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Isolation and Screening of *Lactobacillus Plantarum* Strains with Potential Probiotic Aptitudes from Neglected Edible Vegetable and Fruits of Côte D'Ivoire

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ABSTRACT

Background: Lactofermentation by probiotics would help valorize ENUS (edible neglected and underutilized species). Probiotic lactic acid bacteria (LAB) are considered as vectors of molecules of nutrition-health interest. This study has isolated and screened *Lactobacillus plantarum* strains with potential probiotic aptitudes from neglected edible vegetable and fruits of Côte D'ivoire.

Methods: Three *L. plantarum* strains (Pa6, A6, and Pe3) isolated from Ivorian ENUS (passion fruit, garlic and parsley) were isolated and screened for potential probiotic aptitudes from neglected edible vegetable and fruits of Côte D'ivoire.

Results: The screening of three strains revealed that they presented interesting probiotic potentialities (hydrophobicity values higher than 65% and pH=2, 0.3% bile salt, antibiotic and intestinal microbial pathogens resistances, respectively). They also presented antioxidant (14.51±0.39 -39.48±0.88%), anti-inflammatory (8.88±00.00%-78.61%±00.00%) and exopolysaccharide aptitudes, respectively. They synthetized degrading-anti-nutritional- factor enzymes (phytase and tannase) and cell-wall degrading enzymes (amylase and cellulase). They fermented the indigestible sugar raffinose, survived at 6.5% NaCl for Pa6 and Pe3, pH=9 and 45°C for Pe3.

Conclusion: These strains with such interesting technological properties would be suitable for industry in general, and particularly as starters for the controlled fermentation of Ivorian ENUS.

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Introduction

Indigenous fruits and vegetables are rich in vitamins, minerals, fibers, antioxidants (vitamin C, carotenoids, flavonoids) and are used both for food and as treatments against several diseases in sub-Saharan Africa (1). These plants could be valorized as a sustainable solution as affordable medicinal, to achieve food and nutritional security owing of their unleash high nutritional value, fight persistent poverty in remote regions, counter soil

depletion and create more sustainable farm systems (2). Despite these super-foods potentialities recognized by the scientist community and their usefulness to local communities around the world, ENUS are neglected and not taken into account in research- development. After harvest, they decompose quickly, because of naturally occurring microorganisms and enzymes, leading to short duration conservation and many losses. In Africa, their most consumed organs (fruits, leaves and seeds) collected in the surrounding ecosystems after their spontaneously growing are therefore very cheap. In Côte D'Ivoire, they are moringa, soursop, eggplant and baobab fruit (*Adansonia digitata* L.) which are unanimously accepted as natural ready-to-eat functional foods. They are also water lily, black plum, jujube, cocoma, mangosteen, Garcinia Kola, tamarind fruit, guava, etc. (3).

Among vegetables, they are *Celosia argentea* (soko), *Ipomea batatas* (potato), *Hibuscus sabdariffa* (dah), *Amaranthus hybridus* (borombrou), *Adansonia digitata* (baobab), *Vigna unguiculata* (bean), *Corchorus olitorius Linn* (Kplala), *Basella alba* (Spinach). In addition, there are wild mushrooms, spices (ginger, garlic, turmeric, etc.), herbs, *V. unguiculata* (L.) Walp, and *H. sabdariffa* (4). *V. unguiculata* (L.) (Cowpea), and *H. sabdariffa* are traditionally fermented in West Africa against hypertension. In most cases, stabilization treatments (bleaching, pasteurizing, drying) involving high temperature which are applied, generally decrease their functional properties (vitamins, phenolic compounds, etc.) (5). Other processes without negative effects are lactofermentation (LA) by probiotics which has gained a great interest in naturotherapy, phytotherapy and in the Worldin diet (6). Probiotification of foods into functional foods has revolutionized nutrition in the last twenty years towards therapeutic applications (7). Currently, there is a great interest in the isolation of new probiotic LAB strains from unconventional sources, such as fermented staple foods (8). Addition of active bacterial starter cultures would be an added value and a marker of high quality (9). Therefore, this work aims for the first time to select potential probiotic lactic bacteria as starters for fermentation of current Ivorian ENUS. This study has isolated and screened *L. plantarum* strains with potential probiotic aptitudes from neglected edible vegetable and fruits of Côte D'ivoire.

Materials and Methods

ENUS used in this work are those commonly used as food and against malaria, diabetes, obesity, hypertension and other cardiovascular diseases in Côte D'Ivoire according to ethnobotanical investigations (Table 1). They are purchased at the Gouro markets in Adjamé (Abidjan), carefully washed in water, manually rid of all susceptibilities of rot and cut into thin strips (1cm thick), crushed and left to ferment for three days at room temperature (27-30°C). These pasties are then used as isolation source of potential fermentative probiotic lactic bacteria.

After the room fermentation, 1 g of each fermented sample was aseptically homogenized in a stomacher with 9 mL of sterile peptone buffered water (Oxoid, Basingstoke, United Kingdom) previously adjusted with a 3 M hydrochloric acid solution to pH of 2 and incubated at 30°C for 3 h to obtain the widest possible pH=2 resistant fermentative LAB (10). Isolation of LAB was carried out by serial dilutions (10-1-10-5) and inoculations on MRS plates then incubated anaerobically at 30°C for 48 h. After incubation, presumptive LAB were identified as Gram positive, oxidase negative and catalase negative and 5 colonies were randomly grown in the MRS agar before were stored in MRS broth containing 20 % of glycerol at -80°C. Probiotic properties screened in this work concerned the effects of acid and bile salt resistance and hydrophobicity characters of the new strains, respectively. The acidity resistance of each LAB was determined in 10 ml of MRS broth adjusted at pH of 2 for 3 hours (11).

To neutralize the acidity in the culture medium, dilutions for counting bacterial cells were made in 0.1 M phosphate buffer, and pH=6.2. Cell counts were made on MRS agar and the survival rate was determined comparatively to the control. Bile salts tolerance was obtained by the resistance rate comparatively to a control after 18 h of preculture of a new colony in the presence of 0.3 and 0.5% of bile salts (Oxgall Powder, B-3883, Sigma). The Cell surface hydrophobicity of bacteria was screened by the ability of bacteria to adhere to hydrocarbons with xylene as the hydrophobic substance (12). One milliliter of each young culture of bacteria (18 h) was centrifuged at 8000 rpm for 15 min, were washed twice with PBS (pH=7.4) and resuspended in 3 mL of PBS. The optical density (A0) was determined at 600 nm. A proportion of xylene was added equally, then vortexed for 5 min and was incubated at 37°C for 1 hour. The absorbance at 600 nm of organic matter (A1) was read. The adhesion capacity of bacteria (BATH) was calculated as follows: BATH%=[(A0–A1)/A0]×100. After their selection, three strains codified Pe3, Pa6 and A6 were then identified after PCR (model T gradient, Germany). On colonies by amplification of their hypervariable region (HV) of the 16S gene (approximately 500 bp). Obtained

Table 1: Common and botanical names of the ENUS used in this work.

Common name	Botanical name	Organ
Bissap or Dah	*Hibiscus sabdariffa*	Corolla
Bissap or Dah	*Hibiscus sabdariffa*	Leaf
Garlic	*Allium sativum*	Bulb
côcôta	*Saba senegalensis*	Fruit
Pepper	*Capsicum annuum*	Fruit
Passion	*Passiflora edulis*	Fruit
Parsley	*Petroselinum crispum*	Leaf
Thousand diseases	*Phyllanthus amarus*	Leaf
Mamichou or african spinach	*Talinum triangulare*	Leaf
Black nightshade or Foué	*Solanum nigrum*	Leaf
False basil	*Ocimum gratissimum*	Leaf
Moringa	*Moringa oleifera*	Leaf
Moringa	*Moringa oleifera*	Fruit
Baobab	*Adansonia digitata*	Pulp
Gnangnan	*Solanum torvum*	Fruit
Diabetes pod	*Picralima nitida*	Seed
Néré	*Parkia biglobosa*	Pulp
Néré	*Parkia biglobosa*	Grain
Little cola	*Garcinia Kola*	Fruit
Soursop	*Annona muricata*	Fruit

amplicons were then sequenced to determine their species by comparison with the National Center for Biotechnology Information (NCBI) database. The partial 16S rRNA gene sequences were determined in the Microbiology, Adaptation, Pathogeny laboratory (Lyon, FRANCE) and were deposited in the NCBI database.

Antibiotic resistance was tested on MRS by the standardized technique of diffusion of the antibiogram Committee of the French Society of Microbiology (CASFM). Isolates of LAB were cultured for 24 hours at 37°C on MRS broth and 100 µL of the bacterial culture was spread on MRS agar and then dried at room temperature for 15 to 20 minutes under a laminar flow hood. Each antibiotic (chloramphenicol, penicillin, erythromycin, kanamycin, sulfamin, cephalosporin, rifampin, nalidixic acid and tygecycline.) was deposited on the agar previously inoculated with bacterial strains. Antimicrobial potentialities of the strains were studied from their culture supernatant towards pathogenic microorganisms (*Staphylococcus, Pseudomonas, Escherichia coli, Salmonella, Klebsiella, Candida, Aspergillus*). LAB and pathogenic strains were grown in MRS broth and incubated for 18 h at 37°C. Pathogens were plated in the mass (100 µL of homogenized precultures in MRS agar) and after solidification, 6 mm wells were pierced with a Pasteur pipette handle under sterile conditions. Each well was filled with 50 µL of lactic acid bacteria inoculum. The whole set was refrigerated at 4°C for 2 h to allow good diffusion of the substance. The diameters of the inhibition zones were measured after 24 h of incubation at 37°C (13).

For antimicrobial activity of the new strains, MRS Broth cultures of every pathogenic (American Type Culture Collection) microorganism and fermenting LAB were previously done separately for 24 hours at 37°C. Then, broth of the fermenting LAB was put on the MRS agar plates previously inoculated with pathogenic strains. The inhibition halo's presence indicted an antagonistic activity of the fermenting LAB bacteria against the pathogenic (14). Exopolysaccharide (EPS) production was determined using MRS sucrose (40 g/L) agar as previously described by Fessard (5). Anti-inflammatory activity of each LAB was determined from their culture supernatant by the (BSA) bovine serum albumin (protein) inhibition test. Reaction mixture (0.5 mL) consisted of 0.45 mL of BSA (5% aqueous solution) and 0.05 mL of bacterial culture supernatant at different concentrations or diclofenac (reference drug) prepared at five different concentrations (63- 1000 µg/mL). The pH was adjusted to 6.3 using hydrochloric acid (1N). Then samples were incubated at 37°C for 20 min, heated to 57°C for 3 min. After cooling, 2.5 mL of phosphate buffer solution (PBS, pH=6.3) was added to each test tube. Absorbance was measured at 416 nm. For the control test tubes, distilled water replaced the extracts while the product control tube did not contain BSA. The percent inhibition of protein denaturation was then calculated as follows: Inhibition (%)=100-[(Sample OD−Control OD/Control OD)]x10 (15)

For antioxidant activity, 1 mL of bacterial culture supernatants was added in 2 mL of ethanol (70%) and centrifuged (6000 rpm for 10 min) and the supernatant was collected. Totally, 50 µL of the supernatant was added to 1950 µL of 2,2 diphenyl-1-picryl hydrazyl (DPPH•) (0.6 mg/mL). The negative control was prepared by mixing 50 µL of 70% ethanol with 1950 µL on of DPPH at the same concentration. After incubation in the dark for 30 min, absorbance was read at 517 nm and the percentage of DPPH decolorization determined by the formula below: I(%)=((OD control-OD sample))/OD control×100 (16)

Acidification capacity of each strain was screened by single batch with 5 mL MRS broth at 37°C. Acid production was measured by the culture pH after 6, 12, 18, 24, 30, 36, 42 and 48 h following the pH measurement of MRS broth by a pH meter. Biomass evolution was analyzed through absorbance at 600 nm (17). The diluted supernatant was used for the quantification of residual glucose, L-lactate and titratable acidity. Concentrations of glucose (g/L) and L-lactate (g/L) were measured by using a glucose and lactate automatic analyzer YSI2700S ELECT (Yellow springs Instruments Co., Inc.) equipped with two membranes, one for glucose (2365) and another for L-lactate (2329). Total titratable acidity was determined by titrating the sample with 0.1N NaOH using phenolphthalein as indicator and the result was expressed as percentage of lactic acid. The ability of the isolates to produce amylase was performed as described before (18). Many spots of each isolate were made with an inoculation needle on modified MRS agar without glucose but with 2 % of soluble starch (w⁄v) as the only carbon source. The plates were incubated at 30°C for 48 h in an anaerobic jar. After incubation, the culture plates were flooded with Lugol's iodine and a color less area around the growth indicated a positive test. The ability of the isolates to produce, cellulase, phytase and tannase was detected as previously described (18), but with slight modification. Thus, the soluble starch of MRS was replaced by carboxy-methyl-cellulose, phytic acid and tannic acid as only carbon source for research of cellulase, phytase and tannase, respectively.

For the effect of temperature and initial pH growth**,** 24-hours colonies of each isolate were cultivated for 48 hours in sterile MRS broth at 4°C, 23°C, 30°C, 37°C, 45° and 55°C. To assess the ability of isolates to grow at different pHs, MRS broth adjusted to 3; 4; 5; 6; 7; 8 and 9 using acetic acid were inoculated as before. The growth of the strains at 37°C was monitored by measuring the OD at 600 nm (19). Fermentative type of LAB was determined by the ability of strains to produce gas from glucose by using MRS agar supplemented with 0,005% of bromocresol against a negative control (not inoculated). Medium was put in tube and sterilized for 15 min at 121°C. After cooling, each strain was cultivated at 30°C for 48 h. The ability of each strain to produce acid was assessed by the change of the medium color. Presence of gas at the bottom of the tube indicates heterofermentative LAB. Otherwise (absence of gas), the strain was homofermentative (20). Fermentation profile of each sole carbon source (glucose, galactose, raffinose, soluble starch, Sorbitol, Trehalose, Xylose) (20 g/l) was tested at 30°C with 0.004 g/L of bromocresol purple in tubes. An 18 h culture of each isolate was used as the inoculums. If the sugar was fermented after the growth of the strain, the purple would turn yellow (visual evaluation) (21). Effects of NaCl on the isolate growth were studied on MRS supplemented with NaCl (2%, 4% and 6.5%) and with 0,005 % of bromocresol, respectively (19).

All experiments were carried out at least twice independently. The data were analyzed using analysis of variance (one-way Anova) and Tukey's Honestly Significant Difference test (HSD) (the levels of significance 5%) by the XLSTAT statistical software (version 16.0). Principal component analysis (PCA) is a technique for identifying patterns in data, reducing the dimensions of dataset, and increasing interpretability but at the same time minimizing information loss (22).

Results

Twenty organs of ENUS (seven fruits and eight vegetables) were fermented for three days at room temperature to isolate their potential fermentative probiotic LAB. Four strains able to survive at pH=2 and characterized as Gram positive, Oxydase negative and Catalase negative were isolated from garlic, parsley and passion fruit, respectively (Table 2). Their survival rate at pH=2 during three hours was 48-80%. The salt bile tolerance was a determining criterion for probiotic selection. After their exposure to 0.3 % bile salts, three strains codified A6, Pe3 and Pa6 isolated from garlic, parsley and passion presented survival rates of 72.25±0,81%, 83.10±0.14% and 84.67±0.19 %, respectively. The homofermentative isolate A6 was associated in a clusters form while the two other heterofermentative isolates Pe3 and Pa6 were grouped in a chain. Their hydrophobicity (*In vitro* adhesion to mucosal epithelial cells) were from 65% to 70% that was more than 50%. The protective effects of isolates against heat-induced protein denaturation were shown in the Figure 1. A6, Pe3 and Pa6 inhibited the denaturation of BSA

Table 2: Health properties of isolated strains.

Strain	Origin	Shape	Group	Survival (%) at pH=2	Survival (%) at 0.3% bile salts	Hydrophobicity (%)	Anti-oxidant activity (%)	Anti-inflammatory activity (%)
A6	Garlic	Rod	Cluster	50±2^{c}	72.25±0.81^{b}	70.64±0.06^{a}	14.51±1.36^{d}	78.61±3.25^{a}
Pa6	Passion	Cocci	Chain	70±3^{b}	84.67±0.19^{a}	70.92±0.01^{a}	30.65±2.01^{b}	8.88±2.87^{d}
Pe2	Parsley	Cocci	Chain	80±5^{a}	0	36.77±0.04^{c}	39.48±1.47^{a}	20.27±2.51^{c}
Pe3	Parsley	Cocco-bacille	Chain	48±1^{c}	83.10±0.14^{a}	65.93±0.03^{b}	24.06±1.78^{c}	30.9±2.16^{b}
Pi5	Pepper	Cocci	Chain	54±3^{c}	0	nd	nd	nd
Pa4	Passion	Cocci	Chain	85±1^{a}	0	nd	nd	nd
Pi3	Pepper	Cocci	Chain	72±4^{b}	0	nd	nd	nd

Data were shown as mean±standard error of the mean (SEM). Along the column, means were similar (p<0.05), were indicated by the same letter "a". nd: not defined.

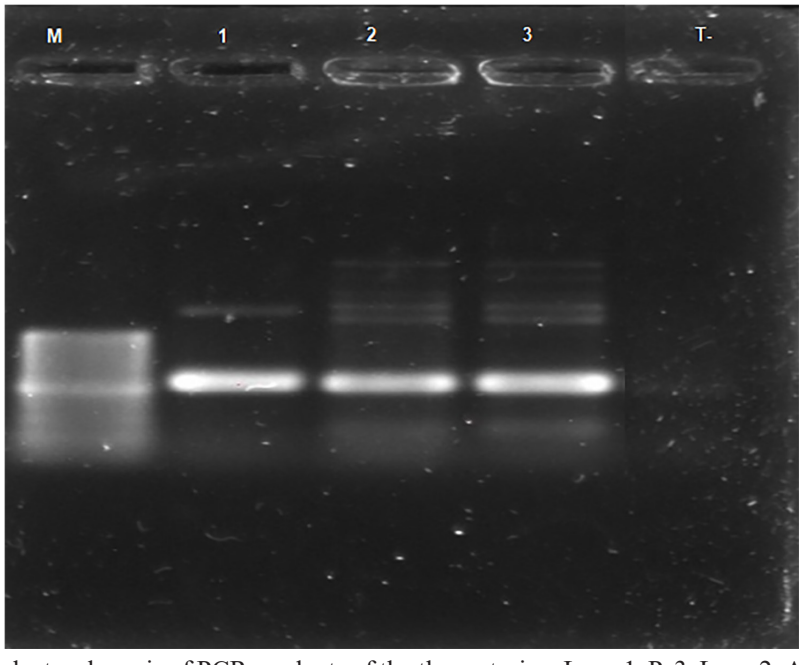

Figure 1: Agarose gel electrophoresis of PCR products of the three strains: Lane 1: Pe3; Lane 2: A6; Lane 3: Pa6. , DNA size markers (GeneRuler DNA ladder mix).

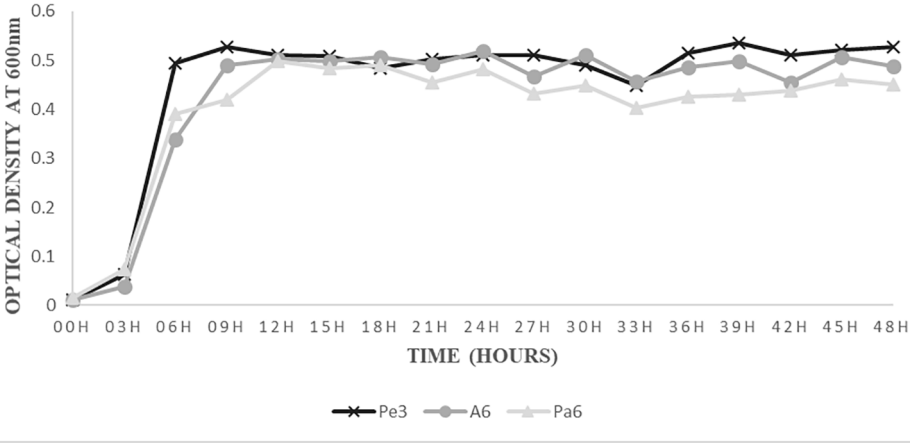

Figure 2: Growth of the three strains over 48 h.

at rates from 08.88±02.87% to 78.61%±03.25%. The greatest inhibitory effect was obtained with A, while the weakest was obtained with Pa6. The three isolates supernatants reduced DPPH from 14.51±1.36 to 39.48±1.47. The greatest reducing effect was obtained with Pa6 (30.65%), while the weakest value (14.51%) was seen with A6) (Figure 2 and Table 2).

The three strains A6, Pa6 and Pe3 were able to survive at pH of 3, 5, 7 and 9 with a weak growth at pH=3. But from pH=5, a faster increase was observed up to pH=9 for all the isolates. Pe3

reached its maximum at pH=7, while Pa6 reached its maximum at pH=9. The three isolates were facultative anaerobic (grew as well-in the absence and presence of oxygen), with growth ability at 23, 30 and 37°C. None of these isolates tolerated 4 and 55°C, but isolate Pe3 supported 45°C and were resistant to 2 and 4% NaCl and only A6 was sensitive to 6.5%. They all fermented raffinose (an indigestible sugar), showed similar fermentation profile excepted for soluble starch of which the fermentation was strain-dependent. Strain A6 fermented soluble starch in contrast to Pa6 and Pe3 (Table 3).

The resulting sequencing and sequence analysis of ribosomal 16S gene indicated that Pe3, Pa6 and A6 were *L. plantarum* with 99.58%, 99.38% and 100% of similarity, respectively (Figure 1).

At 24 h of fermentation, Pe3 and Pa6 decreased the pH of 1.96 and 1.94 units and were considered as fasters acidifying LAB, with pH values of 4.24 and 4.42 and titrable acidities of 1.68±0.02% and 1.72±0.11%, respectively. The second faster acidifying LAB (A6) decreased after 24 h, the pH of 1.86 unit with a pH value of 4.34 and a titrable acidity of 2.13±0.11%. The third acidifying LAB could not decrease the pH of 1 unit in less than 24 h of fermentation. After 24h, the titrable acidity remained constant for the three strains (Table 4). The three strains synthesized amylase, cellulase, phytase and tannase. Except for tannase of which the synthesis was similar for the three strains, A6 was more amylase and phytasee producer and Pa6 was more cellulase producer (Table 4).

Suitability for growth of isolates showed that Pe3 had the better growth followed by A6 and Pa6. From 0 h to 3 h, it was observed the latency phase for the three strains and then until 6 h, the exponential phase was noticed. From 6 h to 9 h, the slowing down phase was observed and a part from 9 h, began the stationary

Table 3: General characteristics of the three potential probiotic LA starters.

Isolate			
Environmental conditions	**A6**	**Pa6**	**Pe3**
Temperature (°C)			
4	-	-	-
23	++	++	++
30	+++	+++	+++
37	++	+++	+++
45	-	-	+
Sugar (20 g/L) fermentation			
Raffinose	+	+	+
Glucose	+	+	+
Galactose	+	+	+
Soluble starch	+	-	-
Sorbitol	+	+	+
Threhalose	+	+	+
Xylose	+	+	+
NaCl concentration (%, W/v)			
2	+	+	+
4	+	+	+
6.5	-	+	+
Initial pH			
3	+	+	+
5	+	++	++
7	+++	+++	+++
9	+	+	+

The data were presented in the form of (+) for tolerance and (-) for no tolerance.

Table 4: Enzymatic synthesis and acidification abilities of isolates.

Isolate	Amylases (cm)	Cellulase (cm)	Phytase (cm)	Tannase (cm)	Titrable acidity (%) at 24 h	ΔpH at 24h	Titrable acidity (%) at 48 h	ΔpH at 48 h
A6	2.0/H	1.5/H	2.1/H	0.6/M	2.13±0.11^{b}	1.86±0.02^{a}	2.13±0.02^{a}	1.87±0.03^{b}
Pe3	0.6/M	2.0/H	0.6/M	0.6/M	1.68±0.02^{a}	1.96±0.01^{a}	1.64±0.20^{b}	2.07±0.01^{a}
Pa6	0.6/M	2.1/H	1.9/H	0.6/M	1.72±0.11^{a}	1.94±0.01^{a}	1.84±0.00^{b}	1.87±0.01^{b}

Data were shown as mean±standard error of the mean (SEM) of 3 strains. Means are similar (p<0.05), were indicated by the same letter "a".

phase until 21 h followed by a slow and long phase of decline until the end of the growth. The microbial growths were accompanied by the synthesis of antioxidant and anti-inflammatory molecules (Figure 3). The antioxidant activity gradually increased until it reached a peak after 24 hours of culture and then, gradually decreased before stabilizing until 48 hours. The highest peak (51.51%) was seen for the Pa6 strain. The anti-inflammatory activity was maximal at the sixth hour and tended to stabilize. Furthermore, an adding of 10 g of glucose was detrimental to the synthesis of antioxidants, while favoring a significant increase in the synthesis of anti-inflammatory activities (Figure 4). Also, the three strains synthetized exopolysaccharides as indicated by their sticky and shiny colonies forms on MRS sucrose (40 g/L).

Discussion

Recently, probiotic LA bacteria with antioxidant and anti-inflammatory abilities are highly exploited in the global search for new therapeutic leads against various pathologies. So their isolation and selection are of great importance. Many scientific discoveries corroborate the fact that their health potentialities are even under-exploited. The three strains A6, Pa6 and Pe3 selected in this study are interesting probiotic potentialities which could allow them to survive and grow in the drastic conditions of the gastrointestinal tract after adhesion. Indeed, their survival rate at pH of 2 for 3 hours and their resistance to bile salts are greater than 50%, as are their hydrophobicity values more than 50%. In addition, considering environmental conditions, the three strains tolerated high pH and presented an osmotolerant property (growth at 6.5% NaCl) for strains Pe3 and Pa6 and Pe3 was also thermotolerant, with abilities of growth and acidification up to 45°C. These two properties suggest they could be suitable for industrial applications, especially for the thermotolerance in tropical regions where ambient temperature is generally higher than 30°C (23).

Indeed, this property makes it possible to reduce cooling energy during production in bioreactor. Moreover, a high fermentation temperature also reduces contamination by other microorganisms. Fermentation of fruits and vegetables is most often carried out in a salty environment. Salt has many functions in the fermentation processes. It decreases the risk of undesirable growth of spoilage germs of yeasts and molds, allows obtaining crisper fermented products by hardening vegetable pectins and decreases the activity of pectinase. On the other hand, the isolates could remain viable during the shelf life of the fermented products with regard to the salt content. These results show that the strains are immediately favored in a salty environment, which is an advantage for a successful lacto-fermentation. Therefore, these LAB strains would be of interest for

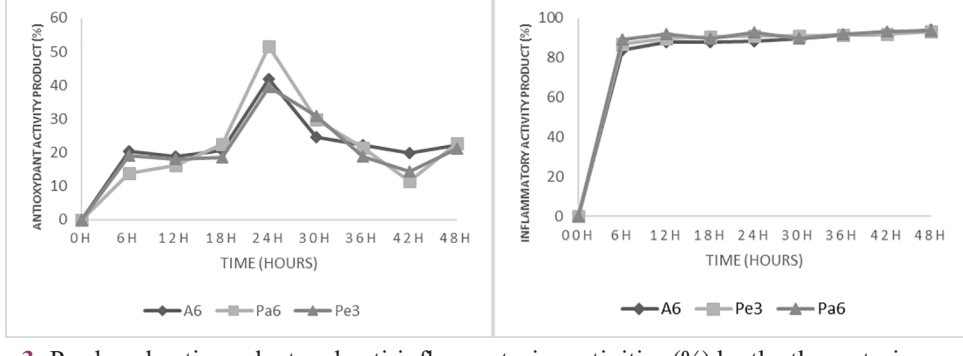

Figure 3: Produced anti-oxydant and anti-inflammatories activities (%) by the three strains over 48 h

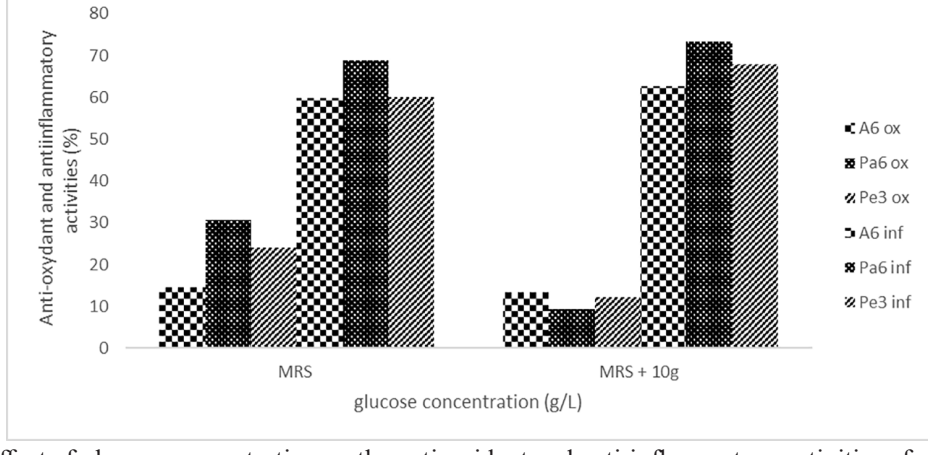

Figure 4: Effect of glucose concentration on the anti-oxidant and anti-inflammatory activities of selected LAB.

functional food development. They ferment several sugars including raffinose (an indigestible sugar) into lactic acid to prevent indigestion and stomach bloating, source of gastrointestinal disorders. An important criteria for potential microbial starter strains is the acidification ability and the pH decrease to extend the lag phase of foodborne pathogens (23).

The three LAB strains were considered as fast acid producers compared to Kostinek *et al.* (2007) who demonstrated that this potentiality is mainly found among *L. plantarum*. In general, these three strains induced a rapid and significant decrease in pH of MRS broth (below 4.5 after 24 h) accompanied by production of lactic acid. Total titrable acidity increased significantly from 6 to 12 h. At the end of 24 h, it reached more than 1%, and after, it remained constant. A pH of less than 4.2 to inhibit pathogens is an important food safety parameter (23). Thus, from both a quality and safety perspective, the use of starter cultures is recommended (23). Regarding such technological properties, these three isolates would be confirmed in further steps by pilot plant fermentations. These results also are in agreement with literature considering *L. plantarum* as suitable starter culture for fruits and vegetable lacto-fermentation. Hydrolytic enzymes are an interesting technological property for fermentation of fruits and vegetables (5). These enzymes contribute to the softening of plants (5). However, only a few amylolytic LAB have been isolated from starchy fermented foods in Africa (20).

In addition, tannase and phytase synthesis is useful to eliminate anti-nutritional factors during the fermentation process. Exogenous and endogenous enzymes produced during domestic processing have been reported to significantly reduce the levels of antinutritional factors such as phytate and tannin of some fruits, cereals, and legumes. These isolates would therefore be of interest for lacto-fermentation because of their ability to colonize the intestinal tract and control undesirable intestinal bacteria. Most of these three strains are highly resistant to antibiotics. In addition, these three strains have healthier properties as probiotics of *L. plantarum* 299v and *L. salivarius* which attenuated colitis in placebo-controlled trials by their anti-inflammatory activity (24). The antioxidants activities of these three strains would act in the body by scavenging harmful free radicals involved in degenerative diseases such as cancer, arthritis, and aging (5). Oxidative damage is part of the pathogenesis of chronic diseases. Antioxidant and/or anti-inflammatory and/or hypocholesterolemia and/or prebiotic properties have been reported for EPS producing strains for functional food formulations (25, 26). Thus, A6, Pa6 and Pe3 could have healthier beneficial effects to be industrially exploited.

Conclusion

The harmful effects of the nutritional transition (mainly the high prevalence of metabolic diseases in the world) have led in recent years to the need for good health of the intestinal microbiota. Many functional foods have thus emerged through their probiotification. This nutritional revolution could also take place with ENUS, which are available, accessible and less expensive, but neglected in Sub-Saharan Africa. As ENUS are associated with local communities and religious ceremonies, it would be an interesting tool in the fight against poverty and to improve the daily lives of the local populations who use them and to revitalize local gastronomy and celebrate the identity of the terroirs. Strains A6, Pe3 and Pa6 with such interesting properties could also be of interest to the food, nutrition and medicinal industries in general.

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Conflict of Interest

None declared.

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