IDENTIFICATION AND PROBIOTICS PROPERTIES OF LACTOBACILLUS STRAINS FROM POULTRY CROP

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Abstract

Sixteen lactobacilli strains were isolated and identified from poultry crop and examined for their potentiality probiotic properties. Results showed that the strain tested were resistant to pH3, resistant to a number of antibiotics and were also bile tolerant. During inhibitory activity screening, *Lactobacillus bifermentans* PC_1 showed the best inhibition of a range of indicator strains tested. Culture supernatants and neutral supernatants exhibited varying degrees of inhibitory activity against strains of enterobacteria.

Keywords: Poultry Crop; lactobacilli; Probiotic properties.

Introduction

The gastrointestinal tract of monogastric animals harbours a large collection of different microbial species. Recent research has suggested that the chicken gastrointestinal microflora may contain more that 650 species of which more than half are from previously unknown bacterial genera (Apajalahti *et al.,* 2004).

The crop serves as a storage organ which partially regulates the entry of the ingested food into the gizzard. Considerable microbial growth occurs in the crop which might contribute to feed digestion and is therefore beneficial to the bird (Champ *et al.,* 1983).

Some of the bacterial species isolated from the crop of chickens include *Escherichia coli,* enterococci*,* staphylococci, lactobacilli, *Campylobacter* spp. and *Salmonella* spp. (Frei *et al.,* 2001), however many of these are transient species and after the first week the dominant species found in the crop are lactobacilli (van der Wielen *et al.,* 2002).

Many reports showed that lactic acid bacteria (LAB) could be used as probiotics for animals (Chen *et al.,* 2005). The selection criteria for probiotic LAB include: safety, viability/activity in delivery vehicles, resistance to acid and bile, adherence to gut epithelial tissue, ability to colonise the GIT, production of antimicrobial substances, ability to stimulate a host immune response and the ability to influence metabolic activities (Salminen *et al.,* 1996).

The present study was carried out to isolate lactobacilli from poultry crop and investigate their probiotic properties.

Materials and Methods

Poultry crop samples, *isolation and identification of lactobacilli strains*: three crop samples of poultry were used. For each sample aliquots of 10g was used for lactic acid bacteria enumeration.

From each sample aliquots, five 10-fold dilutions were prepared and these were inoculated on plates of MRS agar (Pasteur institute, Algeria), acidified with glacial acetic acid to pH5.7 and incubated anaerobically for 48h at 37°C.

Sixteen (16) colonies were picked up from the higher dilutions (10^{-5}) and sub-cultured in MRS broth. The identification of the sixteen isolates was performed according to the criteria of Bergey's Manual of Determinative Bacteriology (Holt *et al.,* 1994) and using the methods and criteria of Sharpe (1979).

Sensitivity of lactobacilli isolates to several parameters: To determine the acid sensitivity or resistance of lactobacilli isolates, MRS agar was prepared in accordance with the manufacturer's instructions. pH were adjusted

to pH3, pH4 and pH5 with glacial acetic acid, the media were autoclaved at 121°C for 15 min. Plates were inoculated from serial decimal dilution 10^{-5} and incubated under anaerobic conditions at 37°C for 24 hours. After incubation, colonies were counted and identified by cell morphology.

To establish the bile salt tolerance of lactobacilli isolates, the overnight cultures were inoculated (1%) into MRS broth with or without 0.5% (w/v) oxgall. Bacterial cell in the MRS broth was measured by reading the optical density (OD) at 620 nm after 4 h incubation at 37°C (Lin *et al.,* 2007).

Screening for antimicrobial activity: The inhibitory activity was screened by the agar spot agar (Schillinger and Lucke, 1989) in MRS agar, under anaerobic conditions at 37°C. The indicator strains used were of poultry crop origin. These Gram negative bacteria were cultured on Luria Bertani broth. A well diffusion assay with the inhibitory strain was performed.

The inhibitory activity of supernatant culture fluid and neutral supernatant pH6 were tested.

Results

Results showed that the counts of lactic acid bacteria are higher in all samples; count of this microflora is between 3.2×10^7 to 6.7×10^7 cfu/ g. Samples 01 and 03 contained the highest count of lactic acid bacteria.

Sixteen (16) strains were isolated, purified and further identified from poultry crop. The isolates were found to be Gram-positive, catalase-negative rods, non motile, which phenotypically correspond to the genus *Lactobacillus*. According to the physiological and biochemical tests and to the carbohydrate fermentation pattern analyzed by API strip system software, four isolates strains were recognized as *Lb.bifermentans* (PC_1, PC_5, PC_{11} and PC_{15}), four as *Lactobacillus viridescens* (PC_3, PC_9, PC_{10} and PC_{13}), three as *Lb.delbrueckii* ssp *delbrueckii* (PC_7, PC_{12} and PC_{16}), two as *Lb.helveticus* (PC_6 and PC_{14}), one as *Lb.plantarum* (PC_2), one as *Lb.brevis* (PC_4) and one as *Lb.fermentum* (PC_8).

Results from Table 1 showed reductions of LAB populations following growth with decrease of pH media. These results showed that the growth of LAB strains decrease about -92.81% to -98. 75% on the MRS agar pH3 compared to the same media pH5. In comparison to the acid tolerance of the lactobacilli species which we isolated from the poultry crop, strains of *Lb helveticus* (PC_{14}); *Lb.plantarum* (PC_2); *Lb. bifermentans* (PC_1) and *Lb.viridescens* (PC_3) seem to have better acid tolerance.

The presence of bile disrupts the growth of some LAB strains, and high dose lead to a loss of the growth (Table 3). It appear that strains coded PC_4 and PC_{14} corresponding to *Lb brevis* and *Lb helveticus* respectively were inhibited dramatically by bile salt. In contrary, some other LAB strains coded PC_2, PC_6, PC_7 and PC_{13} exhibited good resistance to bile salt since the number of cells was important on MRS agar with 0.5% of bile salt.

The influence of some lactobacilli strains, as supernatant and supernatant pH6 on particular enterobacteria isolated from poultry crop is reported in tables 3 and 4. Table 4 shows that six out of nine lactobacilli strains tested are inhibitory to enterobacteria, although inhibitory extents are variable.

Lb.bifermentans PC_1 showed the best inhibition of a range of indicator strains tested. The diameter of zones of growth inhibition for enterobteria isolates were between 7mm and 19mm. Lactobacilli coded PC_2; PC_3; PC_6; PC_7 and PC_8 presented antimicrobial activity against the same enterobacteria isolates. The supernatant from lactobacilli strains culture gave zones of inhibition onto the indicator strains tested. The diameters of inhibition range from 5mm to 20mm. In our study, supernatant broths were neutralized to pH6; the inhibition activity to enterobacteria isolates became lower.

Discussion

In our study, lactobacilli strains were isolated and identified from poultry crop. Our results are in accordance with previous study. From chicken crop and using physiological and biochemical tests Garriga *et al.* (1998) have identified isolates strains as *Lactobacillus salivarius*. Lin *et al.* (2007) have also isolated LAB strains from the gastrointestinal tracts of swine and poultry, and found that *Lb.fermentum* was one of a major LAB species.

The results showed that strains of *Lb helveticus* (PC_{14}); *Lb.plantarum* (PC_2); *Lb. bifermentans* (PC_1) and *Lb.viridescens* (PC_3) seem to have better acid tolerance. These results were in accordance with previous study. The results found by Lin *et al.* (2007) showed that *Lb.acidophilus* and *Lb.bulgaricus* from chicken were less stable in the pH2.6 chicken gizzard extract, although Idoui *et al.* (2007) reported that *Lb.plantarum* BJ0021 was resistant to pH3 and this strain shows a good resistance to rabbit gastric juice.The presence of high dose of bile leads to a loss of the growth of lactic acid bacteria. The results of this study were in accordance with those found by Lin *et al.* (2007). In similar study, Garriga *et al.* (1998) found that the selected LAB strains were resistant to 4% of bile salts.

Results obtained in this study proved the antibacterial activity of some lactobacilli and their supernatants. This activity is one of the major probiotic properties for probiotic LAB. The results of this study were in accordance with those found by Lin *et al.* (2007). In other study conducted by Garriga *et al.* (1998), 77 lactobacilli strains showing inhibition against one or more enteric indicator strains (*E.coli*, *Salmonella.enteritidis*). In addition, Xanthopoulos *et al.* (2000) indicated that *Lb.paracasei* subsp.*paracasei* and *Lb.acidophilus* strains isolated from infant feces had weak antibacterial activity on *E.coli* and *Yersinia enterocolitica*.

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Table 1 Survival of some lactobacilli strains at different pH values

Strains	Number of colonies (cfu / ml)			Percentage of reduction (%)
	pH3	pH4	pH5	
PC1	5.48×10^5	2.00×10^6	1.92×10^7	-97.14
PC2	7.40×10^5	2.80×10^6	2.32×10^7	-96.81
PC3	6.76×10^5	6.40×10^6	2.72×10^7	-97.51
PC4	3.32×10^5	3.20×10^6	2.40×10^7	-92.81
PC6	3.60×10^5	5.80×10^6	2.28×10^7	-98.42
PC7	2.96×10^5	4.28×10^6	2.00×10^7	-98.52
PC8	3.72×10^5	8.08×10^6	1.20×10^7	-96.9
PC13	3.00×10^5	4.40×10^6	2.40×10^7	-98.75
PC14	5.68×10^5	5.45×10^6	1.60×10^7	-96.45

Table 2 Effects of bile salt on the growth of some lactobacilli strains

Strains	OD at 620 nm after 4h incubation at 37°C		Percentage of tolerance (%)
	Without bile salt (BS)	With 0.5% BS	
PC1	0.942	0.931	98.83
PC2	0.793	0.505	63.68
PC3	0.797	0.495	62.11
PC4	0.480	0.102	21.25
PC6	0.723	0.401	55.46
PC7	0.830	0.425	51.20
PC8	0.951	0.814	85.59
PC13	0.751	0.502	66.84
PC14	0.445	0.121	27.19

Table 3 Growth inhibition zones of enterobacteria caused by some lactobacilli strains.

Enterobacteria Inhibition zone (mm)	PC1	PC2	PC3	PC4	PC6	PC7	PC8	PC13	PC14
Obsumbacterium spp. (C1)	3	3	3	-	3	3	3	-	-
Obsumbacterium spp. (C2)	3	3	3	-	2	3	3	-	-
Obsumbacterium spp. (C3)	3	3	3	-	2	2	3	-	-
Obsumbacterium spp. (C4)	1	3	1	-	3	2	3	-	-
Obsumbacterium spp. (C5)	3	1	3	-	1	3	3	-	-
Obsumbacterium spp. (C6)	2	-	-	-	3	1	2	-	-
Erwinia spp. (C7)	2	3	3	-	2	2	2	-	-
Erwinia spp. (C8)	3	3	3	-	2	3	2	-	-
Erwinia spp. (C9)	2	3	3	-	3	3	3	-	-
Erwinia spp. (C10)	1	2	3	-	-	-	-	-	-
Enterobacter spp. (C11)	3	3	2	-	3	2	2	-	-
Enterobacter spp. (C12)	2	2	2	-	2	2	3	-	-
E.coli (C13)	3	3	3	1	3	2	3	-	-
E.coli (C14)	3	3	3	1	2	2	2	-	-

-: No inhibition; 1: inhibition zones smaller than 8mm; 2: inhibition zones between 8 and 12mm; 3: inhibition zones larger than 12mm.

Table 4. Growth inhibition zones of enterobacteria caused by supernatants of some lactobacilli

Inhibition zone (mm)	PC1		PC2		PC3		PC6		PC7		PC8	
Enterobacteria	S	S.pH6	S	S.pH6	S	S.pH6	S	S.pH6	S	S.pH6	S	S.pH6
Obsumbacterium spp. (C1)	3	2	2	1	2	2	3	1	3	2	3	3
Obsumbacterium spp. (C2)	3	2	3	2	3	2	2	1	1	1	3	3
Obsumbacterium spp. (C3)	1	1	3	2	3	2	2	2	1	1	3	2
Obsumbacterium spp. (C4)	3	3	2	1	3	2	2	2	3	3	3	2
Obsumbacterium spp. (C5)	3	2	1	1	3	1	1	-	2	1	3	3
Obsumbacterium spp. (C6)	3	2	-	-	-	-	2	-	-	-	2	1
Erwinia spp. (C7)	3	3	2	1	3	2	2	1	3	3	2	1
Erwinia spp. (C8)	3	2	2	1	3	2	2	1	1	1	2	1
Erwinia spp. (C9)	2	1	2	1	3	2	1	-	2	1	3	2
Erwinia spp. (C10)	3	2	1	-	3	3	0	-	-	-	-	-
Enterobacter spp. (C11)	3	2	2	2	1	-	2	1	-	-	2	-
Enterobacter pp. (C12)	3	2	2	1	3	3	2	2	-	-	3	1
E.coli (C13)	3	2	3	2	2	1	2	1	2	1	3	2
E.coli (C14)	3	2	3	2	2	1	1	1	2	1	2	1

S : supernatant ; S.pH6 : Neutral supernatant ; -: No inhibition; 1: inhibition zones smaller than 8 mm; 2: inhibition zones between 8 and 12mm; 3: inhibition zones larger than 12mm.