

Selection and characterization of the probiotic potential of some lactic acid bacteria isolated from infant feces

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Abstract

Despite the important progress made in the field of probiotics, prediction of the precise mechanism of action is difficult, due, on one side to the complex interactions that can occur between food, microbes and host cells and on the other side, to the fact that the probiotic effect is strain dependent. Most of probiotic products are containing lactic acid bacteria (LAB), microorganisms considered as commensal and harmless for the host organisms.

The purpose of the present work was to characterize the probiotic effects of three *Lactococcus lactis* strains isolated from breast fed infant feces (resistance to pH variation and taurocholic acid sodium salt concentrations, antimicrobial activity, ability to metabolize lactose and cholesterol, the ability to adhere to eukaryotic cells and competition with enteropathogenic *Salmonella enteritidis*, *Shigella flexnerii* and enteropathogenic *Escherichia coli*, immunomodulatory effect).

The tested strains showed an increased resistance to a wide pH range of 3.0 to 8.0, as well as to thauroglicolate concentrations, from 0.5 to 3%, these features representing great advantages for the survival of these bacteria, once introduced in the gastrointestinal tract. Our strains reduced the serum cholesterol with an average of 45%. The three strains lacked mutual inhibition and high inhibitory activity of pathogenic or potentially pathogenic strains growth, demonstrating their potential use in the treatment of pediatric gastro-intestinal disorders, as an alternative or in association with antibiotics. The immunomodulatory studies demonstrated that the probiotic culture fractions are modulating the expression of the most important cytokines in the development of the anti-infectious immunity against enteric pathogens, expressed by the stimulation of TNF-alpha and INF-gamma pro-inflammatory cytokines and the inhibition of IL-6 and IL-8 cytokines, known to be implicated in the occurrence of lesional effects upon the infected host.

Keywords: probiotics, LAB, antimicrobial activity, strain resistance, cytokines

Introduction

The last decades knowledge boom in microbial biodiversity, in the mechanisms involved in microbial interactions with host cells and in immunology has led to the identification of molecules or systems that can drive health or disease [9, 22].

In the recent years, based on *in vitro* and *in vivo* studies, many probiotic products have been developed in order to improve the organism's health [25]. Probiotics are "viable nonpathogenic microorganisms that, when supplied in adequate amounts, confer health benefits to hosts" and they are representing a possible alternative to conventional treatment [19]. The importance of these microorganisms on human and animal health was underlined by international commissions and organizations [18]. In May 2002 an expert panel commissioned by FAO (The Food and Agriculture Organization of the United Nations) and

WHO (World Health Organization) published a Guidelines for the evaluation of probiotics for animal and human use (Report of FAO/WHO, May 2002) [28, 29].

The scientific results obtained up to now demonstrate that the probiotic bio-therapeutic effect is multifactorial, including: maintaining the intestinal microbial balance in human and animal hosts [11, 15, 23, 27], ameliorating lactose intolerance [10], suppression of cancer [12], improved digestion [6], reductions in serum cholesterol levels [3, 4, 30, 10] and modulation of the host immune system [20, 8, 26].

The purpose of the present work was the isolation from specific niches of some indigenous lactic acid bacteria, followed by strains identification and characterization of probiotic effects (resistance to pH variation and taurocholic acid sodium salt concentrations, antimicrobial activity, ability to metabolize lactose and cholesterol, the ability to adhere to eukaryotic cells and competition with pathogenic strains, immunomodulatory activity).

Material and Methods

Microbial strains and culture conditions

Three LAB strains have been freshly isolated from human baby feces. 1 g of sample was thoroughly mixed with 9 mL of 0.9% NaCl, serial dilutions being further prepared and from each dilution, an amount of 0.1 mL was plated on Man, Rogosa and Sharpe (MRS) [17] broth supplemented with 1% calcium carbonate, pH 6.5 and incubated for 24 - 48 hours at 37°C under microaerophilic conditions. The selected colonies were submitted to three successive sub-cloning passages from a single colony for purification purposes. The biochemical identification of LAB strains was made on the basis of phenotypical features using conventional tests (Gram staining, catalase reaction, acid production) and Biolog MicroLog System, release 4.2 (Biolog Inc., Hayward, USA). The three strains were stored at -70°C in appropriate medium represented by MRS supplemented with 20% glycerol, in the Microbial Collection of Center for Research, Education and Consulting in Microbiology, Genetics and Biotechnology – MICROGEN (under the acronym CMGB no. 30, 31, 32).

Indicator strains used for the antimicrobial activity assay were represented by *Listeria innocua* CMGB 218 and *Bacillus cereus* CMGB 215. The strains were cultivated in Brain Heart Infusion (BHI) liquid broth at 37°C, under static conditions for *Listeria* and with 150 rpm shaking for *Bacillus cereus* CMGB 15.

Microbial strains used for the adherence and invasion studies were *Salmonella enteritidis* 361, *Shigella flexnerii* 29833 and *Escherichia coli* O126:B16 isolated from acute diarrhea. Pathogenic microbial strains were cultivated in Luria Bertani (LB) liquid broth at 37°C with 150 rpm shaking.

All strains were stored at -70°C in specific cultivation broth for each strain supplemented with 20% glycerol.

pH resistance of lactic acid bacteria

Resistance of lactic acid bacteria strains to different pH values (1.0; 2.0; 3.0; 4.0; 5.0, 6.0, 7.0; 8.0 and 9.0) was assayed. 1 ml of over night culture (10^9 CFU/ml) was centrifuged (5 minutes at 7000 rpm), cellular sediment was re-suspended in 1ml sterile distilled water with mentioned pH values and then incubated for 2h at 37°C. The number of viable cells was determined by plating 10-fold serial dilutions on MRS agar medium.

Resistance to various taurocholic acid sodium salt concentrations

In order to determine resistance to taurocholic acid sodium salt, 1ml of overnight culture (10^9 CFU/ml) was centrifuged (5 minutes at 7000 rpm) and sediment was resuspended in 1ml phosphate buffer solution (pH 7.4) with various taurocholic acid sodium salt concentrations (0.5%, 1%, 1.5%, 2%, 3% and 4%). The samples were incubated for 2h at

37°C. The number of viable cells was determined by plating 10-fold serial dilutions on MRS agar medium.

Antibiotic susceptibility testing was performed using the standardized disc diffusion method (following CLSI recommendations). The following Oxoid discs were used: gentamicin (GM 10µg), vancomycin (VA 30µg), erythromycin (E 30µg), imipenem (IMP 30µg), neomycin (NE 30µg), chloramphenicol (C 30µg), amoxicillin (AMX 25µg), cefoperazone (CFP 30µg), kanamycin (K 30µg), and aztreonam (ATM 30µg). *Enterococcus faecalis* ATCC 29212 and *Staphylococcus aureus* ATCC 29213 were used as reference strains.

Assimilation of cholesterol

Lactic acid bacteria strains were cultivated in 10 ml MRS broth (1% vol: vol) and respectively in 10ml MRS supplemented with 50mg/ml water-soluble form of cholesterol (Sigma - Aldrich). After 24h of growth at 37°C, bacterial cells were removed by centrifugation (10000 rpm, 10 min, 4°C) and supernatants were then assayed for their cholesterol content. The total cholesterol content of the samples was determined by the enzymatic assay using DIALAB kit (Vienna, Austria). In order to estimate cholesterol concentrations absorption values at 500nm (Spectrophotometer ULTROSPEC 3000) were introduced in following equation:

$$\text{Cholesterol (mg/dl)} = (\text{A sample} / \text{A control}) \times \text{control concentration}$$

Lactose assimilation

The ability of lactic acid bacteria strains to metabolize lactose was tested by strains cultivation in MRS (seeding ratio 1:100 vol: vol) with 2% lactose as carbon source for 24h at 37°C. Cultures obtained on same conditions but on MRS with 2% glucose as carbon source were used as control. Strains growth was estimated by viable cell counts after plating 10-fold serial dilutions on MRS agar medium.

Inhibitory compounds assay

Newly isolated LAB strains were screened for the production of inhibitory compounds by testing cell-free culture supernatants against some pathogenic and potentially pathogenic strains: *Salmonella enteritidis* 361, *Shigella flexnerii* 29833, *Escherichia coli* O126:B16, *Listeria innocua* CMGB 218 and *Bacillus cereus* CMGB 215. Antimicrobial activity of each *Lactococcus* strain against the other two was assessed. Cell-free culture supernatants were obtained by centrifugation (10.000 g, 10 min) of 1.5 ml of culture. Antimicrobial activity was detected by the agar spot assay [13]. 100µl of the indicator cell culture (OD₆₀₀ 0.4 – 0.5) were added to 3.5 ml of overlay LB agar medium (0.5% agar), and to MRS respectively. Plates were incubated for 16 to 20h at the appropriate growth temperature. Growth inhibition was visually detected by observing clear inhibition zones or reduction of growth on the top of the agar (agar spot assay).

Adherence and invasion capacity to the cellular substrate represented by HeLa cells (Cravioto's adapted method)

For this purpose, HeLa cells were routinely grown in Eagle's minimal essential medium (Eagle MEM) supplemented with 10% heat-inactivated (30 min at 56°C) fetal bovine serum (Gibco BRL), 0.1 mM nonessential amino acids (Gibco BRL), and 0.5 ml of gentamicin (50 µg/ml) (Gibco BRL) and incubated in a 5% CO₂ humidified atmosphere, at 37°C for 24 hrs [16].

HeLa cell monolayers grown in 6 multi-well plastic plates were used at 80-100% confluence. Bacterial strains from an overnight culture on 2% nutrient agar were diluted at 10⁷ CFU/ml in Eagle MEM with no antibiotics. The HeLa cell monolayers were washed 3 times with Phosphate Buffered Saline (PBS) and 2 ml from the bacterial suspension were inoculated in each well. The inoculated plates were incubated for 3 hrs at 37°C. After incubation, the

monolayers were washed 3 times with PBS, briefly fixed in cold ethanol (3 minutes), stained with Giemsa stain solution (1:20) (Merck, Darmstadt, Germany) for 30 min. The plates were washed, dried at room temperature overnight, examined microscopically (magnification, $\times 2500$) with the immersion objective (IO) and photographed with a Contax camera (Company, City, Country) adapted for Zeiss (Axiolab 459306) microscope (Zeiss, City, Country).

For the quantitative assay of adhesion and invasion capacity, the infection step was performed in duplicates for each strain, and after 3 hrs incubation of the HeLa monolayer in the presence of microbial strains, the first well plates were washed four times in PBS, the cells were permeabilized by Triton X 1% (Sigma) and incubated for 5 minutes at 37°C for the release of intracellular invasive bacteria. Thereafter, serial ten-fold dilutions in saline solution were performed and 20 μ l from each dilution was spotted in triplicates on solid media; in the second plate, after 2 hrs of incubation the monolayer was washed 4 times in PBS and 1 ml of 100 mg/ml gentamycin solution was added; the plates were further incubated for one hour, in order to kill all adherent extra-cellular bacteria. Thereafter, the second plate was treated as the first one.

It is to be mentioned that in the case of lactic acid bacteria, the adherence capacity was investigated in three variants: 1) integral mid-logarithmic phase cultures; 2) microbial suspensions obtained from the washed cell sediment (CS); 3) heat inactivated cell suspensions (CI) (for 30 minutes at 100°C).

In order to investigate how the probiotic strains influence the adherence and invasion of the HeLa cells by the pathogenic strains, the adherence and invasion assays were performed following the steps mentioned above, the monolayer infection being done in the presence of equal volumes of different LAB culture fractions (i.e. integral cultures, washed cell sediments and heat-inactivated cell suspension).

Immunomodulatory activity of LAB strains

Immunomodulatory activity of LAB strains was tested using probiotic culture fractions (supernatant and heat inactivated bacterial cells). The levels of the main soluble pro- and anti-inflammatory cytokines, i.e. IL-1, IL-2, IL-6, IL-8, IL-10, TNF α and INF- γ induced by different fractions of probiotic cultures were assessed by ELISA (Pierce Endogen kit) according to manufacturer's indications.

Results

Isolated LAB strains proved to be microaerophilic, Gram-positive, catalase – negative, non-spore-forming cocobacilli in pairs or in short chains. They produced lactic acid as major fermentation product from glucose. Selected clones were identified using the Microlog System 4.2 and the results were automatically interpreted by computer software Biolog420. All three strains presented similarity between 0.642 and 0.762 with *Lactococcus lactis ssp lactis*. Identified strains were introduced in the Culture Collection of the Center for Research, Training and Consultancy in Microbiology, Genetics and Biotechnology (MICROGEN) with acronyms CMGB 30, CMGB 31 and CMGB 32.

pH resistance of lactic acid bacteria

In order to establish the capacity of LAB strains to survive in the gastrointestinal tract *Lactococcus lactis* strains were tested for their sensitivity to different pH values. The results showed that resistance to acid, respectively alkaline pH is strain dependent. Maximum growth ratio for all strains was obtained at pH 6.0 and 7.0. The most resistant strain to acid pH range between 1.0 and 3.0 was *Lactococcus lactis ssp lactis* CMGB 32 (viability between 4.85% and 35.46%). For the pH range 3.0 – 5.0 an increased resistance and maximum viability rates were

observed for *Lactococcus lactis ssp. lactis* CMGB 30 (75.24% at pH 5.0). At pH 8.0 the strain viability was strain dependent and viability values were between 62.5% to 90% (Figure 1).

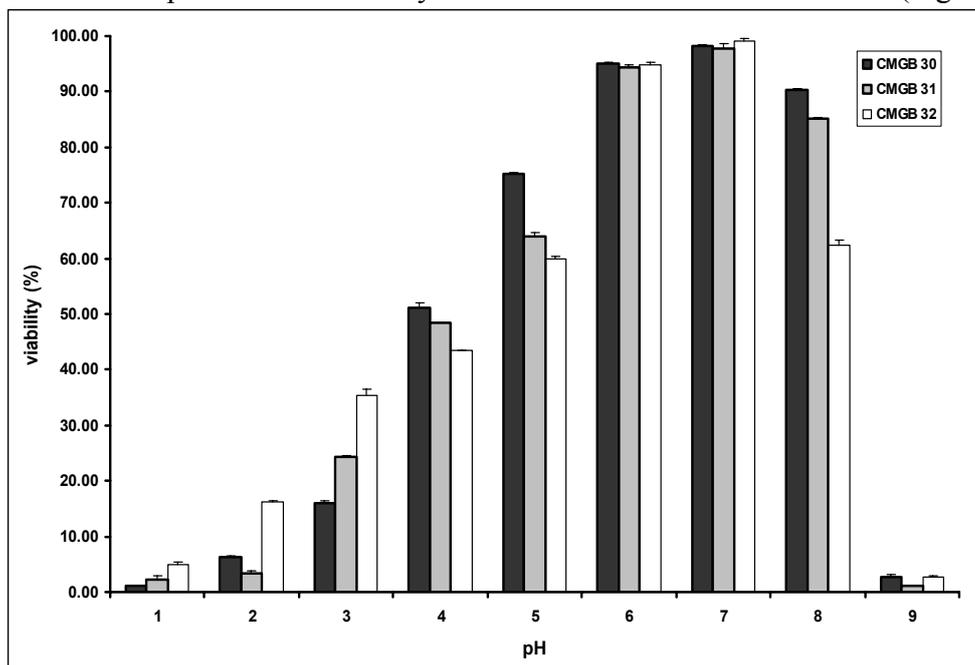


Figure 1. Viability of lactic acid bacteria strains at different pH values. Error bars indicate standard deviations.

At extreme pH values, namely acid (pH 1.0) and alkaline (pH 9.0), all strains showed increased sensitivity (viability between 1.05 and 4.89). The optimum growth for all strains was obtained at pH 6 and 7.

Resistance to various taurocholic acid sodium salt concentrations

The three strains were resistant to all tested taurocholic acid sodium salt concentrations (i.e. 0.5%, 1%, 1.5%, 2%, 3% and 4%) (Figure 2), the greatest resistance being obtained for the strain *Lactococcus lactis ssp lactis* CMGB 30 and *Lactococcus lactis ssp lactis* CMGB 31.

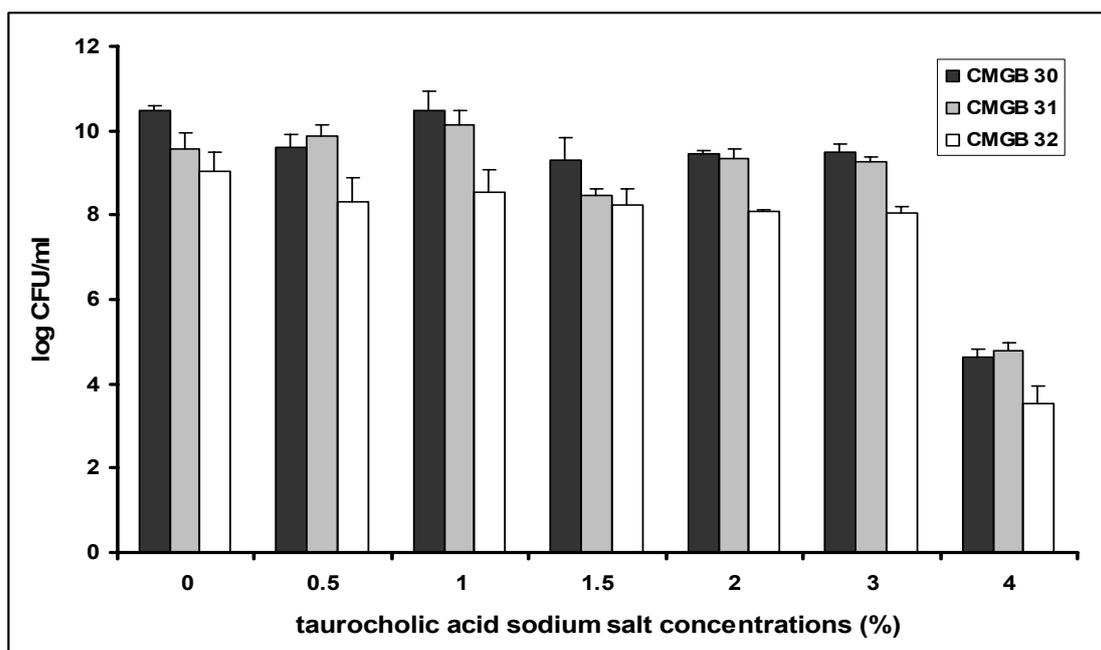


Figure 2. Resistance to taurocholic acid sodium salt concentrations. Error bars indicate standard deviations.

Susceptibility to antibiotics

Our results have demonstrated that all strains exhibited natural resistance to neomycin, gentamicin, erythromycin, cloramfenicol, kanamycin, pefloxacin and aztreonam and were susceptible to imipinem, amoxicillin, cefoperazone and vancomycin (Table no. 1).

Table 1. Susceptibility of *Lactococcus lactis* ssp. *lactis* to different antimicrobial substances

ANTIMICROBIAL COMPOUNDS	Neomicin	Imipinem	Gentamicin	Cloramfphnic	Ceftazidime	Amoxicillin	Cefoperazone	Pefloxacin	Kanamycin	Aztreonam	Erythromycin	Vancomycin
<i>Lactococcus lactis</i> ssp. <i>lactis</i> CMGB 30	R	S	R	R	S	S	I	I	R	R	R	S
<i>Lactococcus lactis</i> ssp. <i>lactis</i> CMGB 31	R	S	R	R	S	S	I	I	R	R	R	S
<i>Lactococcus lactis</i> ssp. <i>lactis</i> CMGB 32	R	S	R	R	S	S	S	R	R	R	R	S

Cholesterol reduction

The results shown in Figure 3 demonstrated that all strains have the potential to decrease the cholesterol concentration by an average of 45%.

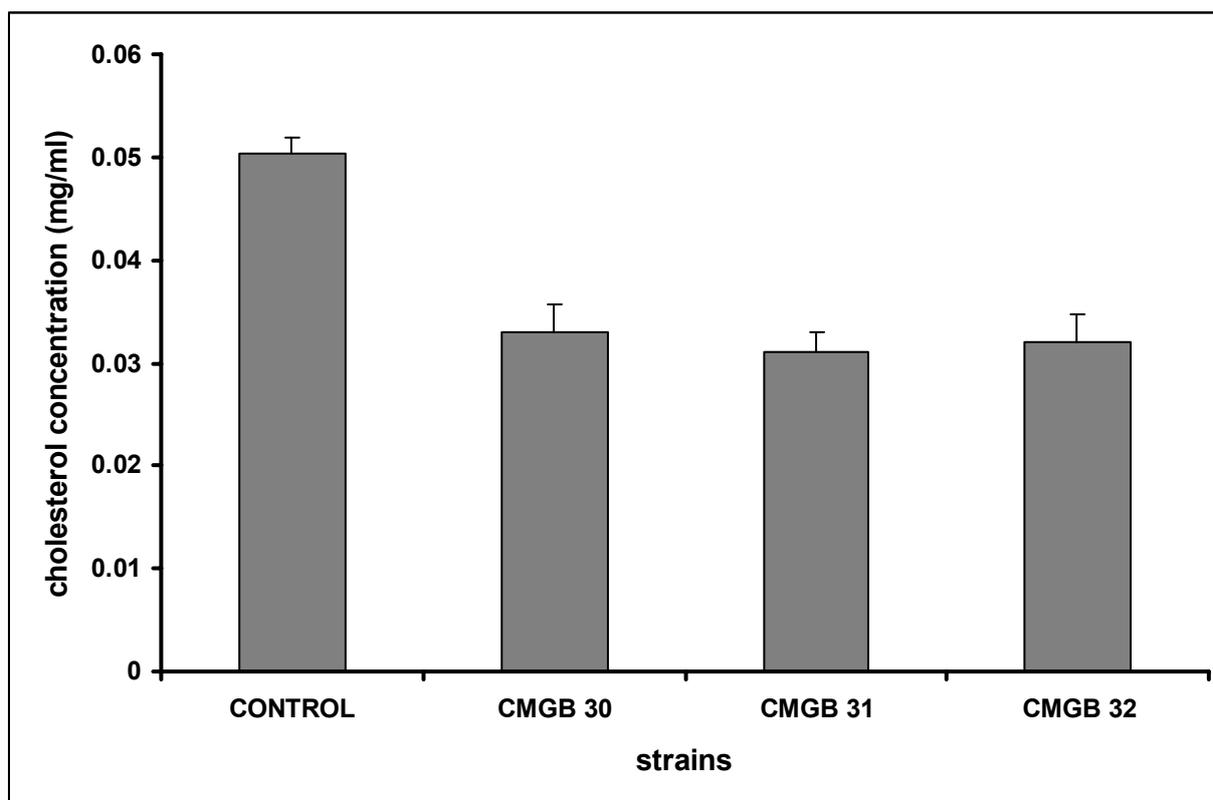


Figure 3. Cholesterol reduction by lactic acid bacteria Error bars indicates standard deviations.

Lactose metabolism

As one of the most frequent problems encountered in newborns pathology is lactose intolerance, the LAB strains were also tested for their ability to metabolize lactose. As

shown in Figure 4 all LAB strains proved to metabolize lactose. *Lactococcus lactis ssp lactis* CMGB 31 and CMGB 32 grew better on MRS with glucose instead *Lactococcus lactis ssp lactis* CMGB 30 showing maximum biomass accumulation (9×10^8 CFU/ml) on culture medium supplemented with lactose.

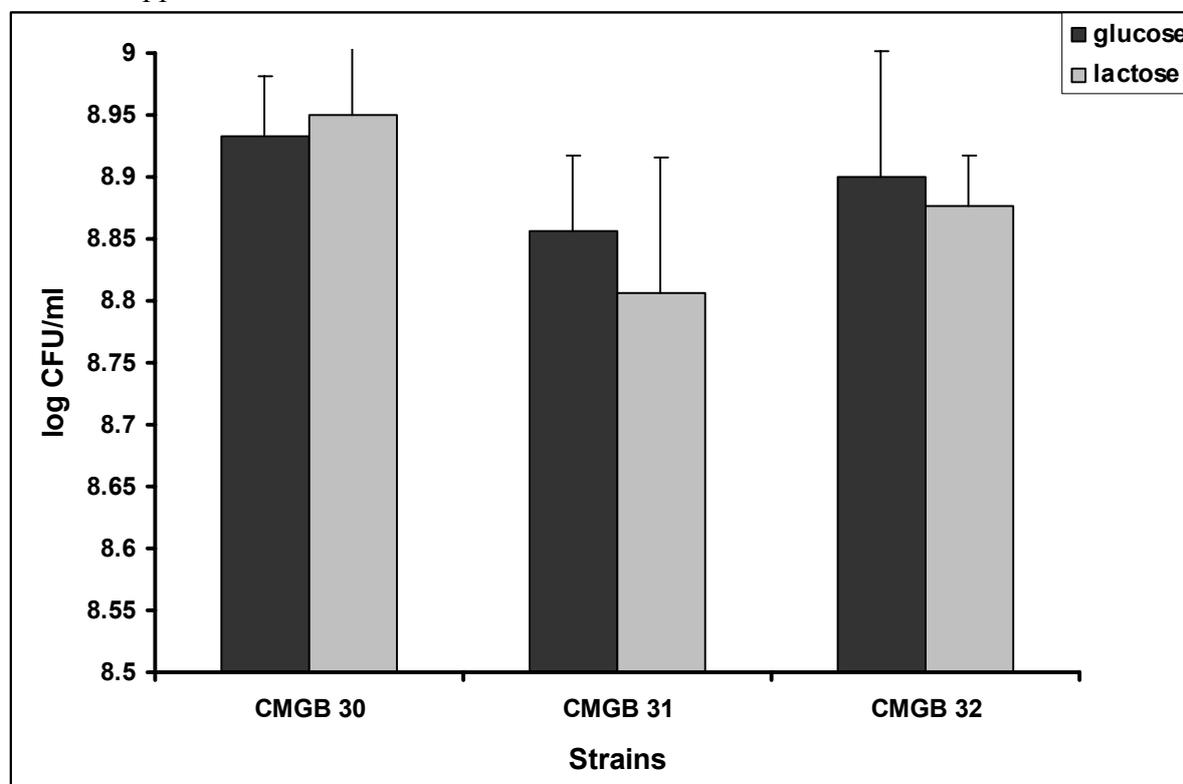


Figure 4. Lactic acid bacteria capacity to metabolize lactose. Error bars indicate standard deviations

Antimicrobial activity

The three strains exhibited a broad spectrum antimicrobial activity as shown in Table 2. It is to be noted that *Lc. lactis ssp lactis* CMGB 31 exhibited antimicrobial activity against all pathogenic or potentially pathogenic strains used in this study. The tested strains did not exhibit any intra-species inhibitory activity.

Table 2. Antimicrobial activity of LAB strains towards different pathogenic bacterial strains.

	<i>S. enteritidis</i>	<i>S. flexnerii</i>	<i>E. coli</i>	<i>L. innocua</i>	<i>B. cereus</i>	CMGB 30	CMGB 31	CMGB 32
CMGB 30	++	+++	-	+	+	-	-	-
CMGB 31	++	++	+	+	+	-	-	-
CMGB 32	++	+	-	+	+	-	-	-

Adherence and invasion capacity of pathogenic strains in presence of LAB fraction to the cellular substrate represented by HeLa cells

Concerning the ability of LAB fractions to interfere with the ability of pathogenic strains to colonize the cellular substratum represented by HeLa cells, the total inhibition of the adherence ability was observed in the presence of LAB washed cell sediments as well as in the presence of heat-inactivated cell suspension, the inhibitory effect being in the last case much less evident on *E. coli* and *Salmonella enteritidis* (Fig. 5-7).

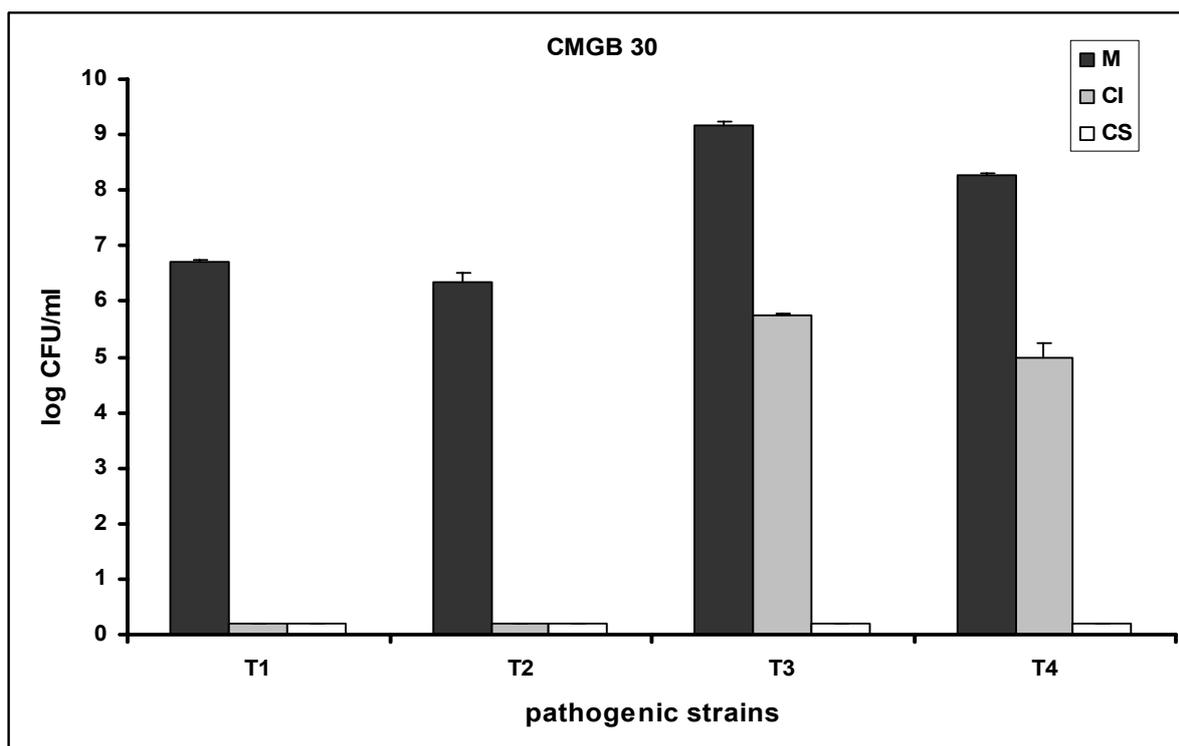


Figure 5. The inhibitory activity exhibited by *Lc. lactis* ssp. *lactis* CMGB 30 probiotic culture fractions against the adherence and invasion of pathogenic strains (T1 – *Shigella flexnerii* 29833; T2 - *Shigella flexnerii* 29834; T3 – *Escherichia coli* enteropatogen - EPEC; T4 – *Salmonella enteritidis* 361; M- control)

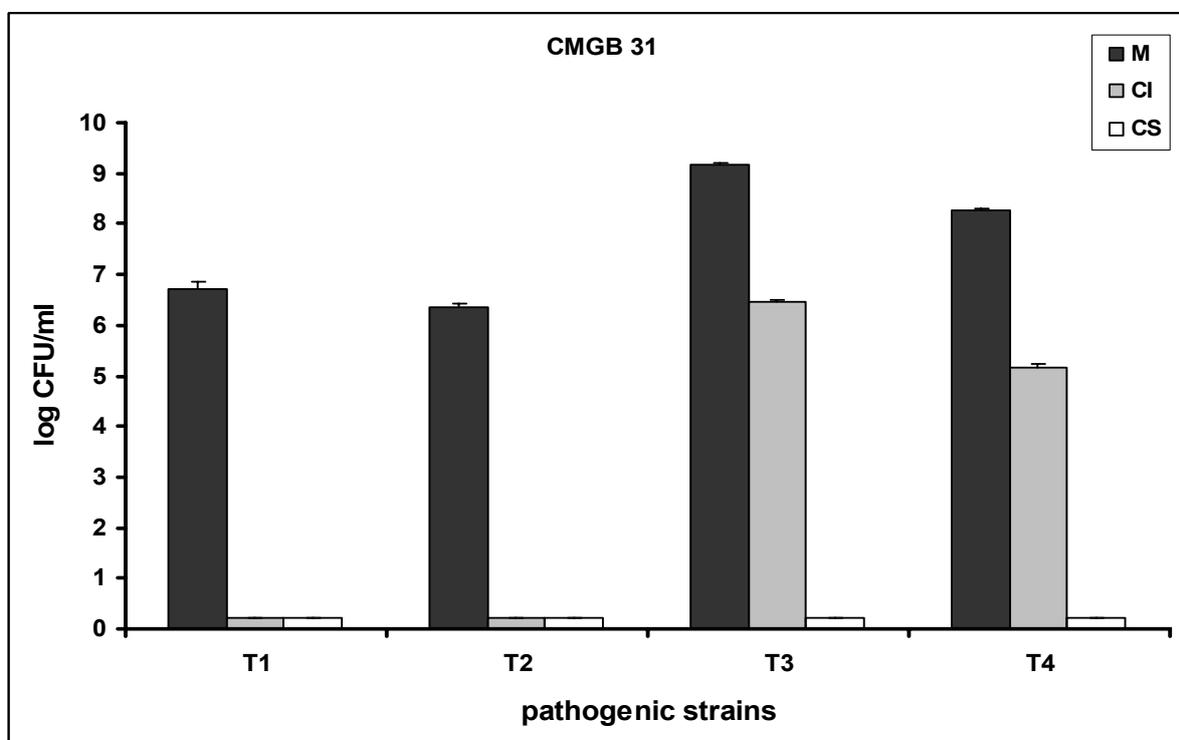


Figure 6. The inhibitory activity exhibited by *Lc. lactis* ssp. *lactis* CMGB 31 probiotic culture fractions against the adherence and invasion of pathogenic strains (T1 – *Shigella flexnerii* 29833; T2 - *Shigella flexnerii* 29834; T3 – enteropatogenic *Escherichia coli* - EPEC; T4 – *Salmonella enteritidis* 361; M- control)

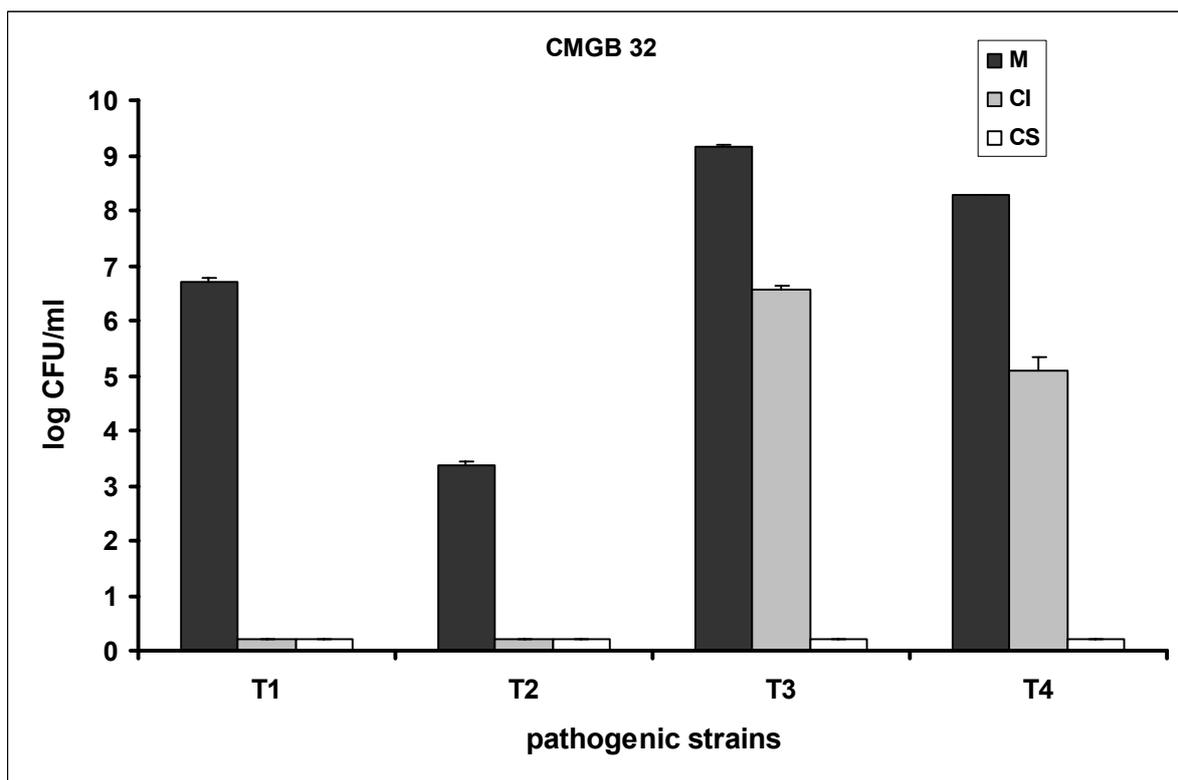


Figure 7. The inhibitory activity exhibited by *Lc. lactis ssp. lactis* CMGB 32 probiotic culture fractions against the adherence and invasion of pathogenic strains (T1 – *Shigella flexnerii* 29833; T2 - *Shigella flexnerii* 29834; T3 – enteropathogenic *Escherichia coli* - EPEC; T4 – *Salmonella enteritidis* 361; M- control)

Immunomodulatory activity of LAB strains

In order to assess the potential immunomodulatory activity of LAB strains, as well as to correlate the obtained results with a certain probiotic culture fraction (supernatant and bacterial sediment) the levels of the main soluble pro- and anti-inflammatory cytokines, i.e. IL-1, IL-2, IL-6, IL-8, IL-10, TNF –alpha and INF-gamma were quantified.

The expression level of each cytokine was strain and tested fraction dependent. However, all fractions of LAB strains generally inhibited the expression of IL-6 and IL-8. The level of IL-2 was affected only by CMGB 32 fractions which decreased its expression, while the level of IFN was slightly increased by CMGB 31 fractions. The majority of tested fractions also stimulated with variable intensities the expression of TNF (Table 3).

Table 3. The level of interleukines induced in Hela cells (quantified by ELISA and expressed in pg/mL) in the presence of different fractions (supernatants –SN and respectively heat-inactivated bacterial cells - CB) of *Lactococcus lactis ssp. lactis* CMGB 30, 31, 32 strains

		IL-2	IL-6	IL-8	IFN	TNF
HeLa Control		41,92	540,91	294,05	0	0
CMGB 30	SN	40,25	527,58	249,29	0	0
	CB	44,42	365,15	243,57	1	16,33
CMGB 31	SN	42,75	497,58	224,05	1,11	69,67
	CB	45,25	316,97	251,67	4,33	19,67
CMGB 32	SN	29,42	508,18	225,95	0	9,67
	CB	32,75	452,42	233,57	0	0

Discussion

The majority of probiotic products used by now are containing lactic acid bacteria (LAB), microorganisms considered as commensal and harmless for the host organisms. In order to exhibit a beneficial effect lactic acid bacteria (LAB) should survive in the human host gastrointestinal tract, this ability requiring resistance to low pH values, bile acid resistance and adherence to mucus or human epithelial cells [29, 1]. In our study, the pH resistance was variable depending on the tested strain, but generally an increased resistance of lactic bacteria to alkaline pH values comparing to acid pH was observed. However, the probiotic strains investigated for the potential use in the prevention and treatment of pediatric gastrointestinal disease are exhibiting different viability rates in a wide pH range of 3.0 to 8.0, suggesting their ability to survive in different parts of the gastro-intestinal tract (GIT), starting with survival in the stomach acid compartment (normally with pH values lower than 2) and continuing with the small bowel where pH is varying from slightly acid (4-7 in the proximal part) to an alkaline one (7.8-9 in its distal part), and finally arriving in large intestine where the pH is turning again towards slight acid (4.0-6.0) [24]. Bile salts (sodium tauroglicolate) are one of the factors that may influence the viability of lactic bacteria in the GIT influencing the health of the host. We have tested tauroglicolate because the taurocholic acid is one of the most important compounds of the bile acids together with deoxycholic acid. Ordinarily the concentration of bile salts in bile is 0.8%, but it concentrates up to 5 times (increasing concentration to 4%), before contracting the walls and releasing it into the duodenum once chyme has entered the small intestine from the stomach. Our strains proved resistance to taurocholic acid sodium salt concentrations, from 0.5 to 3%, this representing an advantage for the survival of these bacteria, once introduced in the GIT, especially at the level of small bowel, where the concentration of bile salts is very high [7].

The natural resistance of *Lc. lactis* strains to multiple classes of antibiotics also represents a great advantage, especially in cases when a probiotic product is administered together with antimicrobials in the treatment of infectious diseases, thereby reducing the likelihood of disbiosis occurrence and the rapid rebalancing of the normal microbiota. An important feature of probiotic lactic bacteria is their capacity to metabolize the serum cholesterol, this ability being present in our strains that reduced the cholesterol with an average of 45%, value situated at the upper level reported for other strains in the literature [3]. As the probiotic product intended to be obtained and used for the prevention and treatment of TGI pediatric diseases is composed of three different LAB strains, studies concerning the growth competition between these species were also required. Regarding growth competition between probiotic strains, our studies proved the lack of mutual inhibition of the three strains proposed for the probiotic product. This result, correlated with performances registered for three different strains concerning different probiotic actions, including inhibition of pathogenic or potentially pathogenic strains growth (*Salmonella enteritidis*, *Shigella flexnerii*, *Escherichia coli*, *Listeria innocua* and *Bacillus cereus*) motivated the inclusion of the three strains in the same product in order to obtain an optimal probiotic effect.

The probiotic arsenal includes multiple mechanisms for preventing infection, one of them being represented by the interaction with the host immune system in a strain-dependent manner. One of the aspects of the probiotic - host communication is the modulation of the epithelial cells secretory pattern by both pathogens and probiotics. It is well known that pathogens are manipulating in different ways the host cell secretory pathways, as a mechanism of invasion, survival and immune response evasion. Therefore, the ability of probiotic fractions (supernatants or bacterial cells) to modulate the cytokine pattern could contribute to the occurrence of an improved host response to pathogenic bacteria aggression [5, 2].

The tested strains fractions (either bacterial cells as well as supernatant) increased the level of tumor necrosis factor (TNF) expression, exhibiting thus a pro-inflammatory effect.

Concerning IL-6, one of the main *in vivo* roles of this cytokine is to initiate the acute phase response, while IL-8 production is triggered by various bacterial products. The decreasing of IL-6 and IL-8 expression by probiotics, could thus prevent the occurrence of an intensive inflammatory response with potential lesional effects on host. Both fractions of *Lactococcus lactis* spp. *lactis* CMGB 31 strain induced the IFN gamma production. Keeping in mind that IFN-gamma is implicated in T cells activation, the induction of this cytokine in the epithelial cells by probiotic fractions might be considered a beneficial aspect, by shortening the time required for the activation of T cells and the subsequent specific immune response.

Taken together, the immunomodulatory studies demonstrated that the probiotic culture fractions are modulating the expression of the most important cytokines in the development of the anti-infectious immunity against enteric pathogens.

Conclusion

Our *in vitro* studies are demonstrating that the selected probiotic strains are exhibiting good antimicrobial activities, inhibiting the adherence and colonization of HeLa cells by the enteropathogenic strains isolated by us from pediatric diarrhea, demonstrating their potential use in the treatment of pediatric gastro-intestinal disorders, as an alternative or in association with antibiotics. A great advantage of the selected probiotic strains is their high pH tolerance, resistance to bile salts ability to reduce the serum cholesterol as well as the ability to induce a beneficial cytokine response in the epithelial cells, expressed by the stimulation of TNF-alpha and INF-gamma pro-inflammatory cytokines and the inhibition of IL-6 and IL-8 cytokines, known to be implicated in the occurrence of lesional effects upon the infected host.

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