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Full Length Research Paper

Phenotypic characterization of *Lactobacillus* strains isolated from different biotopes

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Thirty three strains of *Lactobacillus* were isolated from human milk and infant faeces, animal (cow and goat) milks and from plants (*Anagalis arvensis* and *Bromus mango* species). The various strains were identified based on phenotypic tests. Amongst them, 12 strains belonged to group 1, which comprised *L. acidophilus*, *L. helveticus* and *L. delbrueckii* ssp. *bulgaricus* strains; 16 strains belonged to group 2, which comprised *L. casei* (ssp. *casei* and ssp. *rhamnosus*) and *L. plantarum* strains; and 5 strains belonged to group 3, which comprised *L. brevis* and *L. fermentum*. The variance analysis (with one-fixed criterion classification) of the potential for milk coagulation after 6 and 24 h of growth made possible the characterization of two homogeneous groups, fast and low acidifying strains. The human strains showed a resistance to acidic pH and to bile, indicating a tolerance to gastric acid. The probiotic potential was confirmed for one strain, owing to its antagonistic effect on *Escherichia coli*.

Key words: Lactic acid bacteria, isolation, characterisation, probiotics, statistical analysis.

INTRODUCTION

Since antiquity, lactic acid bacteria (LAB) have aided in the production of numerous human foods, and their strains have always been empirically selected. Nowadays, this selection continues, but in a scientific way. LAB still remains the focus of several studies till date (Kacem et al., 2003; Badis et al., 2005; Kacem and Karem, 2006).

Lactic acid bacteria are usually selected because of their technological properties, such as growth, aroma production, exopolysaccharides production and others. However, to improve the knowledge concerning their benefit for human health (Droult and Corthier, 2000; Ouwehand et al., 2002), additional criteria are taken into account. Indeed, for safety reasons, the trend is to consider human LAB strains tolerance to gastric acidic and bile salts (Chou and Weimer, 1999), and their inhibitory activity against pathogens.

The Algerian dairy industry showed a major development during the last decade. However, it remains dependent upon the importation of the major constituents, namely milk and starter cultures (Ammellel, 2000). Therefore, a rational politics for the dairy industry should take into account both the increase of the milk production and the development of local industrial production of starter cultures, allowing to cut costs and to provide a degree of autonomy for the production of self produced fermented foods.

By considering phenotypic tests, this work aims at identifying new LAB strains isolated from human, animal or vegetal biotopes and to characterize their potential to coagulate milk, as well as the probiotic potential of the human strains, with a view to preparing native starter cultures.

MATERIALS AND METHODS

Biological material

21 samples were used for the essays (Table 1). They were selected from five different biotopes: human milk, cow milk, goat milk, infant faeces and from plants. The considered plants were *Anagalis arvensis* and *Bromus mango* species that are traditionally used to improve lactic acid fermentation.

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Table 1. Number, biotope and location of sampling.

Number of samples	Biotope	Location of sampling
4	Milk from brown race cow from Atlas	Region of Annaba
4	Goat milk of Arabia race	Region of Annaba
3	Human milk from healthy mothers	Region of Annaba
5	Faeces of breast-fed infants feces	Region of Annaba
5	*Anagalis arvensis* and *Bromus mango* plants	Region of Guelma

Strains selection

Successive decimal dilutions of cow and goat milks were carried out. Dilutions 4 to 6 (10^{-4} to 10^{-6}) were considered.

After nipples disinfection, human milk was introduced in sterile test tubes. Faeces of breast-fed infants, which have not received any antibiotherapy, were taken by rectal cleaning out and introduced in test tubes containing MRS (De Mann et al., 1960). These samples were covered by a layer of sterile paraffin to reduce aerobiosis, and then incubated at 37°C during 24 h for enrichment. From these enriched media, isolates were carried out in solid culture on MRS media. All Petri dishes were incubated at 37°C under anaerobiosis in enriched CO_2 atmosphere during 48 to 72 h.

The leaves from the plants, *A. arvensis* and *B. mango* species were rinsed three times with sterile distilled water to remove earth dust and debris. They were cut with sterile scissors before being carried in a sterile plastic bag. They were then ground for 30 s in an ultra turax (Rhema Labortechnik, Hofstein, Germany). The cutting and grinding of the leaves led to the recovery of the juice which contained most of the metabolizable nutrients (Hubert and Dupuy, 1994). Test tubes containing MRS broth at pH 5.0 was inoculated with 2 mL of the recovered juice; they were then covered by a layer of sterile paraffin to reduce aerobiosis, before incubating at 37°C for 24 h for enrichment. From these enriched media, isolates were carried out in solid culture on MRS medium.

Phenotypic identification of strains

Strains were incubated for 48 to 72 h at 30°C and/or 37°C as indicated in an enriched CO_2 atmosphere. The phenotypic characteristics were checked for each strain according to the literature recommendations (Marchal et al., 1982; Leveau et al., 1991; Teuber, 1994; Curk et al., 1994; Bourgeois and Larpent, 1996; Guiraud, 1998; Larpent-Gourgaud et al., 1998; Coeuret et al., 2003).

Acidifying activity

To appreciate the acidifying activity, the tested strain was inoculated in a reconstituted skim milk (10%, w/v). Each collected strain was incubated in a MRS broth at the appropriated temperature at 24 h for enrichment. 100 mL Erlenmeyer flasks containing skim milk were incubated with 2 mL of the enriched medium and then incubated at the appropriate temperature for each strain; 30 and 45°C for the mesophilic and thermophilic strains, respectively.

The acidifying capacity was deduced from pH measurement (pH probe, Hanna Instruments, Tanneries, France). Indeed, pH metric acidity measurement is more accurate than Dornic acidity, owing to its high variation coefficient (Zourari et al., 1991). The acidifying capacity was deduced from the low pH observed after 6 and 24 h incubation. A pH value of 5 according to Huggins and Sandine (1984), or 5.3 according to Cogan et al. (1997) is achieved after 6 h for high acidifying capacity strains.

Evaluation of the probiotic potential

Resistance to acidity: Each strain was cultivated for 18 h in liquid MRS medium, before it was used for inoculation at 1% level MRS medium acidified at pH 2-3 with HCl 10 M. After 0-3 h time-contact, 10 µL of these cultures were spread out on Petri dishes and incubated for 48 h at 37°C under anaerobic conditions. Resistance to acidity was then deduced from the comparison of the culture density with that of control dishes at the initial time t.

Resistance to bile: According to the method of Larpent-Gourgaud et al. (1998), strains were cultivated at 37°C for 18 h in liquid MRS medium. 10 µL of each culture was then sampled and used to inoculate Petri dishes containing MRS medium supplemented with 1 and 2% (w/v) of Oxgall bile. Experiments were duplicated for each bile concentration and for each strain. The Petri dishes were incubated at 37°C for 48 h and then compared to a control culture carried out in the same conditions on bile-free MRS medium.

Antagonistic activity against two Escherichia coli strains: The strains of *E. coli,* EcI and EcII were supplied by a public local hospital (Dorban Hospital, Annaba, Algeria) and were isolated from coproculture. The antagonistic activity against these strains was evaluated by means of the diffusion method in an agar gel after 16 h growth on MRS, according to the "Standard methods for examination of dairy products".

Statistical analysis of the acidifying activity data

Statistical analysis on the pH values, which was achieved after 6 and 24 h of growth, was carried out by means of the variance analysis to one-fixed classification criterion model (Dagnelié, 1999).

When after variance analysis the assumption of equal averages was rejected, the method of the minimum significant difference was applied to highlight homogeneous strain groups according to their acidifying capacity (Dagnelié, 1999). Statistical analysis was carried out using the MINITAB (13.31 version, 2000) software.

RESULTS AND DISCUSSION

Phenotypic identification of strains

Table 2 shows that among the isolated strains, 33 displayed the characteristics of lactic acid bacteria, namely: catalase, Gram+, no mobility, no sporulation, cytochrome oxidase, and nitrate reductase. Creamy white colour colonies of 1 to 3 mm diameter were obtained on MRS medium. These strains were identified, characterized and compared to reference strains (Teuber, 1994; Bourgeois and Larpent, 1996; Leyral et al., 1999). Morphological, biochemical and physiological characteristics

Table 2. Identification of the *Lactobacillus* from the three groups.

Species	***L. acidophilus***		***L. delbrueckii* ssp. *bulgaricus***		***L. helveticus***		***L. casei* ssp. *casei***		***L. casei* ssp. *rhamnosus***		***L. plantarum***		***L. brevis***		***L. fermentum***	
Characteristics	Ref[a]	N=7	Ref[a]	N=1	Ref[a]	N= 3	Ref[a]	N=2	Ref[a]	N=6	Ref[a]	N=8	Ref[a]	N=3	Ref[a]	N=2
Morphology	bacilli	bacilli	bacilli	bacilli	bacilli	bacilli	bacilli	bacilli	bacilli	bacilli	bacilli	bacilli	bacilli	bacilli	bacilli	bacilli
Gram	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Catalase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mobility	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Growth at 15°C	-	-	-	-	-	-	+	+	+	+	+	+	+	+	-	-
30°C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
45°C	+	+	+	+	+	+	-	-	+	+	-	-	-	-	+	+
Nitrate réductase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ADH	-	-	V	-	-	-	-	-	-	-	-		+	+	+	+
CO_2 production from glucose	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
Acid production from/ or hydrolysis of:																
Amidon	V	-	Nd	-	-	-	-	-	-	-	-	-	-	-	Nd	-
Amygdaline	+	+	-	-	-	-	+	+	+	+	+	+	-	-	-	-
Arabinose	-	-	-	-	-	-	-	-	V	+2/-4	V	2+/-6	+	+	V	+/-
Cellobiose	+	+	-	-	-	-	+	+	+	+	+	+	-	-	V	+
Esculine	+	+	-	-	-	-	+	+	+	+	+	+	V	+	-	-
Fructose	V	-6/+1	+	+	V	+2/-	+	+	+	+	+	+	+	+	+	+
Galactose	+	+	-	-	+	+	+	+	+	+	+	+	V	+	+	+
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gluconate	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	-/V[b]	-1/+1	+	+	+	+	V	+1/-2	+	+
Maltose	+	+	-	-	V	+2/-	+	+	+	+	+	+	+	+	+	+
Mannitol	-	-	-	-	-	-	+	+	+	+	+	+	-	-	-	-
Mannose	+	+	-	-	v	+2/-	+	+	+	+	+	+	-	-	V	+/-
Melizitose	-	-	-	-	-	-	+	+	+	+	V	+/-	-	-	-	-
Melibiose	V	-6/+1	-	-	-	-	-	-	-	-	+	+	+/V[c]	+	+	+
Raffinose	V	-	-	-	-	-	-	-	-	-	V	2+/-6	V (+)[d]	+	+	+
Rhamnose	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-

Table 2. Continues

Ribose	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+
Salicine	+	+	-	-	-	-	+	+	+	+	+	+	-	-	-	-
Sorbitol	-	-	-	-	-	-	+	+	+	+	+	+	-	-	-	-
Saccharose	+	+	-	-	-	-	+	+	+/V	+2/-4	+	+	V	-	+	+
Trehalose	V	-	-	-	V	+2/-	+	+	+	+	+	+	-	-	V	-/+
Xylose	-	-	-	-	-	-	-	-	-	-	-/d[e]	-	V (+)[f]	-	-/d[g]	-

+ = Positive reaction; - = negative reaction; V = variable response; d = doubt, no clear response; Nd = Non-determined.
[a]Reference strain (Dellaglio et al., 1994; Teuber, 1994; Bourgeois and Larpent, 1996; Leyral and Joffin, 1999).
[b]Lactose negative according to Dellaglio et al. (1994) and variable response according to Leyral and Joffin (1999).
[c]Mélibiose: positive response according to Leyral and Joffin (1999), while variable response according to Bourgeois and Larpent (1996).
[d]Raffinose: variable or positive Dellaglio et al. (1994), variable (Bourgeois and Larpent (1996) or positive according to Leyral and Joffin (1999).
[e]Xylose: negative response according to Leyral and Joffin (1999), while no clear response according to Dellaglio et al. (1994).
[f]Xylose: variable or positive according to Dellaglio et al. (1994).
[g]Xylose: negative or no clear response according to Leyral and Joffin (1999).

of the isolates (Table 2) showed that they belong to the *Lactobacillus* genus.

Identification of the *Lactobacillus* strains belonging to the group 1

Twelve strains in group 1 of the *Lactobacillus* genus (Table 2) were chosen based on the criteria given in the literature (Teuber, 1994; Bourgeois and Larpent, 1996; Leyral et al., 1999; Larpent, 2000); the criteria include being homofermentative and thermophilic lactobacilli, and inability to ferment gluconate and pentoses.

Identification of the *L. acidophilus* strains

Seven strains isolated from human, cow and goat milks (human, 1; cow, 4; and goat, 2 strains, respectively) were considered to belong to the *L. acidophilus* species, according to the key identification established by Dellaglio et al. (1994) and Bourgeois and Larpent (1996), since they showed the following characteristics: being homofermentative and thermophilic, having no ADH and esculine hydrolysis; being able to acidify with amygdaline, cellobiose, galactose, glucose, lactose, maltose, mannose, saccharose and salicylate. The human strain differs from the other strains, since it metabolizes melibiose and fructose. Only Leyral et al. (1999) consider that *L. acidophilus* type species ferment fructose.

Identification of the *L. helveticus* strains

Three strains isolated from goat milk were considered to belong to the *L. helveticus* species, owing to their growth at 45°C but not at 15°C, not having ADH and esculine hydrolysis, and being able to acidify with galactose, glucose and lactose.

Differences between strains concerning the fermentation of some carbohydrates can be found. The fermentation of fructose, maltose and trehalose by some strains was also previously reported for some *L. helveticus* strains (Torriani et al., 1994; Bourgeois and Larpent, 1996; Leyral et al., 1999).

Identification of the *L. delbrueckii* ssp. *bulgaricus* strains

Two cow milk strains were considered to belong to the *L. delbrueckii* ssp. *bulgaricus* species (Dellaglio et al., 1994; Bourgeois and Larpent, 1996), owing to their growth at 45°C but not at 15°C, not having ADH and fructose, glucose and lactose fermentation.

Identification of the *Lactobacillus* strains belonging to the group 2

Sixteen strains belonging to the group 2 of the *Lactobacillus* genus (Table 2) were chosen based on the criteria given in the literature (Larpent et al., 1994; Leyral et al., 1999), which are the

absence of ADH, esculine hydrolysis, the fermentation of fructose, glucose (without CO_2 production) and mannose, and pentose assimilation.

Identification of the *L. casei* strains

Two cow milk strains were identified as *L. casei* ssp. *casei*, owing to their carbohydrate fermentative profile (Table 2). They differed concerning lactose degradation, in agreement with the variability reported in the available literature (Guiraud, 1998; Leyral et al., 1999). Both strains were totally similar to the type species.

Six trains belonging to *L. casei* ssp. *rhamnosus* were isolated, 3 from human biotopes (2 isolated from infant faeces and 1 from human milk) and 3 from goat milk. Their identification was based on the general features of the *Lactobacillus* from the group 2, namely growth at 15 and 45°C and the carbohydrate fermentative profile. Only the human strains metabolized arabinose and saccharose carbohydrates, showing a relation between the origin of the strains and their fermentative profile. The literature does not show a general agreement concerning the assimilation of saccharose by *L. casei* ssp. *rhamnosus*. Indeed, the result has been reported to be positive (Guiraud, 1998; Leyral et al., 1999), variable (Leveau et al., 1991), or this disaccharide is not included in the fermentative profile (Dellaglio et al., 1994; Bourgeois and Larpent, 1996). Nevertheless, the 6 isolated strains appeared to be close to the type species.

Identification of the *L. plantarum* strains

Eight strains showing the *L. plantarum* characteristics (Bourgeois and Larpent, 1996) were isolated from goat milk (2), cow milk (3) and from plants (3). Both goat milk strains differed from the other strains by their use of arabinose and raffinose. It can be noted that raffinose assimilation by *L. plantarum* has always been reported positive (Larpent et al., 1994, Leyral et al., 1999), or strain dependant (Dellaglio et al., 1994; Bourgeois and Larpent, 1996), in agreement with our findings.

Identification of the *Lactobacillus* strains belonging to group 3

Five strains (3 isolated from cow milk and 2 from plants) from the group 3 were identified, according to the criteria reported in the literature (Larpent et al., 1994; Bourgeois and Larpent, 1996; Leyral et al., 1999); the criteria include the ability to ferment glucose (with CO_2 production) and not having amygdaline, mannitol, rhamnose, salicylate and sorbitol fermentation (Table 2).

Identification of the *L. brevis* strains

The phenotypic characteristics of the 3 strains isolated from cow milk (Table 2) showed their belonging to the *L. brevis* (Bourgeois and Larpent, 1996; Leyral et al., 1999) species. Moreover, a strain dependant response was observed for lactose assimilation (only one strain was lactose +), in agreement with the literature (Bourgeois and Larpent, 1996; Leyral et al., 1999).

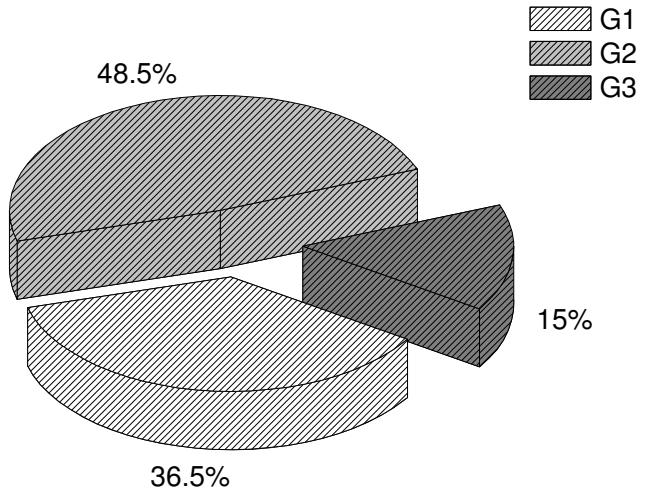

Figure 1. Percentage of the different groups of *Lactobacillus*.

Identification of the *L. fermentum* strains

Two strains isolated from plants belonged to the *L. fermentum* species, owing to their phenotypic characteristics and are in agreement with the literature (Dellaglio et al., 1994; Bourgeois and Larpent, 1996; Leyral et al., 1999). It can be noted that both strains differed in their assimilation of arabinose, mannose and trehalose.

The fermentative profile of the carbohydrates showed therefore an important variability between the isolated species (Table 2), as also previously reported (Chamba et al., 1994). Plasmids are found in most of the lactic acid bacteria, and are responsible for important properties of LAB, like gene coding for sugar assimilation or transport. Plasmids can be lost after successive transfers. The cytoplasmic membrane can be altered by storage treatments, leading to perturbations of the metabolic activities (Chamba et al., 1994).

The above results are in Figures 1 and 2, showing that the identified *Lactobacillus* belonged to 7 species and 3 subspecies. Figure 1 shows that 49, 36 and only 15% belong to the groups 1, 2 and 3 of the *Lactobacillus* genus. The species distribution (Figure 2) showed that the main species were *L. acidophilus*, *L. plantarum* and *L. casei*, including its subspecies, *casei* and *rhamnosus*; while *L. fermentum*, *L. delbrueckii* ssp. *bulgaricus*, *L. brevis* and *L. helveticus* were represented at a lower level.

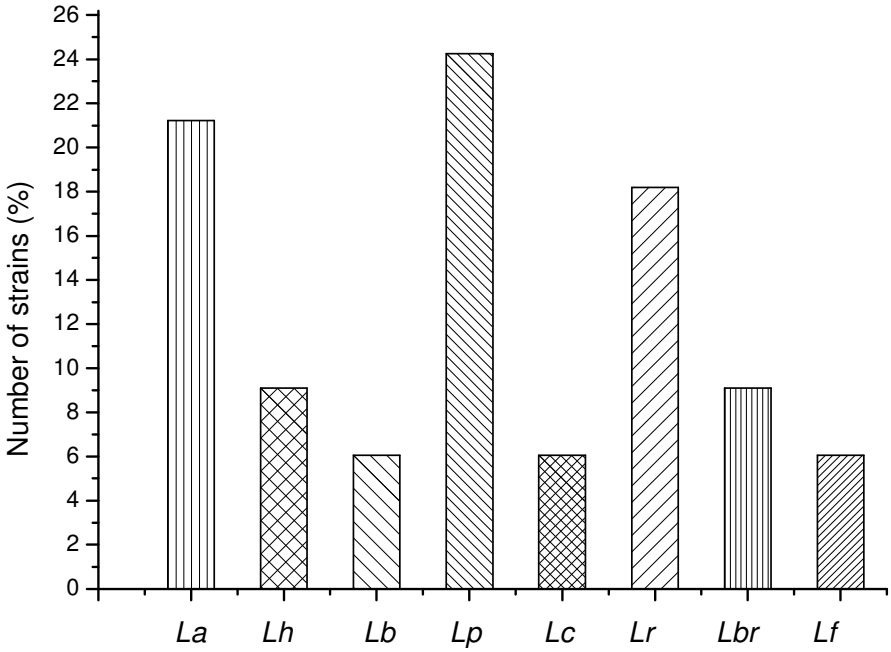

Figure 2. Percentage of the different strains of *Lactobacillus*: *L. acidophilus* (*La*); *L. helveticus* (*Lh*); *L. delbrueckii* ssp. *bulgaricus* (*Lb*); *L. plantarum* (*Lp*); *L. casei* ssp. *casei* (*Lc*); *L. casei* ssp. *rhamnosus* (*Lr*); *L. brevis* (*Lbr*); *L. fermentum* (*Lf*).

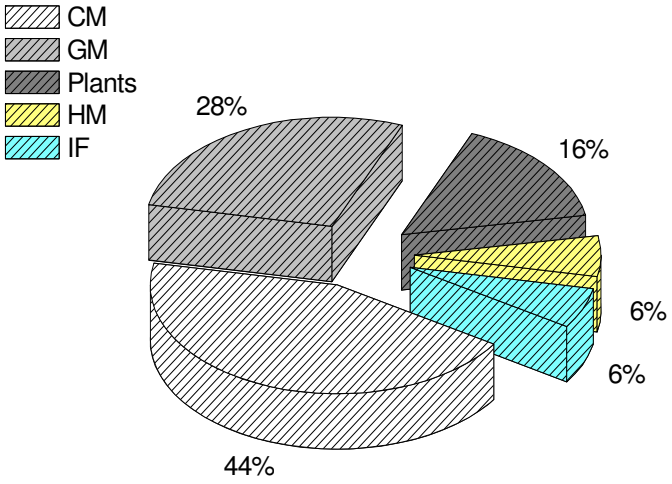

Figure 3. Percentage of the strains according to their various biotopes. CM: Cow milk, GM: Goat milk, P: plants, HM: Human milk, IF: Infant faeces.

The results related to the various biotopes (Figure 3) showed that cow and goat milk samples were characterized by a relatively abundant and diversified microflora, in agreement with the available bibliography (Schmidt et al., 1994; Badis et al., 2005; Casalta et al., 2005). Contrarily, the human samples were qualitatively and quantitatively poor in lactic acid bacteria, including mainly lactobacilli and enterococci with probiotic characteristics. Indeed, it was previously reported that lactobacilli predominate in the intestinal subdominant flora (Larpent et al., 1994) and that human milk and infant faeces contained lactobacilli showing probiotic properties (Rocio Martin, 2005; Olivares et al., 2006). Plant samples showed scarce LAB microflora (Figure 3). This is in agreement with the works of other authors (Hubert and Dupuy, 1994), which showed that LAB are not dominant in plants and that the most often encountered species are *Pediococcus damnosus, Leuconostoc mesenteroïdes, L. brevis, L. fermentum, L. plantarum*.

Evaluation of the acidifying capacity of the *Lactobacillus*

Among the isolated strains, only some *L. casei* ssp. *rhamnosus* and *L. brevis* strains were lactose negative (Table 2); these were only 9.4% of the identified strains. The absence of lactose assimilation had been previously reported for some lactic acid bacteria, belonging to the *Lactobacillus* and *Leuconostoc* genus (Schmidt et al., 1994).

Mean acidification rates were compared by means of the variance analysis with one-fixed criterion classification of the pH achieved after 6 and 24 h (Table 3) of culture of the 29 lactose positive identified *Lactobacillus* strains. This statistical analysis showed very highly significant differences between strains, since the nil probability was below the criterion $\alpha = 0.001$.

Analysis of the acidifying activity by means of the minimum significant difference after 6 h of culture (Table 4) showed two homogeneous groups, fast acidifying

Table 3. Variance analysis with one fixed criterion classification of the mean acidification rate of the 29 *Lactobacillus* strains identified based on the pH achieved after 6 and 24 h of culture.

Time (h)	Cause of the variations	DF[a]	SSE[b]	MS[c]	F[d]	P[e]
6	Differences between strains	28	20.82	0.74367	2009.22	0.000[f]
	Residual variance	58	0.02147	0.00037	-	-
	Total variance	86	20.843	-	-	-
24	Differences between strains	28	20.112	0.71828	2147.45	0.000[f]
	Residual variance	58	0.01940	0.00033	-	-
	Total variance	86	20.13	-	-	-

[a]Degree-of-freedom.
[b]Sum of the squared errors.
[c]Mean square.
[d]Fisher variable.
[e]Probability.
[f]$P \leq \alpha = 0.001$, means that the differences between strains are very highly significant.

Table 4. Identification of homogeneous groups of *Lactobacillus* after 6 and 24 h of growth.

Group	Strain	6 h pH[a]	Biotope	Group	*Strain*	24 h pH[a]	Biotope
G1	*L. helveticus* 1	5.00	Goat milk	G1	*L. acidophilus* 4	3.88	Cow milk
	L. plantarum 7	5.00	Plants		*L. fermentum* 2	3.86	plants
	L. helveticus 2	5.03	Goat milk				
G2	*L. helveticus* 1	5.00	Goat milk	G2	*L. acidophilus* 4	3.88	Cow milk
	L. helveticus 2	5.03	Goat milk		*L. plantarum* 8	3.90	Plants
					L. fermentum 1	3.90	plants
G3	*L. helveticus* 2	5.03	Goat milk	G3	*L. plantarum* 8	3.90	Plants
	L. plantarum 8	5.05	plants		*L. fermentum* 1	3.90	plants
G4	*L. fermentum* 2	5.30	Plants	G4	*L. helveticus* 2	4.04	Goat milk
	L. plantarum 1	5.33	Goat milk		*L. helveticus* 1	4.14	Goat milk
					L. plantarum 6	4.15	plants
G5	*L. fermentum* 1	5.50	Plants	G5	*L. helveticus* 1	4.14	Goat milk
	L. acidophilus 5	5.51	Cow milk		*L. plantarum* 6	4.15	plants
G6	*L. acidophilus* 6	5.60	Cow milk	G6	*L. plantarum* 3	4.59	Cow milk
	L. plantarum 6	5.61	plants		*L. rahmnosus* 2	4.59	infant faeces
	L. acidophilus 7	5.63	Cow milk		*L. acidophilus* 2	4.60	Goat milk
G7	*L. plantarum* 6	5.61	Plants	G7	*L. plantarum* 3	4.59	Cow milk
	L. acidophilus 7	5.63	Cow milk		*L. acidophilus* 2	4.60	Goat milk
					L. acidophilus 7	4.60	Cow milk
					L. plantarum 4	4.60	Cow milk
					L. casei ssp.*casei* 2	4.60	Cow milk
					L. casei ssp. *rhamnosus* 4	4.61	Goat milk
					L. casei ssp. *rhamnosus* 5	4.61	Goat milk
G8	*L. acidophilus* 3	6.00	Goat milk	G8	*L. acidophilus* 2	4.60	Goat milk
	L. plantarum 3	6.00	Cow milk		*L. acidophilus* 7	4.60	Cow milk
	L. casei ssp. *Casei* 2	6.00	Cow milk		*L. plantarum* 4	4.60	Cow milk
	L. plantarum 4	6.02	Cow milk		*L. casei* ssp. *casei* 2	4.60	Cow milk
					L. acidophilus 5	4.61	Cow milk
					L. casei ssp. rhamnosus 4	4.61	Goat milk
					L. casei ssp. *rhamnosus* 5	4.61	Goat milk
					L. acidophilus 3	4.62	Goat milk

Table 4. Continues

G9	*L. plantarum* 3	6.00	Cow milk	G9	*L. acidophilus* 7	4.60	Cow milk
	L. casei ssp. *Casei* 2	6.00	Cow milk		*L. plantarum* 4	4.60	Cow milk
	L. plantarum 4	6.02	Cow milk		*L. casei* ssp. *casei* 2	4.60	Cow milk
					L. casei ssp. *rhamnosus* 4	4.61	Goat milk
					L. casei ssp. *rhamnosus* 5	4.61	Goat milk
					L. acidophilus 5*	4.61	Cow milk
					L. acidophilus 3*	4.62	Goat milk
G10	*L. plantarum* 3	6.00	Cow milk	G10	*L. plantarum* 4	4.60	Cow milk
	L. plantarum 4	6.02	Cow milk		*L. casei* ssp. *casei* 2	4.60	Cow milk
					L. casei ssp. *rhamnosus* 4	4.61	Goat milk
					L. casei ssp. *rhamnosus* 5	4.61	Goat milk
					L. acidophilus 5	4.61	Cow milk
					L. acidophilus 3	4.62	Goat milk
G11	*L. casei* ssp. *rhamnosus* 1	6.11	infant faeces	G11	*L. casei* ssp. *casei* 2	4.60	Cow milk
	L. delbrueckii ssp. *bulgaricus 1*	6.12	Cow milk		*L. casei* ssp. *rhamnosus* 4	4.61	Goat milk
	L. casei ssp. *rhamnosus* 4	6.13	Goat milk		*L. casei* ssp. *rhamnosus* 5	4.61	Goat milk
					L. acidophilus 5	4.61	Cow milk
					L. acidophilus 3	4.62	Goat milk
G12	*L. delbrueckii* ssp. *bulgaricus* 1	6.12	Cow milk	G12	*L. casei* ssp. *rhamnosus* 4	4.61	Goat milk
	L. casei ssp. *rhamnosus* 4	6.13	Goat milk		*L. casei* ssp. *rhamnosus* 5	4.61	Goat milk
	L. casei ssp. *rhamnosus* 5	6.18	Goat milk		*L. acidophilus* 5	4.61	Cow milk
					L. acidophilus 3	4.62	Goat milk
G13	*L. casei* ssp. *rhamnosus* 5	6.18	Goat milk	G13	*L. casei* ssp. *rhamnosus* 4	4.61	Goat milk
	L. acidophilus 2	6.19	Goat milk		*L. casei* ssp. *rhamnosus* 5	4.61	Goat milk
	L. casei ssp. *rhamnosus* 2	6.19	infant faeces		*L. acidophilus* 3	4.62	Goat milk
G14	*L. acidophilus* 2	6.19	Goat milk	G14	*L. casei* ssp. *rhamnosus* 5	4.61	Goat milk
	L. casei ssp. *rhamnosus* 2	6.19	infant faeces		*L. acidophilus* 3	4.62	Goat milk

[a]Each pH value was the average of three measurements.

strains (G1-G7 groups), including *L. helveticus, L. plantarum, L. fermentum and L. acidophilus* strains; and low acidifying strains (G11-G14 groups), including *L. delbrueckii* ssp. *bulgaricus, L. casei* ssp. *rhamnosus* and *L. acidophilus* strains. Similar conclusions can be drawn from the statistical analysis of the acidifying activity after 24 h of growth (Table 4).

Thus, the acidification rate and the acidifying capacity are in close relation and the acidifying activity is strain dependant. It can be noted that strains isolated from goat milk and plants showed higher acidification rates and acidifying capacity, while the lowest acidifying activity was recorded for the human strains, in agreement with the available literature (Elli et al., 1999; Vinderola et al., 2000).

The important differences in the acidifying potential recorded between strains of the same species were in agreement with the available literature (Schmidt et al., 1994). The stress resulting from pH decrease can account for the variability between strains (Kashket, 1987). Medium acidification due to lactic acid production leads to an adaptation of cells, which allows growth until an inhibitory pH and cellular maintenance at this pH level (Guillouard et al., 2004). The protomotive force as well as the production of alkaline compounds causes resistance to acidic stress. Production of alkaline compounds results from decarboxylase activity, but the involved enzymes are not present in all strains, most likely accounting for the significant differences recorded between strains.

Probiotic potential of the human strains

The resistance of 4 human strains to acidic pH, for at least 3 h, and to bile salts indicated a tolerance to gastric acidic (Table 5). Moreover, the transport of bacteria by

Table 5. Evaluation of the probiotic potential of the human strains.

Species	Strain	Resistance to bile (g L^{-1})		Resistance to acidity		Antagonistic *E. coli* (ECI)[a]	activity against *E. coli* (ECII)[a]	Biotope
		10	20	pH3	pH2			
L. acidophilus	L. a1	+	+	+	+	–	–	Human milk
L. casei ssp. *rhamnosus*	L. cr1	+	+	+	+	–	–	İnfant faeces
	L. cr2	+	+	+	+	++	+	İnfant faeces
	L. cr3	+	+	+	+	–	–	Human milk

*Antagonistic activity against *Escherichia coli*: +, + +, + + + corresponds to a diameter of an inhibition zone of 2, 4 and 6 mm, respectively.

food such as milk, and a stomach residence time below 90 min can help in the protection of bacteria against acidic (Berrada et al., 1991). The tolerance to bile salts is also generally considered as an essential property of the probiotic strains due to their ability to survive in the small intestine, and is considered as more decisive than the resistance to acidity (Khalil et al., 2007).

Only one strain, a *L. casei* ssp. *Rhamnosus*, which was resistant to acidity and bile salts, also had an antagonistic effect on *E. coli* strains (Table 5). The antagonistic activity was illustrated by a diffusion diameter of 4 and 2 mm against *E. coli* strains, ECI and ECII, respectively. This inhibitory activity against the pathogenic strain can be due to the production of antagonistic compounds like hydrogen peroxide, short chain volatile fatty acids or bacteriocins (Chou and Weimer, 1999; Salminen et al., 1998; Piard and Desmazeaud, 1992; Desmazeaud, 1996; Ouwehand et al., 2002; Turchet et al., 2003; Wang et al., 2004; Tursi et al., 2004).

Conclusion

The above results showed that some *Lactobacillus* from the collection displayed interesting characteristics and can contribute to the preparation of local fermented products. The production of local starter cultures may help to reduce costs and to provide a degree of autonomy for the production of fermented foods. Some of the selected human strains also showed a probiotic potential. To confirm the potential of the selected strains, additional work is needed concerning taxonomic (genotypic characterization), technological (resistance to freezing and lyophilization), biotechnological (biomass production) and probiotic (trials on animal laboratories) criteria.

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