American Journal of Microbiological Research, 2023, Vol. 11, No. 2, 31-39
Available online at http://pubs.sciepub.com/ajmr/11/2/1
Published by Science and Education Publishing
DOI:10.12691/ajmr-11-2-1

Preliminary Screening of Four Lactic Acid Bacteria with Better Health-Promoting Attributes Isolated from Five Ivorian Stapple Foods for Food Industry

Konan Jean Noel Yao*, Hadja Djeneba Ouattara, Kouadio Eric Donald N'dri, Allah Antoine Assamoi

Laboratory of Biotechnology, Agriculture and Valorization of Biological Resources, University Felix Houphouet Boigny, Cote d'Ivoire (Abidjan)
*Corresponding author: ykjeannoel@gmail.com

Received April 08, 2023; Revised May 13, 2023; Accepted May 21, 2023

Abstract The search for microorganisms of interest for new applications is of paramount importance. Thus, five Ivorian traditional fermented stapple foods (sorghum beer "tchapalo", cocoa beans, cassava paste "placali", culinary Parkia biglobosa Seeds condiment "Soumbala" and palm wine "bandji") were used for the first time as isolate source of probiotic lactic acid bacteria with antioxidant and anti-inflammatory aptitudes comparatively to the two starters strains of an international yogurt marketed in Côte d'Ivoire. This preliminary study resulted in obtaining four strains identified by their ARN sequencage as *Lactobacillus plantarum* (TC4 and TC7), *Pediococcus acidilactili* (PL1), *Enterococcus faecalis* (C13) with excellent in Vitro probiotic aptitudes (hydrophobicity, resistance to pH 2 and bile salt). They all presented antioxidant (25,50 ± 2,36 - 33,05 ± 4,45%), anti-inflammatory (28,35 ± 10,42 - 74,65 ± 8,18%), exopolysaccharides activities, synthetized beneficial enzymes (amylase, cellulase, phytase, tannase), fermented the indigestible sugar raffinose and were strain-dependant resistant to some food pathogens, antibiotic and environmental stresses, respectively. These remarkable technological properties in regarding to the yogurt starters strains position them as good candidates for the agri-food, nutrition and health sectors.

Keywords: *technological properties, lactic acid bacteria, antioxidant, antiinfmattaory, traditional fermented stapple foods*

Cite This Article: Konan Jean Noel Yao, Hadja Djeneba Ouattara, Kouadio Eric Donald N'dri, and Allah Antoine Assamoi, "Preliminary Screening of Four Lactic Acid Bacteria with Better Health-Promoting Attributes Isolated from Five Ivorian Stapple Foods for Food Industry." *American Journal of Microbiological Research*, vol. 11, no. 2 (2023): 31-39. doi: 10.12691/ajmr-11-2-1.

1. Introduction

Lactic acid bacteria have (LAB) have been used for several millennia for the manufacture and preservation of food. Generally Recognized as Safe (GRAS), they ferment sugars (mainly glucose) into acid. Their production of various compounds (lactic acid or a mixture of lactic acid, acetic acid, ethanol and CO2) during lactic fermentation extends the shelf life of foods by limiting the growth of contaminating microorganisms and pathogens [1]. LAB represent commercially after yeasts the most important group of microorganisms [2]. As starters, they also occupy a prominent place in the chemical industry for the production of lactic acid and biopolymer. In recent years, they have acquired a growing role in animal and human health in the form of probiotics, symbiotics after mixing with prebiotics, possessing several beneficial effects for the health of the host [3,4]. LAB have several beneficial actions, whether at the level of the product obtained or at the level of the benefits for the consumer by improving the sensory properties and the nutritional properties. They synthesize several interesting molecules such as organic acids, exopolysaccharides, bacteriocins… [5]. Through their enzymatic action, they also release phenolic compounds and thus improve their bioavailability. Lactic fermentation helps to better preserve minerals, vitamins and phenolic compounds, thus preserving the antioxidant properties of the food. It breaks down antinutritional compounds or compounds that are difficult for the body to assimilate, such as phytic acid or tannins [5]. While the beneficial effects of fermented foods on health are widely documented to the point of being sources of isolation of microorganisms of health interest. In Côte d'Ivoire, there is still no work relating to the isolation and selection of probiotic, anti-inflammatory and/or antioxidant strain. This preliminary work aims to fill the gap in this sense from five common Ivorian naturally fermented stapple foods ("tchapalo", cocoa beans, "placali", "Soumbala" and "bandji") which are commonly eaten in Côte d'Ivoire and contribute to a subsistence household economy with low technological innovation. Although they are abundant and available

locally and despite their possible functional potential, these foods are not sufficiently valued.

2. Materials and Methods

2.1. Isolation, Selection and Tentative Identifation of Probiotic Lactic Bacteria

Fresh samples of five (5) Ivorian naturally fermented staple foods (sorghum beer (tchapalo), cassava paste (placali), culinary Parkia biglobosa Seeds broth (soumbala), palm wine (bandji) and cocoa beans were purchased from nine large merchants chosen at random from the Gouro market (Abidjan) as well as an international brand yogurt marketed in Côte d'Ivoire, in pre-sterilized sampling bags, transported to the laboratory in an icebox carrier, stored at 4°C and 1g of each sample was aseptically homogenized in a stomacher with 9 mL of sterile buffered peptone water (Oxoid, Basingstoke, UK) initially adjusted to pH 2 with a 3M hydrochloric acid solution. Isolation of LAB were done by successive dilutions (10^{-1}-10^{-5}) and inoculation onto MRS plates. Plates were spread and 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 picked from the agar plates. A culture of the collected strains was done on MRS agar, then repeatedly spread to check their purity and a morphological description was performed by biochemical tests. Stock cultures of isolates were stored in MRS broth containing 20% glycerol at -80°C. The probiotic properties concerned the hydrophobicity potentiality of the strains and their resistances to acidity, bile salt, antibiotic, intestinal and non intestinal pathogens, respectively.

The acid resistance of each LAB was determined in 10 ml of MRS broth adjusted with 3M hydrochloric acid solution at pH 2 for 3 hours [6]. To neutralize the acidity of the culture medium, dilutions for bacterial cell counts were made in 0.1 M phosphate buffer, pH = 6.2. Cell counts were performed on MRS agar and survival rate was determined relative to the control. Tolerance to bile salts was obtained by the rate of resistance compared to a control after 18 h of preculture of a new colony in the presence of 0.3 and 0.5% of a mixture of conjugated and unconjugated bile salts (Oxgall Powder B-3883 SIGMA) [7]. The hydrophobicity of the bacterial cell surface was assessed by the ability of bacteria to adhere to hydrocarbons (BATS: Bacterial Adhesion to Solvents) [8]. Xylene was taken as the hydrophobic substance for the test. One milliliter of each young bacterial culture (18 h) was centrifuged at 8000 rpm for 15 min and the collected cells were washed twice with PBS (pH 7.4) and resuspended in 3 ml of the same buffer solution. The optical density (A0) is determined at 600 nm. A proportion of xylene is added in equal parts and vortexed for 5 min. The vortexed mixture is incubated at 37°C for 1 hour. The absorbance at 600 nm of the organic matter (A1) is read. The bacterial adhesion capacity (BATH) is calculated as follows: BATH% = [(A0 - A1)/A0] × 100.

Antibiotic resistance was tested on MRS by the standardized technique of diffusion of the antibiogramm committee of the French society of microbiology (CASFM) [9]. 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 sudied 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 (100µL of homogenized precultures in MRS agar) and after sfter solidification, 6 mm wells were pierced with a Pasteur pipette handle under sterile conditions. Each well was filled with 50 uL 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 [10]. From these results, five strains coded TC4, TC7, PL1, and C13 were selcted and then identified after colony PCR by amplification of their hypervariable (HV) region of the 16S gene (approximately 500 bp). The obtained amplicons were then sequenced to determine their species by comparison with the National Center for Biotechnology Information (NCBI) database. For PCR amplification of the 16S ribosomal gene, the 500-bp fragment containing the hyper-variable region of the 16S rDNA from each sample was amplified using two primers: F27 (5'-AGAGAGTTTGATCCTGGCTCAG-3') and R520 (5'-ACCGCGGCTGCTGGC-3') [11]. PCR amplification was performed in a Biometra thermal cycler (model Tgradient, Germany). Reactions were performed in a final volume of 50 µL containing 1 µL of DNA extract, 0.25 µL of 5U Taq polymerase (Go Taq DNA polymerase, Promega®, USA), 5 µL of 10X buffer, 1 µL (10 mM) of deoxynucleoside triphosphate (dNTPs, BioRad® France) and 2 µL (10 mM) of each primer (Eurogentec, Lyon, France). After an initial denaturation at 95°C for 4 min, the reactions were run for 35 cycles, each cycle including: denaturation at 95°C for 1 min, annealing at 56°C for 1 min and extension at 72°C for 1 min. Finally, an extension at 72°C for 10 min was performed. The presence and yield of specific PCR products were checked by 0.8% (w/v) agarose gel electrophoresis at 90 V for 1 h in 1X Tris-Borate-EDTA buffer and made visible by ethidium bromide staining and UV transillumination. PCR products were purified using the nucleospin II extraction kit (Machereye Nagel, Germany) and then sequenced using the F27 primer. Partial sequences of the 16S rRNA gene determined in the Microbiology, Adaptation, Pathogenesis laboratory (Lyon, FRANCE) were deposited in the NCBI databas.

2.2. Screening of the Technological Properties of Strains

EPS (exoploysaccharide) production was determined using MRS sucrose agar (40 g-L-1) as previously described [12]. The ability of the isolates to produce amylase, cellulase, phytase and tannase was detected by

the replacing in the MRS glucose by soluble by starch, carboxy-methyl-cellulose, phytic acid and tannic acid as only carbon source, respectively [13]. All plates were incubated at 30°C for 48 h in an anaerobic jar. After incubation, Lugol's iodine was used for the revelation of pectinase, cellulase and phytase production. For tannase production, appearance of clear zone around the growth attests a positive test. The anti-oxidant, anti-inflammatory and titratable activities of the isolates were screened on their Cell-Free Culture Supernatant (CFCS). After the 48 hours culture of every isolate in MRS broth at 37°C, the culture was centrifuged at 6000 min^{-1} (RCF 3341×g) for 10 min (MPW-56, MPW Med. Instruments, Warsaw, Poland), sterilized with a MF-Millipore™ Membrane Filter (0.22 µm, 13 mm; mixed cellulose esters membrane, hydrophilic; Merck, Darmstadt, Germany) and then the supernatants were obtained. The anti-inflammatory activity of each LAB was determined by the bovine serum albumin (BSA) inhibition assay [14]. The reaction mixture (0.5 ml) consisted of 0.45 ml of BSA (5% aqueous solution) and 0.05 ml of bacterial culture supernatant or diclofenac (reference drug) [14]. The pH was adjusted to 6.3 using hydrochloric acid (1N). The samples were then incubated at 37°C for 20 minutes, followed by heating to 57°C for 3 minutes. After cooling, 2.5 ml of phosphate buffer (pH = 6.3) was added to each test tube. The 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 percentage inhibition of protein denaturation was then calculated as follows: % inhibition = 100- [(O.D. of sample - O.D. of control /O.D. of control)] x 100. For antioxidant activity, 1ml of bacterial CFCS was added in 2ml of ethanol (70%) and centrifuged (6000 rpm for 10 min) and the supernatant was collected. 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 of DPPH at the same concentration [15]. After incubation in the dark for 30 min, the absorbance was read at 517 nm and the percent decolorization of DPPH was determined by the formula below: I (%) = ((DOc-DOex)/DOc) x100. Produced titratable acidity and pH values were measued on 10 ml of CFCS of every strain with a sodium hydroxide solution (NaOH, 0.1 N) in the presence of two drops of phenolphthalein and with a HANNA pH meter [16]. The titratable acidity (AT) was expressed as a percentage of lactic acid according to the formula: AT (%) = (Vol Naoh×N×0.09×100)/ (Vol test) [17].

2.3. Screening of Environmental Stresses Resistance, Fermentative Profile and Type

The effects of NaCl (2%, 4% and 6.5%), temepartures (4°C, 23°C, 30°, 37°, 45° and 55°C) and pH (3; 4; 5; 6; 7; 8 and 9 adjusted with using acetic acid) were studied with a young pre-culture of every LAB at 37°C for 48h in MRS broth supplemented with bromocresol purple (BCP) (0.04g/l) as a pH indicator. Fermentative type of every LAB was studied on MRS containg Durham bells and their fermentation of every sugar (glucose, raffinose, xylose, galactose, soluble starch, trehalose, and sorbitol as sole carbon source) was tried. The production of gas (CO_2) was reflected by the appearance of bubbles in the bell [18]. The growth of the tested strain and its production of acid resulted in a yellow turn of the pH indicator against a control (without inoculum).

2.4. Statiscal Analysis

All experiments were carried out at least twice independently. The data were analysed using analysis of variance (one-way Anova) and Tukey's Honestly Significant Difference test (HSD) (the levels of significance 5%) by the RSTUDIO and IBM SPSS statistical software (version 4.2.3 and 20).

3. Results and Discussion

3.1. Main Characteristics of the Obtained Isolates

Five current Ivorian fermented foods (sorghum beer "tchapalo", cocoa beans, cassava paste ''placali'', culinary Parkia biglobosa Seeds condiment "Soumbala" and palm wine "bandji") were served to isolate healthier strains with probiotic properties for the food, nutrition and health industries in general. After the first isolation tests following incubation of the samples at pH 2 for 48 hours, 58 presumptive LAB (Gram+, catalase-, oxidase-) among which 40 bacillus and 18 cocci were isolated (data not shown). Cocoa beans (32 isolates) was the major isolates source followed by sorghum beer ''tchapalo'' (12 isolates), cassava dought ''placali'' (7 isolates), culinary condiment "Soumbala" (5 isolates) and palm wine ''bandji'' (2 trains). Currently, there is a great interest in the isolation of new probiotic LAB strains from unconventional fermented staple foods (kimchi) [19], agave (Agave angustifolia Haw) [20]. After various successive screening tests (tolerance to pH 2 and 0.1-03% bile salt, cell surface hydrophobicity, synthesis of antioxidants, anti-inflammatories, exopolysaccharides, respectively), four of tthese strains, resistant to 0.3% bile salt (excepted TC7) while they were all resistant to 0.2% bile salt, with anti-oxydant ((25,50 ± 2,36 - 33,05 ± 4,45%), anti-inflammatory (28,35 ± 10,42 -74,65 ± 8,18%), were so selected comparatively to the two probiotic LAB starters strains Lb (a bacillus and therefore probably *Lactobacillus bulgaricus*) and Lc (a cocci and therefore probably *Streptococcus thermophilus*) of an international yogurt marketed in Côte d'Ivoire (Table 1). From these results, cocoa beans were the best source of isolation followed by tchapalo and then cassava dought, respectively. The beneficial effects of *cocobiota* (cocoa fermentative microorganisms) are documented [21]. Palm wine and Parkia biglobosa Seeds condiment were a weak source of isolation, respectively. The one strain isolated from cocoa bean C13 demonstrated hight pH tolerance than the others strains. The tchapalo and cassava strains, on the other hand, were more resistant to 0.3% bile salt. Generally, the first criteria for a probiotic microorganism is acid and bile salt resistances to ensure their survival through the stomach and intestine, respectively. The resistance to this drastic living condition suggests that the

four selected bacteria were able to cross stomach acidity and reach the intestinal environment to multiply there showed that very few lactic acid bacteria strains are able to resist to 0.3% bile salts for 3 hours [22]. This concentration is harmful to most microorganisms and inhibits their probiotic actions. LAB resist to that bile salt concentration by synthesizing a hydrolase (BSH) that deconjugates bile acids. In a recent study, the resistance to acidity of LAB probiotics isolated from the fermentation process of cassava and cocoa beans from Côte d'Ivoire was improved by up to more than 90% at pH 4 while some of these strains could survive up to 1% of bile salt [23]. The denaturation of tissue proteins is a well-documented cause of inflammation which may be at the origin of the production of autoantigens in certain diseases (arthritic, rheumatoid, …). The protective effects of the CFCS against protein denaturation (albumin) caused by heat indicate inhibition was more than 50% for TC7, PL1and C13. Only TC4 had lower anti-inflammatory activity production than both yogurt strains. C13 with the highest rate of synthesis in anti-inflammatories greater than 80% would be industrially exploitable in this direction. This activity is greater at the 48th hour for TC4 and TC7 but remains stable from the 24th hour for C13 and PL1 (Figure 1 a).

Moreover, the ability to trap the free radical DPPH reflects the presence of antioxidant activity. The microbial strains TC4, PL1 had an antioxidant activity greater than 35% and greater than that of the two yogurt strains (Table 1). This activity remain substantially unchanged from the 24th hour for all four LAB (Figure 1b). Probiotic *Limosilactobacillus reuteri FEEL 6901* with the abilty to synthetyse anti-inflammatory substances was considered by its intact cells to have an excellent antioxidant activity with 21.6% DPPH inhibition rate (higher than that of WCFS1 (10.6%) or LGG (17.1%)) [3]. Also, the four strains presented exopolysaccharides (new source of cancer treatment) synthesis ability by their sticky and shiny colonies forms on MRS sucrose (40g/l) agar (Figure 2). Recently, synthetic antioxidants have been used for stabilizing foods from oxidation deterioration [38]. Synthetic oxidants that are being used commonly are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tertbutylated hydroxyquinone (TBHQ). Nonetheless, certain of thse synthetic antioxidants (butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tertbutylated hydroxyquinone (TBHQ) are carcinogenic and toxic to human health. Hence, natural antioxidants due to their safe statut are preferred over synthetic antioxidants and are readily acceptable by consumers as they. Due to the limitation in the use of synthetic antioxidants, the demand for health-promoting natural antioxidants in foods is increasing. Comparatively to the two yogourt starters, the five isolates expressed good adhesion to hydrophobic compounds (xylene) (69.00 ± 0.01% to 77.72 ± 0.02%.), indicating potential good adhesion to the intestinal epithelium to colonize the gastrointestinal tract. These results were similar to those of lactic isolates towards xylene from [24]**.** Also, the antibacterial activity of a probiotic is essential against intestinal pathogenic bacteria. Strain-dependant, the four strains TC4, PL1, TC7 and C13 inhibited all the indicator pathogen strains like the probiotic LAB strain of *Lactiplantibacillus plantarum* isolated from kenyan spontaneously fermented milk, Amabere amaruranu [25]. They got off a total harmfulness towards the yeast (Candida albicans) and the two enterobacteria (Klebsiella pneumoniae and *Pseudomonas aeruginosa*) involved in the complications of diabetes [26,27,28]. These inhibitions could have several origins among which the production of antimicrobial compounds such as organic acids (lactic acid or acetic acid). Also compared to the two yogurt starter strains, the four LAB TC4, PL1, TC7 and C13 had a relatively better resistance to antibiotics. They were resistant to nalidixic acid (unlike the two strains of yogurt) but susceptible to erythromycin and tigecycline (Table 2).

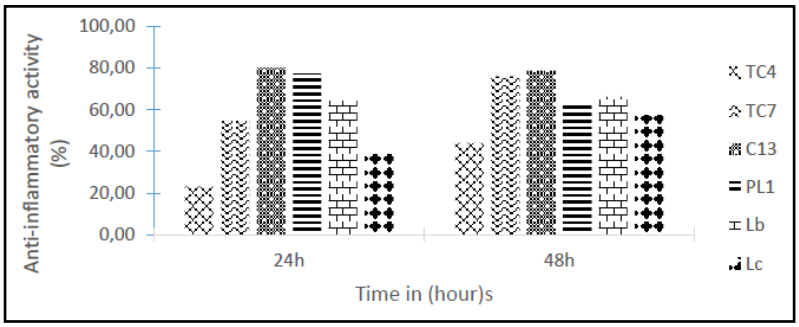

Figure 1 a. Produced anti-inflammatory by the seven strains in MRS broth

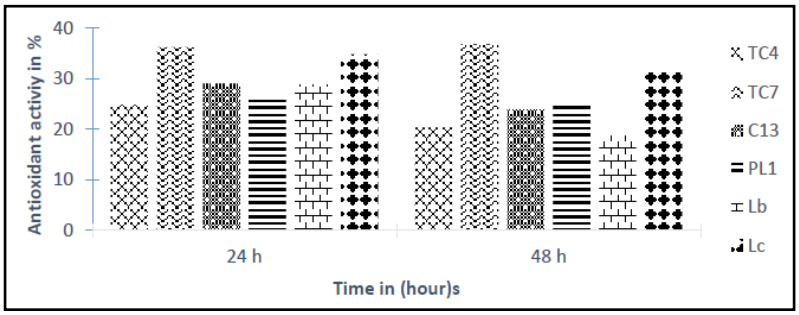

Figure 1 b. Produced antioxidant by the seven strains in MRS broth

Table 1. Health potentialities of selected lactic acid bacteria

Code of isolates	Survival rate (%) to pH 2	Survival rate at 0,3% bile salts	Cell surface hydrophobicity (%)	Activity antioxidant (%)	Anti-activity inflammatory (%)	Exopolysaccharaides synthesis
TC4	$4,01 \pm 0,55^{d}$	$93,34 \pm 0,47^{e}$	$69,00 \pm 0,01^{e}$	$28,60 \pm 8,12^{a}$	$28,35 \pm 10,42^{b}$	+
TC7	$15,78 \pm 1,07^{e}$	$54,50 \pm 0,06^{f}$	$77,72 \pm 0,02^{c}$	$33,05 \pm 4,45^{a}$	$68,46 \pm 10,51^{a}$	+
C13	$31,05 \pm 1,06^{a}$	$75,16 \pm 0,07^{a}$	$71,72 \pm 0,02^{a}$	$25,50 \pm 2,36^{a}$	$70,19 \pm 11,85^{a}$	+
PL1	$11,30 \pm 1,46^{c}$	$85,25 \pm 0,01^{d}$	$74,60 \pm 0,01^{d}$	$31,80 \pm 7,64^{a}$	$74,65 \pm 8,18^{a}$	+
Lb	$47,21 \pm 0,45^{b}$	$51,55 \pm 0,83^{b}$	$66,32 \pm 1,41^{b}$	$28,1 \pm 0,95^{a}$	$63,94 \pm 0,42^{a}$	+
Lc	$45,63 \pm 1,55^{b}$	$71,35 \pm 0,69^{c}$	$76,8 \pm 0,50^{c}$	$34,75 \pm 0,78^{a}$	$38,38 \pm 0,47^{b}$	+

The values are means standard deviations of triplicate measurements. Values with similar superscript alphabet letter along the column are significantly different at $P < 0,05$.

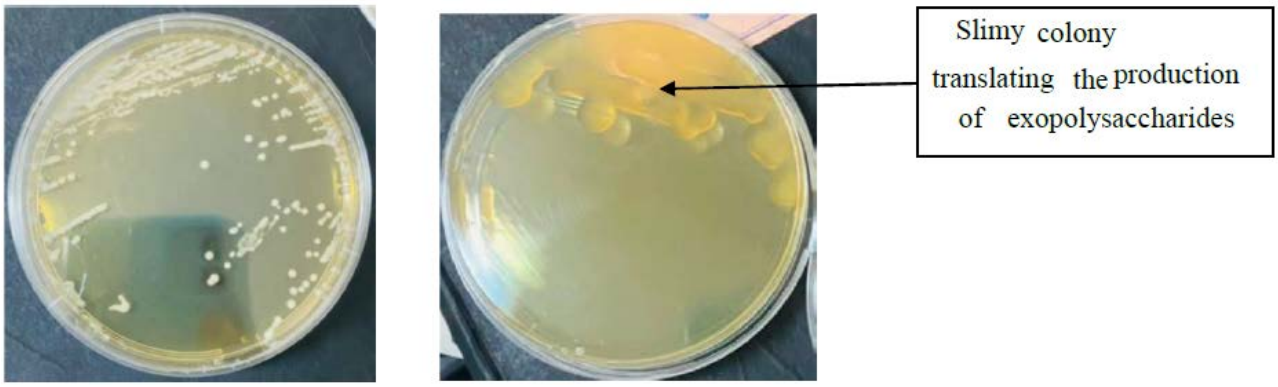

Figure 2. Production of exopolysaccharides by lactic isolate Appearance of the five strains on MRS (left) and (on the right) on MRS-sucrose (40 g•L-1) agar with exopolysaccharides synthesis (sticky and shiny colonies forms)

Table 2. Inhibition diameter obtained after confrontation of lactic isolates with pathogens

Isolates	Asp	Staph	E.Coli	Candida	Klebsiella	Salmo	Pseudo
TC4	$6 \pm 0,82^{c}$	$8,50 \pm 1,30a^{c}$	$8,75 \pm 0,96^{ab}$	$16,5 \pm 1,30^{c}$	$10 \pm 0,81^{a}$	$6,5 \pm 1,30^{c}$	$15 \pm 0,82^{c}$
TC7	$10,5 \pm 1,30^{b}$	$10,5 \pm 0,81^{bc}$	$6,75 \pm 1,70^{a}$	$11,5 \pm 1,30^{a}$	$15,25 \pm 0,96^{bd}$	$6 \pm 0,81^{c}$	$9,75 \pm 0,96^{a}$
C13	$13,5 \pm 1,30^{ab}$	$6 \pm 0,81^{a}$	$6,5 \pm 1,29^{a}$	$10,5 \pm 1,30^{a}$	$12,5 \pm 1,30^{ab}$	$9,5 \pm 1,30^{a}$	$11,5 \pm 1,30^{ab}$
PL1	$6,25 \pm 1,25^{c}$	$13,25 \pm 2,22^{b}$	$7 \pm 1,41^{a}$	$11,75 \pm 1,70^{a}$	$16,5 \pm 1,30^{cd}$	$11 \pm 0,81^{ab}$	$15,75 \pm 0,96^{c}$
Lb	$13,5 \pm 1,30^{ab}$	$5,75 \pm 0,95^{a}$	$6 \pm 0,82^{a}$	$6 \pm 0,82^{b}$	$18,75 \pm 1,5^{c}$	$11 \pm 1,83^{ab}$	$14 \pm 1,41^{bc}$
Lc	$14,25 \pm 2,06^{a}$	$12 \pm 1,83^{b}$	$10,25 \pm 0,96^{b}$	$12,75 \pm 1,5^{a}$	$17,25 \pm 2,06^{cd}$	$13,25 \pm 0,96^{b}$	$14,25 \pm 2,06^{bc}$

The values are means standard deviations of triplicate measurements. Values with similar superscript alphabet letter along the column are significantly different at $P < 0,05$.

Table 3. Diameters obtained by antibiotics on isolate

	Antibiotic diameters in mm								
Isolates	Chl	Pen	Ery	Kan	Sul	Tgc	Nal	Rif	Cef
TC7	20S	12I	26S	12I	6R	26S	6R	6R	18S
TC4	22S	10I	22S	18S	25S	20S	6R	21S	15S
PL1	24S	6R	30S	14I	25S	20S	6R	20S	6R
C13	24S	9R	20S	16S	22S	20S	6R	13I	20S
Lb	31S	6R	6R	6R	26S	25S	31S	6R	6R
Lc	30 S	6R	6R	25S	32S	27S	30S	6R	6R

Chl: Chloramphenicol; Pen: Penicillin G; Ery: Erythromycin; Kan: Kanamycin; Sul: Sulfonamide; Tgc: tigecycline; Nal: Nalidixic acid; Rif: Rifampicin; Cef: Cephalotine.

3.2. Technological Properties and Behaviour to Environmental Stresses of Selected Isolates

The four strains TC4, PL1, TC7 and C13 tolerated high pH (3-9) and presented an osmotolerant property (growth at 6.5% NaCl) with thermotolerant abilities of growth and acidification up to 37-45°C (except C13 at 45°C) (Table 4). None of the strains studied could grow at 4°C, including even curiously the two starter strains isolated from commercial yoghurt. These properties suggest that they could be suitable for industrial applications, especially in tropical regions where ambient temperature is generally higher than 30°C regarding to their thermotolerance. Indeed, this property makes it possible to reduce cooling energy in production scale in bioreactor. Moreover, a high fermentation temperature also reduces microbial contamination. They ferment several sugars including raffinose (an indigestible sugar) into lactic acid to prevent indigestion and stomach bloating, source of gastrointestinal disorders. They all synthesized degrading-anti-nutritional- factor enzymes (phytase and tannase) and cell-wall degrading enzymes (amylase and cellulase), respectively (Table 5). Hydrolytic enzymes are an

interesting technological property for fermentation of food-based plants [29]. These enzymes contribute to the softening of foods. However, only a few amylolytic LAB have been isolated from starchy fermented foods in Africa. 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. Moreover, their ability to synthesize lipase and protease would not be in doubt according to the work of [23] who demonstrated that probiotic LAB isolated from cassava and cocoa synthesize these two enzymes. An important criterion for potential microbial starter strains is the acidification ability and the pH decrease to extend the lag phase of foodborne pathogens. The five LAB strains were considered as fast acid producers compared to [30]. In general, they induced a rapid and significant decrease in pH of MRS broth (below 4.5 after 24 h) followed by synthesis of lactic acid. Total titratable acidity increased significantly from 6 to 12 h. At the end of 24 h, it reached more than 1%, and after, it remains constant (Table 5)). A pH less than 4.2 to inhibit pathogens is an important food safety parameter [13]. These results corroborate those of [31] who showed that probiotic LAB isolated from cassava and cocoa produce slightly more than 1-2.5% titratable acid on MRS. Since the main metabolite of LAB is lactic acid, TC4 could be exploited industrially for the production of lactic acid due to its high titratable acid excretion capacity. Thus, from both a quality and safety perspective, the future use of these strains for industrial applications, specially for pilot scale-controlled fermentation of their own food matrices into functional foods rich in antioxidants and anti-inflammatories is recommended in the current context of sustainable food in the face of the high prevalence of metabolic diseases. These new fermented foods would be more effective against the harmful effects of the nutritional transition in Sub-Saharan Africa where, it is only recently that the selection of LAB including those of [32] is interested in their ability to synthesise protective compounds with health effects (anti-oxidants, anti-inflammatory, etc.). The resulting sequencing and sequence analysis of ribosomal 16S gene revealed identities of C13 (*Enterococcus faecalis*), TC4 and TC7 *(Lactobacillus plantarum*), *PL1 (Pediococcus acidilactili)* with 99.58%, 99.38% and 100% of similarity, respectively. This diversity of species reflects that these fermented foods (cocoa beans, tchapalo, soumbala, placali and Bangui) are favorable environments for the development of most lactic bacteria. Controlled fermentation which is necessary to produce more standardized, hygienic, and stable products with an improved nutritional composition, could be an interesting tool to valorize these staple food-based plants. Recently, probiotic LAB bacteria with anti-oxydant and anti-inflammatory abilities are highly exploited in the global search for new therapeutic leads against various pathologies [33]. Probiotic lactic acid bacteria, whether or not metabolizing cholesterol and/or producing exopolysaccharides and/or antioxidants and/or anti-inflammatories are industrially highly sought for functional food formulations [34,35]. So, their isolation and selection are of great importance. Many scientific discoveries corroborate the fact that their health potentialities are even under-exploited. These strains are also favored in a salty environment, which is an advantage for a successful lacto-fermentation. Certainly, due to the liquid nature of tchapalo, strains originating from this medium grew faster in MRS liquid broth than other microorganisms. The latency phase was generally brief until the sixth hour for these five LABs, then the exponential phase between the sixth and the twelfth hour. The growth rate was then lower from the twelfth hour. Compatratetively to the two strains Lb and Lc, these four strains showed best growth performance.

Table 4. Main characteristics of the five potential probiotic LAB starters

	Isolates					
Environmental conditions	TC4	TC7	C13	PL1	Lb	Lc
Temperatures (°C)						
4	-	-	-	-	-	-
23	++	+++	++	+	+	+
30	++++	+++++	+++	+++++	+++++	++++
37	+++	++++	++	+++	+++	++
45	++	+	-	++	+	+
Sugar (20 g/l) fermentation						
Raffinose	+	+	+	+	+	+
Glucose	+	+	+	+	+	+
Galactose	+	+	+	+	+	+
Soluble starch	-	-	+	-	+	+
Sorbitol	+	-	+	+	+	+
Threolose	+	+	+	+	+	+
Xylose	+	-	+	+	+	+
Nacl concentration (%, W/v)						
2	+++	+++	++	+	+++	+++
4	++	+++	+	++	++	++
6.5	++	+++	+	+	+	+
Initial pH						
3	+	++	+	++	+	+
5	+	+	+	++++	++	+
7	+++	++	++	++++	+	+
9	+++	++	++	+++	+	+

Table 5. Enzymatic profile and acidification abilities of isolates

Isolates	Amylases (cm)	Cellulase (cm)	Phytase (cm)	Tannase (cm)	pH at 24h	Titratable acidity (%)
TC4	0.6/M	0.6/M	1.5/H	0.6/M	3,62 ± 0,01^{d}	2,78 ± 0,05^{d}
TC7	0.6/M	1.0/H	1.6/H	0.6/M	4,24 ± 0,15^{e}	1,75 ± 0,04^{c}
C13	0.6/M	2.0/H	2.0/H	0.6/M	4,69 ± 0,02^{a}	1,50 ± 0,02^{a}
PL1	0.6/M	1.8/H	2.1/H	1.1/H	4,02 ± 0,04^{c}	1,84 ± 0,04^{b}
Lb	1.5/M	1.5/M	2.0/M	0.6/M	3,14 ± 0,09^{b}	1,77 ± 0,01^{bc}
Lc	2.1/M	3.1/M	2.0/M	0.6/M	4,01 ± 0,02^{c}	1,83 ± 0,02^{bc}

High producers (d ≥ 1 cm), Medium producers (d ≥ 0.5 cm), Low producers (d < 0,5 cm).
The values are means standard deviations of triplicate measurements. Values with similar superscript alphabet letter along the column are significantly different at P < 0,05.

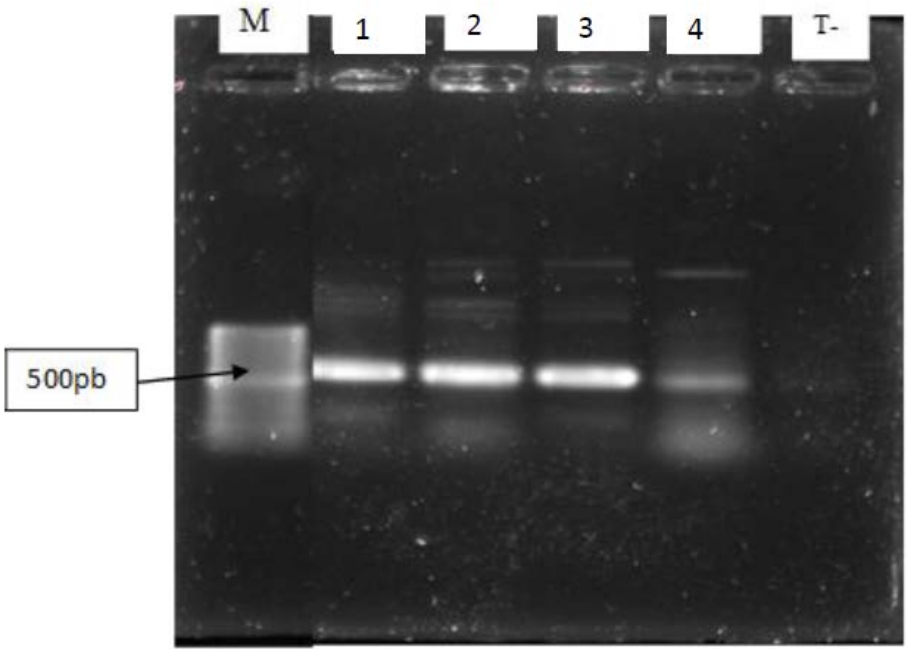

Figure 3. Electrophoretic profile of PCR amplification products of the 16 S RNA gene selected lactic isolates. M: Marker molecular; weight 1 kb (+) (Eurogentec, SmartLadder); 16 S gene positive amplicons (1: TC4; 2: TC7; 3: C13; 4: PL1) have the expected size of 500pb

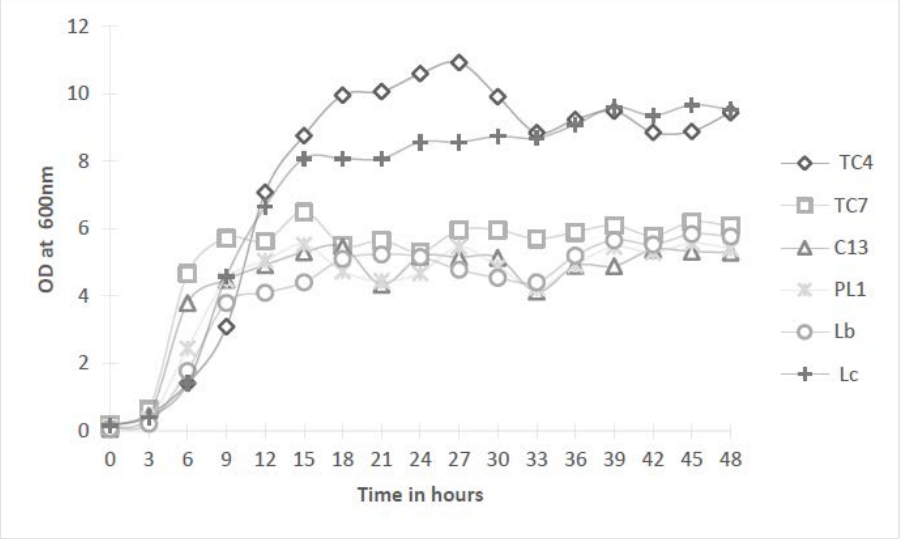

Figure 4. Growth of the seven strains in MRS during 48 hours

4. Conclusion

The new strains (TC4, TC7, C13, PL1) of this study are potential candidates for future use as food supplements, starters or for the production of molecules of health interest. Their GRAS status «Generally Recognized As Safe» could not be in doubt since their isolation matrices are usually consumed without any danger. However, additional data are necessary about these strains such their viability behavior during storage and the chemical nature of their antioxidant and anti-inflammatory substances. They should be tested in animal models against the effects of oxidative stress and/or inflammatory syndrome to confirm their probable health nutrition claims

and by clinical evaluations in diabetics or hypertensives humans.

Declaration of Conflicting Interests

The authors had no potential conflicts of interest in the research, writing and/or publication of this article.

Ethical Approval

The Laboratory of Biotechnology, Agriculture and Valorization of Biological Resources of the University Félix Houphouet Boigny of Cocody approved the study protocol. The work had the written or oral consent of all participants.

Funding

The authors have stated that they received the following financial support for the research, writing and/or publication of this article: This study was fully funded by the Fund for Science, Technology and Innovation (FONSTI).

Authors' Consent

The authors after several readings and corrections of the manuscript have given their approval for the publication of the article.

Acknowledgements

The authors would like to thank the FONSTI (fund for science, technology and innovation) for having financed the research project from which this work stems.

References

[1] S. Fan, T. Xue, B. Bai, T. Bo, and J. Zhang, "Probiotic Properties Including the Antioxidant and Hypoglycemic Ability of Lactic Acid Bacteria from Fermented Grains of Chinese Baijiu," 2022.

[2] H. N. J. Shangpliang, S. Sharma, R. Rai, and J. P. Tamang, "Some technological properties of lactic acid bacteria isolated from Dahi and Datshi, naturally fermented milk products of Bhutan," *Front. Microbiol.*, vol. 8, no. FEB, pp. 1-6, 2017.

[3] K. Abdisa, D. Atalel, G. Berhanu, and V. Kandi, "Probiotics in Health and Disease: A Review of Emerging Evidence of Potential Benefits and Harm," *Am. J. Microbiol. Res.*, vol. 10, no. 1, pp. 23-33, 2022.

[4] C. Hector Momo Kenfack, F. Zambou Ngoufack, P. Marie Kaktcham, Y. Rui Wang, T. Zhu, and L. Yin, "Safety and Antioxidant Properties of Five Probiotic *Lactobacillus plantarum* Strains Isolated from the Digestive Tract of Honey Bees," *Am. J. Microbiol. Res.*, vol. 6, no. 1, pp. 1-8, 2018.

[5] A. Fessard, E. Bourdon, B. Payet, and F. Remize, "Identification, stress tolerance, and antioxidant activity of lactic acid bacteria isolated from tropically grown fruits and leaves," *Can. J. Microbiol.*, vol. 62, no. 7, pp. 550-561, 2016.

[6] B. Hyronimus, C. Le Marrec, A. Hadj Sassi, and A. Deschamps, "Acid and bile tolerance of spore-forming lactic acid bacteria," *Int. J. Food Microbiol.*, vol. 61, no. 2-3, pp. 193-197, 2000.

[7] S. E. Gilliland, C. R. Nelson, and C. Maxwell, "Assimilation of cholesterol by Lactobacillus acidophilus," *Appl. Environ. Microbiol.*, vol. 49, no. 2, pp. 377-381, 1985.

[8] L. Mattsson *et al.*, "Nutritional improvement of beans (Phaseolus vulgaris) by natural fermentation," *Trends Food Sci. Technol.*, vol. 6, no. 1, pp. 46-49, 2017.

[9] SFM, "Recommandations 2019 V.1.0 Janvier," *Clin. Microbiol. Infect.*, vol. 2, p. 144, 2019.

[10] G. Tadesse, E. Ephraim, and M. Ashenafi, "Assessment of the antimicrobial activity of lactic acid bacteria isolated from Borde and Shamita, traditional Ethiopian fermented beverages, on some food- borne pathogens and effect of growth medium on the inhibitory activity," *Internet J. Food Saf.*, vol. V, no. 5, pp. 13-20, 2005.

[11] A. M. O. Leite *et al.*, "Probiotic potential of selected lactic acid bacteria strains isolated from Brazilian kefir grains," *J. Dairy Sci.*, vol. 98, no. 6, pp. 3622-3632, 2015.

[12] M. Abdellah, H. Ahcne, Y. Benalia, B. Saad, and B. Abdelmalek, "Evaluation of biofilm formation by exopolysaccharide-producer strains of thermophilic lactic acid bacteria isolated from Algerian camel milk," *Emirates J. Food Agric.*, vol. 27, no. 6, p. 513, 2015.

[13] R. E. Krabi, A. A. Assamoi, F. Ayawovi Ehon, and S. L. Niamke, "Screening of lactic acid bacteria as potential starter for the production of attiéké, a fermented cassava food," *J. Fac. Food Eng.*, vol. XIV, no. 1, pp. 21-29, 2015.

[14] M. V Anoop and A. R. Bindu, "In-vitro Anti-inflammatory Activity Studies on Syzygium zeylanicum (L.) DC Leaves," *Int. J. Pharma Res. Rev.*, vol. 4, no. 8, p. 18, 2015.

[15] N. Benhammou, F. Atik, and T. K. Panovska, "Antioxidant and antimicrobial activities of the Pistacia lentiscus and Pistacia atlantica extracts," *African J. Pharm. Pharmacol.*, vol. 2, no. 2, pp. 22-8, 2008.

[16] M. J. R. Nout, F. M. Rombouts, and G. J. Hautvast, "Accelerated natural lactic fermentation of infant food formulations," *Food Nutr. Bull.*, vol. 11, no. 1, pp. 65-73, 1989.

[17] O. CB, A. AM, B. AY, A. DL, A. OA, and B. SY, "Proximate Analysis and Mineral Content Determination of Traditionally Processed Locust Bean (Parkia biglobosa) Fruit Pulp for Possible Industrial Application," *Edelweiss J. Biomed. Res. Rev.*, vol. 4, no. 1, pp. 10-13, 2021.

[18] A. Badis, D. Guetarni, B. Moussa Boudjema, D. E. Henni, and M. Kihal, "Identification and technological properties of lactic acid bacteria isolated from raw goat milk of four Algerian races," *Food Microbiol.*, vol. 21, no. 5, pp. 579-588, 2004.

[19] M. A. Lee *et al.*, "Influence of salinity on the microbial community composition and metabolite profile in Kimchi," *Fermentation*, vol. 7, no. 4, pp. 1-13, 2021.

[20] R. Martin, N. C. Hernández-delgado, E. Torres-maravilla, L. Mayorga-, P. Langella, and R. R. Pérez-pastén-borja, "Propriétés antioxydantes et anti-inflammatoires des probiotiques Souches candidates isolées pendant la fermentation d ' agave (Agave angustifolia Haw)," pp. 1-14, 2021.

[21] I. M. Petyaev and Y. K. Bashmakov, "Cocobiota: Implications for Human Health," *J. Nutr. Metab.*, vol. 2016, pp. 10-13, 2016.

[22] Y. Jeong *et al.*, "The antioxidant, anti-diabetic, and anti-adipogenesis potential and probiotic properties of lactic acid bacteria isolated from human and fermented foods," *Fermentation*, vol. 7, no. 3, 2021.

[23] E. E. Akpa, C. S. Djoman, B. G. Goualié, O. H. Djeneba, L. Samagassi, and D. Y. N'Guessan, "Selection of Lactic Acid Bacteria Isolated from Cocoa and Cassava Fermentation as Potential Probiotic for Pathogenic Microorganisms Control in Poultry Farming," *Annu. Res. Rev. Biol.*, vol. 37, no. 3, pp. 31-40, 2022.

[24] Z. C. Dlamini, R. L. S. Langa, O. A. Aiyegoro, and A. I. Okoh, "Safety Evaluation and Colonisation Abilities of Four Lactic Acid Bacteria as Future Probiotics," *Probiotics Antimicrob. Proteins*, vol. 11, no. 2, pp. 397-402, 2019.

[25] M. M. Katiku, J. W. Matofari, and J. M. Nduko, "Preliminary evaluation of probiotic properties and safety profile of Lactiplantibacillus plantarum isolated from spontaneously fermented milk, Amabere amaruranu," *Heliyon*, vol. 8, no. 8, p. e10342, 2022.

[26] A. B. Benjamin *et al.*, "Efficacy of Cathelicidin-Mimetic Antimicrobial Peptoids against Staphylococcus aureus," *Microbiol. Spectr.*, vol. 10, no. 3, 2022.

[27] S. Rasoulpoor *et al*., "Candida albicans skin infection in patients with type 2 diabetes: a systematic review and meta-analysis," *J. Diabetes Metab. Disord.*, vol. 20, no. 1, pp. 665-672, 2021.

[28] F. Waibel *et al*., "Optimization of the antibiotic management of diabetic foot infections: Protocol for two randomized controlled trials," *Trials*, vol. 21, no. 1, pp. 1-12, 2020.

[29] A. A. Assamoi, E. R. Krabi, A. F. Ehon, G. A. N'guessan, L. S. Niamké, and P. Thonart, "Isolation and screening of Weissella strains for their potential use as starter during attiéké production," *Base*, vol. 20, no. 3, pp. 355-362, 2016.

[30] M. Kostinek *et al*., "Characterisation and biochemical properties of predominant lactic acid bacteria from fermenting cassava for selection as starter cultures," *Int. J. Food Microbiol.*, vol. 114, no. 3, pp. 342-351, 2007.

[31] E. E. Akpa, C. S. Djoman, O. H. Dj, L. Samagassi, G. Gouali, and D. Yevi, "Recherche examen annuels en biologie Sélection de bactéries lactiques isolées de fermentation du cacao et du manioc comme potentiel Probiotique pour le contrôle des micro-organismes pathogènes dans Aviculture Machine Translated by Google," 2022.

[32] B. T. Fossi *et al*., "Probiotic lactic acid bacteria isolated from traditional cameroonian palm wine and corn beer exhibiting cholesterol lowering activity," *Heliyon*, vol. 8, no. 11, 2022.

[33] B. A. Tegegne and B. Kebede, "Probiotics, their prophylactic and therapeutic applications in human health development: A review of the literature," *Heliyon*, vol. 8, no. 6, p. e09725, 2022.

[34] I. Mahmoudi, O. Ben Moussa, and M. Hassouna, "Symbiotic, Hypocholesterolemic and Antioxidant Effects of Potential Probiotic Lactobacilli Strains Isolated from Tunisian Camel Milk," *Adv. Microbiol.*, vol. 07, no. 04, pp. 328-342, 2017.

[35] Z. Wang, Y. Zhao, Y. Jiang, and W. Chu, "Prebiotic, Antioxidant, and Immunomodulatory Properties of Acidic Exopolysaccharide From Marine Rhodotorula RY1801," *Front. Nutr.*, vol. 8, no. August, pp. 1-12, 2021.