ISSN 1682-8356
ansinet.com/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

Science Alert
scialert.net

ANSInet
an open access publisher
http://ansinet.com

OPEN ACCESS International Journal of Poultry Science
ISSN 1682-8356
DOI: 10.3923/ijps.2022.119.128

Research Article
Probiotic Potential of Lactic Acid Bacteria Isolated from Broiler Chickens in Côte d'Ivoire

A.E. Sika-Kadji, T.B. Kéhi, F.K. N'Guessan and R.A. Koffi-Nevry

Department of Food Science and Technology, Laboratory of Biotechnology and Food Microbiology, Nangui Abrogoua University, 02 BP 801, Abidjan 02, Côte d'Ivoire

Abstract
Objective: The objective of this study was to characterize and assess lactic acid bacteria isolated from the gastrointestinal tract of broilers for their use in poultry farming as potential probiotic in Côte d'Ivoire. **Materials and Methods:** For this purpose, 90 colonies of lactic acid bacteria isolated from the crop and cecum of broilers were subjected to several probiotic tests: Thermoresistance, tolerance to acid pH and bile salts, self-aggregation and co-aggregation, hydrophobicity, antibacterial activity and sensitivity to antibiotics. **Results:** The results of this study showed that out of the 90 isolates 44 were resistant to pH 3 and 0.3% bile salt. Of the 44 isolates, 15 showed high probiotic potential. These isolates belong to the species *Pediococcus acidilactici* (4), *Lactobacillus pentosus* (4), *Weissella confusa* (2) *Enterococcus faecium* (2), *Pediococcus pentosaceus* (2) and *Enterococcus faecalis* (1). The heat map analysis also showed that the species with the best probiotic potential were, *Lactobacillus pentosus* JK (51 and 55) and *Enterococcus faecium* JK 96. **Conclusion:** The species *Lactobacillus pentosus* JK (51 and 55) and *Enterococcus faecium* JK 96 could be used for the production of probiotic feed for poultry farming in Côte d'Ivoire thus reducing the use of antibiotics.

Key words: Lactic acid bacteria, probiotic, broiler, antibiotics, gastrointestinal tract

Citation: Sika-Kadji, A.E., T.B. Kéhi, F.K. N'Guessan and R.A.Koffi-Nevry, 2022. Probiotic potential of lactic acid bacteria isolated from broiler chickens in Côte d'Ivoire. Int. J. Poult. Sci., 21: 119-128.

Corresponding Author: A.E. Sika-Kadji, Department of Food Science and Technology, Laboratory of Biotechnology and Food Microbiology, Nangui Abrogoua University, 02 BP 801, Abidjan 02, Côte d'Ivoire Tel: +2250707711732

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

For more than fifty years, antibiotics have been added to the ration of factory-farmed animals not only to prevent certain infectious diseases but also to promote growth and improve feed efficiency. However, application of antibiotics growth promoters (AGPs) in poultry has been linked to the development and spread of resistant bacteria. The presence of antimicrobial residues in chicken products can affect human health. European countries banned their application in poultry feed in 2006[1]. This situation has led researchers to develop alternatives to antibiotics such as organic acids, plant extracts, prebiotics and probiotics[2,3]. However, probiotics are the most widely used live microorganisms that confer a health benefit to the host (humans and animals) when administered in adequate amount[4]. They reduce enteric pathogens, improve the immune system or promote growth[5]. Probiotics can be isolated from the GIT of poultry that has abundant and dominant microbiota. Microbiota from the crop, gizzard, duodenum, jejunum, ileum, caecum and feces/excreta and colon have been studied. Many microbial species are used as probiotic agents. However, the most widely used belong to the group of lactic acid bacteria (LAB), mainly in the genera *Lactobacillus*, *Bifidobacterium*, *Pediococcus*, *Streptococcus*, *Enterococcus* and *Lactococcus*[6,7]. These microbial species can be used alone or in combination of two, three or more species. Many studies regarding various probiotics for poultry have been done in Europe but Africa particularly Côte d'Ivoire did not do such studies. Therefore, this study was designed to characterize and assess LAB strains isolated from broilers GIT with optimal probiotic properties for their use in poultry farming.

MATERIALS AND METHODS

Lactic acid bacteria and pathogenic strains: A total of 90 LAB isolated from gastrointestinal tract (crop and caecum) of broilers chickens were used in this study. The LAB cultures were prepared in the laboratory of biotechnology and food microbiology at Nangui Abrogoua University (Abidjan, Côte d'Ivoire). They were identified by MALDI-TOF MS method as *Enterococcus faecium* (3 isolates), *Ent. faecalis* (3 isolates), *Pediococcus acidilactici* (41 isolates), *Pediococcus pentosaceus* (19 isolates), *Weissella confusa* (3 isolates) and *Lactobacillus pentosus* (21 isolates). They were kept at -20°C in Man Rogosa and Sharp (MRS, Oxoid, France) broth with 40% glycerol.

Thermoresistance and NaCl tolerance assay: The LAB cultures were inoculated into MRS broth, then placed in a water bath at 63.5°C for 30 min. After sudden cooling, they were incubated at 30°C ±1°C for 48-72 hrs. Similarly, LAB cultures were inoculated into MRS broth containing increasing concentration of NaCl (2.0, 4.0 and 6.5%) and incubated at 37°C for 24 hrs. Cloudiness of the solution indicates positive result[8].

Acid tolerance test: The 90 isolates were subjected for different pH tolerance (pH 2.0, 2.5 and 3) according to the method descried by Ramos *et al.*[9] with slight modifications. Briefly, each isolate was grown on MRS broth for 24 hrs 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 10^8 cell mL^{-1} and 1 mL was inoculated into 5 mL of PBS adjusted to pH 2.0, 2.5 and 3 with 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 different pH (pH 2.0, 2.5 and 3) was indicated by subsequent growth on MRS agar plates after 48 hrs of incubation at 37°C.

Bile salt tolerance test: The ability of the isolates to tolerate bile salts was determined according to the modified method described by Handa and Sharma[10]. The washed cells obtained above were inoculated into sterilized 10 mL of MRS broth containing 0.3, 1 and 2% (w/v) bile salt (Merck, Germany) respectively and incubated at 37°C for 72 hrs. The optical density (OD) at 620 nm was measured and compared to a bile salt-free MRS culture. The percent survival of cells was calculated using formula given below:

$$\text{Survival (\%)} = \frac{\Delta\text{OD 0\%BS-OD (0.3, 1, 2)\%BS}}{\Delta\text{OD 0\%BS}} \times 100$$

where, ΔOD 0%BS and ΔOD (0.3, 1, 2)%BS correspond to absorbance of cells cultivated in the presence of 0% and 0.3, 1 and 2% bile salt, respectively.

Cell surface hydrophobicity test: 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.*[11] with slight modifications. Cells cultivated in MRS broth at 37°C for 24 hrs 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.

$$\text{Hydrophobicity (\%)} = \frac{\text{A0-A}}{\text{A0}} \times 100$$

where, A0 and A measure cells optical density at the beginning and the end of the experiment, respectively.

Auto-aggregation and co-aggregation test: Auto-aggregation and co-aggregation assays were performed according to Kos *et al.*[12] with slight modifications. The LAB and two pathogen strains (*Salmonella enteritidis* and *Escherichia coli*) were separately cultured at 37°C for 24 hrs 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 hrs without agitation. After 5 hrs, absorbance was determined at 600 nm and percentage of auto-aggregation was calculated using the following formula:

$$\text{Auto-aggregation (\%)} = \frac{\text{1-At}}{\text{A0}} \times 100$$

where, A0 and At measured at 600 nm, represent the absorbance of the mixture at 0 and 5 hrs, 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 hrs without agitation. Absorbance was determined at 600 nm and percentage of co-aggregation was calculated as followed:

$$\text{Co-aggregation (\%)} = (((Ax+Ay)/2) - (Axy/(Ax+Ay))/2) \times 100$$

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

Antimicrobial activity test: 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.*[13] was performed with some modifications. 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 hrs of incubation at 37°C, the diameters of the zones of growth inhibition were measured.

Antibiotic sensitivity test: Antibiotic susceptibility testing of LAB was carried out according to the method described by Bauer *et al.*[14]. The antibiotic discs were chosen according to their importance in the different treatments in humans 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 hrs. The zones of inhibition around disc were measured. Inhibition diameters were measured and strains were classified as susceptible (S) or resistant (R) according to the recommendations of the Committee of Antibiogram of the French Society of Microbiology[15].

Statistical analyses: All the experiments were performed in duplicate and repeated twice. The XLSTAT-2017 statistical software was used to calculate the mean and standard deviation as well as Principal Component Analysis (PCA) and heatmap.

RESULTS

Viability of lactic acid bacteria on inhibitory substances conditions: Of the 90 LAB probiotic strains isolated from the gastrointestinal tract of broilers chickens tested for acid and temperature tolerances, 15 strains were resistant to 63.5°C, 6.5% NaCl, pH 2 and 0.3% bile salt. They were: *Weissella confusa* (2), *Pediococcus acidilactici* (4), *Pediococcus pentosaceus* (2), *Lactobacillus pentosus* (4), *Enterococcus faecalis* (1), *Enterococcus faecium* (2) (Fig. 1).

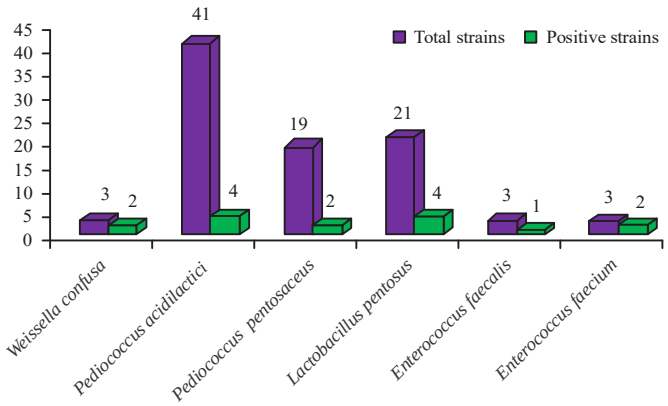

Fig. 1: Lactic acid bacteria strains resistant on inhibitory substances

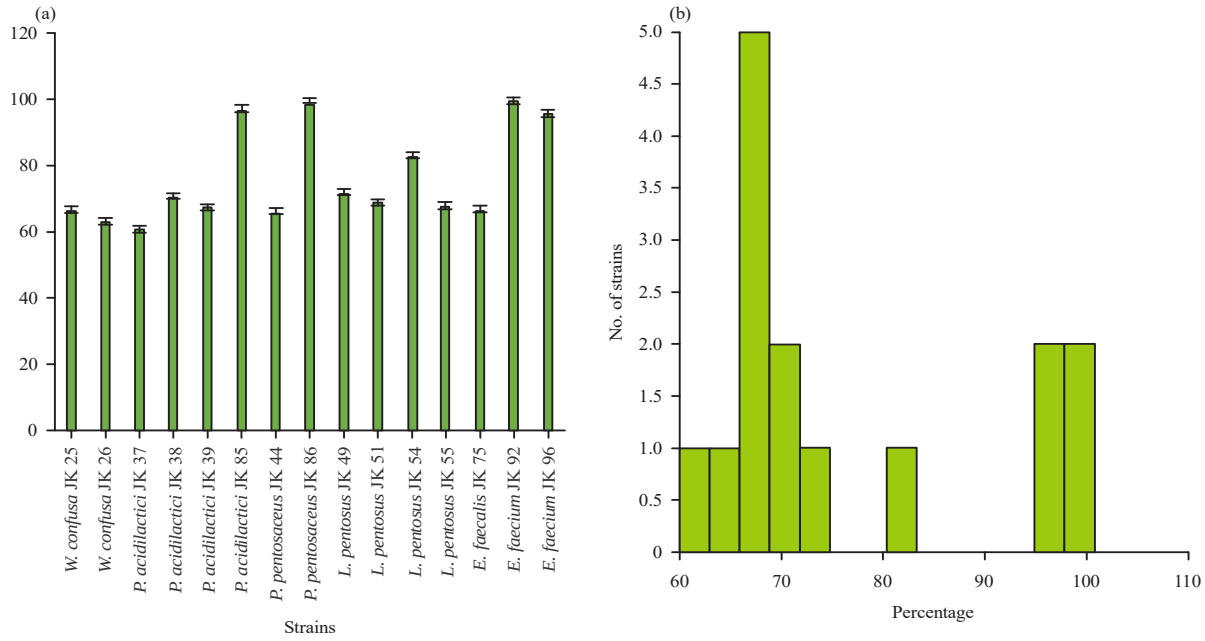

Fig. 2(a-b): Cell surface hydrophobicity of lactic acid bacteria strains

Cell surface hydrophobicity of lactic acid bacteria strains isolated from the gastrointestinal tract of broilers chickens: All the 15 LAB strains showed high hydrophobicity ranged from 61-99.75%. *Enterococcus faecium* JK 92 was the most hydrophobic strains, followed by *Pediococcus pentosaceus* JK 86 (99.40%). *Pediococcus acidilactici* JK 37 was the least hydrophobic strains (61%) (Fig. 2). Statistical analyses showed 3 groups of lactic acid bacteria according to their hydrophobic properties. The first group of 10 LAB strains had values between 60 and 75%, the second of 1 LAB strain between 80 and 85% and the last group of 4 LAB strains between 95 and 100%.

Auto-aggregation properties of lactic acid bacteria strains isolated from the gastrointestinal tract of broilers chickens: All the 15 strains showed low auto-aggregation ability with values ranged from 0 to 20.22%. *Enterococcus faecium* JK 92 showed the most auto-aggregation. *Pediococcus acidilactici* JK 38 and *Enterococcus faecalis* JK 75 showed the least auto-aggregation (Fig. 3). Statistical analyses showed 3 groups of lactic acid bacteria according to their auto-aggregation properties. The first group of 9 LAB strains had values between 0 and 6%, the second of 4 LAB strains between 10 and 16% and the last group of 2 LABs strains between 18 and 21%.

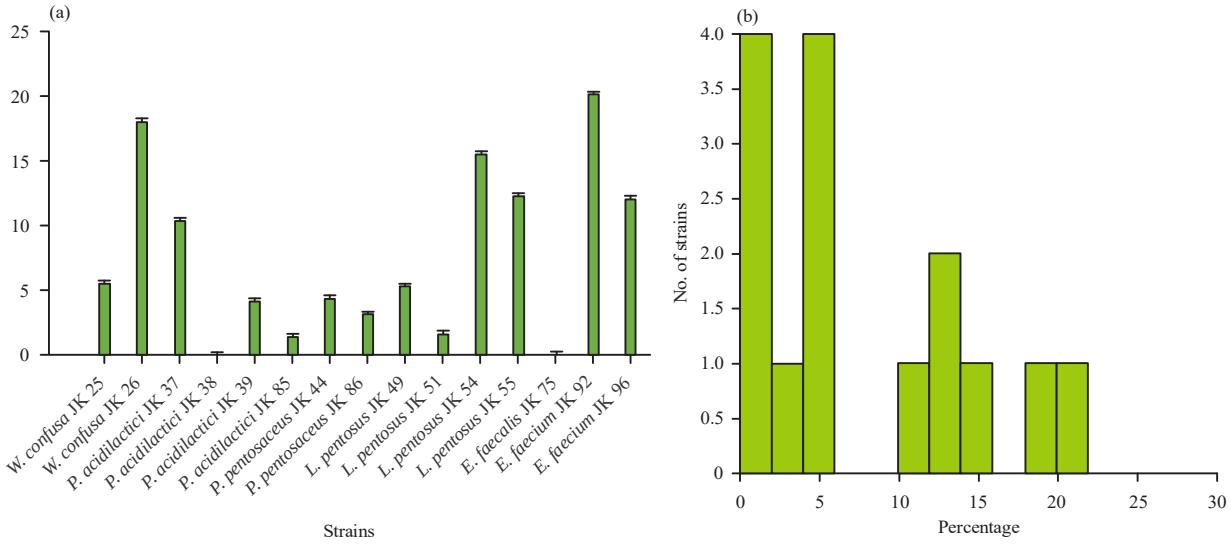

Fig. 3(a-b): Auto-aggregation properties of lactic acid bacteria strains

Co-aggregation properties of lactic acid bacteria strains isolated from the gastrointestinal tract of broilers chickens: All the tested LAB strains showed co-aggregation ability with the pathogens *Salmonella enteritidis* and *Escherichia coli*. With *Salmonella enteritidis*, values were between 47.97% (*Pediococcus pentosaceus* JK 44) and 58.80% (*Pediococcus pentosaceus* JK 86) while with *Escherichia coli*, they varied from 49.21% (*Lactobacillus pentosus* JK 49) to 53.66% (*Weissella confusa* JK 25) (Fig. 4). Statistical analyses showed 2 groups of lactic acid bacteria according to their co-aggregation properties. The first group of 1 LAB strain had value between 30 and 35% for the co-aggregation with *S. enteritidis* and 35 and 40% for the co-aggregation with *E. coli* respectively. The second of 14 LAB strains had value between 45 and 60% for the co-aggregation with *S. enteridis* and 47 and 53% for the co-aggregation with *E. coli*, respectively.

Antimicrobial activity: The results for antimicrobial activity of the 15 LAB isolated from the gastrointestinal tract of broilers chickens against pathogenic bacteria is presented in Table 1. All 15 LAB strains showed antagonistic effects against *Salmonella typhimurium*, *Pseudomonas aeruginosa* and *Bacillus cereus*. The diameters of inhibition were ranged from 10-19 mm. The highest activity towards *S. typhimurium* was obtained by *Lactobacillus pentosus* JK 51 (18 mm). For *Bacillus cereus*, the highest activity was obtained by *Lactobacillus pentosus* JK 54 (18 mm) and *Pediococcus pentosaceus* JK 86 (18 mm). The highest activity towards *Pseudomonas aeruginosa* was obtained by *Lactobacillus pentosus* (JK 51 and 55) (19 mm). *Salmonella enteritidis* was inhibited by *Enterococcus faecium* JK 96 (19 mm). *Escherichia coli* was inhibited *Lactobacillus pentosus* (JK 51 and 55) (15 mm), *Pediococcus acidilactici* JK 38 (15 mm) and *Weissella confusa* JK 25 (12 mm). All 4 *Lactobacillus pentosus* strains and *Enterococcus faecium* JK 96 showed antagonistic activity towards all pathogens.

Lactic acid bacteria strains isolated from the gastrointestinal tract of broilers chickens with high probiotic potential: To assess the similarity and variability between the probiotic strains in order to select candidate probiotic isolated for the next set of *in vitro* and *in vivo* studies, the data of the probiotic phenotypes (cell surface hydrophobicity, aggregation, co-aggregation and antimicrobial activity) were subjected to multivariate principal component analysis using XLSTAT-2017 software. Further, heat map was generated to cluster probiotic strains (Fig. 5 and 6). Figure 5 presents the Principal Component Analysis (PCA). The first (F1) and the second (F2) principal components represented 34.83 and 20.82% of the total 10 variables. Further, the Principal Component Analysis (PCA) indicated that the strain specific difference exists in the probiotic attributes among all the 15 LAB strains. Figure 6 shows the probiotic phenotype heatmap clustered 14 probiotic isolates into 4 clusters C1, C2, C3 and C4. Of the 4 groups, group C4 shows the highest values of the probiotic parameters studied. This group composed of: *Pediococcus pentosaceus* JK 86, *Pediococcus acidilactici* JK 85, *Enterococcus faecium* JK 92 and *Enterococcus faecium* JK 96 was used to perform the antibiotic susceptibility test.

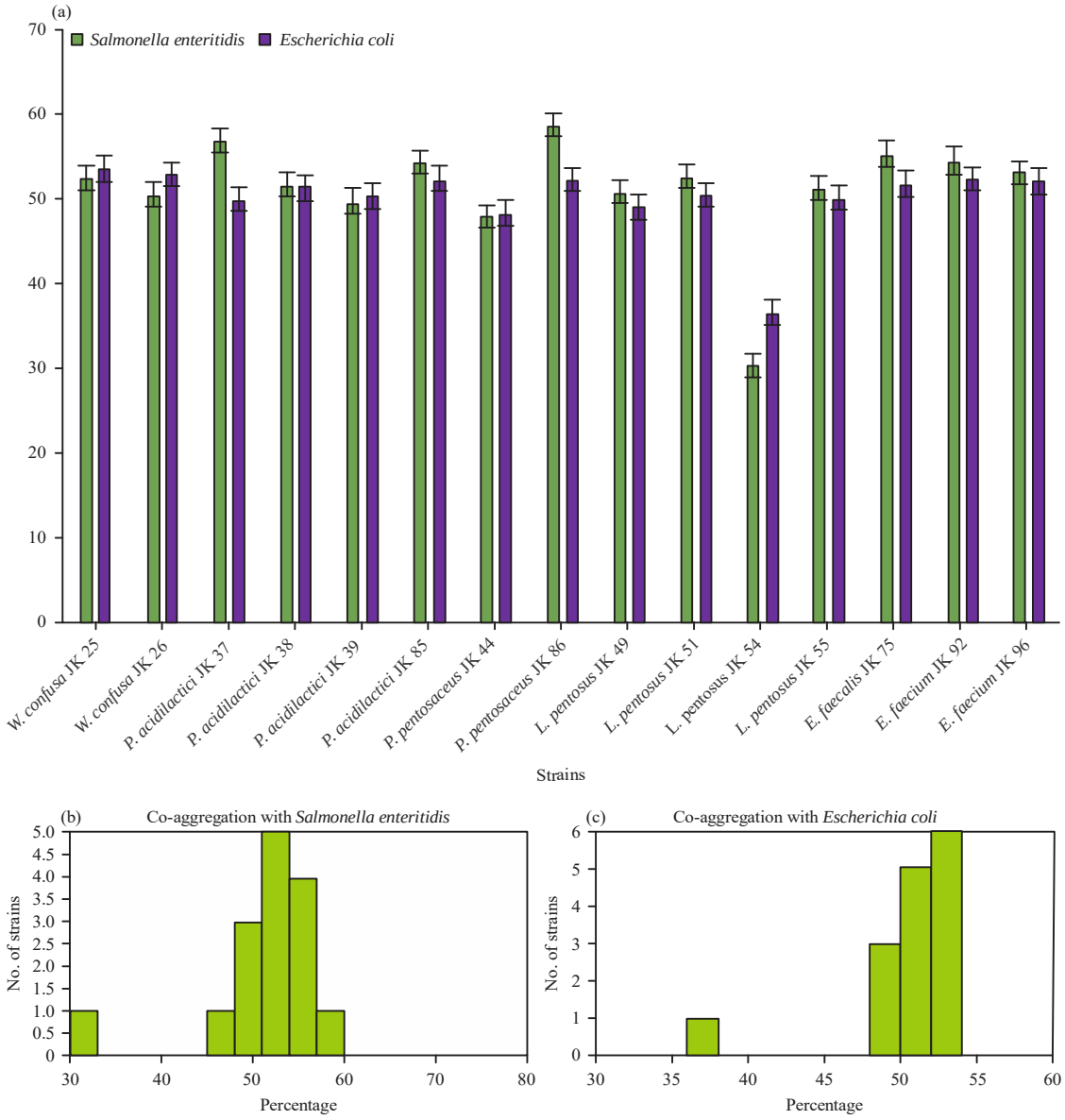

Fig. 4(a-c): Co-aggregation properties of lactic acid bacteria strains

Antibiotic susceptibility: The antibiotic susceptibility test was carried out for five selected LAB strains against nine antibiotics and the results are shown in Table 2. All the selected strains (100%) showed resistance to Colistin, Oxacillin and Kanamycin. On contrary, they were all sensitive to Imipenem and Erythromycin. Overall, *Enterococcus faecium* JK 96 was the most resistant strain while *Pediococcus acidilactici* JK 85 was the most sensitive strain.

DISCUSSION

The application of probiotics in the poultry industry as a suitable alternative to antibiotics as well as to improve their performance and productivity has received considerable attention in recent years. In addition to the other beneficial properties of probiotics, probiotic strains derived from their natural host is most preferred, as these microbial strains are already familiar with the gastrointestinal tract and can spontaneously proliferate and express the desired beneficial effects better than strains isolated from other sources. In this study, we characterized LAB strains from the gastrointestinal tract of broilers to identify candidate probiotics for these birds. All LAB strains tested showed excellently tolerance to 6.5% NaCl concentration and 63.5°C. These results are desirable features from potential LAB probiotics which could increase bacterial growth and production of beneficial metabolites.

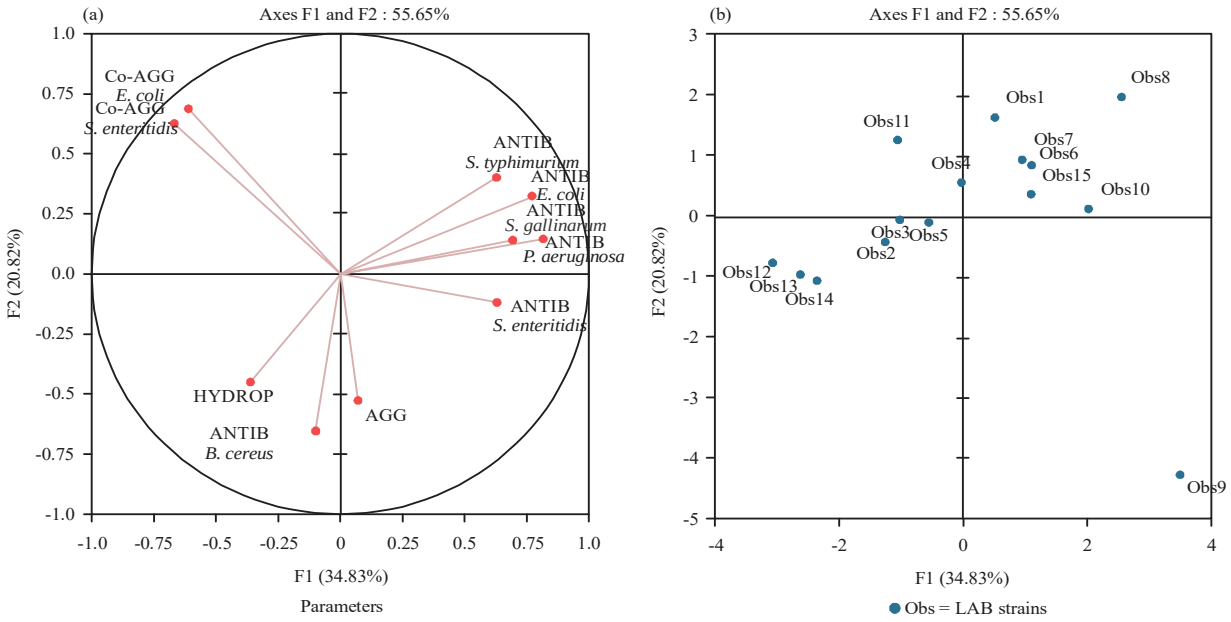

Fig. 5(a-b): Principal component analysis (PCA) of probiotics parameters of LAB strains

Table 1: Diameters of inhibition (mm) of lactic acid bacteria strains against test pathogens

Strains	*Salmonella typhimurium*	*Pseudomonas aeruginosa*	*Bacillus cereus*	*Salmonella enteritidis*	*Staphylococcus gallinarum*	*Escherichia coli*
W. confusa JK 25	15±0.1^{c}	17±0.1^{c}	10±0.1^{a}	0	(10±0.1)^{a}	12±0.1^{c}
W. confusa JK 26	17±0.2^{d}	13±0.1^{a}	14±0.1^{b}	0	0	0
P. acidilactici JK 37	17±0.1^{d}	15±0.1^{b}	16±0.1^{bc}	0	0	0
P. acidilactici JK 38	15±0.1^{c}	17±0.1^{c}	16±0.1^{bc}	0	0	15±0.1^{d}
P. acidilactici JK 39	16±0.2^{cd}	17±0.1^{c}	15±0.1^{b}	0	0	0
P. acidilactici JK 85	11±0.1^{a}	13±0.1^{a}	14±0.1^{b}	0	0	0
P. pentosaceus JK 44	17±0.1^{d}	16±0.1^{bc}	10±0.1^{a}	0	10±0.1^{a}	8±0.1^{a}
P. pentosaceus JK 86	12±0.1^{a}	16±0.1^{bc}	18±0.1^{c}	0	0	0
L. pentosus JK 49	16±0.1^{ab}	17±0.1^{c}	10±0.1^{a}	0	12±0.1^{bc}	8±0.1^{a}
L. pentosus JK 51	18±0.2^{e}	19±0.2^{d}	10±0.1^{a}	10±0.1^{a}	12±0.1^{bc}	15±0.1^{d}
L. pentosus JK 54	15±0.1^{c}	17±0.1^{c}	18±0.1^{c}	10±0.1^{a}	14±0.1^{c}	8±0.1^{a}
L. pentosus JK 55	15±0.1^{c}	19±0.1^{d}	15±0.1^{b}	10±0.1^{a}	11±0.1^{b}	15±0.1^{d}
E. faecalis JK 75	15±0.1^{c}	13±0.1^{a}	10±0.1^{a}	0	11±0.1^{b}	0
E. faecium JK 92	12±0.1^{a}	15±0.1^{b}	10±0.1^{a}	0	0	0
E. faecium JK 96	17±0.2^{d}	15±0.1^{b}	10±0.1^{a}	19±0.1^{b}	10±0.1^{a}	10±0.1^{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 Tukey test, W: *Weissella*, P: *Pediococcus*, L: *Lactobacillus*, E: *Enterococcus*

Table 2: Diameters of inhibition (mm) showed by the selected LAB strains with different antibiotics

Strains	OX 5	IMP 10	CN 10	KF 30	AML 10	E 15	C 30	K 30	CST 30
P. acidilactici JK85	00(R)	26(S)	10(S)	15(S)	15(S)	24(S)	26(S)	00(R)	00(R)
P. pentosaceus JK86	00(R)	30(S)	00(R)	22(S)	15(S)	22(S)	24(S)	00(R)	00(R)
E. faecium JK92	00(R)	24(S)	00(R)	14(S)	12(S)	22(S)	24(S)	00(R)	00(R)
E. faecium JK96	00(R)	30(S)	00(R)	00(R)	00(R)	20(S)	00(R)	00(R)	00(R)

P: *Pediococcus*, Ent: *Enterococcus*, OX: Oxacillin, IMP: Imipenem, CN: Gentamicin, KF: Cephalotin, AML: Amoxicillin, E: Erythromycin, C: Chloramphenicol, K: Kanamycin, CST: Colistin

Also, these traits exhibited by these LAB strains are of industrial and technological relevance as well as for preservation[16].

An important characteristic that must be possessed by lactic acid bacteria with probiotic ability were viability and survival ability on stress condition in digestive tract. In this study, 15 of 90 strains had ability to survive on pH 3 after 90 min incubation and had high cell viability on 0.3% (b/v) bile salt. Most of bacteria grow slower at low pH, due to the presence of an acid that can damage and decrease its viability.

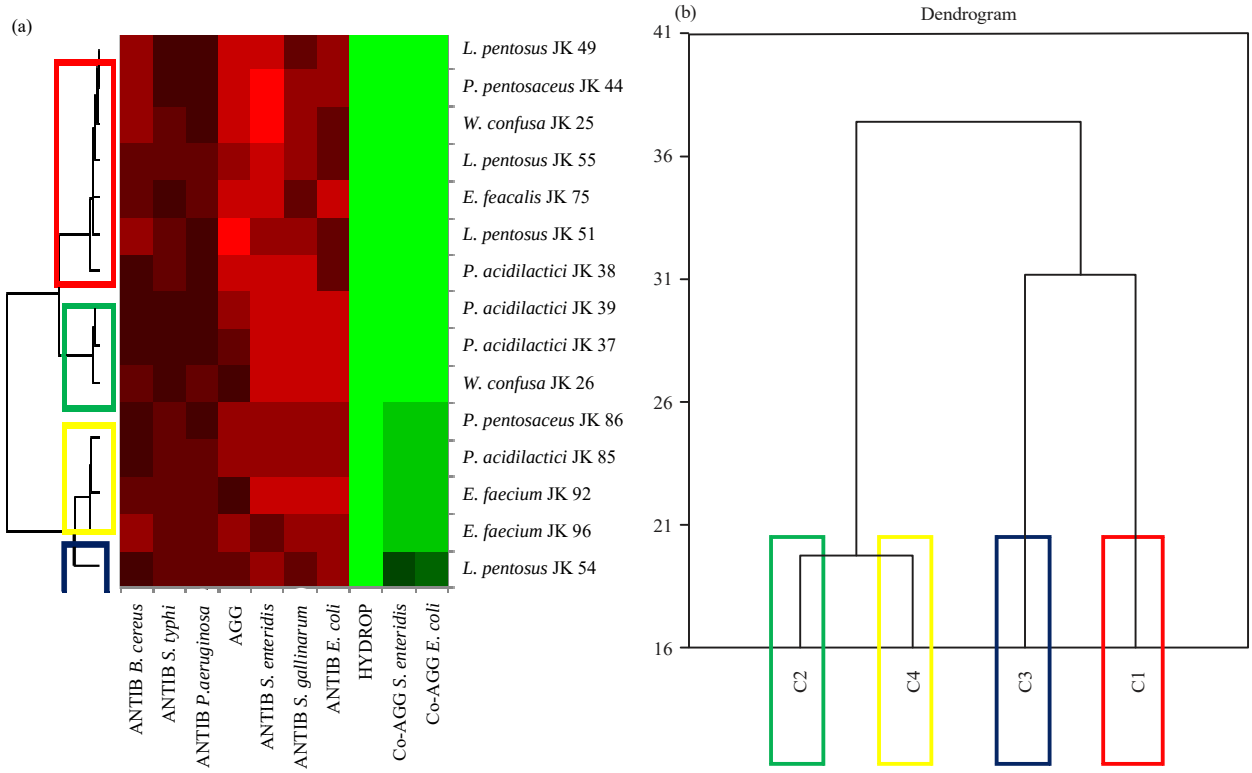

Fig. 6(a-b): Heat map of probiotics parameters of LAB strains

However, LAB has the ability to regulate their cytoplasmic or intracellular pH around neutral pH, even when it is in low extracellular pH during growth or in storage[17]. In the chicken GIT, the duodenum and cecum have a total bile salt concentration of 0.175 and 0.008%[18]. However, the average level of 0.3% bile salt has been considered in many studies for bile salt tolerance of potential probiotic LAB[19,20]. These results were similar to previous studies conducted by Jannah *et al.*[1] and Reuben e*t al.*[16], who observed good tolerance to pH 3 and 0.3% bile salts.

A highly sought-after property of probiotics is the hydrophobicity of the strain's surface. The hydrophobicity of probiotics directly measures their ability to adhere to enterocyte cell lines[21]. All LAB strains examined showed good hydrophobicity capabilities with values ranging from 61-99.75%. Similar results were reported by Ehrmann *et al.*[22] who obtained high hydrophobicity among LAB strains isolated from poultry. Previous reports have shown a correlation between high hydrophobicity of LAB strains with their attachment to intestinal mucosal and epithelial cells[11,22].

The cell-binding properties: Auto-aggregation and co-aggregation are generally considered for selecting potential probiotic strains. Auto-aggregation (inter-isolate coaggregation ability) and co-aggregation (aggregation between different microbial strains) support bacterial adhesion to epithelial cells of the host GIT and avoid adhesion of pathogen on host intestinal cells[16]. All the tested LAB strains showed low auto-aggregation but high co-aggregation ability with the pathogens *Salmonella enteritidis* and *Escherichia coli*. These results disagree with those of Reuben *et al.*[16] who recorded auto-aggregation ability of 32-56.5% for LAB strains from broilers chickens. These results are also different from those of Jannah *et al.*[23] who observed weak coaggregation with *S. enteritidis* and *E. coli*.

All fifteen LAB strains showed antagonistic effects against *Salmonella typhimurium*, *Pseudomonas aeruginosa* and *Bacillus cereus*. Antagonistic activity by LAB are sustained by the secretion of different antimicrobial substances including organic acids (lactic, acetic etc), bacteriocins, bacteriocin-like components alcohols, with a consequent reduction in pH, or to the production of hydrogen peroxide[24,25]. Lactic acid is the major organic acid in LAB fermentation where it is in equilibrium with its undissociated and dissociated forms and the extent of the dissociation depends on pH. The probiotic candidates should be able to inhibit the growth of bacteria associated with infections to overcome one of the main loses of the poultry industry[25]. These results were similar to previous studies conducted by Kizerwetter-Swida *et al.*[26], Oyewole *et al.*[27]; Reuben *et al.*[16] who reported antagonistic

activity against *Salmonella typhimurium* and *Pseudomonas aeruginosa* by LAB isolated from poultry. In addition, Shamsudin *et al*.[28] and Reuben *et al*.[16] showed that all *Lacto bacillus* strain exhibited antimicrobial properties towards all pathogenic strains tested in this study.

Heatmap shows that group C4 has the highest values of the probiotic parameters studied. This group composed of: *Pediococcus pentosaceus* JK 86, *Pediococcus acidilactici* JK 85, *Enterococcus faecium* JK 92 and *Enterococcus faecium* JK 96. *P. acidilactici* isolated from warapossesses desirable probiotic properties *in vitro* with the inhibition of pathogens and high adhesion abilities. Denev *et al*.[29]; Olajugbagbe *et al*.[30]; Ayyash *et al*.[31]; Jiang *et al*.[32]; Franz *et al*.[33] and Zommiti *et al*.[34] showed that *Pediococcus acidilactici*, *Pediococcus pentosaceus* and *Enterococcus faecium* had desirable probiotic properties *in vitro* with the inhibition of pathogens and high adhesion abilities, respectively.

The determination of antimicrobial susceptibility profile is an important criterion for potential probiotics evaluation. Microbial strains to be considered as probiotics should not serve as antibiotic resistance genes reservoir, which may further be transferred to intestinal pathogens[25]. All the five strains were sensitive to Imipenem and Erythromycin. All the strains showed resistance to Colistin, Oxacillin and Kanamycin. It has been reported in literature that strains of LAB are resistant to β-lactam antibiotics including oxacillin, because they harbor of β-lactamase[35,36]. According to Kim and Austin[37], the intrinsic antibiotic resistance nature of LAB probiotics suggests their application for both therapeutic and preventive purposes in the treatment and control of intestinal infections.

CONCLUSION

Gastrointestinal tract is a good source of lactic acid bacteria. In this study, 15 lactic acid bacteria strains having probiotic potentials were isolated from Ivoirian broilers chickens' gastrointestinal tract. 4 LAB strains from poultry were found to possess suitable *in vitro* probiotic properties. They are: *Pediococcus acidilactici* JK85, *Pediococcus pentosaceus* JK86, *Enterococcus faecium* JK92and *Enterococcus faecium* JK96. This study indicated that Ivoirian broilers chickens gut is a good resource to isolate lactic acid bacteria with good characteristics as probiotics.

ACKNOWLEDGMENT

The authors would like to thank all the chicken farmers who contributed to this research. This study was supported by the International Foundation for Science (IFS), Sweden "Grant No. I3-B-6527-1".

REFERENCES

1. Jannah, S.N., T.R. Saraswati, D. Handayani and S. Pujiyanto, 2018. Antibacterial activity of lactic acid bacteria isolated from gastrointestinal tract of "Ayam Kampung" chicken against food pathogens. J. Phys.: Conf. Ser., Vol. 1025, 10.1088/1742-6596/1025/1/012082.
2. Vondruskova, H., R. Slamova, M. Trckova, Z. Zraly and I. Pavlik, 2010. Alternatives to antibiotic growth promoters in prevention of diarrhoea in weaned piglets: A review. Vet. Med., 55: 199-224.
3. Kazemi, S.A., H. Ahmadi and M.A.K. Torshizi, 2019. Evaluating two multistrain probiotics on growth performance, intestinal morphology, lipid oxidation and ileal microflora in chickens. J. Anim. Physiol. Anim. Nutr., 103: 1399-1407.
4. FAO/WHO, 2002. Guidelines for evaluation of probiotics in food. Drafting Guidelines for the Evaluation of Probiotics in Food London, Ontario, Canada,
5. Dicks, L. and M. Botes, 2010. Probiotic lactic acid bacteria in the gastro-intestinal tract: Health benefits, safety and mode of action. Beneficial Microbes, 1: 11-29.
6. Park, J.H. and I.H. Kim, 2015. The effects of the supplementation of Bacillus subtilis RX7 and B2A strains on the performance, blood profiles, intestinal *Salmonella concentration*, noxious gas emission, organ weight and breast meat quality of broiler challenged with *Salmonella typhimurium*. J. Anim. Physiol. Anim. Nutr., 99: 326-334.
7. Abdelbasset, A., 2016. Development of a Prebiotic Matrix for the Encapsulation of Bacteriocinogenic Probiotics, Intended for Animal Feed: From Physicochemistry to Biopharmacy. PhD Theses, Laval University, Canada.
8. Badis, A., N. Laouabdia-Sellami, D. Guetarni, M. Kihal and R. Ouzrout, 2005. Phenotypic characterization of lactic bacteria isolated from raw goat milk from two local goat populations "Arabia and Kabyl. Sci. Technol. C, Biotechnol., 23: 30-37.
9. Ramos, C.L., L. Thorsen, R.F. Schwan and L. Jespersen, 2013. Strain-specific probiotics properties of *Lactobacillus fermentum*, *Lactobacillus plantarum* and *Lactobacillus brevis* isolates from Brazilian food products. Food Microbiol., 36: 22-29.
10. Handa, S. and N. Sharma, 2016. *In vitro* study of probiotic properties of *Lactobacillus plantarum* F22 isolated from chhang-A traditional fermented beverage of Himachal Pradesh, India. J. Genet. Eng. Biotechnol., 14: 91-97.
11. Taheri, H.R., H. Moravej, F. Tabandeh, M. Zaghari and M. Shivazad, 2009. Screening of lactic acid bacteria toward their selection as a source of chicken probiotic. Poult. Sci., 88: 1586-1593.
12. Kos, B., J. Suskovic, S. Vukovic, M. Simpraga, J. Frece and S.J. Matosic, 2003. Adhesion and aggregation ability of probiotic strain *Lactobacillus acidophilus* M92. J. Appl. Microbiol., 94: 981-987.

13. Arici, M., B. Bilgin, O. Sagdic and C. Ozdemir, 2004. Some characteristics of *Lactobacillus* isolates from infant faeces. Food Microbiol., 21: 19-24.
14. Bauer, A.W., W.M.M. Kirby, J.C. Sherris and M. Turck, 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol., 45: 493-496.
15. CASFM, 2018. Comité de l'antibiogramme de la Société Française de Microbiologie. https://www.sfm-microbiologie.org/wp-content/uploads/2018/12/CASFMV2_SEPTEMBRE2018.pdf
16. Reuben, R.C., P.C. Roy, S.L. Sarkar, R.U. Alam and I.K. Jahid, 2019. Isolation, characterization and assessment of lactic acid bacteria toward their selection as poultry probiotics. BMC Microbiol., Vol. 19. 10.1186/s12866-019-1626-0.
17. Konings, W.N., J.S. Lolkema, H. Bolhuis, H.W. van Veen, B. Poolman and A.J.M. Driessen, 1997. The role of transport processes in survival of lactic acid bacteria, Energy transduction and multidrug resistance. Antonie van Leeuwenhoek, 71: 117-128.
18. Lin, J., O. Sahin, L.O. Michel and Q. Zhang, 2003. Critical role of multidrug efflux pump cmeabc in bile resistance and *In vivo* colonization of *Campylobacter jejuni*. Infec. Immun.., 71: 4250-4259.
19. García-Hernández, Y., T. Pérez-Sánchez, R. Boucourt, J.L. Balcázar and J.R. Nicoli *et al*., 2016. Isolation, characterization and evaluation of probiotic lactic acid bacteria for potential use in animal production. Res. Vet. Sci., 108: 125-132.
20. Shokryazdan, P., R. Kalavathy, C.C. Sieo, N.B. Alitheen, J.B. Liang and M.F. Jahromi, 2014. Isolation and characterization of *Lactobacillus* strains as potential probiotics for chickens. Pertanika J. Trop. Agric. Sci., 37: 141-157.
21. Onifade, A.A., 1997. Growth performance, carcass characteristics, organs measurement and haematology of broiler chickens fed a high fibre diet supplemented with antibiotics or dried yeast. Nahrung, 41: 370-374.
22. Ehrmann, M.A., P. Kurzak, J. Bauer and R.F. Vogel, 2002. Characterization of lactobacilli towards their use as probiotic adjuncts in poultry. J. Applied Microbiol., 92: 966-975.
23. Jannah, S.N., A. Dinoto, K.G. Wiryawan and I. Rusmana, 2014. Characteristics of lactic acid bacteria isolated from gastrointestinal tract of cemani chicken and their potential use as probiotics. Media Peternakan, 37: 182-189.
24. Gonzalez, L., H. Sandoval, N. Sacristan, J.M. Castro, J.M. Fresno and M.E. Tornadijo, 2007. Identification of lactic acid bacteria isolated from Genestoso cheese throughout ripening and study of their antimicrobial activity. Food Control, 18: 716-722.
25. Nallala, V., V. Sadishkumar and K. Jeevaratnam, 2017. Molecular characterization of antimicrobial *Lactobacillus* isolates and evaluation of their probiotic characteristics *in vitro* for use in poultry. Food Biotechnol., 31: 20-41.
26. Kizerwetter-Swida, M. and M. Binek, 2005. Selection of potentially probiotic *Lactobacillus* strains towards their inhibitory activity against poultry enteropathogenic bacteria. Polish J. Microbiol., 54: 287-294.
27. Oyewole, O.F., C.O. Maria, P.S. Tope and O.O. Funmi, 2018. *In vitro* study of potential probiotic lactic acid *Bacteria* isolated from the gut of chickens in Abeokuta, Nigeria. Alexandria J. Vet. Sci., 58: 73-84.
28. Shamsudin, W.N.F., S.S. Loo, H.W. Ho, N. Abdullah, W.Z. Saad and K.L. Wan, 2019. Probiotic properties of *Lactobacillus* isolates from chicken intestines. J. Bio. Microbiol. Biotech., 7: 8-13.
29. Denev, S., E. Chevaux and V. Demey, 2013. Efficacy of the Probiotic *Pediococcus acidilactici* on Laying Hens' Performances. Actes des 10èmes Journées de la Recherche Avicole et Palmipèdes à Foie Gras du 26 au 28 mars, 2013, La Rochelle, France. March 26-28 Institut Technique de l'Aviculture, 943-946.
30. Olajugbagbe, T.E., O.E. Elugbadebo and B.O. Omafuvbe, 2020. Probiotic potentials of *Pediococuss acidilactici* isolated from wara; a Nigerian unripened soft cheese. Heliyon, Vol. 6 10.1016/j.heliyon.2020.e04889.
31. Ayyash, M., B. Abu-Jdayil, A. Olaimat, G. Esposito and P. Itsaranuwat *et al*., 2020. Physicochemical, bioactive and rheological properties of an exopolysaccharide produced by a probiotic *Pediococcus pentosaceus* M41. Carbohydr. Polym., Vol. 229, 10.1016/j.carbpol.2019.115462.
32. Jiang, S., L. Cai, L. Lv and L. Li, 2021. *Pediococcus pentosaceus*, a future additive or probiotic candidate. Microb. Cell Fact., Vol. 20, 10.1186/s12934-021-01537-y.
33. Franz, C.M.A.P., M. Huch, H. Abriouel, W. Holzapfel and A. Galvez, 2011. Enterococci as probiotics and their implications in food safety. Int. J. Food Microbiol., 151: 125-140.
34. Zommiti, M., M. Cambronel, O. Maillot, M. Barreau and K. Sebei *et al*., 2018. Evaluation of probiotic properties and safety of *Enterococcus faecium* isolated from artisanal Tunisian meat "Dried Ossban". Front. Microbiol., Vol. 9. 10.3389/fmicb.2018.0168.
35. Hoque, M.Z., F. Akter, K.M. Hossain, M.S.M. Rahman, M.M. Billah and K.M.D. Islam, 2010. Isolation, identification and analysis of probiotic properties of *Lactobacillus* spp. from selective regional yoghurts. World J. Dairy Food Sci., 5: 39-46.
36. Jose, N., C. Bunt and M. Hussain, 2015. Comparison of microbiological and probiotic characteristics of lactobacilli isolates from dairy food products and animal Rumen contents. Microorganisms, 3: 198-212.
37. Kim, D.-H and B. Austin, 2008. Characterization of probiotic *Carnobacteria* isolated from rainbow trout (*Oncorhynchus mykiss*) intestine. Lett. Appl. Microbiol., 47: 141-147.