Probiotic Potential of Lactic Acid Bacteria Isolated from Human Gut

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Abstract: The aim of this study was to characterize human gut lactic acid bacteria group strains on the basis of their phenotypic profiles. In addition, their *in vitro* potential probiotic properties were evaluated with a view to identifying potential interesting application. Fifteen strains of lactic acid bacteria were isolated and identified. Among the strains, both biochemical and physiological characteristics differed noticeably and also showed a remarkable heterogeneity. The strains were grouped into the species *Lactobacillus gasseri, Lb.casei* ssp *casei, Lb. delbrueckii* ssp *lactis, Lb. fermentum* and *Lb. delbrueckii* ssp *bulgaricus*. These strains have an inhibitory activity against enterobacteria including *Escherichia coli* and *Salmonella* sp. A major part of these strains survived at pH 2.5 and in 0.3% bile salts. Additionally they produced no haemolysis, were resistant to kanamycin and adhered to epithelial cells.
The results showed that the strains *Lb. gasseri* HG8 and *Lb. fermentum* HG3 have the best potential probiotic properties.

Key words: Human gut, Lactic acid bacteria, Probiotic.

Introduction

Intestinal microflora is a complex ecosystem formed by a number of bacterial populations, which are crucially important for the host. Most of the bacteria (>400 species) colonizing the intestine are strict anaerobes (*Bacteroides* sp., bifidobacteria, eubacteria, propionibacteria, clostridia) while aerobes (enterobacteria, especially *E. coli*, enterococci) and facultative anaerobes form a minor part (2–5 %) (Fanaro et *al.*, 2003).
The large intestine in the human body is where the highest numbers of microbes are housed. It is believed that the general well-being of humans depends on the number and type of microbes associated with the gastrointestinal (GI) tract, especially those colonizing the large intestine (Mitsuoka, 1992).
Among colon biota, lactobacilli have drawn considerable attention among researchers in last four to five decades. Since their lactobacilli have attracted the attention of researchers because of their probiotic and therapeutic advantages (Mayur et *al.*, 2002). The contemporary definition of a probiotic is “a microorganism which, when administered in adequate amounts, confers a health benefit on the host” (FAO/WHO 2002).

To exert a beneficial effect, it is generally considered essential that probiotic cells remain viable during transit of the gastrointestinal tract (GIT) in sufficiently high numbers to either establish residence (i.e. to colonise) or to benefit the host (Sheehan et *al.*, 2007).
However, the ability of lactobacilli to survive in the GIT varies considerably between different species and strains. For probiotic cells to accumulate in the GIT, they must adhere to mucus or tissue cells and then survive exposure to the relatively harsh conditions imposed by gastric acids, bile and digestive enzymes and by the highly diverse and competitive commensal microbiota of the GIT (Saulnier et *al.*, 2008).
Adhesion to the intestinal mucosa is considered an essential trait of probiotic bacteria and is thought to be a possible mechanism where by probiotics provide protection against pathogens through competition for binding sites and by localised production of antimicrobial substances (Coconnier et al., 1993).

The objective of the current study was to isolate identify and characterize a number of lactic acid bacteria (LAB) isolated from adults faeces. The strains were further characterized by tolerance to low pH and bile, and adhesion to intestinal mucous of poultry. The competitiveness of selected strains with Gram negative strains was also evaluated.

Material and Methods

Samples:
Fresh faecal samples were obtained from five healthy adults’ person (region of Chekfa, Taher and Texana, Algeria). Samples were transported to the laboratory at 4°C.

Isolation and identification of lactobacilli strains:
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.
The identification of isolates was performed according to the criteria of Bergey’s Manual of Determinative Bacteriology and using the methods and criteria of Sharpe (1979).

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 48 hours 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.

Sensitivity of isolates to several parameters:

***Bile salt tolerance*:** The overnight cultures of LAB cells were inoculated into MRS broth with or without 0.3% bile salt (Pasteur institute; Alger's, Algeria).
Initial bacterial cell in the culture broth was measured by reading the optical density (OD) at 620nm and numeration using the Thomas Cell. After 4 h incubation at 37 °C, the same parameters were determined. The percentage of bile tolerance was calculated by comparison of the OD values of the bacteria cultures in MRS broth with bile salt to those in MRS broth without bile salt (Lin et *al.*, 2007).

***Acid tolerance*:** To determine the acid sensitivity or resistance of lactobacilli isolates, MRS broth was prepared in accordance with the manufacturer's instructions. pH were adjusted to pH2, pH3 and pH4 with glacial acetic acid, the media were autoclaved. The overnight cultures of LAB cells were inoculated into MRS broth and incubated for 3h at 37°C.
Viable bacterial counts were determined by plating serial dilutions (in PBS, pH 7.2) on MRS agar followed by incubation at 37 °C for 48 h (Lin et *al.*, 2007).

***Antibacterial activity*:** The inhibitory activity was screened by the agar spot agar (Schillinger and Lucke, 1989) in MRS agar at 37°C. The indicator strains used were of human gut origin (*Escherichia coli, Klebsiella* sp, *Enterobacter* sp, *Salmonella* sp and *Citrobacter* sp). A well diffusion assay with the inhibitory strain was performed.
The neutral supernatant culture fluid was tested. The plates were incubated overnight at 37°C. The diameters of the inhibition zones were measured.

Adherence of LAB to the epithelial cell: The method described by Annika et *al.* (1983) was used for the preparation of epithelial cells. Segment of poultry ileum was washed with sterilized phosphate-buffer saline (PBS, pH 7.2). It was held at 4°C for 30 min and then washed three times with PBS. The epithelial cell concentration was adjusted to approximately 5.10^4 cells/ml.
Briefly, cell pellet from overnight culture of LAB was resuspended to approximately 1.10^8 cells/ml in PBS (pH 7.2). One ml of such bacteria suspension was mixed with 1 ml 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, 200) after stained with 0.5% crystal violet for 5 min.

Results and Discussion

Isolation and identification of LAB:
Forty isolates, primarily lactobacilli were recovered from faecal samples of healthy adults' person (figure1). Only fifteen isolates were identified. From this collection, five of the identified *Lb.*strains was *Lb. gasseri*, which accounted for 33.33 % of the total isolated strains. The species *Lb.delbrueckii* ssp *bulgaricus*, *Lb.casei* ssp *casei*, *Lb.delbrueckii* ssp *lactis* and *Lb.fermentum* represent respectively 26.66%, 20%, 13.33% and 6.66% of the total isolated strains.

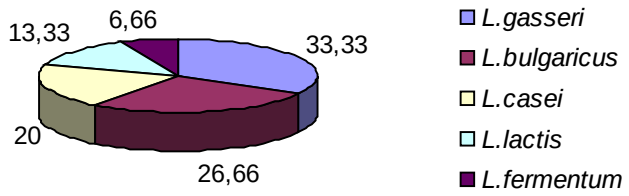

Figure1. Repartition of the identified isolates of lactic acid bacteria

Bile salt Tolerance:
The bile in intestine is an important factor which affects the viability of LAB cells. Although the composition of human bile juice is not exactly the same as that of the 0.3% oxgall solution, most studies used oxgall as one substitute for human bile because of their similarity (Lin et *al.*, 2006)

The best results were obtained, only with 6 out of 15 strains tested. From the results (table1), *Lb. fermentum* HG3 exhibited the highest bile acid tolerance (98.83%) followed by *Lb.gasseri* HG 8 (85.59%). The lowest bile acid tolerances were observed by *Lb. delbrueckii* subsp. *lactis* (21.25%). The results of this study were in accordance with those found by some authors. In similar study, Garriga et *al.* (1998) found that the selected LAB strains were resistant to 4% of bile salts. Gilliland et *al.* (1984)

observed a great variability among *Lb.acidophilus* strains isolated from calf intestinal contents in their ability to grow in vitro in the presence of bile salts

Table1. Effects of bile salt on the growth of some lactobacilli strains

Strains	$OD_{620\ nm}$		Percentage of tolerance
	Without bile salt (BS)	With 0.3 % BS	
Lb.fermentum HG3	0.942	0.931	98.83
Lb.bulgaricus HG2	0.793	0.505	63.68
Lb.casei ssp *casei* HG3	0.797	0.495	62.11
Lb. delbrueckii ssp *lactis* HG4	0.480	0.102	21.25
Lb.gasseri HG8	0.951	0.814	85.59

Tolerance to Low pH:

The effect of acidity on the viability of the isolates was assessed by adjusting the growth medium to different pH values. At pH 2, the strains' viability was affected, where this pH value was considered as the lethal for all cultures (data not shown).

The results showed (table 2) that the five isolates were able to grow on the MRS broth with different pH. The viable bacterial counts for these LAB strains changed after incubation in the pH 2. After 3 h incubation in the pH 2 or pH 3, the viable LAB counts decreased about 1–2 log value for a few strains.

In comparison to the acid tolerance of the *Lactobacillus* species which we isolated from the gastrointestinal tracts of human, strains of *Lb. fermentum*, *Lb.gasseri* and *Lb.delbrueckii* ssp *bulgaricus* seem to have better acid tolerance. These results were in accordance with previous study. The results of study conducted by Idoui et *al.* (2007) showed that *Lb.plantarum* BJ0021 was resistant to pH3 and this strain shows a good resistance to rabbit gastric juice.

Table2. Survival of some lactobacilli strains at different pH values

Strains	Number of cell / ml			Percentage of reduction (%)
	pH2	pH3	pH4	
Lb.fermentum HG3	5.48×10^5	2.00×10^6	1.92×10^7	97.14
Lb.bulgaricus HG2	7.40×10^5	2.80×10^6	2.32×10^7	97. 18
Lb.casei ssp *casei* HG3	6.76×10^5	6.40×10^6	2.72×10^7	96.51
Lb. delbrueckii ssp *lactis* HG4	3.32×10^5	3.20×10^6	2.40×10^7	92.81
Lb.gasseri HG8	3.60×10^5	5.80×10^6	2.28×10^7	98.42

Antibacterial activity evaluation of the isolated strains:

One of the major probiotic properties for probiotic LAB is its inhibitory effect on the growth of pathogenic bacteria. In our study, the bacteria used as indicator is a Gram negative bacteria, such as *E. coli*, *Salmonella* spp., *Klebsiella* spp., *Enterobacter* spp and *Citrobacter* spp from human origine.

Table shows that LAB exhibited an inhibitory activity to the indicator bacteria, although inhibitory extents are variable and we observed that *Klebsiella* spp strain seem to be more tolerant to LAB inhibition when compared to the other bacteria, such as *E. coli*. In the other hand, the antagonistic effect of the isolate was more pronounced on *E. coli* (table 3).

In 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*

Table3. Growth inhibition zones of enterobacteria caused by some lactobacilli strains.

Inhibition zone (mm)	*Lb.fermentum* HG3	*Lb.bulgaricus* HG2	*Lb.casei*ssp *casei* HG3	*Lb.delbrueckii* ssp *lactis* HG4	*Lb.gasseri* HG8
E.coli	3	3	3	2	3
Enterobacter spp	3	3	2	2	2
Salmonella spp	3	3	2	0	2
Klebsiella spp	2	3	1	1	2
Cirobacter spp	3	2	3	1	1
diameter of inhibition zone: 3 >10mm; 10 mm >2 > 5 mm; 1 < 5 mm; 0 < 0 mm					

In our study, supernatant broths were neutralized to pH6.5; the inhibition activity to enterobacteria isolates became lower. The result obtained with neutral supernatant, showed that the inhibition was not related to lactic acid but might be due to other antimicrobial substances.

The results indicate that neutral supernatant inhibited the tested indicator strains (table4). Excepted the strain *Lb.casei*, *In vitro* inhibitory capability of neutral supernatant of the other strains against indicator bacteria seems to be a good probiotic property. Daeschel, (1989) 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.

Table4. Growth inhibition zones of enterobacteria caused by neutral supernatants of some lactobacilli strains

Inhibition zone (mm)	*Lb.fermentum* HG3	*Lb.bulgaricus* HG2	*Lb.casei*ssp *casei* HG3	*Lb.delbrueckii* ssp *lactis* HG4	*Lb.gasseri* HG8
E.coli	2	2	1	1	2
Enterobacter spp	2	2	1	0	2
Salmonella spp	2	2	1	0	2
Klebsiella spp	2	1	1	0	1
Cirobacter spp	1	1	0	0	1
diameter of inhibition zone: 3 >10mm; 10 mm >2 > 5 mm; 1 < 5 mm; 0 < 0 mm					

Assay of the adherence capability for LAB isolates:

The ability to adhere to host intestinal mucosa is considered as an important selection criterion for LAB strains intended for probiotic use. It should be reminded that for LAB strain, only more than 15 LAB cells adhered on one epithelial cell, it could be considered as ‘‘positive’’ adherent strain.

In our case, we found that solely *Lb. fermentum* HG3 and *Lb.gasseri* HG8, showed the adherence specificity to the chicken intestinal epithelium (fig.2). We don’t know if they are able to adhere to the human intestinal epithelium.

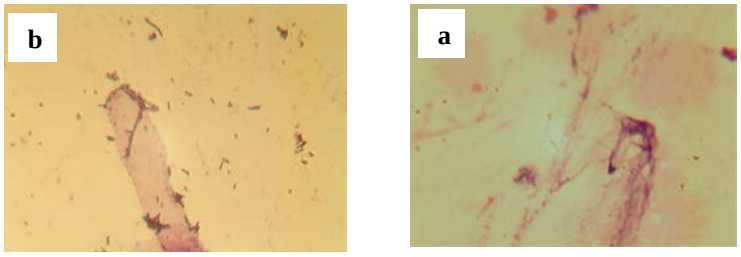

Fig. 2. Adherence of isolated *Lactobacillus gasseri* on the epithelium cells: (a) negative adhesion (control) (b) positive adhesion of *Lb. gasseri* HG8

Conclusion

From the results of the study reported here, potential lactobacilli strains isolated from human gut able to be used as probiotics may be found. Strains *Lb.fermentum* HG3 and *Lb.gasseri* HG8 were able to adhere to the intestinal epithelium from poultry. In addition, they were resistant to acid and were also bile tolerant. As the antagonistic effects of strains against enterobacteria, the isolates HG3 and HG8 might be the most preferential strain of choice.

Therefore, this study provides the information that human digestive tract offer a potential source for the isolation of probiotic LAB strains suitable for use as feed supplements**.**

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