, V. 2012; Hempel et al., 2012; Tham, Peh, Bhat, & Liong, 2012; Bautista- Gallego et al., 2013). Some of these LAB have been reported to produce technologically important substances such as EPS (Adesulu-Dahunsi, Sanni, & Jeyaram, 2018a; Adesulu-Dahunsi et al., 2018b). The EPS-producing LAB confers rheological and functional properties on foods and also improves the technological properties of sourdough and fermented milk and beverages (De Vuyst & Degeest, 1999; Ruas-Madiedo & de los Reyes-Gavilán, 2005). The EPS produced have been reported to improve gastrointestinal (GI) colonization of harmless bacteria in the human GI tract and thus, also play a significant role as prebiotics (Welman & Maddox, 2003). Their unique and complex chemical structures make them valuable and important in food industries and for other industrial applications, they have also been reported to be used as a substitute for antitumor, immunostimulatory, immunomodulatory and antioxidant agents in pharmaceutical industries (Liu & Pan, 2010; Pan & Mei, 2010).

In this study, EPS-producing LAB strains isolated from cereal-based indigenous fermented foods were evaluated for their *in vitro* probiotic and technological properties as an impetus towards the development of functional cereal foods and beverages and potential starter culture for improvement of traditional fermented cereal production.

## 2. Materials and methods

### 2.1. Bacterial strains

A total of 63 LAB isolates from spontaneously fermented *ogi* beverages were qualitatively screened for mucoid appearance on sucrose modified de Man Rogosa and Sharpe (mMRS) media, colonies showing highly viscous slimy growth on the agar plate were selected. Evaluation of EPS production by the selected LAB isolates was performed as previously reported Adesulu-Dahunsi et al. (2018a), each isolates were incubated at 30 °C/37 °C for 24 h in MRS broth, 100 mL of the culture was inoculated into 900 mL modified MRS (m-MRS) broth (glucose was replaced with sucrose as carbon source at a concentration of 20 g/L). The EPS yield was determined by drying to a constant weight in an oven at 60 °C overnight. The total genomic DNA of the EPS producing LAB isolates was extracted using lysozyme-heat lysis method as described by Jeyaram et al. (2011), the DNA lysate were amplified by PCR using universal forward fD1 (5′-AGAGTTTGATCCTGGCTCAG-3′) and reverse primer rD1 (5′-AAGGAGGTGATCCAGCCGCA-3′) as previously reported by Adesulu-Dahunsi et al. (2017a). The PCR products were purified using NucleoSpin® Extract II gel extraction kit (Machery-Nagel, Germany) and sequenced at Merck Specialties Private Limited, Banglore, India. The indicator organisms for antimicrobial activity include; *Bacillus cereus* MTCC 430, *Staphylococcus aureus* subsp *aureus* ATCC 11632, *Listeria monocytogens* ATCC7644, *Escherichia coli* ATCC 11229 and *Enterococcus faecium* ATCC 35667, they were cultured and maintained in Luria–Bertani media.

### 2.2. Probiotic properties of EPS-producing LAB strains

#### 2.2.1. Tolerance to low pH

The LAB tolerance to low pH was determined according to Guo, Kim, Nam, Park, and Kim (2010), with slight modifications. Freshly grown bacteria cultures were centrifuged at 5000 × g for 15 min at 4 °C, the cells were washed twice with phosphate buffered saline (PBS) (g/L) (NaCl: 9; $Na_2HPO_4 \cdot 2H_2O$: 9; $KH_2PO4$:1.5; pH 7.0) and were adjusted to obtain approximately 8.0 $\log_{10}$ CFU/ml, and resuspended in MRS broth with pH 2.0 and 2.5 respectively. Samples were taken at 0 min and after 4 h, and were plated on MRS agar plate after serial dilutions and incubated at 30/37 °C for 48 h, and then the total viable counts were enumerated.

The survival rate of the bacteria (%) was calculated as follows:

$$\text{Survival rate}(\%) = \frac{\text{logcfu}V_1}{\text{logcfu}V_2}$$

$V_1$: total viable count survived.$V_2$: initial viable count inoculated.

#### 2.2.2. Bile resistance

The ability of the LAB isolates to grow in bile salt was performed according to Guo et al. (2010). As described above, the cells were resuspended in MRS broth containing 0.3% of bile salt (Oxgall, Himedia) and the one without bile salt serves as a control. The samples were taken at 0 min and after 4 h incubation and were plated on MRS agar plate after serial dilutions and incubated at 30/37 °C for 48 h, and then the total viable counts were enumerated. Survival rate (%) of the bacteria was calculated according to (1).

#### 2.2.3. In vitro determination of cholesterol activity

The LAB isolates were assayed for BSH activity following the method of Dashkevicz and Feighner (1989). Freshly grown bacterial cultures were streaked on MRS agar medium containing 0.5% (w/v) taurodeoxycholic acid (TDCA) (Sigma) and 0.37 g/l $CaCl_2$, and incubated anaerobically at 30 °C/37 °C using Anaerocult R A (Merck, Germany) for 48 h. The BSH activity was quantified by measuring the diameter of precipitation zones.

#### 2.2.4. Tolerance to gastric acid

Tolerance of the EPS-producing LAB isolates to gastric acid was determined (Schillinger, Guigas, & Holzapfel, 2005). A simulated gastric juice was prepared by dissolving 3 mg/mL of pepsin in 0.5% sterile saline (pH 2.0) (the pH was adjusted with concentrated HCl), 1.5 mL of the overnight cultures were centrifuged at 9500 × g for 5 min at 4 °C and washed twice in quarter strength Ringer's solution (QSRS), 0.3 mL of the washed suspension was added to 1.5 mL prepared simulated gastric juice and vortexed for 60 s. Aliquots of 100 μL were removed after 90 and 180 min at 37 °C and serial dilutions were plated on MRS agar and incubated at 30 °C/37 °C for 24–48 h, the colony forming units were enumerated. The number of colony forming units was expressed as log values.

*2.2.5. Cell surface hydrophobicity (CSH)*

The *in vitro* CSH was performed by bacterial adherence to hydrocarbon (BATH) assay using two hydrocarbons; n-hexadecane and xylene (Schillinger et al., 2005). Overnight grown LAB cultures were harvested by centrifugation at 10,000 × g for 10 min, the cells were washed two times to remove non adherent cells and then re-suspended in 1.2 mL PUM buffer pH 7.1, with an optical density at 560 nm ($OD_{560}$) of 1.0 ($A_0$), 0.3 ml of each hydrocarbon separately was added to 1.2 ml bacterial suspension (4:1). The mixture was vortexed (Spinix, India) for 2 min and was allowed to stand by incubating at 37 °C for 20 min to allow the phase to be separated. Hydrocarbon layer was left to rise completely by keeping undisturbed. The lower aqueous phase was carefully removed and transferred to 1 ml cuvette; the absorbance (A) was measured. Percentage CSH (H %) of the LAB isolates adhering to each hydrocarbon was calculated:

$$H\ \% = [(A_0 - A)/A_0 \text{ x } 100]$$

where $A_0$ and A are the absorbance before and after extraction with the hydrocarbons.

*2.2.6. Antimicrobial activity*

The antimicrobial activity was determined by agar spot test as described by (Schillinger & Lucke, 1989) and modified by Gaudana, Dhanani, and Bagchi (2010). Briefly, 5 μl cells harvested ($1 \times 10^8$ CFU/ml) from each overnight grown LAB strain were spotted on the surface of MRS agar plates, air dried and incubated for 14–18 h at 37 °C for spots to develop. A 100 μl of each indicator organisms grown overnight under shaking condition at 37 °C were vigorously mixed with 10 ml soft agar containing 0.75% agar (w/v) and were overlaid on MRS plates with developed colonies, the plates were then incubated for 24 h at 37 °C, at the end of incubation, diameters of the zone inhibition (surrounding the spotted isolates) were measured. The antimicrobial activity was expressed by measuring the zone of inhibition as (diameter of the zone of inhibition - diameter of the colony) mm/2. Each assay was performed in triplicate.

*2.3. Technological properties of the EPS-producing LAB strains*

*2.3.1. Amylolytic activity*

Twenty-four hours (24 h) old LAB cultures were streaked on modified MRS agar plates without glucose but containing soluble starch (0.2%), incubated at 30 °C for 48–72 h. The plates were flooded with Gram's iodine solution and were examined for clear halo zones indicating the α-amylase production.

*2.3.2. Acidifying activity*

The LAB isolates were inoculated into MRS broth (pH adjusted to 6.5) and are incubated at 37 °C. The acidifying activities were determined by measuring the pH of the culture supernatant after 6, 12, 24 and 36 h.

*2.3.3. Qualitative assessment of biogenic amine (BA) production*

To establish the safety status of the LAB isolates, qualitative assessment for the production of BA using five amino acids, L-Histidine, L- Tyrosine, L-Ornithine, L-Arginine, and L-Lysine was performed following Maijala, Eerola, Aho, and Hirn (1993). Change of the bromocresol purple indicator to purple in the decarboxylase broth inoculated with actively growing cultures after incubation at 37 °C after 48 h indicates amino acid decarboxylase activity.

*2.4. Statistical analysis*

Statistical analyses of the data obtained were done using SPSS17.0. Data are expressed as a mean value with standard deviation from three independent replications, and the comparisons of differences between the means of the experiments were tested using one-way Analysis of variance (ANOVA) at the significance level of $P < 0.05$.

## 3. Results and discussion

*3.1. Screening and identification of EPS-producing LAB isolates*

Exopolysaccharide producing LAB species have been frequently isolated from various fermented foods. They have found their wide usage in traditional and industrial fermentation processes due to their GRAS status (Adesulu et al., 2018b; Galle, Schwab, Arendt, & Ganzle, 2010). The EPS produced by these LAB have some physiochemical and rheological properties, such as viscosifying, stabilizing, gelling or emulsifying which have motivated their exploitation in food applications. Researchers have reported that EPS have unique physicochemical effects in which they improve the technological properties of sourdough preparations (Brand, Roth, &

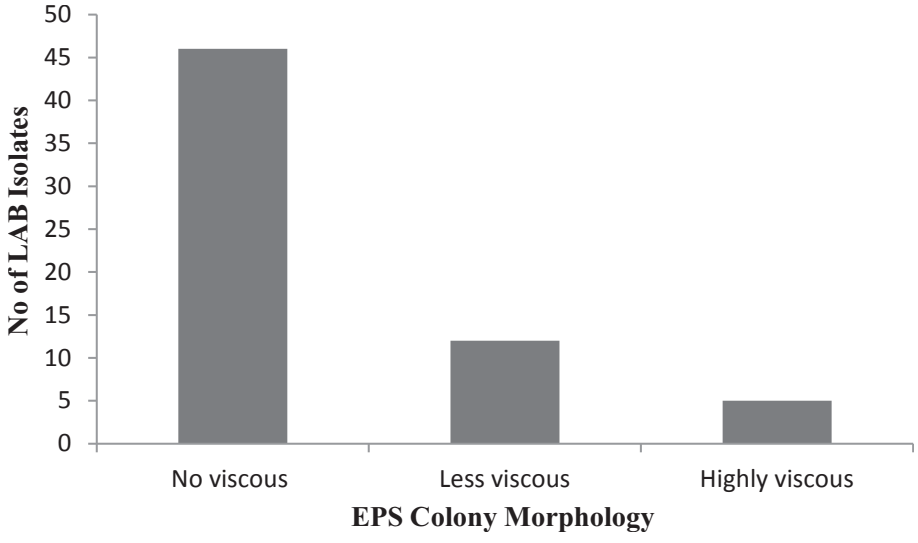


**Fig. 1.** Colony morphology of EPS producing isolates.

**Table 1**
Biochemical and Molecular characterization of the EPS producing LAB isolates.

| Isolates code | Source of isolation | Biochemical Identity | Molecular Identity | No. base pair | % Similarity | | GenBank Accession No† | EPS Production (g/L) |
|---|---|---|---|---|---|---|---|---|
| | | | | | NCBI | RDB | | |
| OF101 | *Ogi-funfun* | *L.plantarum* | *L. plantarum*[a] | 1429 | 99 | 0.974 | KU892393 | 2.18 |
| YO175 | *Ogi-pupa* | *L.plantarum* | *L. plantarum*[a] | 1034 | 99 | 0.987 | KU892395 | 1.36 |
| OF31 | *Ogi-funfun* | *Pediococcus* sp. | *P. pentosaceus*[b] | 1399 | 99 | 0.979 | KU892403 | 5.30 |
| OF126 | *Ogi-funfun* | *Weissella* sp. | *W. confusa*[c] | 1448 | 99 | 0.995 | KU892398 | 3.00 |
| WS90 | *Ogi-baba* | *Weissella* sp. | *W. confusa*[c] | 900 | 99 | 0.977 | KU892399 | 4.78 |

†NCBI GenBank Accession number of the LAB strains (http://www.ncbi.nlm.nih.gov/Genbank).
[a] % Sequence similarity of 16SrDNA gene with the type strain *L. plantarum* NRRL B-14768[T].
[b] % Sequence similarity of 16SrDNA gene with the type strain *P. pentosaceus* DSM 20336[T].
[c] % Sequence similarity of 16SrDNA gene with the type strain *W. confusa* JCM 1093[T].

**Table 2**
Survival of the EPS producing LAB isolates in low pH (2.0 and 2.5), 0.30 %bile concentration after 4 h incubation and their BSH activity.

| LAB Isolates | Viable counts (log10 CFU/mL) | | | | BSH activity |
|---|---|---|---|---|---|
| | Control | Tolerance to low pH | | Bile resistance | |
| | pH 7.0 | pH 2.5 | pH 2.0 | 0.30% | |
| *L. plantarum* YO175 | $8.06 \pm 0.07^{a}$ | $7.98 \pm 0.08^{b}$ (99.0)[1] | $7.82 \pm 0.04^{b}$ (97.0) | $8.10 \pm 0.13^{b}$ (100.4) | + |
| *L. plantarum* OF101 | $8.16 \pm 0.06^{a}$ | $8.03 \pm 0.05^{b}$ (98.4) | $7.92 \pm 0.07^{b}$ (97.1) | $7.79 \pm 0.30^{ab}$ (96.9) | + |
| *P. pentosaceus* OF31 | $8.11 \pm 0.13^{a}$ | $8.08 \pm 0.00^{b}$ (99.6) | $7.95 \pm 0.98^{b}$ (98.0) | $7.97 \pm 0.11^{b}$ (98.3) | + |
| *W. confusa* OF126 | $8.75 \pm 0.03^{b}$ | $7.98 \pm 0.31^{b}$ (91.2) | $7.21 \pm 0.19^{b}$ (82.4) | $7.96 \pm 0.09^{b}$ (90.9) | − |
| *W. confusa* WS90 | $8.09 \pm 0.34^{a}$ | $6.09 \pm 0.59^{a}$ (75.3) | $5.28 \pm 0.33^{a}$ (65.3) | $7.59 \pm 0.20^{a}$ (93.8) | − |

Values are means of three independent experiments (mean ± SD). [a,b]Means in the same column with different superscript letters represent significant difference ($P < 0.05$) by Duncan's post hoc comparisons.
+, positive; - negative.
[1] - figures in bracket represent survival rate (%) of each strain.

Hammes, 2003; Di Cagno et al., 2006; Galle et al., 2010). Also, these EPS-producing LAB influence the texture of food, as the texture is an essential characteristic related to the consumption of fermented foods. Sixty-three (63) LAB isolates were screened for the production of EPS as showed in (Fig. 1), 46 (73.01%) isolates showed no viscous colonies or EPS production, 12 (19.04%) isolates showed less viscous colonies and 5 (7.94%) isolates showed highly viscous slimy colonies on sucrose m-MRS agar plates. The 5 LAB isolates that showed highly viscous slimy morphology were found to produce EPS ranges from 1.36 to 5.30 g/L in m-MRS broth and were genotypically characterized using 16S rRNA sequencing with their GenBank accession numbers shown in Table 1. These LAB isolates were further selected to study their probiotic and their technological characteristics *in-vitro*.

### 3.2. Probiotic properties of EPS-producing LAB strains

#### 3.2.1. Tolerance to low pH

Before reaching the intestinal tract, probiotic bacteria must pass through acidic stomach condition where the pH can be as low as 1.5 during fasting and rises to pH 3 or even higher after a meal (Jacobsen et al., 1999). All the examined strains were tolerant to low pH showing more than 65% survival rate after incubation at pH 2.5 and 2.0 for 4 h (Table 2). *L. plantarum* strains YO175 and OF101 and *P. pentosaceus* OF31 did not show any significant decrease in the viable count (7.98, 8.03, 8.08 and 7.82, 7.92, 7.95 $\log_{10}$ CFU/ml) from the initial log CFU/ml as the survival rate are greater than 97%, while *W. confusa* strain OF126 and WS90 showed marginal decrease in the viable cell counts from initial count 8.75 and 8.09, to 7.98, 7.21 and 6.09, 5.28 $\log_{10}$ CFU/ml, at pH 2.5 and 2.0 respectively. Previous works from other authors who investigated LAB strains from food, human or animal origin showed that some LAB were able to retain their viability when exposed to low pH (Bao et al., 2010; Gu, Yang, Li, Chen, & Luo, 2008; Lee et al., 2012).

#### 3.2.2. Bile resistance

Resistance of bacteria to the intestinal bile salts is also an important factor to be considered during probiotic selection. Zavaglia, Kociubinski, Perez, and DeAntoni (1998) and Dunne et al. (1999) reported that the relevant physiological concentration of human bile ranges from 0.3 to 0.5%. The ability of the LAB isolates to grow in MRS broth supplemented with 0.3% bile salt was performed. The result showed good resistant to bile salts with more than 90% survival rate (Table 2). Studies on the resistance to different bile concentrations by some LAB from food origin have being reported by other researchers (De Palencia et al., 2009; Gu et al., 2008).

**Table 3**
Effect of simulated gastric juice on the viability of the EPS producing LAB isolates at different incubation times.

| Isolates | Viable counts ($\log_{10}$ CFU/mL) | | |
|---|---|---|---|
| | 0 min | 90 min | 180 min |
| *L. plantarum* YO175 | $8.11 \pm 0.11^{b}$ | $7.22 \pm 0.24^{c}$ | $6.27 \pm 0.36^{c}$ |
| *L. plantarum* OF101 | $8.20 \pm 0.29^{b}$ | $6.80 \pm 0.23^{bc}$ | $5.41 \pm 0.34^{b}$ |
| *P. pentosaceus* OF31 | $7.83 \pm 0.36^{ab}$ | $6.61 \pm 0.14^{bc}$ | $5.59 \pm 0.55^{bc}$ |
| *W. confusa* OF126 | $7.58 \pm 0.24^{a}$ | $6.38 \pm 0.35^{b}$ | $6.06 \pm 0.31^{bc}$ |
| *W. confusa* WS90 | $7.72 \pm 0.17^{ab}$ | $4.22 \pm 0.61^{a}$ | − |

Values are means of three independent experiments (mean ± SD). [a,b,c]Means in the same column with different superscripts represent significant difference ($P < 0.05$) by Duncan's post hoc comparisons; viable counts ($\log_{10}$ CFU/ml) of each strain at 90 and 180 min were compared with 0 min.

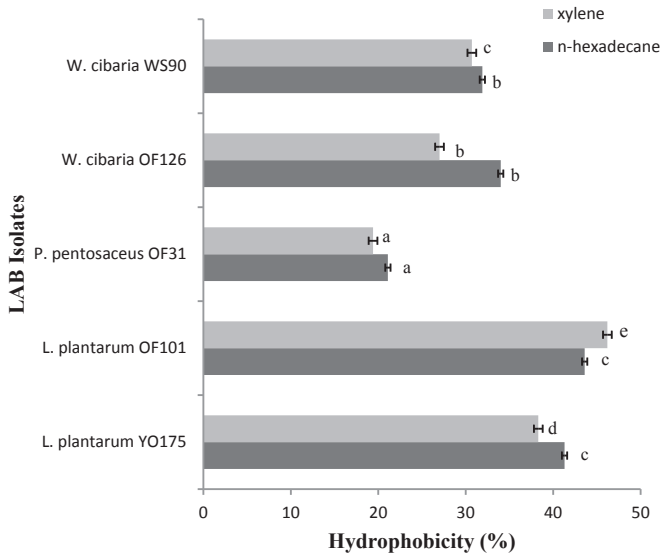


**Fig. 2.** Cell surface hydrophobicity of the EPS producing LAB isolates.

### 3.2.3. Cholesterol-lowering effect

Secretion of bile salt hydrolase by LAB strains is an important probiotic marker which assists the organisms to resist toxic bile environment in the GI tract and also increases the intestinal survival of the LAB strains (Begley, Gahan, & Hill, 2005; Begley, Hill, & Gahan, 2006). The results showed that among the isolated strains *L. plantarum* YO175, OF101 and *P. pentosaceus* OF31 were BSH positive, showing precipitation zone around the spotted culture, this suggests their ability of lowering serum cholesterol level (Table 2).

### 3.2.4. Tolerance to gastric acid

The LAB isolates displayed good tolerance to simulated gastric juice condition (pH 2.0) (Table 3). They all survived after 180 min incubation, except *W. confusa* WS90 that lost its viability. These results agreed with those obtained by Mechai, Debabza, and Kirane (2014) who reported that tolerance of probiotic strains to passage through the gastrointestinal tract is strain-dependent. Other strains from Lactobacillus and Pediococcus genera have also been reported to show good tolerance to gastric juice conditions (Bove et al., 2012; Jensen, Grimmer, Naterstad, & Axelsson, 2012; Turchi et al., 2013).

### 3.2.5. Cell surface hydrophobicity (CSH)

The surface properties, like autoaggregation and hydrophobicity, are used as a measurement directly related to ability to adhere to cell monolayers (Bautista-Gallego et al., 2013; Iniguez-Palomares, Perez-Morales, & Acedo-Felix, 2008). The percentage hydrophobicity of the LAB isolates were determined by using two hydrocarbons namely; n-hexadecane and xylene (Fig. 2). The isolates showed varied hydrophobicity values ranging from 19.4 to 46.2%. The results revealed that highest hydrophobicity value for n-hexadecane and xylene were observed in *L. plantarum* OF101 at 43.6 and 46.2% and the least in *P. pentosaceus* OF31 with 21.1 and 19.4% respectively. Mehmet, GokGen, Simel, Nurdan, and Filiz (2015) reported higher hydrophobicity values ranges from 82.41% to 97.96% among the *Lactobacillus* species.

### 3.2.6. Antimicrobial activity

Production of antimicrobial substances such as organic acids (lactic and acetic acids) and bacteriocins by LAB have been reported as one of the probiotic properties for strain selection (Fuller, 1989). The assay of antimicrobial activity against five food-borne pathogens was performed using agar spot test (Table 4), the results showed varying inhibitory activity against *Bacillus cereus* MTCC430 (with diameter of inhibition zones between 15 and 24 mm), *Staphylococcus aureus* ATCC11632 (8–15 mm), *Listeria monocytogenes* (10–14 mm), *Enterococcus faecium* ATCC35667 (3–10 mm) and *Escherichia coli* ATCC11229 (2–9 mm). *P. pentosaceus* OF101 exhibited strongest antibacterial activity against all the food-borne pathogens with the diameter of inhibition zones greater than 9 mm. The *W. confusa* strain showed less inhibitory activity against *Escherichia coli* ATCC11229. The high inhibitory effect observed could essentially due to lowering of pH due to the production of various organic acids by these LAB strains during the traditional cereal fermentation. Similarly, broad spectrum of antimicrobial activity observed among the *Lactobacillus* and *Pediococcus* species have been reported (Lavilla-Lerma, Perez-Pulido, Martınez-Bueno, Maqueda, & Valdivia, 2013; Patel, Prajapatia, Holst, & Ljungh, 2014). The antibacterial activity exhibited by these isolates may be useful to control the undesirable microbiota either in food system or during GI tract application.

## 3.3. Technological properties of the EPS-producing LAB strains

### 3.3.1. Amylolytic activity

Amylolytic activities of the LAB strains are shown in Table 5. *L. plantarum* strains showed weak amylolytic activity and no activity were detected in the *P. pentosaceus* and *W. confusa* strains. Few Literature have reported amylolytic activity from Lactobacillus species isolated from fermented cereals in Africa (Sanni et al., 2002). Amylolytic LAB from traditional fermented foods are desirable and can be applied as starter culture during traditional cereal fermentation.

### 3.3.2. Acidifying activity

The acidifying activity of the EPS-producing LAB isolates at 0 h and after 6, 12, 24 and 36 h of incubation is shown Table 6. In all the LAB isolates, the pH of the culture medium decreased to values lower than 4.00 after 12 h. *L. plantarum* YO175 and *P. pentosaceus* OF31 showed the high acidifying capacity throughout the incubation period. *W. cibaria* WS90 showed a relatively low acidifying capacity after 12 h.

**Table 4**
Antimicrobial activity of the EPS producing LAB isolates against food borne pathogens.

| Isolates | Indicator organisms | | | | |
|---|---|---|---|---|---|
| | *Bacillus cereus* MTCC 430 | *Staphylococcus aureus* ATCC 11632 | *Listeria monocytogenes* ATCC 7644 | *Enterococcus faecium* ATCC 35667 | *Escherichia coli* ATCC 11229 |
| *L.plantarum*YO175 | +++[a] | +++ | +++ | + | + |
| *L. plantarum*OF101 | +++ | ++ | +++ | + | + |
| *P.pentosaceus*OF31 | +++ | +++ | +++ | ++ | ++ |
| *W. confusa*OF126 | +++ | ++ | ++ | – | – |
| *W. confusa*WS90 | +++ | + | ++ | + | – |

[a] Diameter of inhibition zones (mm): +++ > 15 mm; ++ > 10 mm; + > 5 mm; - < 5 mm.

**Table 5**
Amylolytic activity of the EPS producing LAB isolates.

| Isolates | Amylase activity |
|---|---|
| | Clear zone around colonies |
| *L. plantarum* YO175 | + |
| *L. plantarum* OF101 | + |
| *P. pentosaceus* OF31 | – |
| *W. confusa* OF126 | – |
| *W. confusa* WS90 | – |

- No clear zone around colony.
+ diameter of clear zone ‘ 2 mm.

**Table 6**
Acidifying activity (pH) of the EPS producing LAB isolates.

| Isolates | Incubation time (h) | | | | |
|---|---|---|---|---|---|
| | 0 | 6 | 12 | 24 | 36 |
| *L.plantarum* YO175 | 6.50 | 4.98 | 4.07 | 3.21 | 3.07 |
| *L.plantarum* OF101 | 6.50 | 4.44 | 4.12 | 3.81 | 3.53 |
| *P. pentosaceus* OF31 | 6.50 | 4.31 | 3.19 | 2.85 | 2.71 |
| *W. cibaria* OF126 | 6.50 | 4.76 | 4.00 | 3.93 | 3.27 |
| *W. cibaria* WS90 | 6.50 | 5.21 | 5.03 | 4.61 | 4.06 |

### 3.3.3. Safety evaluation

None of the strains tested produced BA (result not shown). Our result is in agreement with other works (Lavilla-Lerma et al., 2013).

## 4. Conclusion

In Nigeria, LAB are consumed in large numbers via many fermented cereals. Thus, the implementation of carefully selected strains as starter cultures or co-cultures during fermentation processes provides better quality and consistency to the food. High EPS-producing LAB starter culture gives several advantages over non EPS producer, as the former affect the texture, rheology and improves the technological properties of the food produced. Three isolates, *L. plantarum* YO175, *L. plantarum* OF101 and *P. pentosaceus* OF31 showed significant probiotic and technological potentials by their ability to withstand and adapt to GI conditions and broad antibacterial activity. These multifunctional LAB strains under study can bring about delivery of functional food with added health benefits and can also serve as potential for exploitation in the development of indigenous functional cereal-based foods with enhanced health benefits, as well as improve food safety by improving the cereal substrates to meet contemporary market demands. This study was able to select some promising strains as suitable functional starter cultures for cereal food fermentation. As the preliminary *in vitro* tests have shown definite probiotic potential for these strains, further *in vivo* investigation for their performance studies is ongoing.

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