Original Research Article

Screening for the Probiotic Activities of Lactic Acid Bacteria Isolated from the Gastrointestinal Tract of Traditional Poultry in Côte d'Ivoire

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ABSTRACT

In the view to overcome the problem of antibiotic multi-resistance of pathogens encountered in poultry farming, the most appropriate strategy was suggested to be the use of probiotic lactic acid bacteria (LAB) strains. Thus, this study aimed to evaluate the probiotic properties of LAB isolated from gastrointestinal tract of Ivorian traditional chickens for potential application as probiotic in broiler production. So, after a preliminary screening for pH 2.0 tolerance, the probiotic characters like tolerance to bile salt, hydrophobicity, auto and co-aggregation properties, antimicrobial activity and antibiotic susceptibility were evaluated. The results showed that amongst the 90 LAB investigated, a total of 15 isolates were able to grow after 90 min exposed to pH 2.0. The resistance of these 15 isolates to 0.3% bile salt was more than 50%. A test of their adhesion ability to host epithelial cells using cell surface hydrophobicity produced a range of 50.25% to 92.57%. The isolates had weak auto-aggregation ability and high coaggregation with the pathogens *Salmonella enteritidis* and *Escherichia coli*. They had wide spectrum inhibitory activity against challenging zoonotic and foodborne pathogens. Furthermore, all the five selected strains showed resistance to Colistin while 60-80% resistance was demonstrated against Oxacillin, Gentamicin and Kanamycin. Based on above characteristics, the LAB strains *Pediococcus acidilactici* JK148 and *Lactobacillus pentosus* JK151 which showed excellent probiotic potentials were selected as chicken probiotic candidates.

Keywords: Probiotic, lactic acid bacteria, traditional chicken, gastrointestinal tract, in vitro

1. INTRODUCTION

The poultry industry has become an important economic activity in many countries. So, several farming aspects such as building design, equipment, prevention of infectious diseases, bird genetics and feeding have been improved in order to optimize farm performance [1]. Antibiotics have been also used to help maintain overall health, prevent certain infectious diseases, but also for economic purposes to promote growth in broilers [2, 3], enhance egg production in laying hens [4] and improve feed efficiency [5]. However, repeated use of antibiotics in poultry diets resulted in severe problems like resistance of pathogen to antibiotics, accumulation of antibiotic residues in animal products and environment, imbalance of normal microflora, and reduction in beneficial intestinal microflora [6]. Thus, in Europe, the Regulation (EC) No 1831/2003 stated that “Antibiotics, other than coccidiostats or histomonostats, shall not be authorized as feed additives” and these are now banned in the EU [7]. Other countries such as Mexico, New Zealand, South Korea, United States, Canada, and Japan have adopted the EU approach or have established guidelines and recommendations to reduce the use of antibiotics as growth promoters in animal productions [8-10].

This situation has led research to develop alternatives to antibiotics. Thus, different agents such as prebiotics, antimicrobial peptides, bacteriophages and their gene products, antibodies, vaccines, polyphenols and probiotics have been proposed as alternatives to antibiotics in poultry production among which probiotics have emerged [11-13].

Probiotics are pure or mixed cultures of living microorganisms that, when administered in adequate amounts, confer a health benefit on the host [14]. Numerous beneficial effects of probiotics administered to broilers have been reported, e.g. improvement in growth performance, increased digestion of nutrients, modulation of intestinal microflora, inhibition of pathogens, competitive exclusion of pathogens and antagonism, and modulation of gut mucosal immunity [15]. Many microbial species are used as probiotic agents. However, the most widely used belong to the group of lactic acid bacteria (LAB), mainly species of the genera *Lactobacillus, Bifidobacterium, Pediococcus, Streptococcus, Enterococcus* and *Lactococcus* [16, 17]. Nevertheless, *Lactobacillus* species remain the upmost studied and used ones [18]. LAB are suitable as probiotics because of their capabilities to modify the environment in which they have been delivered by producing different metabolites among which a wide range of inhibitory substance (bacteriocins, organic acids, ethanol, diacetyl, carbon dioxide, and hydrogen peroxide) and even competitive exclusion [19, 20]. Yirga [21] and Seal et al. [13] reported and argued on the use of LAB-probiotics in promoting the growth and reproduction performances and the survival rate and health status of animals.

The most common routes of administration of probiotics are food supplementation, drinking water supplementation and, much less frequently, *in ovo* injections, sprays and oral administration [22, 23]. In all applications of the probiotics mentioned above, microorganisms must pass through the gastrointestinal tract to their targets, such as the lower intestines and caeca. Essentially, these microorganisms must survive at low pH and high concentrations of bile salts available in the upper parts of the gastrointestinal tract to reach the posterior intestine in an active and functional form and exercise their beneficial properties [24]. Thus, to be selected as probiotic, the strain should meet the following criteria: be acid and bile tolerant; be able to adhere to the host's intestinal epithelium; show antagonistic activity against pathogenic bacteria; and maintain its viability during processing and storage [25, 26].

While many studies in Europe have led to the marketing of various probiotics for poultry farming, this is not yet the case in Africa and more particularly in Côte d'Ivoire. Thus, this study aimed to evaluate the probiotic properties of LAB isolated from gastrointestinal tract of Ivorian traditional chickens for potential application as probiotic in broiler production. The isolates were tested for their tolerance to low pH and bile, surfaces properties (aggregation, co-aggregation and hydrophobicity), antagonistic activity towards pathogenic bacteria and antibiotic sensitivity.

2. MATERIAL AND METHODS

2.1. Lactic acid bacteria and pathogenic strains

A total of 90 LAB isolated from gastrointestinal tract (crop and caecum) of traditional chickens were used in this study. They belonged to the culture collection of the laboratory of biotechnology and food microbiology of Nangui Abrogoua University (Abidjan, Côte d'Ivoire). They were identified by MALDI-TOF MS method as *Enterococcus faecium* (31 isolates), *Ent. faecalis* (29 isolates), *Ent. hirae* (3 isolates), *Pediococcus acidilactici* (14 isolates), *Leuconostoc mesenteroides* (7 isolates), *Weissella confusa* (4 isolates) and *Lactobacillus pentosus* (2 isolates) (data not published). They were kept at -20°C in Man Rogosa and Sharp (MRS, Oxoid, France) broth with 40% glycerol.

The pathogenic strains *Salmonella enteritidis* ATCC 9186, *Salmonella typhimurium* ATCC 14028, *Escherichia coli* ATCC 25922, *Staphylococcus gallinarum* ATCC 35539, *Pseudomonas aeruginosa* ATCC 10145 and *Bacillus cereus* ATCC 10702 obtained from the culture collection of ''Institut Pasteur de Côte d'Ivoire'' (IPCI, Abidjan) were employed in the antagonistic assay. They were grown in Brain Heart Infusion (BHI, Oxoid, France) broth, 37°C for 24 h and stored as described above.

2.2. Tolerance to pH 2.0

The 90 isolates were subjected to a pH 2.0 tolerance assay according to the method descried by Ramos et al. [27] with slight modifications. Briefly, each isolate was grown on MRS broth for 24 h at 37°C. The cells were harvested by centrifugation at 5000 rpm for 10 min at 4°C and washed twice in sterile phosphate buffer saline (PBS, pH 7.0). Then, the washed cell density was adjusted to 0.2 optical density (OD) at 600 nm in PBS corresponding to approximately 108 cell/mL and 1 mL was inoculated into 5 mL of PBS adjusted to pH 2 with 1 N HCl. Cultures were incubated for 90 min at 37°C. Samples (0.1 mL) were obtained at time 0 and after 90 min and inoculated in MRS agar plates. Tolerance to pH 2.0 was indicated by subsequent growth on MRS agar plates after 48 h of incubation at 37°C. The experiment was performed in duplicate. Among the 90 isolates, 15 were selected for further studies according to their tolerance to pH 2.0.

2.3. Bile salt tolerance

The ability of the isolates to tolerate bile salts was determined according to the modified method described by Handa and Sharma [28]. The washed cells obtained above were inoculated into sterilized 10 mL of MRS broth containing 0.3% (w/v) bile salt (Merck, Germany) and incubated at 37°C for 72 h. The optical density (OD) at 620 nm was measured and compared to a bile salt-free MRS culture. The assay was performed in duplicate and repeated twice. The percent survival of cells was calculated using formula given below:

$$\text{Survival (\%)} = (\Delta OD\ 0\%BS - \Delta OD\ 0.3\%BS) / (\Delta OD\ 0\%BS) * 100 \quad (1)$$

2.4. Cell surface hydrophobicity

Bacterial cell surface hydrophobicity was assessed for the 15 acid tolerant isolates by measuring microbial adhesion to the non-polar solvent as described by Taheri et al. [29]. Cells cultivated in MRS broth at 37°C for 24 h were washed twice in PBS and suspended in the same buffer. The optical density of the suspension was adjusted to 0.5 at 600 nm (A0). Then, 3 mL of cell suspension was mixed with 1 mL of toluene (VWR, France). The mixture was vortexed for 2 min and the phases were allowed to separate for 1 h at 37°C. The lower aqueous phase was carefully removed with a sterile Pasteur pipette and final optical density (A) was recorded at 600 nm to calculate cell hydrophobicity. The assay was performed in duplicate and repeated twice.

$$\text{Hydrophobicity (\%)} = (A0 - A)/A0 * 100 \quad (2)$$

2.5. Auto-aggregation and co-aggregation assays

Auto-aggregation and co-aggregation assays were performed according to Kos et al. [30]. The LAB and two pathogen strains (*Salmonella enteritidis* and *Escherichia coli*) were separately cultured at 37°C for 24 h in MRS broth and BHI broth. The pellet was washed twice in PBS and re-suspended in similar solution. The optical density of the suspension was adjusted to 0.3 at 600 nm. For auto-aggregation, the LAB suspension was vortexed and incubated at 37°C for 5 h without agitation. After 5 h, absorbance was determined at 600 nm and percentage of auto-aggregation was calculated using the following formula:

Auto-aggregation (%) = (1-At/A0) *100 (3)

where, A0 and At measured at 600 nm, represents the absorbance of the mixture at 0 h and 5 h respectively.

For co-aggregation, equal volume of the LAB and pathogenic strain cultures (1:1 v/v) were mixed and incubated at 37°C for 5 h without agitation. Absorbance was determined at 600 nm and percentage of co-aggregation was calculated as followed:

Co-aggregation (%) = (((Ax+Ay)/2) - (Axy/(Ax+Ay))/2) *100 (4)

where, Ax, Ay and Axy represent the absorbance of individual pathogen, LAB and their mixture after incubation for 5 h, respectively.

2.6. Antagonistic activity against pathogens

Six strains that are pathogenic to chickens were used as test pathogens to investigate the antagonistic activity of the LAB strains. They were *Salmonella enteritidis* ATCC 9186, *Salmonella typhimurium* ATCC 14028, *Escherichia coli* ATCC 25922, *Staphylococcus gallinarum* ATCC 35539, *Pseudomonas aeruginosa* ATCC 10145 and *Bacillus cereus* ATCC 10702. For detection of antimicrobial activity, the well diffusion assay described by Arici et al. [31] was performed. Briefly, the pathogenic strains were grown in BHI broth at 37°C for overnight. Simultaneously, the LAB strains were grown anaerobically overnight in MRS broth at 37°C. The cultures obtained were centrifuged and the supernatants were recovered and then filter-sterilized (0.45 mm, Millipore, BioRad, France). Aliquots of 60-80 µL of the sterile cell free supernatant were placed in 7 mm diameter wells on Muller-Hinton-agar plates previously seeded with the respective pathogenic strains. After 18 h of incubation at 37°C, the diameters of the zones of growth inhibition were measured. The assay was performed in triplicate.

2.7. Antibiotic sensitivity test

Antibacterial susceptibility testing was performed using the disk diffusion method. In this study, various antibiotics were supplied in the form of dodeca discs (Oxoid, England) which included Cephalothin (KF, 30 µg), Colistin (CST, 30 µg), Chloramphenicol (C, 30 µg), Oxacillin (Ox, 5 µg), Gentamycin (CN, 10 µg), Kanamycin (K, 30 µg), Imipenem (IPM, 10

µg), Amoxicillin (AML, 10 µg) and Erythromycin (E, 15 µg). The 100 µL of LAB strains were inoculated on MRS agar plate. The antibiotic discs were put on MRS agar surface and then incubated at 37°C for 24 h. The zones of inhibition around disc were measured. The assay was repeated twice.

2.8. Statistical analyses

The quantitative data were analyzed by using One-way analysis of variance (ANOVA) with Tuckey test to distinguish the treatment means. The statistical software used was R 4.0.2 version and differences were considered significant for values of $P < 0.05$. Hierarchical clustering analysis (HCA) was performed with an R environment (http://www.r-project.org/) using the heatmap package.

3. RESULTS

3.1. Selection of isolates

Tolerance to pH 2.0 in MRS broth was chosen as selection criteria for isolates to be included in further experiments. Amongst the 90 LAB investigated, a total of 15 isolates were able to grow after 90 min exposed to pH 2.0. Thus, 16.67% of LAB isolated from GIT of traditional chickens showed survival at pH 2.0. They were composed of five isolates of *Ent. faecium*, two *Ent. hirae*, two *P. acidilactici*, three *Leuc. mesenteroides*, two *W. confusa* and one *Lb. pentosus*.

3.2. Bile salt tolerance, hydrophobicity, auto and co-aggregation properties

All the 15 LAB strains selected by tolerance to pH 2.0 were evaluated according to their bile tolerance, hydrophobicity, auto and co-aggregation properties. The attained results suggest that the 15 LAB strains were resistance to 0.3% bile salt during the incubation period of 90 min. The values were ranged from 51.5% to 70% (Table 1). The highest bile tolerance was demonstrated by *Leuc. mesenteroides* JK139 (70%), followed by *Lb. pentosus* JK151 (66.05%) and *P. acidilactici* JK148 (61.50%). The 15 LAB strains exhibited high hydrophobicity that was determined by microbial adhesion to toluene in the range of 50.25% to 92.57%. *Weissella confusa* JK157 was the most hydrophobic strains, followed by *Ent. hirae* JK115 (77.25%). Regarding auto-aggregation, excepted for *Ent. faecium* JK110 which highly aggregated (auto-aggregation value of 24.57%), all other strains showed moderate or low auto-aggregation ability with values ranged from 3.75% to 14.61%. Furthermore, all the tested LAB strains showed co-aggregation ability with the pathogens *S. enteritidis* and *E. coli*. With *S. enteritidis*, values were between 48.8% (*Ent. faecium* JK132) and 60.15% (*Leuc. mesenteroides* JK139) while with *E. coli*, they varied from 50.15% (*Ent. faecium* JK136) to 58.40% (*Leuc. mesenteroides* JK139).

Table 1. Tolerance to bile salt, hydrophobicity, auto and co-aggregation of LAB isolated from gastrointestinal tract of traditional chicken*

LAB strains	Bile salt (%)	Hydrophobicity	Aggregation	Co-	Co-

		(%)	(%)	aggregation with *S. enteritidis* (%)	aggregation with *E. coli* (%)
Enterococcus faecium JK110	53.50±1.41^{a}	64.05±0.35^{c}	24.57±0.25^{a}	53.37±0.38^{b}	51.57±0.10^{ab}
Enterococcus faecium JK132	57.00±1.41^{b}	61.45±0.35^{bc}	9.37±0.10^{c}	48.80±0.28^{a}	51.20±0.14^{ab}
Enterococcus faecium JK134	53.82±1.16^{a}	54.49±0.22^{a}	7.45±0.28^{d}	53.95±0.07^{b}	53.45±0.07^{b}
Enterococcus faecium JK136	51.50±0.70^{a}	56.30±0.56^{ab}	10.71±0.04^{c}	52.75±0.07^{b}	50.15±0.07^{a}
Enterococcus faecium JK144	51.50±0.70^{a}	68.32±0.10^{cd}	4.70±0.00^{e}	53.20±0.14^{b}	51.85±0.07^{ab}
Enterococcus hirae JK113	55.25±1.06^{b}	52.50±0.14^{a}	15.86±0.12^{b}	54.17±0.10^{b}	52.50±0.14^{b}
Enterococcus hirae JK115	57.50±1.41^{b}	77.25±0.21^{e}	6.70±0.17^{d}	53.52±0.16^{b}	50.60±0.56^{a}
Leuconostoc mesenteroides JK137	52.50±3.53^{a}	66.80±3.32^{c}	13.79±0.15^{b}	52.30±1.55^{b}	52.90±1.83^{b}
Leuconostoc mesenteroides JK139	70.04±0.07^{d}	71.62±0.67^{d}	13.42±0.03^{b}	60.15±0.21^{c}	58.40±0.14^{c}
Leuconostoc mesenteroïdes JK147	52.50±0.70^{a}	56.35±0.27^{ab}	11.62±0.17^{c}	54.85±0.07^{b}	53.50±0.14^{b}
Pediococcus acidilactici JK148	61.50±0.70^{c}	58.51±0.33^{b}	10.00±0.35^{c}	52.85±0.07^{b}	53.25±0.14^{b}
Pediococcus acidilactci JK150	52.00±1.41^{a}	58.85±0.21^{b}	3.75±0.03^{e}	53.10±0.14^{b}	52.70±0.14^{b}
Lactobacillus pentosus JK151	66.05±0.07^{d}	68.72±0.31^{cd}	10.77±0.10^{c}	54.27±0.10^{b}	54.30±0.28^{b}
Weissella confusa JK157	52.00±2.82^{a}	92.57±0.60^{f}	12.67±0.11^{c}	51.50±0.14^{b}	53.57±0.10^{b}
Weissella confusa JK160	52.33± 0.94^{a}	50.25±0.63^{a}	14.61±0.04^{b}	53.75±0.20^{b}	53.90±0.00^{b}

* Presented values are means of two determinations ± standard deviations. Mean values (± standard deviation) within the same column followed by different superscript letters differ significantly ($p < 0.05$) by Tuckey test. *S. enteritidis = Salmonella enteritidis; E. coli = Escherichia coli*

3.3. Antimicrobial activity

The results for antagonistic activity of the LAB against pathogenic bacteria are shown in Table 2. All fifteen LAB strains showed antagonistic effects against *Salmonella typhimurium*, *Pseudomonas aeruginosa* and *Bacillus cereus*. The diameters of inhibition were ranged from 12 to 22 mm. The highest activity towards *S. typhimurium* was obtained by the species *Ent. hirae, P. acidilactici* and *Ent. faecium*. For *B. cereus*, the highest activity was obtained by *W. confusa* (JK157 and JK160), *Ent. hirae* (JK115 and JK113) and *Ent. faecium* (JK110 and JK132). Concerning pathogenic bacteria *Salmonella enteritidis* and *Escherichia coli*, they were inhibited by eight and ten LAB strains, respectively. The diameters of inhibition were ranged from 6 to 22 mm for *Salmonella enteritidis* and from 6 to 15 mm for *Escherichia coli*. Furthermore, only the three LAB strains *Ent. faecium* JK144, *P. acidilactici* JK148 and *P. acidilactici* JK150 showed antagonistic activity towards *Staphylococcus gallinarum*. Overall, the LAB strains *Ent. faecium* JK144 and *P. acidilactici* JK148 exhibited inhibition against all indicator microorganisms tested while *Ent. faecium* JK132, *Ent faecium* JK135 and *Leuc. mesenteroides* JK139 inhibited only three indicators out of the six.

Table 2. Diameters of inhibition (mm) of LAB strains against test pathogens

LAB strains	*Salmonella typhimurium* ATCC 14028	*Pseudomonas aeruginosa* ATCC 10145	*Bacillus cereus* ATCC 10702	*Salmonella enteritidis* ATCC 9186	*Staphylococcus gallinarum* ATCC 35539	*Escherichia coli* ATCC 25922
Enterococcus faecium JK110	20	14	20	00	00	06
Enterococcus faecium JK132	19	15	19	00	00	00
Enterococcus faecium JK134	19	17	16	00	00	15
Enterococcus faecium JK135	15	12	12	00	00	00
Enterococcus faecium JK136	18	16	16	10	00	00
Enterococcus faecium JK144	18	16	15	06	08	10
Enterococcus hirae JK113	20	16	20	00	00	12
Enterococcus hirae JK115	20	15	21	00	00	12
Leuconostoc mesenteroïdes JK137	14	14	18	21	00	00
Leuconostoc mesenteroïdes JK139	14	14	12	00	00	00
Leuconostoc mesenteroïdes JK147	15	14	16	17	00	12
Pediococcus acidilactici JK148	20	17	16	10	10	15
Pediococcus acidilactci JK150	20	14	17	00	08	00
Lactobacillus pentosus JK 151	14	16	18	15	00	12
Weissella confusa JK 157	16	16	22	20	00	12
Weissella confusa JK 160	16	18	20	22	00	14

3.4. Heatmap analysis

Heatmap was used to simplify the interpretation of the data. It depicts values for a variable across two axis variables as grid of colored squares. Each cell's color indicates the value of the variable in the corresponding cell range (Fig. 1). The red color indicates high activity and on contrary the blue color shows weak property. The pattern in cell colors across strain shows that the highest activity of each probiotic property varies according to the strain. Thus, *Leuconostoc mesenteroïdes* JK139 showed the highest bile salt tolerance, *Weissella confusa* JK157 the highest hydrophobicity, *Pediococcus acidilactici* JK150 the weakest auto aggregation and *Leuconostoc mesenteroïdes* JK139 the highest coaggregation with pathogenic strains. However, *Pediococcus acidilactici* JK148 and *Lactobacillus pentosus* JK151 showed good activity with the maximum probiotic characters.

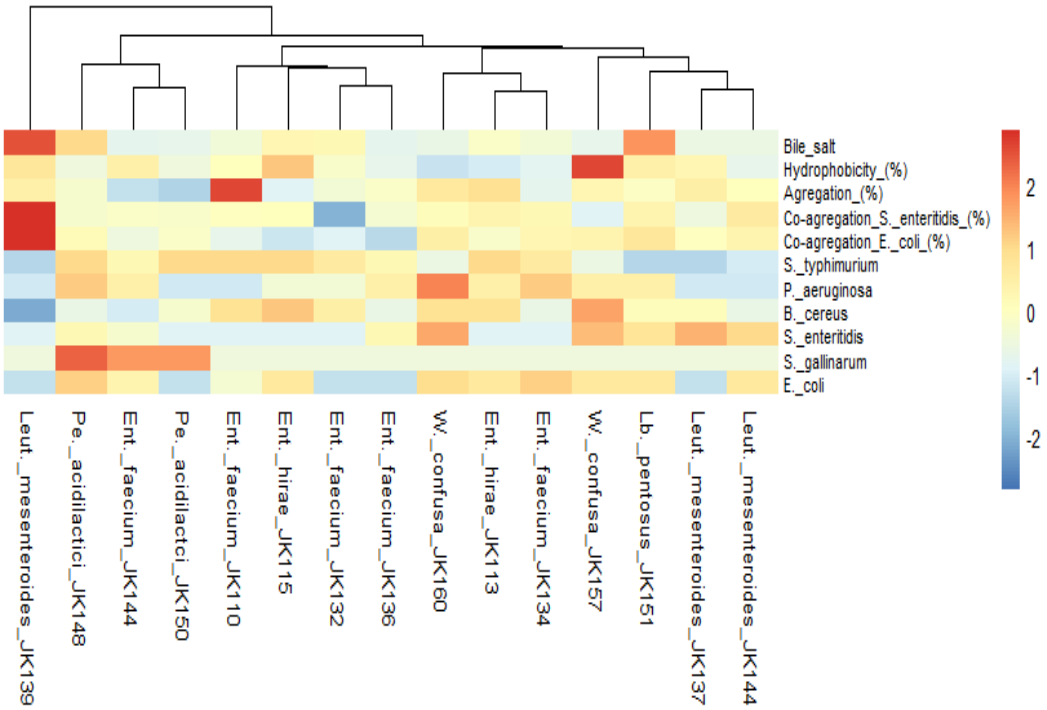

Fig. 1. Cluster analysis of probiotic properties of LAB strains using the heatmap package in the R software

3.5. Antibiotic susceptibility

The antibiotic susceptibility test was carried out for five selected LAB strains against nine antibiotics and the results are shown in Table 3. All the selected strains (100%) showed resistance to Colistin while 60-80% resistance was demonstrated against Oxacillin, Gentamicin and Kanamycin. On contrary, they were all sensitive to Imipenem as 80% were sensitive to Chloramphenicol, Erythromycin, Cephalothin and Amoxicillin. Overall, *P. acidilactici* JK148 and *Lb. pentosus* JK151 were the most resistant strains. They showed resistance to seven and five antibiotics, respectively, out of the nine tested. Their resistant phenotypes were Oxacillin-Gentamicin-Cephalotin-Amoxicillin-Erythromycin-Kanamycin-Colistin and Oxacillin-Gentamicin-Chloramphenicol-Kanamycin-Colistin.

Table 3. Diameters of clearance zone (mm) showed by the selected LAB strains with different antibiotics

LAB strains	Oxacillin (OX 5)	Imipenem (IMP 10)	Gentamicin (CN 10)	Cephalotin (KF 30)	Amoxicillin (AML 10)	Erythromycin (E 15)	Chloramphenicol (C 30)	Kanamycin (K 30)	Colistin (CST 30)
Enterococcus hirae JK113	13 (S)	22 (S)	12 (S)	18 (S)	12 (S)	16 (S)	20 (S)	14 (S)	00 (R)
Enterococcus hirae JK115	00 (R)	24 (S)	10 (S)	18 (S)	12 (S)	17 (S)	23 (S)	00 (R)	00 (R)
Pediococcus acidilactici JK148	00 (R)	18 (S)	00 (R)	00 (R)	00 (R)	00 (R)	27 (S)	00 (R)	00 (R)
Lactobacillus pentosus JK151	00 (R)	32 (S)	00 (R)	15 (S)	16 (S)	20 (S)	00 (R)	00 (R)	00 (R)
Weissella confusa JK157	00 (R)	26 (S)	00 (R)	22 (S)	20 (S)	23 (S)	30 (S)	00 (R)	00 (R)

(S) = Susceptible, (R) = Resistant.

4. DISCUSSION

Before reaching the intestinal tract, probiotic bacteria have to survive in the transit through the stomach where pH can be as low as 1.5–2 [32]. According to Ruben et al. [33], the ability of potential LAB probiotic strains to tolerate an acidic environment is important not only in overcoming GIT stresses, but also serves as a major requirement for their application as dietary supplements, enabling them to survive longer in acidic environments and still be effective when ingested. In this study, 15 LAB strains (out of 90 tested strains) exhibited resistance to pH 2. A similar result was reported by Jannah et al. [34] and Blajman et al. [35] who showed that several LAB isolated from GIT of chickens were resistant to pH 2.

When evaluating the potential of using microorganisms as effective probiotics, it is generally considered necessary to evaluate their ability to resist to the effects of bile acids as it was reported that tolerance to bile salts is a prerequisite for colonization and metabolic activity of bacteria in the small intestine of the host [28]. From this study, all the LAB strains that previously survived to pH 2 showed resistance to 0.3% bile salt during the incubation period of 90 min with values ranging from 51.50% to 70.04%. Mathara et al. [36] established a limit of 0.3% bile to select strains considered to have good resistance. Therefore, all the tested strains were able to grow in very high concentrations of bile salts. Similar findings have been earlier reported for LAB strains obtained from different environments [27, 37, 38]. Nallala et al. [39] reported that bile salt tolerance of LAB was associated with bile salt hydrolase which deconjugates bile acids.

Probiotics are administered orally and must be able to colonize and competitively exclude pathogens in the GIT [40]. Adhesion of LAB is a multiplex phenomenon but bacterial cell surface hydrophobicity is often applied to an indirect examination of the adhesion ability to host epithelial cells [29]. It was reported as one of the most important diagnostic ways to detect the attachment of bacteria to host tissue [41]. The 15 LAB strains hydrophobicity was determined by microbial adhesion to toluene and values were in the range of 50.25% to 92.57%. Based on Colloca et al. [42] who classified hydrophobicity into three groups, 12 strains of the study exhibited moderate hydrophobicity (36-70%) and three strains demonstrated high hydrophobicity (71-100%). Similar studies were reported by Blajman et al. [35] where two and nine strains exhibited, respectively, high and moderate degree of hydrophobicity determined by microbial adhesion to n-hexadecane. While it is believed that high values of hydrophobicity indicate a greater ability of the bacteria to adhere to epithelial cells, a moderate level of hydrophobicity does not necessarily imply that the microorganism is less likely to adhere to the intestinal epithelium of the host, because hydrophilic domains could also be involved in the adhesion of bacteria [35].

Furthermore, the potential probiotic strains were examined for cell-binding properties; that is, auto-aggregation and co-aggregation. These two properties are generally considered when selecting potential probiotic strains. Auto-aggregation (aggregation between the same microbial strains) and co-aggregation (aggregation between different microbial strains) support bacterial adhesion to epithelial cells of the host GIT and the prevention of pathogen colonization [33]. The auto-aggregation of the LAB examined ranged from 3.75% to 24.57% while co-aggregations with *S. enteritidis* and *E. coli* were 48.80-60.15% and 50.60-58.40%, respectively. Auto- and co-aggregation abilities of LAB show discrepant results in the literature. In fact, Reuben et al. [43] recorded auto-aggregation ability of 32-56.5% for LAB strains from chickens while Espeche et al. [44] reported no auto-aggregation for LAB strains isolated from milk. In addition, Reuben et al. [43] stated that co-aggregation ability of LAB isolated from GIT of broilers was strain-specific.

It is expected that potential probiotic strains can adhere to GIT cells and competitively exclude or inhibit pathogens that may cause harm to the host. Thus, one of the crucial properties for selecting probiotic strains is their antimicrobial activity [33]. Wider zones of inhibition were recorded against *B. cereus* (12-21 mm) and *S. typhimurium* (14-20 mm). The broad spectrum of antagonistic activity against both Gram-negative and Gram-positive pathogens exhibited by the LAB strains examined here agrees with previous findings [33, 45]. On contrary to our finding, Ayodeji et al. [46], Oyewole et al. [47] and Reuben et al. [43] reported antagonistic activity against wide spectrum of pathogens by LAB isolated from poultry. So, weak or no antibacterial activity of examined LAB strains was found against *Staph. gallinarum* and only five strains exhibited activity against *S. enteritidis*. Among all selected strains, only the LAB strains *Ent. faecium* JK144 and *P. acidilactici* JK148 exhibited inhibition against all tested indicator pathogens. This finding highlighted the probiotic capabilities of these strains. Antimicrobial compounds including bacteriocins, organic acids (e.g., acetic, lactic, propionic, succinic acids), short-chain fatty acids, hydrogen peroxide, and other low-molecular-weight substances produced by LAB might be responsible for their antimicrobial activity [48].

Five strains (*Ent. hirae* JK113 and JK115, *P. acidilactici* JK148, *Lb. pentosus* JK151 and *W. confusa* JK157) that exhibited good probiotic properties were selected and subjected to antibiotic susceptibility. According to Reuben et al. [43], the assessment of antimicrobial susceptibility profile is a major criterion for potential probiotics evaluation. In this study, most of strains showed resistance to Oxacillin, Gentamicin, Kanamycin and colistin. Taheri et al. [29] earlier showed that resistance to antimicrobial substance is a very important selection property for probiotic used for poultry, as antimicrobial resistant strains can be co-administered with antimicrobial compound for treatment of diseases. Lee et al. [49] also reported that LABs, in general, must be somewhat resistant to antibiotics, in order to survive in the intestine and allow for successful preventive antibiotic treatment. But, microbial strains to be considered as probiotics should not serve as antibiotic resistance genes reservoir, which may further be transferred to intestinal pathogens [43]. In accordance to our results, high resistance to Gentamicin, Oxacillin and Kanamycin has also been reported for LAB strains isolated from broilers by several authors [43, 50]. Resistance to Gentamycin as an aminoglycoside is attributed to the absence of cytochrome-mediated electron transport, which mediates drug uptake [51].

5. CONCLUSION

In conclusion, the two LAB strains isolated from GIT of Ivorian traditional chicken, *P. acidilactici* JK148 and *Lb. pentosus* JK151, were found to possess suitable in vitro probiotic properties. Thus, they were able to survive in acidic environment (pH 2), capable of tolerating high bile salt, had strong hydrophobicity and co-aggregation ability. In addition, these isolates showed broad spectrum of antimicrobial activity against zoonotic and foodborne pathogens and were found resistant to most of the antibiotics. These LAB strains are ideal probiotic candidates which can be used in vivo for both biocontrol of intestinal pathogens and to increase poultry performance.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because

we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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