Novel Isolates of Lactobacilli from Crop of Algerian Poultry as Potential Probiotic for Food Industry

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Abstract: This study was aimed at selecting novel strains of *Lactobacillus* from crop of Algerian poultry. One hundred forty (140) lactobacilli strains were isolated and examined for their potentiality probiotic properties. From these isolated strains, nine appear to possess a probiotic value and highlighted a noticeable heterogeneity. The isolate *L. plantarum* G_1 showed the best inhibitory activity against several indicator strains. Furthermore, the results showed that culture and neutralized supernatants exhibited varying degrees of inhibitory activity against strains of enterobacteria from poultry origin. The tested strains were acid resistant and were also bile tolerant. Antibiotic resistance, co-aggregation activity and hydrophobicity percentage were strain-dependent. Moreover, six strains were able to adhere to epithelial cells. Finally, six *Lactobacillus* strains, such as strain *L. plantarum* G_1, *L. plantarum* PC_2, *L. viridesencs* G_3, *L. helveticus* PC_6, *L. delbrueckii* ssp *delbrueckii* G_7 and *L. fermentum* PC_8, showed essential probiotic properties. The identity of the best strain *L. plantarum* G1 was confirmed by 16S rRNA gene sequence using PCR.

Keywords: *Lactobacillus*, Poultry crop, Probiotic properties, Adhesion.

INTRODUCTION

The gastrointestinal (GI) tract of animals is a very complex microbial ecosystem, and it is colonised by a large number of different microbial species [1-2]. The community structure and the diversity of the microflora of the GI tract are continuously affected by factors associated with the host health and nutrition and the outside environment [2-3]. The crop as a part of the GI tract contains considerable amount of bacterial species which might play an important role in host performance, for example to assist the digestion of food, to produce micronutrients and to modulate the immune system… [4-6].

Lactobacilli dominate the intestinal microbiota of chickens. The presence of *Lactobacillus* species in the chicken crop is reported. Many of these bacterial species contribute to maintaining the ecological balance of the GI tract ecosystem [6]. Several works reported the properties of lactic acid bacteria (LAB) isolated from animals such as antimicrobial activity against pathogens [7] and their possible use as probiotic in animal feed [8]. The identification and selection of probiotic bacteria is generally based on their ability to resist to acid and bile acids and adherence to epithelial cells, safety, production of antimicrobial activity... [5, 9].

Sahnouni *et al.* [10] reported the isolation and characterization of LAB from the intestinal microbiota of marine fish of Algerian coast with important properties such as the antimicrobial and antifungal activities against several microbial strains. The present study was designed to characterize and select novel isolates of lactobacilli from crop of Algerian poultry as potential probiotic based on essential probiotic characteristics. The best isolated strain was identified by API50 CHL test kits and confirmed by partial sequencing of 16S rRNA.

MATERIALS AND METHODS

Poultry Crop Samples

In this study, a total of 3 crop samples of healthy Algerian poultry were used. For each sample the pH measurements was obtained with a pH meter (Hanna Instruments, Padova, Italy), calibrated with two standard solutions buffered at pH 4.0 and pH 7.0. For each sample aliquots of 10g was used for enumeration of crop microflora. The media and the conditions using for microflora numeration were the following: Plate count agar (PCA) incubated at 37°C for 48h for total bacteria; violet red bile glucose agar (VRBG), incubated at 37°C for 24h for enterobacteria; violet red bile lactose agar (VRBL), incubated respectively at 37°C and 44°C for 24h for coliforms and thermotolerant coliforms; MRS agar (Pasteur institute, Algeria), incubated at 32°C for 48h to 72h in anaerobiosis for lactic acid bacteria.

Isolation of Lactobacilli Strains

The isolation of lactobacilli strains was carried out by preparing aliquots of five 10-fold dilutions from each

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sample and used to inoculate MRS agar (Pasteur institute, Algeria) plates acidified with glacial acetic acid to pH 5.7, then plates were incubated anaerobically for 48h at 37°C. One hundred forty (140) colonies were picked up from the higher dilutions (10^{-5}) and sub-cultured in MRS broth. The pure isolates were initially subjected to the Gram staining and the catalase test (3% H_2O_2). Only the rods, Gram positive, catalase negative isolates were further used. The purified cultures were stored in MRS broth plus 10% (v/v) glycerol.

Poultry Crop Enterobacteriaceae and Indicators Strains

Fourteen strains which were isolated from the crop and intestine of poultry and two pathogenic *E. coli* were used as indicator strains. All strains are listed in Table **1**. Prior to use, the indicators strains were transferred twice in Luria Bertani broth (LB) and incubated at 37°C for 24h.

Screening for Antimicrobial Activity

The antimicrobial activity of the selected strain was screened using the agar spot agar in MRS agar under anaerobic conditions at 37°C [11, 12]. The used indicator strains were of poultry crop origin and they were cultured on LB broth. The well diffusion method was applied with strains exhibited inhibitory activity. The supernatant culture fluid was tested. In order to minimize the effect of pH, supernatant pH was adjusted to pH 7.0 using 5N NaOH. Ten µl (10µl) of filtrate was spotted onto MRS agar. Soft agar (7 ml; 0.75%) was poured onto the surface of pre-poured agar plates. The plates were incubated overnight at 37°C and the inhibition zone diameters were measured [11, 12]. The isolates with the highest inhibitory activity were selected for further study.

Sensitivity of Lactobacilli Isolates to Gastric Juice

The method described by Lin *et al.* [13] was used. The crop digesta from local poultry (body weight, 2.2± 0.35 kg) were collected and mixed with two volumes of sterile water. To obtain the sterile gastric juice, the mixture was centrifuged at 3000g for 30min and the supernatants, after measuring the pH value, was passed through a sterile 0.45 mm-pore-size filter. For the test essay, 1 ml of the overnight of each culture was centrifuged at 7000rpm for 10min. The cell pellet was then mixed with 1ml of the sterile gastric juice prepared as described above. Each mixture was then incubated at 37°C for 3h. After incubation, viable bacterial counts were determined by plating serial dilutions in phosphate-buffer saline (PBS, pH 7.2) on MRS agar followed by incubation at 37°C for 48h. For the preparation of sterile gastric acid from gizzard digesta, the gizzard contents collected from the same poultry were used, and the procedure is the same as that described earlier for crop gastric juice.

Bile Salt Tolerance

The method used was modified from that of Yu and Tsen [14]. To determine the resistant of lactobacilli strains to bile salt, serial decimal dilution was spread on MRS agar containing 0.5% and 2% of bile salt. The strains were counted after incubation for 24h at 37°C. The percentage of bile tolerance was calculated by comparing of the number of colonies cultivate in MRS agar with bile salt to those in MRS agar without bile salt.

Co Aggregation Assay

The bacterial cells were harvested by centrifugation at 5000 g for 15min after the incubation at 37°C for 18h, washed twice and resuspended in PBS to give

Table 1: Origin of Indicator Strains

Origin	indicator strains	code	number	reference or laboratory
Crop of poultry	*Obsumbacterium* spp.	C_1 to C_4	four	L.B.E.S
Intestine of poultry	*Obsumbacterium* spp.	C_5 and C_6	two	L.B.E.S
Crop of poultry	*Erwinia* spp.	C_7 and C_8	two	L.B.E.S
Intestine of poultry	*Erwinia* spp.	C_9 and C_{10}	two	L.B.E.S
Crop of poultry	*Enterobacter* spp.	C_{11} and C_{12}	two	L.B.E.S
Crop of poultry	*Escherichia coli*	C_{13} and C_{14}	two	L.B.E.S
Poultry	*E. coli*	0604 RPOS	one	INRA
Poultry	*E. coli*	TK3	one	Pourbakhsh *et al.* 1997

L.B.E.S: Laboratory of Biotechnology, Environment and Health, University of Jijel, Jijel, Algeria.
INRA: National Institute for Agricultural Research, Clermont-Ferrand, France.

viable counts of approximately 10^8 CFU /ml. Equal volumes (2ml) of each cell suspension were mixed together in pairs by vortexing. Control tubes were set up at the same time, containing 4 ml of each bacterial suspension alone. The absorbances (A) at 600 nm of the suspensions were measured after mixing and after 5h of incubation [13]. The percentage of coaggregation was calculated using the equation:

$$\text{Coaggregation (\%)} = [(A_x + A_y)/2) - A(x+y)] / [(A_x + A_y)/2] \times 100$$

Where x and y represent each strain in the control tubes, and (x + y) the mixture.

Cell Surface Hydrophobicity and Adherence Assay to Epithelial Cell of Local Poultry

For the cell surface hydrophobicity, Xylene and Toluene were used as hydrocarbons. The cell surface hydrophobicity was measured according to the method described by Rosenberg *et al.* [15]. Segment of poultry ileum was washed with sterilized PBS (pH 7.2). It was held at 4°C for 30min and then washed three times with PBS. The epithelial cell concentration was adjusted to approximately $5x10^4$ cells ml^{-1}. Briefly, cell pellet from overnight culture of LAB was resuspended to approximately 1.10^8 cells ml^{-1} in PBS. One ml of such bacterial suspension was mixed with 1ml of the cell suspension of epithelial cells. The mixture was incubated at 37°C for 30 min. The adhesion was observed using phase contrast microscopy (magnification fold x 200) after stained with 0.5% crystal violet for 5min [13].

Resistance to Antibiotics

In order to examine the antibiotic sensitivity, the standardized single disk method described by Beaur *et al.* [16] was used. The antibiotiques (Sigma Aldrich Co., St. Louis, USA) used are Penicillin G, Ampicilin, Erythromycin, Streptomycin, Amoxicillin, Gentamicin, Kanamycin, Oxytetracycline, Tetracycline, Vancomycin, Ofloxacin, Amikacin and Nalidixic acid. After inoculation of the bacterial culture on MRS agar and placement of disks, plates were incubated at 37°C for 18h. Zones of inhibition were measured.

Identification of the Finally Selected Strains

The selected isolates were firstly submitted to Gram staining, catalase reaction, motility and cell morphology. The ability of the isolated strains to produce acid from different carbohydrates was determined by API 50 CHL test kits (Bio Merieux, S.A., France). The API test strips were prepared as recommended by the kit supplier and scored after incubation for 24 and 48h at 37°C. The results were loaded on the API system software, which used the phenotypic data to predict a species identity (%) for each isolate. The identity of the best selected isolate was further confirmed by partial sequencing of 16S rRNA gene.

RESULTS

Enumeration of Crop Microflora

The results for the population of crop bacterial groups are presented in Table **2**. Results indicate that crop samples reveal a diversity of microflora and the highest total bacterial count was observed in samples 1 and 3. This diversity could be linked to environmental conditions. Count of total bacteria is between 7.5×10^6 to 9.0×10^6 cfu g^{-1}. The enterobacteria counts ranged from 0.60×10^4 cfu g^{-1} to 1.85×10^4cfu g^{-1}. Coliforms and thermotolerant coliforms were detected in all samples. Also, counts of lactic acid bacteria were between 3.2 $\times10^5$ to 7.6×10^5cfu g^{-1}.

Antibacterial Activity of Lactobacilli Strains

The influence of some lactobacilli strains, supernatant and neutralized supernatant (pH 7.0) on particular enterobacteria isolated from poultry crop is reported in Tables **3** and **4**. Results of Table **3** showed that six of nine tested lactobacilli strains exhibited inhibitory activity against enterobacteria, although inhibitory degrees are variable.

Table 2: Total Counts of Microflora Detected in Crop of Poultry

Microflora (cfu g^{-1})	Total bacteria	Enterobacteria	Coliforms	Thermotolerant coliforms	Lactic acid bacteria
Sample 1	8.8×10^6	0.85×10^4	0.70×10^3	0.55×10^3	5.8×10^5
Sample 2	7.1×10^6	0.60×10^4	0.40×10^3	0.30×10^3	3.2×10^5
Sample 3	9.0×10^6	1.85×10^4	0.87×10^3	0.76×10^3	7.6×10^5

Table 3: Growth Inhibition Zones of Enterobacteria Caused by Lactobacilli Isolates

Enterobacteria Inhibition zone (mm)	G_1	PC_2	G_3	PC_4	PC_6	G_7	PC_8	PC_{13}	PC_{14}
Obsumbacterium spp. (C_1)	16.2	14.2	15.2	0.0	13.0	14.6	13.1	0.0	0.0
Obsumbacterium spp. (C_2)	17.0	14.5	15.0	0.0	10.0	14.2	13.5	0.0	0.0
Obsumbacterium spp. (C_3)	16.8	12.8	15.1	0.0	10.2	11.1	13.2	0.0	0.0
Obsumbacterium spp. (C_4)	16.6	12.5	7.0	0.0	11.5	11.5	13.0	0.0	0.0
Obsumbacterium spp. (C_5)	16.7	7.0	12.5	0.0	7.0	14.0	13.0	0.0	0.0
Obsumbacterium spp. (C_6)	11.2	0.0	0.0	0.0	12.2	7.0	11.2	0.0	0.0
Erwinia spp. (C_7)	15.4	13.2	14.2	0.0	9.5	11.0	11.0	0.0	0.0
Erwinia spp. (C_8)	15.6	13.0	13.9	0.0	9.4	13.2	11.0	0.0	0.0
Erwinia spp. (C_9)	10.2	12.5	12.8	0.0	12.0	13.6	13.2	0.0	0.0
Erwinia spp. (C_{10})	15.0	10.0	14.0	0.0	0.0	0.0	0.0	0.0	0.0
Enterobacter spp. (C_{11})	13.8	12.2	8.8	0.0	12.5	10.2	10.3	0.0	0.0
Enterobacter spp. (C_{12})	10.8	8.6	8.3	0.0	11.2	10.5	12.8	0.0	0.0
E.coli (C_{13})	17.0	15.3	15.0	7.0	15.3	11.0	12.8	0.0	0.0
E.coli (C_{14})	17.0	15.0	15.6	7.0	11.8	11.2	9.5	0.0	0.0
E.coli RPOS0604	16.4	15.2	15.2	ND	7.0	7.0	9.5	ND	ND
E.coli TK3	9.5	8.1	8.2	ND	7.0	7.0	9.6	ND	ND

ND: note determined.

Table 4: Growth Inhibition Zones of Enterobacteria Caused by Supernatants of some Isolates

Inhibition zone (mm)	G_1		PC_2		G_3		PC_6		G_7		PC_8	
Enterobacteria	**S**	**S. pH7**	**S**	**S. pH7**	**S**	**S. pH7**	**S**	**S. pH7**	**S**	**S. pH7**	**S**	**S. pH7**
Obsumbacterium spp. (C_1)	16.0	8.2	8.0	5.0	8.0	7.6	11.2	5.0	12.1	8.0	10.3	8.4
Obsumbacterium spp. (C_2)	16.0	8.2	12.1	8.0	11.0	9.5	8.0	5.0	5.4	5.1	11.2	9.2
Obsumbacterium spp. (C_3)	7.0	5.5	12.0	8.0	10.2	8.1	8.0	7.0	5.2	5.0	12.2	8.0
Obsumbacterium spp. (C_4)	20.0	12.5	8.0	5.0	10.0	8.0	8.0	7.0	12.0	8.3	11.4	8.0
Obsumbacterium spp. (C_5)	20.0	10.0	7.8	5.2	10.3	5.9	5.5	0.0	8.0	5.2	11.5	8.2
Obsumbacterium spp. (C_6)	14.0	9.2	0.0	0.0	0.0	0.0	8.0	0.0	0.0	0.0	8.0	5.1
Erwinia spp. (C_7)	13.0	10.2	8.2	5.1	10.3	8.0	8.0	5.2	11.2	8.0	7.2	5.0
Erwinia spp. (C_8)	12.8	8.2	8.2	5.2	10.3	8.1	8.2	5.4	5.0	5.0	8.0	5.3
Erwinia spp. (C_9)	9.5	5.5	8.0	5.0	10.0	8.0	5.1	0.0	8.0	5.0	11.0	6.0
Erwinia spp. (C_{10})	13.2	8.2	7.0	0.0	10.4	8.6	0.0	0.0	0.0	0.0	0.0	0.0
Enterobacter spp. (C_{11})	12.5	8.3	8.0	5.2	5.0	0.0	8.2	5.2	0.0	0.0	8.0	0.0
Enterobacter pp. (C_{12})	12.8	8.2	8.0	5.0	9.2	8.5	8.0	7.0	0.0	0.0	10.0	5.2
E. coli (C_{13})	13.5	8.0	9.0	5.0	7.5	5.1	8.5	5.3	8.1	5.0	10.2	6.0
E. coli (C_{14})	13.8	8.0	9.0	5.0	7.5	5.1	5.8	5.0	8.0	5.2	7.8	5.0
E. coli RPOS0604	9.0	7.0	7.0	0.0	5.5	5.1	5.3	0.0	0.0	0.0	5.2	5.1
E. coli TK3	5.6	5.0	7.0	0.0	5.8	5.0	5.4	0.0	0.0	0.0	5.0	5.0

S: supernatant; S. pH7: Neutral supernatant.

Table 5: Viability of Lactobacilli Cells in Different Gastric Juices

Strains	Poultry crop extract (pH 2.8) (Number of colonies :cfu × 10^5 ml^{-1})			Poultry gizzard extract (pH 2.3) (Number of colonies : cfu × 10^5 ml^{-1})		
	0h	3h	Viability (%)	0h	3h	Viability (%)
G_1	9.48 ± 0.10	8.18 ± 0.02	86.28 ± 0.2	9.48 ± 0.10	7.20 ± 0.13	75.94 ± 1.3
PC_2	8.40 ± 0.21	7.68 ± 0.10	91.42 ± 0.4	8.40 ± 0.21	7.32 ± 0.41	87.14 ± 1.9
G_3	9.76 ± 0.32	8.48 ± 0.20	86.88 ± 0.6	9.76 ± 0.32	7.52 ± 0.32	77.04 ± 1.0
PC_4	7.32 ± 0.01	6.46 ± 0.10	88.25 ± 1.0	7.32 ±0.01	3.27 ± 0.50	44.67 ± 5.0
PC_6	8.60 ± 0.30	6.48 ± 0.32	75.34 ± 1.0	8.60 ± 0.30	5.01 ± 0.61	58.25 ± 2.0
G_7	10.96 ± 0.15	7.81 ± 0.23	71.25 ± 1.5	10.96±0.15	6.51 ± 0.03	59.39 ± 0.2
PC_8	9.72 ± 0.20	8.12 ± 0.30	83.53 ± 1.5	9.72 ± 0.20	6.02 ± 0.42	61.93 ± 2.1
PC_{13}	10.00 ± 0.08	7.38 ± 0.08	73.80 ± 1.0	10.00±0.08	6.27 ± 0.32	60.70 ± 4.0
PC_{14}	9.68 ± 0.32	8.37 ± 0.45	86.46 ± 1.4	9.68 ± 0.32	7.41 ± 0.05	76.54 ± 0.1

Isolate G_1 showed the best inhibition of a range of the tested indicator strains. Isolates PC_2; G_3; PC_6; G_7 and PC_8 presented antimicrobial activity against the enterobacteria isolates; they inhibited 13 of 14 tested indicator strains. The use of the supernatant of the lactobacilli strains culture exhibited inhibition zones onto the indicator strains tested. The highest diameter inhibition was obtained against *Obsumbacterium* spp. C_4 and C_5. The neutralized supernatant broths (pH 7.0) showed an inhibitory activity against enterobacteria isolates but with low efficacy comparing with the supernatant broth. The result obtained with the neutral supernatant indicated thatt the inhibition was not related to lactic acid only but it might be due to other antimicrobial substances such as bacteriocins.

Tolerance of Lactobacilli Strains to Acid

This preliminary experiment was realised to determine the ability lactobacilli strains to resist to acids. Results of Table **5** showed that a major part of isolates are acid stable. These results showed that the growth of LAB strains decrease after 3h of incubation. In comparison between the acid tolerance of the isolated lactobacilli species it is clear that strains PC_{14}, PC_2, G_1 and G_3 seem to have the better acid tolerance to poultry crop extract and poultry gizzard extract.

Tolerance of Lactobacilli Isolates to Bile Salt

The presence of bile disrupts the growth of some LAB strains, and high dose lead to a loss of the growth (Table **6**). It appears that strains PC_4 and PC_{14} were inhibited dramatically by bile salt. In contrast, some other LAB strains such as PC_2, PC_6, G_7 and PC_{13} exhibited good resistance to bile salt since the number of cells was important on MRS agar supplemented with 2% of bile salt.

Antibiotic Susceptibility of some Lactobacilli Strains

Results concerning the determination of antibiotic resistance of some LAB isolates are given in Table **7**. The results indicated that lactobacilli strains tested were resistant to 6µg Penicillin G, 10µg Ampicilin, 10µg

Table 6: Effects of Bile Salt on the Growth of Lactobacilli Isolates

Strains	Number of colonies (cfu ml^{-1})			Percentage of tolerance (%)	
	Without bile salt (BS)	With 0.5% BS	With 2% BS	0.5% BS	2% BS
G_1	54.40× 10^5	31.60× 10^5	13.16× 10^5	58.08	24.19
PC_2	9.62× 10^5	9.60× 10^5	9.60× 10^5	99.79	99.79
G_3	23.20× 10^5	3.52× 10^5	4.80× 10^5	15.17	20.68
PC_4	12.80× 10^5	0.22× 10^5	0.20× 10^5	01.71	01.56
PC_6	11.06× 10^5	11.04× 10^5	11.04× 10^5	99.81	99.81
G_7	7.12× 10^5	7.06× 10^5	7.03× 10^5	99.15	98.73
PC_8	21.60× 10^5	4.76× 10^5	1.48× 10^5	22.03	06.85
PC_{13}	10.21× 10^5	1.10× 10^5	0.75× 10^5	10.77	07.34
PC_{14}	10.24× 10^5	1.92× 10^5	0.80× 10^5	18.75	07.81

Table 7: Antibiotic Susceptibility of Lactobacilli Strains

Strains	G_1	PC_2	G_3	PC_4	PC_6	G_7	PC_8	PC_{13}
Penicillin G (6µg)	R	R	R	R	R	R	R	R
Ampicilin (10µg)	R	R	R	R	R	R	R	R
Erythromycin (30µg)	S	S	S	S	S	S	S	S
Streptomycin (10µg)	S	S	S	S	S	S	S	S
Tetracycline (10µg)	R	R	R	R	R	R	R	R
Vancomycin (30µg)	R	R	R	R	R	R	R	R
Amikacin (30µg)	R	R	R	R	S	R	R	R
Amoxicillin(10µg)	S	S	S	S	S	S	S	S
Oxytetracycline(30µg)	S	S	S	S	S	S	S	S
Ofloxacin (10µg)	R	ND	ND	ND	ND	R	R	R
Gentamicin (10µg)	S	S	S	S	S	S	S	S
Nalidixic acid (5µg)	S	S	ND	S	S	S	ND	ND

R: ressistance, S: sensitive, ND: not determined.

Table 8: Co-Aggregation Ability, Hydrophobicity and Adherence Efficiency of the Best Isolates

Strain	Co-aggregation (%)			Hydrophobicity (%)	Adhesion efficiency
	Erwinia spp.	*E. coli*	*Enterobacter* spp.		
G_1	20.00	22.49	20.21	70.24	+
PC_2	20.45	22.20	19.56	70.24	+
G_3	18.69	21.29	19.00	55.58	+
PC_6	17.90	19.56	18.98	45.45	+
G_7	14.36	16.41	15.78	36.80	+
PC_8	14.56	17.86	15.56	35.60	+

+: mean number of adherent bacteria on poultry epithelial cells is more than 15 CFU/cell.

Tetracycline, 30µg Vancomycin and sensitive to 30µg Erythromycin, 30µg Oxytetracycline, 10µg Gentamicin, 5µg Nalidixic acid and 10µg Streptomycin.

Coaggregation

Coaggregation of isolates with some enterobacteria was examined (Table **8**). Results are expressed as the percentage of absorbance reduction after 5h of the mixed suspension compared with the individual suspension. The best co-aggregation properties were obtained with ***isolates*** PC_2 and G_1.

Assay of the Adherence Capability for LAB Isolates

The target cells used for adhesion study were the epithelial cells isolated from the poultry. It was found that some lactobacilli strains from poultry crop, such as isolates G_1, PC_2, G_3, PC_6, G_7 and PC_8, showed adherence capability to the poultry intestinal epithelium. Figure **1** showed the adherence of *L. plantarum* G_1.

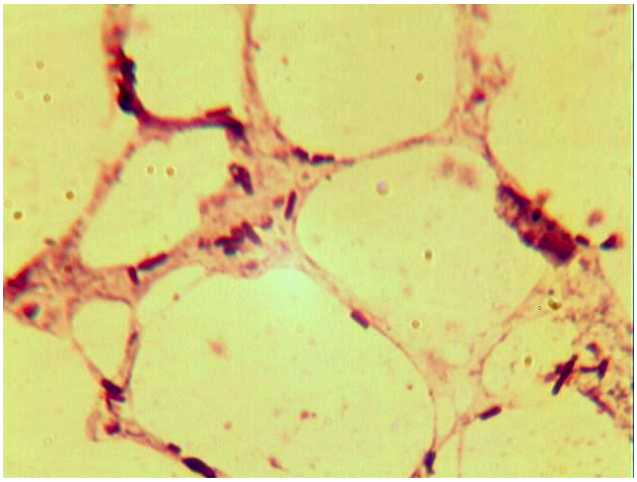

Figure 1: Adherence of *L. plantarum* G_1 to the epithelium cells (Microphotography × 100).

Identification of the Selected Isolate

According to the physiological and biochemical tests and to the carbohydrate fermentation pattern analyzed

by API strip system software, the selected isolates were identified as *L. bifermentans* PC_1, *L. viridescens* G_3, *L. delbrueckii* ssp *delbrueckii* G_7, *L. helveticus* PC_6, *L. plantarum* G_1 and *L. fermentum* PC_8.

The best isolate G1 was firstly identified as *L. plantarum* by API 50 CHL test kits and API system software. The identification was confirmed by 16S rRNA gene sequence and showed high similarity to *L. plantarum*. The GenBank Accession number for 16S rRNA gene sequence of this strain is KC965107.

DISCUSSION

These results show that lactic acid bacteria are the dominant microflora in the crop samples. These results are in agreement with previous studies. Barnes [17] has reported that lactobacilli represent an important component of the intestinal flora of chickens, reaching $10^9 g^{-1}$ of the caecal content. As host specificity of bacterial strains is well recognized and documented [18, 19], isolation of lactobacilli from poultry crop was undertaken in this study as a first step towards developing a probiotic. Garriga *et al.* [20] identified isolated strains such as *L. salivarius* from chicken crop using physiological and biochemical tests. *L. fermentum* was found to be one of the major LAB species isolated from the gastrointestinal tracts of swine and poultry [13]. Furthermore, Fons *et al.* [21] have reported that *L. fermentum* was commonly found in the digestive tract of pigs, rodents and humans. Herein, we also found that *L. fermentum* was one of the LAB species in the crop of local poultry.

Barrow *et al.* [19] pointed out that growth of most microorganisms, such as coliform bacteria, could be inhibited when pH of the medium was bellow 4.5. Daeschel [22] has reported that the antimicrobial effect exerted by LAB is due to the production of lactic acid and reduction of pH, acetic acid, diacetyl, hydrogen peroxide, fatty acids, aldehydes and other compounds. One of the major probiotic properties of LAB is their ability to produce antimicrobial activity against pathogens. The obtained results confirm the ability of some isolated strains and their supernatant to inhibit the growth of the indicator strains. These results are in agreement with those reported by several researchers [13, 20]. LAB of aquatic origin displayed a broad antimicrobial/bacteriocin activity against the main Gram-positive and Gram-negative fish pathogens [7]. In contrast, Xanthopoulous *et al.* [23] reported the weak antibacterial activity of *L. paracasei* subsp. *paracasei* and *L. acidophilus* strains isolated from infant feces against *E. coli* and *Yersinia enterocolitica*.

Concerning the stability of LAB to acid pH, Lin *et al.* [13] showed that *L. acidophilus* and *L. bulgaricus* isolated from chicken were less stable in chicken gizzard extract at pH 2.6, although Conway *et al.* [24] reported that *L. acidophilus* strains isolated from human digestive tract, showed an acid tolerance to gastric juice at pH 2.5. In the same way, Garriga *et al.* [20] found that the selected LAB strains were resistant to pH 3. The results of a study conducted by Idoui *et al.* [25] demonstrated that *L. plantarum* BJ0021 showed a good resistance to the rabbit gastric juice and resists to pH 3.

The ability to survive in the presence of bile salt is an absolute need of probiotic bacteria, and it is generally included among the criteria used to select potential probiotic strains. The bile in animal intestine is also an important factor which affects the viability of LAB cells [14]. Lin *et al.* [13] reported that some *L. fermentum* strains isolated from swine and poultry showed 100% bile tolerance and some one were inhibited in the bile condition. In a similar study, selected LAB strains were found to be resistant to 4% of bile salts [20]. Gilliland *et al.* [26] observed a great variability among *L. acidophilus* strains isolated from calf intestinal contents in their ability to grow *in vitro* in the presence of bile salts.

For the resistance to antibiotics, our results were not in accordance with those found by Garriga *et al.* [20]. These authors reported that lactobacilli strains isolated from crop and intestinal content of chicks showed a higher resistance to erythromycin and streptomycin but they found more resistant strains to Ampicillin. In the same way, Idoui *et al.* [25] found that *L. plantarum* BJ0021 was resistant to streptomycin. On the other hand, intrinsically antibiotic-resistant probiotic strains may benefit to patients whose normal intestinal microbiota has become unbalanced or greatly reduced in numbers due to the administration of various antimicrobial agents.

Fuller [18] reported that the dominance of the lactobacilli in the crop is maintained by their ability to adhere to the crop epithelial cells. When the food moves on from the crop to the gizzard, large numbers of lactobacilli are left behind attached to the crop epithelium. These are available for inoculation of the incoming food. Under *in vitro* conditions, 10^7 lactobacilli ml^{-1} are required to suppress the growth of *E. coli*.

Adhesion is a strain-specific property as shown with lactobacilli strains isolated from dairy products where

all strains are able to adhere to Caco-2 cells to various levels [27]. The lactobacillus flora attached to the crop wall is an important factor in the control of the composition of the chicken gut flora and attempts have been made to describe the mechanisms by which lactobacilli become associated with the crop epithelium. Most clinical studies of probiotic persistence and colonization show that probiotic organisms do not permanently colonize the GI tract and continue providing their hosts benefits only for brief periods after they have stopped being administered [28]. Bacteria adhere initially to GI surfaces by nonspecific physical interactions, such as steric and hydrophobic interactions, which result in reversible attachment. Several researchers have reported that there is a degree of correlation between hydrophobicity and adhesion to the hydrophobic mucosal surface. However, other studies indicated that there was no correlation between cell surface hydrophobicity and adhesion to intestinal mucus [29]. In these studies, highly adhesive bacteria demonstrated fairly low surface hydrophobicities.

CONCLUSION

Results indicate that some *Lactobacillus* strains isolated from poultry crop are potential probiotic candidates due to their ability to adhere to epithelial cells, to produce antimicrobial agent and to resist to acid and bile salts conditions. These properties could be valuable for the use of these strains as food and feed additives to promote human and animal health. Further and deep studies are needed on the properties of the selected strains to be used as food and feed additives.

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