

# Evaluation of probiotic properties in *Lactobacillus* isolated from small intestine of piglets

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**ABSTRACT.** *Lactobacillus* has been associated with beneficial effects in human and animal health. Oral administration of probiotic bacteria must resist gastrointestinal transit, in order to colonize the intestinal mucus and offer an antagonistic effect against pathogenic microorganisms. The aim of this work was to select *Lactobacillus* strains isolated from small intestine of piglets based on the characteristics of resistance to low pH and bile salts, surface properties and antagonistic effect against *Escherichia coli* K88. To identify *Lactobacillus* species, a fragment of 16S rRNA gene of the strains was sequenced. Low pH, bile salts resistance and antagonistic activity were quantified by viable count in plates. Surface properties were measured using a spectrophotometer at 600 nm. Sixty-two *Lactobacillus* strains were isolated from small intestine of piglets. Species *Lactobacillus salivarius*, *Lactobacillus reuteri* and *Lactobacillus mucosae* were identified. We found that 20 *Lactobacillus* strains resisted low pH and bile salt, 8 of them were adherents and they inhibited *in vitro* the growth of *E. coli* K88. In conclusion, our results showed that 8 strains have potential probiotic value, according resistance to gastrointestinal tract, surface properties and antagonistic characteristics. *Lb. salivarius* was the species that fulfilled the criterion to be identified as a possible probiotic microorganism.

**Key words:** Piglets, *Lactobacillus*, probiotic characteristics, antagonism.

**RESUMEN.** Con frecuencia los lactobacilos se han relacionado con diversos efectos benéficos en la salud humana y animal. En la administración oral de bacterias benéficas, éstas deben resistir el tránsito gastrointestinal para que colonicen la mucosa y ejerzan un efecto antagónico contra diversas bacterias patógenas. Por lo anterior el objetivo de este trabajo fue seleccionar cepas de *Lactobacillus* aisladas del intestino delgado de lechones de acuerdo a sus características de resistencia al pH bajo y sales biliares, propiedades de superficie y efecto antagónico en contra de *Escherichia coli* K88 para utilizarlas a futuro como probióticos. Se aislaron sesenta y dos cepas de *Lactobacillus* del intestino delgado de seis lechones. Se identificaron las especies *Lactobacillus salivarius*, *Lactobacillus reuteri* y *Lactobacillus mucosae*. Encontramos que 20 cepas de *Lactobacillus* resistieron la presencia de pH bajo y sales biliares, 8 de ellas fueron adherentes e inhibieron *in vitro* el crecimiento de *E. coli* K88. En conclusión, nuestros resultados mostraron que 8 cepas tienen valor probiótico potencial de acuerdo a la resistencia al tránsito gastrointestinal, propiedades de superficie y efecto antagónico. *Lb. salivarius* fue la especie que cumple con los criterios para ser identificada como un probiótico potencial para aplicarse en cerdos recién destetados.

**Palabras clave:** Lechones, *Lactobacillus*, características probióticas, antagonismo.

## INTRODUCTION

Pig breeding is a profitable activity in many countries. Sometimes, the economy of this activity is affected by infectious diarrhea in neonatal pigs (Gusils *et al.*, 2002). *Escherichia coli* K88 has been identified as one of the main causal agents of this illness. Those bacteria invade mucosal cells and produce enterotoxins that cause diarrhoea and death of infected pigs (Meng *et al.*, 1998). Commonly antibiotics are used to prevent and/or eliminate these infections, unfortunately low control or antibiotic misuse are frequent practices. The worst disadvantage of these practices

is bacterial resistance developed to these substances, for this reason it is important to look for alternatives to antibiotic usage (Gibson and Wang, 1994). Probiotics can be administered to prevent infectious diseases, to strengthen the barrier function of the gut microflora and for a non-specific enhancement of the immune system (Gusils *et al.*, 2002).

Probiotic microorganisms are those capable of colonizing their hosts with beneficial effects. Oral administration of these bacteria helps to maintain microbiota balance, preventing or remedying the appearance of gastrointestinal infections (Gómez-Gil *et al.*, 1998). *In vitro* studies have shown that *Lactobacillus* can offer protection against some human pathogens, like *E. coli* O157:H7, *Salmonella* and *Clostridium* (Yusof *et al.*, 2000, Gopal *et al.*, 2001). Also it has been found that lactic acid producer species like *Bifidobacterium infantis* and *Bifidobacterium choerinum*, isolated from the colon of pigs, have an antagonistic *in vitro* effect against *E. coli* K88 and *Salmonella choleraesuis* (Corona, 2003). Previous facts suggest that beneficial bacteria can be a feasible solution to diminish incidence of intestinal diseases in piglets.

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*Lactobacillus* and *Bifidobacterium* belong to the normal human and animal microbiota. In this way their species are widely studied as probiotic. In animals, it has been observed that the rate of growth has increased with a better food conversion, and probiotics are helpful for this conversion (Jonson and Conway, 1992). Probiotics neither generate antimicrobial resistance nor produce toxic compounds in carcass (Fuller, 1999). Strains need to survive acidic conditions in the stomach and bile salts in duodenum, in order to exert their beneficial effects in the gut. Therefore, bile tolerance is considered one of the most important properties of probiotic microorganisms, because it allows them to survive and to colonize the gastrointestinal tract in mucus and/or by enterocytes adhesion (Gómez-Zavaglia. *et al.*, 2002).

Adherence to the intestinal mucus layer is another important selection criterion for probiotic microorganisms, because it is a requirement for the bowel colonization. Adherence constitutes the first defence mechanism against pathogen invasion. Passing through the small intestine takes about 2.5 hours, but it is faster in the duodenum than the colon, therefore bacterial adherence to mucus and/or enterocytes is necessary for colonization (Yusof *et al.*, 2000; Rinkinen *et al.*, 2003).

The aim of this work was to select *Lactobacillus* strains isolated from small intestines of piglets, based on their characteristics of resistance to low pH and bile salts, surface properties and antagonism against *E. coli* K88 as a first requirement for their possible use as probiotics.

## MATERIALS AND METHODS

### *Isolation of Lactobacillus strains*

Six Landrace piglets breed crossed with Large White were slaughtered, all of them from the same farm. They were healthy and weaned Small intestine was divided into three portions in aseptic conditions: duodenum, jejunum and ileum. Each portion was separately placed in tubes with MRS broth (Difco-Becton Dickinson & Company, Sparks, MD, USA) pH 6.0 with cysteine hydrochloride 0.5 gr/L (J.T. Baker, Phillipsburg, NJ), 2,3,5-tripheniltetrazolium chloride (TTC) 25 ppm (Merck, Darmstadt, Ge), sodium propionate 0.3% w/v (Sigma, St. Louis MO, USA), lithium chloride 0.2% w/v (Sigma) and antibiotics, as nalidixic acid 20 ppm (Sanofi-synthelabo Edo. México, Mex.), kanamycin 50 ppm (Sigma) and polymyxin B sulphate 8.5 ppm. (Sigma). Cultures were incubated at 37° C in 5% CO<sub>2</sub> by 48 h. After that cultures were seeded in plates with MRS agar (Difco-Becton Dickinson & Company) pH 6.0 with cysteine, without antibiotics and incubated as described above (Corona, 2001). From those plates,

white and creamy colonies were selected. Gram stain, motility and catalase assay were done as a first screening.

### *Phenotypic and Genotypic identification by 16S rRNA gene sequence analysis*

To identify the species of *Lactobacillus*, the strains were tested for the fermentation of carbohydrates. Sugars tested were L-arabinose, lactose, celobiose, melezitose, raffinose, sorbitol, starch, xylose, mannose, fructose, galactose, sucrose, maltose, trehalose, melibiose, mannitol, inulin and salicin (Sigma). Fermentation test was done in tryptone peptone yeast broth (TPY) (Difco-Becton Dickinson & Company) supplemented with 1% carbohydrate and bromocresol purple as pH indicator (Kandler and Weiss, 1986).

In the genotypic identification, DNA from strains was isolated (De los Reyes *et al.*, 1992). PCR was performed in a Thermal cycler (Perkin Elmer, Wellesley MA, USA). PCR primers used for this experiment were 27F and 519R reported previously. They amplified a 492 bp fragment from 16S rRNA gene (Lane, 1996). A typical reaction used the following programme involving a initial denaturation of 3 min at 94° C, 30 cycles of 94° C for 30 s, 55° C for 60 s and 72° C for 30 s. The final cycle was 72° C for 10 min. The PCR products were analyzed on 1.2% agarose gels (Sigma). They were stained with ethidium bromide and observed in a UV transilluminator (Vilbert Loumart, Marne La Vallee, Francia). PCR products were purified with a GFX PCR DNA and Gel Band Purification Kit (Amersham, Piscataway NJ, USA). The purified products were sent to Arizona Research Laboratories (Tucson AZ, USA) for sequencing. Sequenced DNA was compared with information in the data base available in basic BLAST (Altschul *et al.*, 1997). Partial sequences were manually aligned using DNAMAN (4.03 Lynnon BioSoft, Quebec Canada). A distance matrix and phylogenetic tree was generated using the Observed Divergency method.

### *Resistance to pH 3.0 and bile salts*

To test the resistance to pH 3.0 and conjugated porcine bile salts (CPBS) (0.5% w/v) (Sigma), a modification of the proposed technique by Rodríguez *et al.* (2003) was performed. Strains were inoculated in 4 mL of MRS broth pH 6.0 and incubated in 5% CO<sub>2</sub> at 37° C for 48 h, then 2 mL were used for viable count on MRS agar pH 6.0 (Control). Remaining 2 mL were harvested 2,600 x g at 4° C (GS-6R Beckman, USA). Supernatant was discarded and the pellet was resuspended in 2 mL MRS broth pH 3.0 with cysteine. Cultures were incubated during 1 h at 37° C in aerobic conditions. After incubation, viable count was done using

MRS agar pH 6.0 in previously described conditions. At the same time, the cultures were inoculated for a viable count in plates of MRS with CPBS (0.5% w/v). In both cases, the plates were incubated in 5% CO<sub>2</sub> at 37° C for 48 h. All plates were inoculated by duplicate.

Percentage of resistance to pH 3.0 and CPBS was determined using the equation of Kociubinsky *et al.* (1999) % Resistance =  $100(\text{CFU}_{(\text{pH } 3.0 \text{ or CPBS})} / \text{CFU}_{\text{control}})$ .

#### Autoaggregation assay

Strains were grown as described above in 3 ml of MRS broth pH 6.0 with cysteine and they were harvested at 2400 x g. Supernatant was retained in a different tube. The pellet was washed twice with phosphate buffered saline (PBS) 0.02 M pH 7.4 and resuspended in same buffer until an optical density (O. D.) of 0.5 units at 600 nm (Spectronic 21D Milton Roy, USA) was reached. From this suspension 3 mL were harvested at 2,400 x g. Supernatant was eliminated and cells were resuspended in their original broth. They were incubated by 2 h at 37° C and then, 1 ml was taken from the superior part of the culture and the O.D. was measured. Finally, culture was shaken and total O. D. was measured. The autoaggregation (% A) is expressed in the following equation  $1 - (\text{O. D. superior culture} / \text{O. D. total}) \times 100$  (Del Re *et al.*, 2000). This experiment was done in triplicate.

#### Hydrophobicity (microbial adhesion to hydrocarbons)

Strains were grown as described above in 3 ml of MRS broth pH 6.0 with cysteine. Cultures were washed with PBS buffer and resuspended as described previously. 2 ml of bacterial suspension were transferred into another tube and 0.4 mL of xylene was added (Fluka, GmbH, Switzerland). Tubes were shaken for 2 min and reposed for 15 min. After that O.D. of aqueous phase at 600 nm was measured. O.D. decrease in aqueous phase was considered as a measurement of cells surface hydrophobicity (%H). %H was calculated according to the following equation  $[(A_0 - A) / A_0] \times 100$ . Where A<sub>0</sub> and A, were the absorbance before and after xylene extraction respectively (Del Re *et al.*, 1998; Gusils *et al.*, 2002; Mishra and Prasad, 2005).

#### Antagonism against *Escherichia coli* K88

*Lactobacillus* strains and *E. coli* K88 were inoculated separately in MRS broth, pH 7.0 with cysteine. Cultures were incubated for 24 h at 37° C in 5% CO<sub>2</sub>. All the *Lactobacillus* cultures were adjusted with tube number 5 of a MacFarland nephelometer. *E. coli* K88 culture was adjusted with the number 3. K88 was massively inoculated over

MRS plates pH 7.0 with cysteine according to Hernández (2003). Four holes of 6 millimeters (mm) of diameter at similar distances were punched and filled with 70 µl of *Lactobacillus* culture. Plates were incubated at 37° C for 24 h and growth inhibition was measured in millimeters (De Martinis *et al.*, 2002).

On the other hand, mixed cultures were prepared according to González *et al.*, (1993). 24 h cultures of *Lactobacillus* and *E. coli* K88 in MRS broth pH 7.0 with cysteine were adjusted with tube number 3 of MacFarland nephelometer. Pathogenic bacterium was diluted three times by serial dilutions. Equal volumes of both cultures in proportion 1,000:1 (*Lactobacillus*: *E. coli* K88) were mixed in 3 mL MRS broth pH 7.0 with cysteine and incubated at 37° C for 6 h in 5% CO<sub>2</sub>. A control tube was made containing just *E. coli* K88. After 6 h, a viable count in ENDO agar (Difco, Mexico) was done. Determination of antagonism percentage was calculated according the equation: % I =  $100 [(T_{6 \text{ control}} - T_{6 \text{ mixed culture}}) / T_{6 \text{ control}}]$ , where % I was the percentage of bacterial inhibition of each strain, T<sub>6 control</sub> was the viable count obtained from the control and T<sub>6 mixed culture</sub> was the viable count obtained from the mixed culture (González *et al.* 1993; Gusils *et al.*, 2002; García-Galaz *et al.*, 2004).

#### Statistical Analysis

ANOVA and Tukey-Kramer test were used for mean comparisons (p<0.05) in all experiments, with statistical package NCSS 6.0 (Hintze, 1997). Comparisons were carried out for species and strains.

## RESULTS

#### Isolation of *Lactobacillus* strains

Sixty-two *Lactobacillus* strains were isolated from small intestine of six healthy piglets. All of them grew in aerobic and 5% CO<sub>2</sub> conditions, were Gram positive rods, non-motile and catalase negative as preliminary characteristics. From jejunum, 33 strains were isolated, significantly different (p<0.05) from duodenum (10 strains) and ileum (19 strains) in bacterial gut distribution in piglet.

#### Phenotypic and Genotypic identification by 16S rRNA gene sequence analysis

At least a 492 bp fragment of the 5' region of the 16S rRNA gene was sequenced for all the strains. We found that by comparison of sequences in the NCBI data base, 35 strains showed 99% of identity with *Lactobacillus salivarius* subsp. *salivarius*, 2 strains had 99% of identi-

ty with *Lactobacillus salivarius* subsp. *salicinus*, 5 strains had 99% of identity with *Lb. salivarius*, 19 strains showed 99% of identity with *Lactobacillus reuteri* and 1 strain showed 98% of identity with *Lactobacillus mucosae*. With the phylogenetic tree we could observe three species groups. Group I corresponds to *Lb. salivarius* (subsp. *salivarius* and *salicinus* together), Group II to *Lb.*

*mucosae* and Group III to *Lb. reuteri*. It is important to notice that the phylogenetic analysis was not enough to differentiate subsp. *salivarius* from subsp. *salicinus* (Figure 1). There were no statistical differences ( $p>0.05$ ) in species distribution, according to three analyzed portions in small intestine.

The isolates identified by partial sequence of 16S rRNA gene, were characterized by carbohydrate fermentation and it confirmed the genotypic identification (data not shown).

### Resistance to pH 3.0 and bile salts

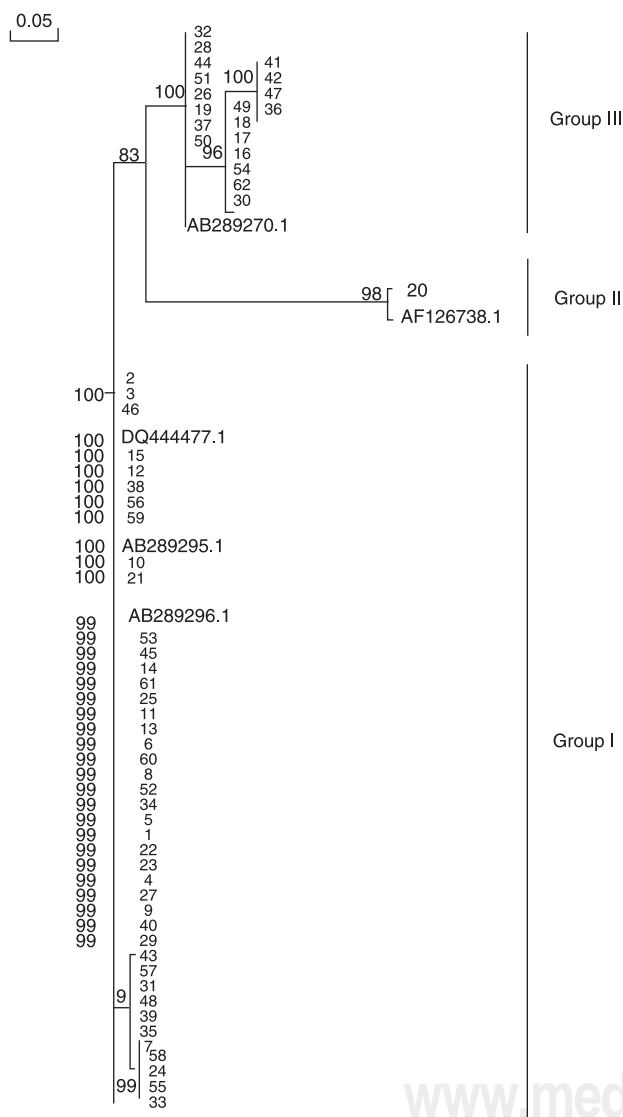
From all isolated strains, just 20 survived at pH 3.0 and CPBS conditions in 45% or more. Data of strains which did not survive are not shown. Survival at pH 3 is significant because ingestion of probiotic bacteria with food or dairy products raises the pH in stomach to 3.0 or higher. Resistant strains belonged to three identified species, *Lb. salivarius* being the most common with 15 strains (Figure 2). Others strains showed good survival to low pH (more than 50%), but they were discarded, because the resistance to CPBS was less than 0.1%. There were no statistical differences in the survival percentage ( $p>0.05$ ), between the two main 1 species isolated (Table 1).

### Autoaggregation and Hydrophobicity (microbial adhesion to hydrocarbons)

20 strains that survived to pH 3.0 and CPBS conditions were included to further characterization. They showed significant differences ( $p<0.05$ ) in their autoaggregation and hydrophobicity properties. Strains 5, 6, 8, 9, 10, 11, 13, 18 and 20 showed an autoaggregation percentage superior to 40%, but strain 13 had less than 30% for hydrophobicity, for which reason it was discarded as a potential probiotic. For hydrophobicity, strains 7, 15 and 19 showed less than 10% (Figure 3). Altogether, 8 strains showed autoaggregation and hydrophobicity percentages superior to 40%, from these, 6 correspond to *Lb. salivarius*, 1 to *Lb. reuteri* and 1 to *Lb. mucosae*. This indicates that these strains possess autoaggregative and hydrophobic characteristics which are related to adhesion to epithelia.

### Antagonism against *Escherichia coli* K88

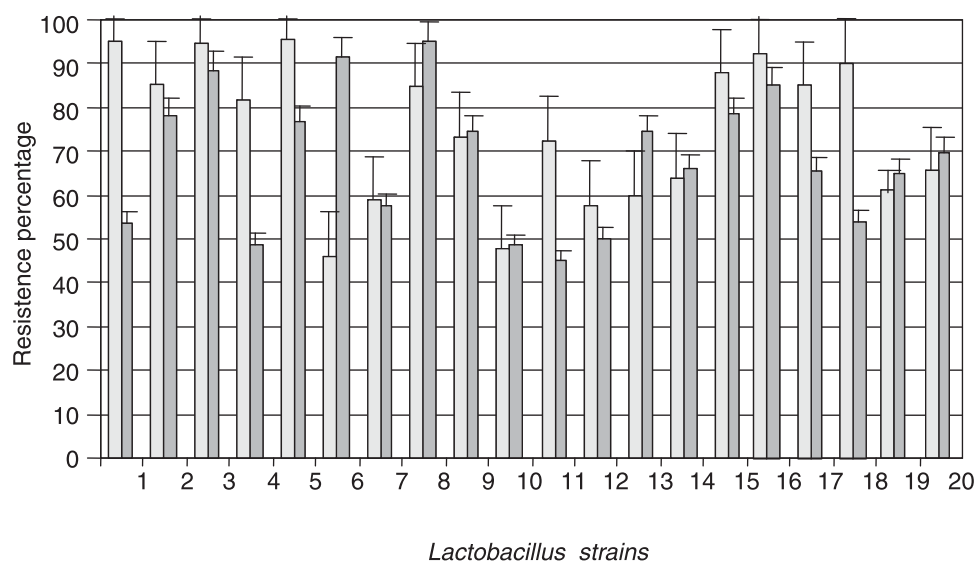
8 strains that displayed superior autoaggregation and hydrophobicity properties were included in this experiment for antagonism. Whole cultures were used and halos of inhibition more than 20 mm against *E. coli* K88 were observed. There were no significant differences ( $p>0.05$ ) between strains.



Accession numbers of sequences obtained from NCBI correspond to the following species: AB289296.1 *Lb. salivarius* subsp. *salivarius*, AB289295.1 *Lb. salivarius* subsp. *salicinus*, DQ444477.1 *Lb. salivarius*, AB289270.1 *Lb. reuteri* and AF126738.1 *Lb. mucosae*.

**Figure 1.** Unrooted phylogenetic tree based on the observed divergency method of partial 16S rRNA sequences of *Lactobacillus* isolates from small intestine of piglets. Horizontal bar represents 1% sequence divergence. Numbers indicate bootstrap values for branch point.





Lb. salivarius from 1 to 15, Lb. reuteri from 16 to 19 and Lb. mucosae 20.

**Figure 2.** Resistance to pH 3.0 (%) and conjugated porcine bile salts (%) (CPBS) of *Lactobacillus* strains isolated from small intestine of piglets.

**Table 1.** Percentage of resistance to pH 3.0 and conjugated porcine bile salts (CPBS) of predominant species of *Lactobacillus* strains isolated from small intestine of piglets.

Species	Resistance to pH 3.0	Resistance to CPBS
<i>Lb. salivarius</i>	66.5 ± 30	29 ± 35.5
<i>Lb. reuteri</i>	48.7 ± 39.2	16 ± 24.8

± indicates standard deviation

Quantitative antagonistic analysis, including all 8 strains showed pathogen growth decrease of 3 Log (Figure 4). There were no differences between the effects of the *Lactobacillus* strains tested ( $p > 0.05$ ). Antagonistic activity of *Lb. reuteri* strains, which were not resistant to low pH or CPBS and did not show surface properties in the required percentage, was quantified. They showed a decrease of *E. coli* K88 growth of approximately 3 Log (data not shown).

## DISCUSSION

*Lactobacilli* are established early in piglet intestine, and although succession occurs throughout lifetime of the pigs, they may remain as one of the predominant elements of the bacterial community (De Angelis *et al.*, 2006).

In this work, we isolated one facultative heterofermentative and two obligate heterofermentative *Lactobacillus* species: *Lb. salivarius*, *Lb. reuteri* and *Lb. mucosae*, respectively. Similar results were reported by Robredo and Torres (2000) and Roos *et al.*, (2000) in intestinal tract of

healthy adult pigs. Those results indicated that isolated species are maintained in the intestine throughout the whole life of pigs (Saarela *et al.*, 2000; De Angelis *et al.*, 2006).

Probiotic microorganisms need to resist the adverse factors in the gastrointestinal tract when they pass through it, like the stomach acidity and bile salts, excreted in duodenum. For this investigation it was decided to select strains with a resistance more than 45%, to assure that bacteria arrive in suitable concentration (6 to 8 logarithms/g of consumed food) to the intestine, and exert their probiotic effect (Shah *et al.*, 1999).

In this work, all of three species were resistant to pH 3.0 and CPBS. Maxwell and Stewart (1995) found that *Lb. acidophilus*, *Lb. fermentum* and *Lb. lactis* were resistant to these adverse conditions in adult pigs. From those species, 20 strains survived the gastrointestinal transit more than 45%. Gómez-Zavaglia *et al.* (1998) and Kociubinski *et al.* (1999) obtained resistant strains to gastrointestinal transit over 23%. Those species were *B. pseudolongum*, *B. infantis*, *B. animalis* and *B. breve*. Aside, Ibrahim and Bezko-rovainy (1993) worked with strains of *B. bifidum*, *B. breve*, *B. infantis* and *B. longum*, which were resistant to the adverse conditions of digestive tract. In general, variable results have been documented in respect the resistance of low pH and bile salts of the *Lactobacillus* and *Bifidobacterium* strains (Clark and Martin, 1994; Chung *et al.*, 1999; Mishra and Prasad, 2005).

The *Lactobacillus* genus has optimal growth in pH 6.0. It is characterized by its capacity to produce lactic acid mainly, which creates environments with pH up to 4.0, where they are able to remain viable for variable periods,

depending on the activity of their H<sup>+</sup>-ATPase (Matsumoto *et al.*, 2004).

The greater adverse effect was observed for CPBS. Some authors have reported that the conjugated salts, mainly the glycodeoxicolic acid, are lethal for this bacterial genus and the mortality rate increases as pH diminishes. It has been found that biliary salts hydrolases produced by some *Lactobacillus* strains, are involved in the resistance (Grill *et al.*, 2000; Tanaka *et al.*, 2000; Kim *et al.*, 2004).

Another desirable property of probiotic bacteria is the colonization in intestinal wall. This colonization is necessary in order to exert its beneficial effects (Tuomola *et al.*, 2001). In probiosis, it is important to evaluate surface properties, like autoaggregation and hydrophobicity, because they are used as a measurement directly related to adhesion ability to enterocytic cellular lines (Pérez *et al.*, 1998; Del Re *et al.*, 2000).

Autoaggregation besides determines the capacity of the bacterial strain to interact with itself, in a nonspecific way. Aside, when that hydrophobicity is high (more than 40%), it indicates the presence of hydrophobic molecules in the bacterial surface, like surface array proteins; wall intercalated proteins, cytoplasmic membrane protein and lipids. (Ofek and Doyle, 1994; Pérez *et al.*, 1998; Bibiloni *et al.*, 1999; Bibiloni *et al.*, 2001).

In this work, 20 strains were resistant to gastrointestinal transit, 8 had values of autoaggregation and hydrophobicity superior to 40%. According to Del Re *et al.* (1998) and Pérez *et al.* (1998), this percentage is the minimum necessary for considering a strain with adhesion abilities.

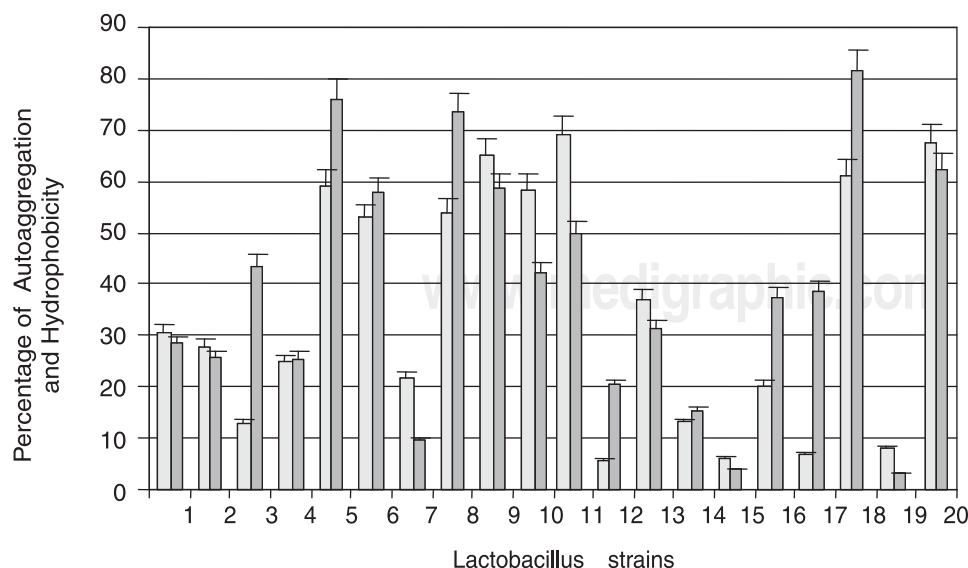
Pérez *et al.* (1998) found that *B. breve* strains isolated from humans were not autoaggregatives or hydrophobics.

Neither were adherent to cellular lines Caco-2. Time later, Bibiloni *et al.*, (2001) related these non adherent strains with poor presence of protease-sensitive non polar like proteins molecules in their surface.

Del Re *et al.* (1998) concluded that the adhesion property is characteristic of each strain and cannot be generalized to species. Ability to autoaggregate together with cell surface hydrophobicity could be used for preliminary screening to identify potentially adherent bacteria.

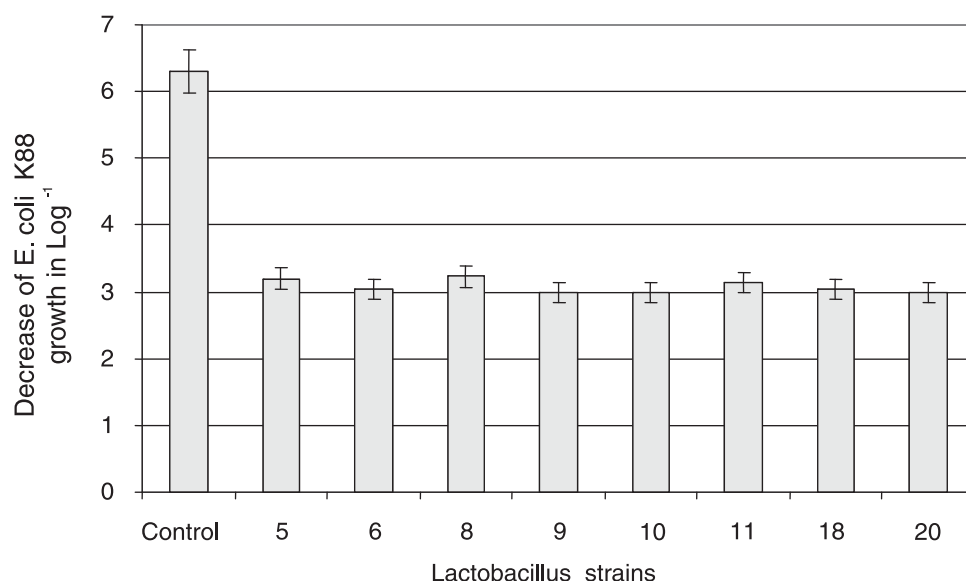
To improve the probiotic characterization, 8 strains that showed surface properties over 40%, were tested for their antagonism *in vitro* against *E. coli* K88. All strains showed inhibition zones and decreased the pathogen growth. Gusils *et al.* (2002) found that *Lactobacillus* and *Enterococcus* strains isolated from pig feces, do not inhibit the growth of *Yersinia enterocolitica*, *Salmonella choleraesuis*, *Salmonella typhimurium* and *Salmonella enteritidis* after 24 hours of plates incubation. The quantification of antagonism of the *Lactobacillus* strains in this work showed an *E. coli* K88 inhibition in 3 Log in 6 hours. According to antagonistic values, resistance to gastrointestinal transit and adherence factors of isolated strains, it is possible to infer that these bacteria could be used as an alternative for the treatment of diarrhea in piglets. Nevertheless, *in vivo* studies are necessary to confirm it. Recent work in other countries, using probiotic bacteria in pigs, that showed positive results in performance, decrease of intestinal infections and viral diseases (Casey *et al.*, 2007, Schierack *et al.*, 2007) encourage us to continue investigating our strains.

*E. coli* K88 has been identified as one of the main bacterial producers of diarrhea in new born pigs. It has been



Lb. salivarius from 1 to 15, Lb. reuteri from 16 to 19 and Lb. mucosae 20.

**Figure 3.** Percentages of autoaggregation (%) and hydrophobicity (%) of *Lactobacillus* strains that survived pH 3.0 and CPBS.



Control = *E. coli* K88 culture without *Lactobacillus* added

*Lb. salivarius* from 5 to 11, *Lb. reuteri* 18 and *Lb. mucosae* 20.

**Figure 4.** Antagonism against *E. coli* K88 of *Lactobacillus* strains isolated from small intestine of piglets.

**Table 2.** *Lactobacillus* strains with the best values to be a probiotic potential.

Strain	Percentage of resistance to pH 3.0	Percentage of resistance to CPBS	Percentage of autoaggregation	Percentage of hydrophobicity	Percentage of inhibition against K88
5	95.4	76.6	59.1	76.1	97.2
6	46.0	91.4	52.9	57.8	98.4
8	84.6	95.0	54.0	73.5	98.0
9	73.4	74.4	64.9	65.5	98.2
10	47.7	48.5	58.4	42.1	98.3
11	65.4	69.1	69.8	49.7	98.3
18	72.5	45.0	61.2	81.5	97.9
20	90.2	53.8	67.6	62.1	97.8

suggested that probiotics can coaggregate pathogenic bacteria and release antagonistic substances, like organic acids (lactic mainly) and bacteriocins. Also there are studies that indicate that they can compete for adhesion sites with several microorganisms, but this is still not verified (Mulder *et al.*, 1997; Meng *et al.*, 1998; Ouwehand *et al.*, 1999; Doyle, 2001).

In conclusion, our results showed that 8 selected strains have potential probiotic value (Table 2). We found that the predominant species, *Lb. salivarius*, shows the best characteristics to fulfill the criteria of a probiotic strain. In addition, it is recommended that these strains be further analyzed according to the selection criteria like stimulation of the immunological system and adhesion to the pig mucosa and/or epithelium intestinal.

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