

Research Paper

## Bacteriocinogenic *Lactococcus lactis* subsp. *lactis* DF04Mi isolated from goat milk: Evaluation of the probiotic potential

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### Abstract

Lactic acid bacteria capable of producing bacteriocins and presenting probiotic potential open innovative technological applications in the dairy industry. In this study, a bacteriocinogenic strain (*Lactococcus lactis* subsp. *lactis* DF4Mi) was isolated from goat milk, and studied for its probiotic potential. *Lc. lactis* DF4Mi was resistant to acidic pH and oxbile, presented co-aggregation with *Listeria monocytogenes*, and was not affected by several drugs from different generic groups, being sensitive to most tested antibiotics. These properties indicate that this *Lc. lactis* strain can be used for enhancement of dairy foods safety and quality, in combination with potential probiotic properties.

**Key words:** bacteriocin, probiotic, *Lc. lactis* subsp. *lactis*, biopreservation, goat milk.

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### Introduction

Lactic acid bacteria (LAB) present many important properties in food manufacturing, such as improvement of organoleptic and physical characteristics, production of lactic acid contributing to the extension of the shelf life, and reduction of lactose content. Several LAB are used in dairy industries as starter cultures as well as probiotics. Probiotics are defined as live microorganisms which when administered in adequate amounts confer health benefits to the host (WHO, 2002; Bertazonni-Minelli *et al.*, 2004; Oelschlaeger, 2010; Todorov *et al.*, 2012). Probiotic beneficial effect for the host include suppression of growth of pathogens, control of serum cholesterol level, modulation of the immune system, improvement of lactose digestion, synthesis of vitamins, increase in bio-availability of minerals and possible anti-carcinogenic activity (Gomes and Xavier, 1999; Kailasapathy and Chin, 2000; Chan and Zhang, 2005). Bacteriocin producing LAB may also present a probiotic potential if capable of surviving the harsh conditions in the gastro-intestinal tract (GIT), including low pH and high concentrations of bile salts. As probiotics, these bacteria can confer health benefits to the host such as reduction of gastrointestinal infections and inflammatory bowel

disease, modulation of the immune system, and defense against colonization by pathogenic microorganisms (WHO, 2002; Galdeano *et al.*, 2007; Oelschlaeger, 2010).

Aggregation of LAB is an important feature in evaluation of potential probiotic properties. While auto-aggregation may result in biofilm formation, co-aggregation with pathogens is important for elimination of non-desirable strains from the GIT (Todorov and Dicks, 2008). These capabilities are strain-specific and depend on species-specific surface proteins that recognize or bind to components in the environment. According to Kleerebezem *et al.* (2003), several genes encoding for surface proteins are homologous to genes coding for proteins with predicted functions, such as mucus-binding, aggregation-promotion and intracellular adhesion.

In the evaluation of a possible probiotic potential of LAB it is important to check for antibiotic resistance as they can act as potential reservoirs of resistance genes that can be transferred to other microorganisms, producing multidrug resistant strains (Dicks *et al.*, 2011). Probiotic consumers may be under treatment for a variety of illnesses, and the beneficial effects of the probiotic strain may be hampered by possible interactions with the medicaments used by these consumers. It should be emphasized that the

interaction between antibiotics or other medicaments and probiotic bacteria in the GIT depends on their concentration in this environment (Todorov *et al.*, 2007; Todorov *et al.*, 2008), so establishing the Minimal Inhibitory Concentration (MIC) values play an important role for the proper evaluation of these interactions. Of higher importance are the drugs for treatment of chronic diseases normally used in long course treatments, since they may accumulate in the gastrointestinal tract and affect the viability of probiotic bacteria (Carvalho *et al.*, 2009).

Application of bacteriocinogenic strains for dairy product preservation is in agreement with consumers' demands for foods that are naturally preserved. Additional claims of health-promoting benefits due to probiotic activity bring extra value to these foods. In this study we report results on the evaluation of the probiotic potential of the strain *Lc. lactis* DF04Mi.

## Materials and Methods

### Bacterial strains

The study was conducted with a bacteriocinogenic *Lactococcus lactis* subsp. *lactis* strain (*Lc. lactis* DF04Mi) isolated from raw goat milk (Furtado *et al.*, 2009). *L. monocytogenes* 711 (collection of University of São Paulo, Faculty of Pharmaceutical Sciences, Department of Food Science and Experimental Nutrition) and *E. faecalis* ATCC 19433 and *Lactobacillus sakei* ATCC 15521 (ATCC, Manassas, Virginia, USA) were used as co-aggregation partners. All strains were stored at -80 °C in presence of 15% glycerol.

Auto-aggregation and co-aggregation with *L. monocytogenes* 711, *E. faecalis* ATCC 19433 and *Lb. sakei* ATCC 15521

The cells of a culture of *Lc. lactis* DF04Mi grown in MRS broth (Difco) for 24 h at 37 °C were harvested by centrifugation (7,000 x g, 10 min, 20 °C), washed and re-suspended in sterile saline (0.85% NaCl, w/v). One ml of the cell suspension adjusted to an OD<sub>660nm</sub> = 0.3 was transferred to a 2 mL sterile plastic cuvette and the OD<sub>660nm</sub> recorded, using a spectrophotometer (Ultraspec 2000, Pharmacia Biotech). After 60 min OD<sub>660nm</sub> readings were performed in the supernatant, after centrifuging the cultures at 300 x g for 2 min at 20 °C. Auto-aggregation was determined using the following equation (Todorov *et al.*, 2008):

$$\% \text{ Auto-aggregation} = [(OD_{0\text{min}} - OD_{60\text{min}})/OD_{0\text{min}}] \times 100$$

OD<sub>0min</sub> and OD<sub>60min</sub> refer to the initial OD and the OD determined after 60 min, respectively.

For evaluation of co-aggregation strains *Lc. lactis* DF04Mi, *E. faecalis* ATCC 19433 and *Lb. sakei* ATCC 15521 were grown in 10 mL MRS at 30 °C and *L. monocytogenes* 711 in 10 mL BHI at 37 °C. Cells were harvested after 24 h (7,000 x g, 10 min, 20 °C), washed and

re-suspended in sterile saline (0.85% NaCl). One ml of the cell suspension of *Lc. lactis* DF04Mi (adjusted to an OD<sub>660nm</sub> = 0.3) and 1 mL of co-aggregation partner (adjusted to an OD<sub>660nm</sub> = 0.3) were mixed and transferred to a 2 mL sterile plastic cuvette and the OD<sub>660nm</sub> recorded using a spectrophotometer. OD<sub>660nm</sub> readings were performed in the supernatant after 60 min and after centrifuging the suspension at 300 x g for 2 min at 20 °C. Co-aggregation was calculated using the following equation (Todorov *et al.*, 2008):

$$\% \text{ Co-aggregation} = [(OD_{0\text{min}} - OD_{60\text{min}})/OD_{0\text{min}}] \times 100$$

OD<sub>0min</sub> and OD<sub>60min</sub> refer to the OD determined immediately after mixing the cultures and the OD determined after 60 min, respectively. Experiments were conducted in triplicates on two separate occasions.

### Effect of pH and bile on growth

*Lc. lactis* DF04Mi was grown in MRS broth (Difco) adjusted to pH 3.0, 4.0, 5.0, 6.0, 7.0, 9.0, 11.0 and 13.0 by adding 1 M HCl or 1 M NaOH before autoclaving. If needed, pH was re-adjusted after sterilization by adding sterile 1 M HCl or 1 M NaOH. The strain was also grown in MRS broth containing 0.2%, 0.4%, 0.6%, 0.8%, 1.0%, 2.0% and 3.0% (w/v) oxbile (Sigma). All tests were conducted in sterile flat-bottom 96-well microtiter plates (TPP, Switzerland). Each well was filled with 180 µL of the medium and inoculated with 20 µL of cultures of *Lc. lactis* DF04Mi activated in MRS broth (Difco) at 37 °C until presenting OD<sub>600nm</sub> = 0.2. Growth of *Lc. lactis* DF04Mi in the tested conditions was monitored through determination of OD<sub>600nm</sub> every hour for 12 h, using a microtiter plate reader (Versa-max, Sunnyvale, CA, USA). Cultures grown in non-modified MRS broth served as control. Experiments were done in triplicate.

### Determination of cell surface hydrophobicity in *Lc. lactis* DF04Mi

The test for bacterial adhesion to hydrocarbons (BATH), described by Doyle and Rosenberg (1995) with some modification (Todorov *et al.*, 2008), was used. *Lc. lactis* DF04Mi was grown in MRS broth (Difco) at 37 °C for 18 h. Cells were harvested (6,700 g, 4 °C, 6 min), washed twice with sterile saline solution (0.85% NaCl), re-suspended in the same solution and the optical density (OD<sub>580nm</sub>) was determined. An aliquot of 1.5 mL cell suspension was added to 1.5 mL *n*-hexadecane (Sigma) and vortexed for 2 min. The phases were allowed to separate for 30 min at room temperature. One ml of the watery phase was removed and the optical density (OD<sub>580nm</sub>) determined. The experiment was repeated and the average optical density value determined. The percentage of hydrophobicity was calculated as follows:

% hydrophobicity = [(OD<sub>580</sub> reading 1 - OD<sub>580</sub> reading 2) / OD<sub>580</sub> reading 1] x 100.

Experiments with all strains were conducted in triplicate.

### Resistance to drugs and antibiotics

*Lc. lactis* DF04Mi was tested for resistance to several drugs used in human and animal therapy, purchased in local drugstores (Sao Paulo, SP, Brazil) and solubilized in sterile water to achieve the desired concentration listed in Table 1. An overnight culture of *Lc. lactis* DF04Mi in MRS broth (Difco) was mixed with MRS soft agar (1.0%, w/v, Difco) in order to achieve a population of 10<sup>6</sup> cfu/mL and transferred to empty petri plates. After solidification of the agar 10 µL of each tested drug were spotted onto the surface. The plates were incubated at 37 °C for 24 h, examined for the presence of inhibition zones around the spotted drug, and those presenting inhibition zones larger than 2 mm diameter were subjected to the determination of the minimal inhibition concentration (MIC). For this test, serial two-fold dilutions of the drug were prepared in sterile water and 10 µL were spotted onto the surface of MRS soft agar plates, previously inoculated with strain *Lc. lactis* DF04Mi (approx. 10<sup>6</sup> cfu/mL). The plates were incubated for 24 h at 37 °C and examined for the presence of inhibition zones around the spots. The MIC corresponded to the highest dilution that resulted in inhibition halos of at least 2 mm diameter. In a similar experimental approach, susceptibility of strain *Lc. lactis* DF04Mi to antibiotics listed in Table 2 was tested by the disk diffusion test, using antibiotic disks from CEFAR (Sao Paulo, Brazil). The inhibitory effect of the antibiotics was expressed in millimeters of the inhibition zones.

### Results and Discussion

Bacteriocin produced by *Lc. lactis* DF04Mi presented a large spectrum of activity, inhibiting the growth of many food spoilage bacteria and foodborne pathogens. Similar results were recorded for the cell free supernatant and for the semi-purified bacteriocin. It is important to emphasize the bioactivity against *L. monocytogenes*, a foodborne pathogen of increasing importance. Antimicrobial activity against Gram-negative bacteria is also a relevant characteristic that was previously detected in other microorganisms including bacteriocins produced by *Lc. lactis* DF04Mi (Furtado *et al.*, 2009).

Auto-aggregation of *Lc. lactis* DF04Mi was low (12.2%) (Figure 1). Co-aggregation of *Lc. lactis* DF04Mi with the pathogens *L. monocytogenes* 711 and *E. faecalis* ATCC 19443 was 30.0% and 24.3%, respectively, which are lower values than for *Lb. sakei* ATCC 15521, a non-pathogen. The low levels of auto-aggregation and co-aggregation with pathogens may play an important role in

preventing the formation of biofilms, and in this way eliminating the pathogens from the gastrointestinal tract.

Good growth of *Lc. lactis* DF04Mi was recorded in MRS broth (Difco) when the initial pH was 6.0, 7.0 or 9.0 (Figure 2). Similar results were reported for *Lc. lactis* subsp. *lactis* HV219 (Todorov *et al.*, 2007). In another study, growth of several strains of *Lb. plantarum*, *Lb. rhamnosus*, *Lb. pentosus* and *Lb. paracasei* was suppressed at pH 3.0 and 4.0, while variable results were recorded in medium with an initial pH of 11.0 and 13.0, with poor growth recorded for strains *Lb. paracasei* ST242BZ and ST284BZ, *Lb. rhamnosus* ST462BZ, *Lb. plantarum* ST664BZ and *Lb. pentosus* ST712BZ at pH 13.0 (Todorov *et al.*, 2008).

*Lc. lactis* DF04Mi grew well in the absence of oxbile, or when the concentration was below 3.0% (Figure 2). The bacteriocinogenic *Lc. lactis* HV219 strain was less tolerant to bile salts, as the growth of it was inhibited by concentrations of oxbile above 0.3% (Todorov *et al.*, 2007).

Other studies have also reported similar effects of oxbile and pH for different strains of *Lc. lactis*, such as *Lb. plantarum* 423, *Lb. salivarius* 241 and *Lb. curvatus* DF38 (Brink *et al.*, 2006). Haller *et al.* (2001) reported variable results for different strains of *Lb. plantarum* when exposed to HCl (pH 2.0) and bile salts, and as many as 10% of *Lb. plantarum* cells, but less than 0.001% of *Lb. sakei* and *Lb. paracasei* cells survived these conditions. Although these assays cannot predict the behavior of the microorganism in the human body, the results are valuable in selecting *Lactococcus* spp. for probiotic applications, as resistance to low pH and high concentrations of bile salts is important for growth and survival of bacteria in the intestinal tract (Haveenaar *et al.*, 1992; Carvalho *et al.*, 2009).

It has been suggested that there is a possible correlation between surface hydrophobicity of probiotic strains and the ability to adhere to the intestinal mucosa (Wadstrom *et al.*, 1987). Cell surface hydrophobicity defines a non-specific interaction between microbial and host cells. The initial interaction may be weak, is often reversible and precedes subsequent adhesion processes mediated by more specific mechanisms involving cell-surface proteins and lipoteichoic acids (Granato *et al.*, 1999; Rojas *et al.*, 2002; Roos and Jonsson, 2002). Bacterial cells with a high hydrophobicity usually form strong interactions with mucosal cells. Hydrophobicity values of 52.4% were recorded for *Lc. lactis* DF04Mi. Higher hydrophobicity values of 75% - 80% were recorded for strains *Lb. rhamnosus* ST461BZ, *Lb. rhamnosus* ST462BZ and *Lb. plantarum* ST664BZ and these values were higher than those reported for *Lb. rhamnosus* GG, well known commercial probiotic (55%) (Todorov *et al.*, 2008). Hydrophobicity varies among genetically close related species and even among strains of the same species (Schar-Zammaretti and Ubbink, 2003). Some strains with a high cell surface hydrophobicity were not able to properly adhere to Caco-2 and HT-29 cells

**Table 1** - Effect of drugs on growth of *Lactococcus lactis* subsp *lactis* DF04Mi in MRS agar, presented as diameter of inhibition zones and Minimal Inhibitory Concentration (MIC).

| Brazilian commercial name | Concentration (mg/mL) | Active substance   | Medicament class   | Inhibition zone (mm) [MIC (mg/mL)] |
|---------------------------|-----------------------|--|--|------------------------------------|
| AAS                       | 20                    | Acetylsalicylic acid   | Analgesic / Antipyretic  | -                                  |
| Amoxil                    | 100                   | Amoxicillin  | $\beta$ -Lactam antibiotic (Penicilin)                           | 40 [ $< 0.2$ ]                     |
| Antak                     | 30                    | Ranitidine hydrochloride   | Histamine H2-receptor antagonist (proton pump inhibitor)         | -                                  |
| Arotin                    | 4                     | Paroxetine   | selective serotonin reuptake inhibitor (SSRI) antidepressant     | -                                  |
| Aspirina                  | 100                   | Acetylsalicylic acid   | Analgesic / Antipyretic  | -                                  |
| Atlansil                  | 40                    | Amiodarone   | Antiarrhythmic   | 12 [1.25]                          |
| Cataflam                  | 10                    | Diclofenac potassium   | Non-steroidal anti-inflammatory drug (NSAID)                     | 20 [2.5]                           |
| Celebra                   | 40                    | Celecoxib  | NSAID  | -                                  |
| Clorana                   | 5                     | Hydrochlorothiazide  | Diuretic   | -                                  |
| Coristina R               |                       | Acetylsalicylic acid, Pheniramine maleate, Phenylephrine hydrochloride, Caffeine | Analgesic / Antipyretic / Antihistaminic / Decongestant.         | -                                  |
| Diclofenaco*              | 10                    | Diclofenac potassium   | NSAID  | 18 [0.16]                          |
| Diclofenaco*              | 10                    | Diclofenac potassium   | NSAID  | 14 [0.32]                          |
| Dorflex                   | 10                    | Orphenadrine citrate, Metamizole sodium, Caffeine                                | Analgesic  | -                                  |
| Doxuran                   | 0.8                   | Doxazosin  | Antihypertensive / Treatment of prostatic hyperplasia            | -                                  |
| Dramin                    | 20                    | Dimenhydrinate   | Antiemetic   | -                                  |
| Fenergan                  | 5                     | Promethazine hydrochloride   | Antihistaminic   | -                                  |
| Fluimucil                 | 8                     | Acetylcysteine   | Mucolytic agent  | -                                  |
| Flutec                    | 30                    | Fluconazole  | Antifungal   | -                                  |
| Higroton                  | 10                    | Chlorthalidone   | Thiazide diuretic  | -                                  |
| Omeprazole                | 4                     | Omeprazole   | Proton pump inhibitor  | -                                  |
| Neosaldina                | 60                    | Metamizole sodium, isometheptene mucate, caffeine                                | Analgesic  | -                                  |
| Nimesulida                | 20                    | Nimesulide   | NSAID  | -                                  |
| Nisulid                   | 20                    | Nimesulide   | NSAID  | -                                  |
| Redulip                   | 3                     | Sibutramine hydrochloride monohydrate  | Anorexiant / Sympathomimetic                                     | -                                  |
| Seki                      | 3.54                  | Cloperastine   | Antitussives (central and periferic mode of action)              | -                                  |
| Spidufen                  | 120                   | Ibuprofen arginine   | NSAID  | 20 [7.5]                           |
| Superhist                 | 80                    | Acetylsalicylic acid, Pheniramine maleate, Phenylephrine hydrochloride           | Analgesic / Antipyretic / Antihistaminic / Decongestant          | -                                  |
| Tylenol                   | 150                   | Paracetamol  | Analgesic / Antipyretic  | -                                  |
| Tylox                     | 6                     | Paracetamol, Codeine   | Analgesic  | -                                  |
| Urotrobel                 | 80                    | Norfloxacin  | Antibiotic   | 10 [2.5]                           |
| Yasmin                    | 0.6                   | Ethinylestradiol, drospirenone   | Contraceptive  | -                                  |
| Zestril                   | 4                     | Lisinopril   | Antihypertensive (Angiotensin-converting enzyme (ACE) inhibitor) | -                                  |
| Zocor                     | 2                     | Simvastatin  | Hypolipidemic  | -                                  |
| Zyrtec                    | 2                     | Cetirizine hydrochloride   | Antihistaminic   | -                                  |

\*Produced by two different companies. - = no inhibition.



**Table 2** - Effect of antibiotics on the growth of *Lactococcus lactis* subsp *lactis* DF04Mi, presented as diameter of the inhibition zone.

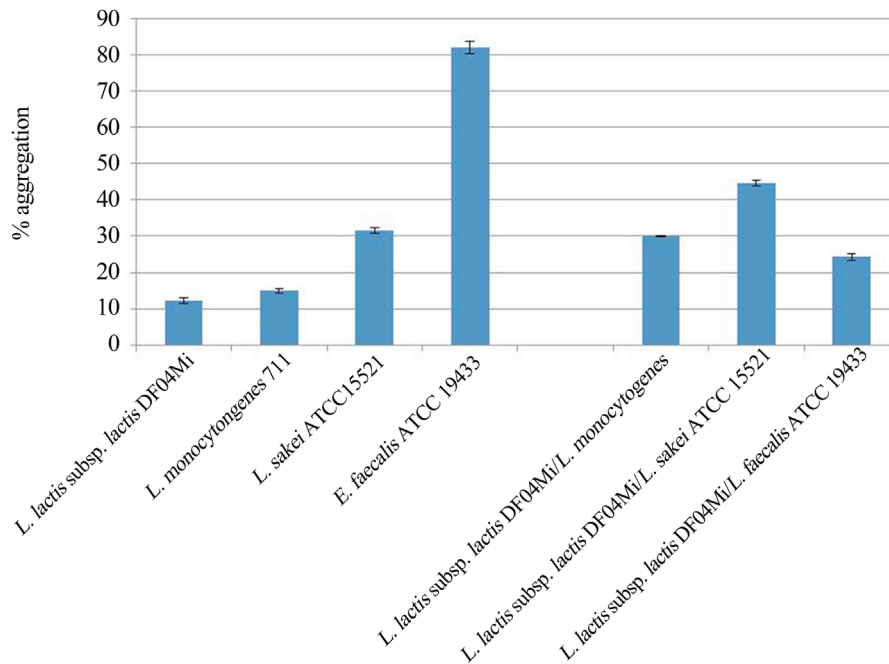
| Antibiotic ( $\mu\text{g}/\text{disk}$ ) | Classification (mode of action)   | Inhibition zone (mm) |
|--|---|----------------------|
| Amicacin (30)                            | Aminoglycoside (inhibits protein synthesis)   | 13                   |
| Ampicilin (10)                           | Penicillin / $\beta$ -Lactam (interferes with bacteria cell wall synthesis)                                   | 30                   |
| Bacitracin (10)                          | Cyclic polypeptide (inhibits bacteria cell wall synthesis)  | 21                   |
| Cefazolin (30)                           | 1 <sup>st</sup> generation cephalosporin / $\beta$ -Lactam (interferes with bacteria cell wall synthesis)     | 24                   |
| Cefepime (30)                            | 4 <sup>th</sup> generation cephalosporin / $\beta$ -Lactam (interferes with bacteria cell wall synthesis)     | 34                   |
| Cefotaxim (30)                           | 2 <sup>nd</sup> generation cephalosporin / $\beta$ -Lactam (interferes with bacteria cell wall synthesis)     | 33                   |
| Ceftazidim (30)                          | 3 <sup>th</sup> generation cephalosporin / $\beta$ -Lactam (interferes with bacteria cell wall synthesis)     | 23                   |
| Ceftriaxon (30)                          | 3 <sup>th</sup> generation cephalosporin / $\beta$ -Lactam (interferes with bacteria cell wall synthesis)     | 30                   |
| Cefuroxim (30)                           | $\beta$ -Lactam (interferes with bacteria cell wall synthesis)  | 30                   |
| Ciprofloxacin (5)                        | Fluoroquinolone (inhibits the bacterial topoisomerase II)   | 18                   |
| Clindamicin (2)                          | Licosamide (inhibits protein synthesis)   | 25                   |
| Cloranfenicol (30)                       | Chloramphenicol (prevents peptide bond formation - inhibits protein synthesis)                                | 28                   |
| Erytromicin (15)                         | Macrolide (inhibits protein synthesis)  | 28                   |
| Furazolidon (10)                         | Antibiotic / antiparasitic  | 21                   |
| Gentamicin (10)                          | Aminoglycoside (inhibits protein synthesis)   | 15                   |
| Imipenem (10)                            | Carbapenem / $\beta$ -Lactam (interferes with bacteria cell wall synthesis)                                   | 32                   |
| Kanamycin (30)                           | Aminoglycoside (inhibits protein synthesis)   | 17                   |
| Metronidazol (50)                        | Nitroimidazole antibiotic (acts on DNA of microorganisms ameba, and protozoa)                                 | 0                    |
| Nalidixic acid (30)                      | Syntetic quinolone antibiotic (acts on DNA gyrase)  | 0                    |
| Neomicin (30)                            | Aminoglycoside (inhibits protein synthesis)   | 15                   |
| Nitrofurantoin (300)                     | Nitrofurane derivative (nucleic acid inhibitor)   | 22                   |
| Ofloxacin (5)                            | Licosamide (inhibits protein synthesis)   | 22                   |
| Oxacilin (1)                             | $\beta$ -Lactam (interferes with bacteria cell wall synthesis)  | 15                   |
| Penicillin G (10)                        | Penicillin / $\beta$ -Lactam (interferes with bacteria cell wall synthesis)                                   | 30                   |
| Rifampicin (30)                          | Semisynthetic compound derived from <i>Amycolatopsis rifamycinica</i> (inhibits DNA-dependent RNA polymerase) | 17                   |
| Rifampicin (5)                           | Semisynthetic compound derived from <i>Amycolatopsis rifamycinica</i> (inhibits DNA-dependent RNA polymerase) | 16                   |
| Streptomycin (10)                        | Aminoglycoside (inhibits protein synthesis)   | 20                   |
| Tetraciclín (30)                         | Tetraciclín (inhibits protein synthesis)  | 35                   |
| Tobramicin (10)                          | Aminoglycoside (inhibits protein synthesis)   | 15                   |
| Trimetoprim (5)                          | Trimetoprim (inhibits folate syntesis)  | 0                    |
| Vancomycin (30)                          | Glycopeptide (inhibits bacteria cell wall synthesis)  | 21                   |

(Todorov *et al.*, 2008). Strain *Lb. pentosus* ST712BZ, characterised with a relatively low hydrophobicity (38%) adhered to HT-29 cells at 63%. Only 32% adherence was recorded for *Lb. rhamnosus* GG (Todorov *et al.*, 2008). Although hydrophobicity may assist in adhesion, it is not a prerequisite for strong adherence to human intestinal cells.

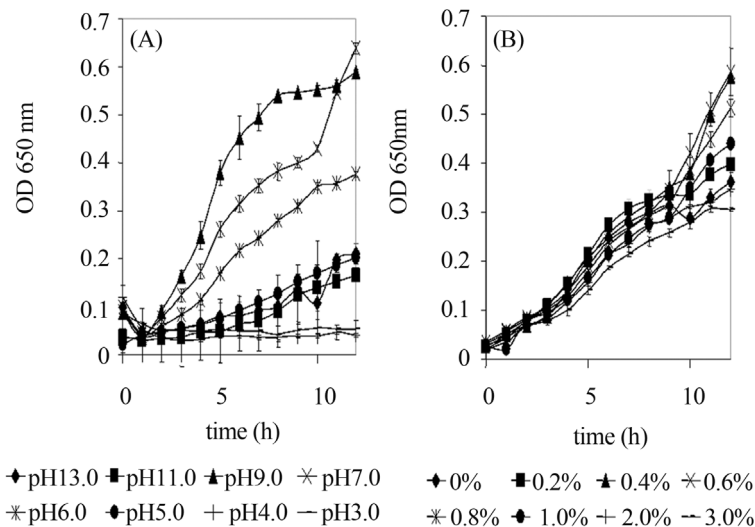
The effect of drugs on the survival of probiotic strains is important for patients being treated for chronic diseases and taking probiotics. As shown in Table 1, *Lc. lactis* DF04Mi was inhibited by antiarrhythmic drugs containing amiodarone, by non-steroidal anti-inflammatory drugs containing potassium diclofenac or ibuprofen arginine, and by the two tested antibiotics (amoxyl and urotrobel). The

interference of anti-inflammatory drugs containing diclofenac on the viability of LAB detected in this study was also reported for other bacteriocinogenic LAB such as *Lb. plantarum* ST8KF and ST341LD, *E. faecium* ST311LD and *Le. mesenteroides* subsp. *mesenteroides* ST33LD (Todorov and Dicks, 2008), *Lc. lactis* subsp. *lactis* HV219 (Todorov *et al.*, 2007), *Lb. casei* Shirota and *Lb. casei* LC01 (Carvalho *et al.*, 2009) and *Lb. casei* Shirota (Botes *et al.*, 2008).

It should be pointed out that the activity of these medicaments against potential probiotic bacteria depends on the amount of the active compound that reaches the GIT, and that the correct evaluation of possible interactions be-



**Figure 1** - Auto-aggregation of *Lactococcus lactis* subsp. *lactis* DF04Mi, *Listeria monocytogenes* 711, *Lactobacillus sakei* ATCC 15521 and *Enterococcus faecalis* ATCC 19433 and co-aggregation of *Lactococcus lactis* subsp. *lactis* DF04Mi with *Listeria monocytogenes* 711, *Lactobacillus sakei* ATCC 15521 or *Enterococcus faecalis* ATCC 19433.



**Figure 2** - Growth of *Lactococcus lactis* subsp. *lactis* DF04Mi in MRS containing increasing concentrations (0 to 3%) of oxbile (B) and at different pH (3 to 13) (A).

tween them depends on the determination of MIC of these medicaments (Todorov *et al.*, 2007, 2008). Due to their long-term application, drugs may accumulate in the GIT and MIC be reached, affecting the viability of the probiotic cultures.

The non-steroidal anti-inflammatory drugs were the medicaments that affected *Lc. lactis* DF04Mi more significantly and potassium diclofenac presented MIC of 0.16 mg/mL and spidufen of 7.5 mg/mL. Considering that the recommended daily doses of these two drugs are 100-150

mg and 600 mg respectively, they will hardly affect the survival of *Lc. lactis* DF04Mi. Similar results were reported in other studies run with other probiotic LAB and gastrointestinal tract related bacteria (Botes *et al.*, 2008; Todorov and Dicks, 2008; Carvalho *et al.*, 2009; Todorov *et al.*, 2007, 2008).

Except for metronidasole, nalidixic acid and trimetoprim, all the antibiotics tested (Table 2) in this study inhibited the growth of *Lc. lactis* DF04Mi to some extent. However, it is important to point out that nalidixic acid is an

antibiotic mostly active against Gram-negative bacteria. Resistance of LAB to antibiotics is a controversial subject. It is important to keep in mind that antibiotic-resistant probiotic LAB may be responsible for horizontal transfer of resistance genes to other bacteria present in the human GIT (Dicks *et al.*, 2011). Resistance may be inherent to a bacterial genus or species, but may also be acquired through exchange of genetic material, mutations or incorporation of new genes (Ammor *et al.*, 2007). Teuber (1999) and Salyers *et al.* (2004) suggested that starter cultures and probiotics may serve as vectors in the transfer of antibiotic resistant genes. Such transfer was documented in other bacterial groups by Levy and Marshall (2004) and Salyers *et al.* (2004).

Results gathered in this study indicate the potential application of *Lc. lactis* DF04Mi isolated from goat milk for manufacturing of novel and functional goat milk products, with increased safety as it is capable to produce bacteriocins with antilisterial activity and presents several features that suggest probiotic activity.

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