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PII: S0717-3458(16)30130-0
DOI: doi:10.1016/j.ejbt.2016.11.008
Reference: EJBT 211

To appear in: *Electronic Journal of Biotechnology*

Received date: 14 July 2016
Accepted date: 11 November 2016

Please cite this article as: García Apolinaria, Navarro Karen, Sanhueza Enrique, Pineda Susana, Pastene Edgar, Quezada Manuel, Henríquez Karem, Karlyshev Andrey, Villena Julio, González Carlos, Characterization of *Lactobacillus fermentum* UCO-979C, a probiotic strain with a potent anti-*Helicobacter pylori* activity, *Electronic Journal of Biotechnology* (2016), doi:10.1016/j.ejbt.2016.11.008

Electronic Journal of Biotechnology
EJBT-D-16-00113 R1
Original Research Article
Received: July 14, 2016
Accepted: November 11, 2016
Areas: Medical Biotechnology; Microbial Biotechnology

Characterization of *Lactobacillus fermentum* UCO-979C, a probiotic strain with a potent anti-*Helicobacter pylori* activity

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Abstract

Background: *Helicobacter pylori* is a microorganism considered the main risk factor in the development of gastric cancer. In this paper we inform a detailed characterization of the probiotic properties and the anti -*H. pylori* activity of a previously isolated lactobacillus strain - *Lactobacillus fermentum* UCO-979C - obtained from human gut.
Results: The strain tolerated pH 3.0, grew in the presence of 2% bile salts, produced lactic acid and hydrogen peroxide, aggregated in saline solution, showed high hydrophobicity, was highly adherent to glass and to Caco-2 and Gastric adenocarcinoma human cells (AGS) cells, showed an efficient colonization in Mongolian Gerbils, and it potently inhibited growth and urease activity of *H. pylori* strains. *L. fermentum* UCO-979C significantly inhibited *H. pylori*-induced IL-8 production in AGS cells and reduced viability of *H. pylori*. Regarding its innocuousness, UCO-979C was susceptible to several antibiotics. The strain did not produce histamine or beta hemolysis in blood agar containing red blood cells from various origins.
Conclusion: The results presented demonstrate that *L. fermentum* UCO-979C is a very good candidate as a probiotic able to protect humans against *H. pylori* infections.

Keywords: efficient colonization; gastric cancer; highly adherent to glass; hydrophobic bacteria; probiotic protection against gastric cancer; probiotic protection against Helicobacter pylori.

1. Introduction

Probiotics are defined as live microorganisms able to confer beneficial properties for health when consumed by individuals [1,2,3]. To be considered as probiotics, microorganisms must fulfill several requirements, including fun

ctional and safety aspects [4,5]. Probiotic bacteria must be non-pathogenic and should produce antibacterial substances to successfully compete with pathogens [6,7]. They also should be tolerant to the gastric acid and bile salts, being able to colonize and persist in the gastrointestinal epithelium [8,9,10]. In addition, potential probiotic bacteria should fulfill safety aspects including the absence of transmissible antibiotic resistance genes [11] and the capacity to synthesize hemolysin or other toxic compounds, such as nocive biogenic amines (histamine, tyramine, putrescine, cadaverine phenylethylamine or tryptamine) [12]. However, if biogenic amines are produced they must be present at low levels [4,13,14].

Human probiotic bacteria that exert beneficial effects in the gastrointestinal tract include members of the genus *Lactobacillus* [2,10,15]. These Gram positive bacteria are members of the normal human microbiota [1] and are considered GRAS (generally recognized as safe) microorganisms [16]. *Lactobacillus* strains have been successfully used in prophylactic or therapeutic treatments of several diseases including gastric disorders, inflammatory syndrome, bowel syndrome, gastric cancer or *Helicobacter pylori* infection [16,17,18]. Due to the gastric localization of *H. pylori* colonization and its relationships with gastric diseases, it is not surprising that several studies were carried out on the effects of probiotics on *H. pylori*. Numerous *in vitro* studies, demonstrating bacterial killing or inhibition, were followed by preclinical and clinical studies. These studies showed promising results in the use of probiotics against *H. pylori* when administered alone, or administered together with the eradication treatment [18,19].

Considering that the Chilean population shows a high prevalence of *H. pylori* infected individuals [20,21] and of gastric cancer [22], our laboratory has centered its attention on the search of new and effective prophylaxis strategies against *H. pylori* infection. Nowadays, no vaccine is available for the prophylaxis against *H. pylori*, and eradication therapy shows increased failure mainly due to clarithromycin resistance of the clinical isolates [23,24,25]. Thus, two fields of research arise as promissory against *H. pylori* infection: the detection of natural compounds with anti-*H pylori* activity [26,27] and the isolation of probiotic bacteria with anti-*Helicobacter* activities, such as *Lactobacillus* spp. [18,24].

The aim of this work was to establish a detailed characterization of the probiotic properties and the anti -*H. pylori* activity of a previously isolated lactobacillus strain -*L. fermentum* UCO-979C - obtained from human gut.

2. Material and methods

2.1. Isolation and identification of *L. fermentum* UCO-979C

Lactobacillus UCO-979C was isolated from a human gastric biopsy in the year 2007 [17].The strain was identified as *Lactobacillus fermentum* (95.0%) through an API 50 CHL V5.2 kit. The final identity of the strain as a *L. fermentum* was confirmed by the complete genome sequencing [28].

2.2. Control strains

Lactobacillus rhamnosus GG (ATCC 53103), *Lactobacillus johnsonii* La1 and *Lactobacillus casei* Shirota reference strains were used as control accordingly to the assay done. The Shirota strain was isolated from a yogurt sample (Yakult) obtained from a local supermarket at Montevideo, Uruguay. Strains GG and La1 were kindly provided by Dr. Martin Gotteland (University of Chile, Santiago, Chile).

Unless otherwise stated, all strains of *Lactobacillus* spp. were grown in MRS (Man-Rogosa-Sharpe, Difco, France) agar or broth at 37ºC under microaerophillic conditions (10% CO_2) for 48 h.

2.3. Physiological and functional properties of *L. fermentum* UCO-979C

2.3.1. Acid and bile tolerance

Acid tolerance was investigated as described by Kaushik et al. [29] with minor modifications. Briefly, 100 µL of a 24 h liquid culture of *L. fermentum* UCO-979C or controls (*L. rhamnosus* GG or *L. casei* Shirota) in MRS broth were inoculated in 10 mL of MRS adjusted to pH 2 or 3. Cultures were incubated at 37ºC under 10% CO_2 atmosphere and aliquots were obtained at 0, 1, 2, 3, and 24 h of incubation to determine viable bacterial counts by the micro drop assay [30]. For studying bile tolerance a similar assay was performed. MRS broth was supplemented with 1.5% or 2.0% Oxgall bile salt (Neogen, USA) [29]. Strains maintaining their viable count or growing ability in the presence of bile salts were considered as tolerant.

2.3.2. Hydrophobicity

An organic solvent partitioning assay (MATH: Microbial Adhesion To Hydrocarbons [31]) was used to reevaluate hydrophobicity as described previously [17]. Briefly, UCO-979C strain or controls (*L. rhamnosus* GG and *L. johnsonii* La1) were grown in MRS broth for 24 h, collected by centrifugation at 5000 *g* for 5 min and suspended in Phosphate buffered saline (PBS) supplemented with 3 M urea and 0.8 mM $MgSO_4$ on glass tubes at OD_{400nm} 0.8-1.0 and OD_{600nm} 0.4-0.6. The bacterial suspension (1.2 mL) was mixed with 0.3 mL of xylene, thoroughly shaken in a Vortex for two minutes and let stand for further 10 min. The aqueous phase was separated and the OD at 400 nm and 600 nm were measured. The hydrophobic percentage (% H) was calculated using Ocaña et al. criteria [32], for classifying the strains as high hydrophobicity (% H: 71-100), medium hydrophobicity (% H: 36-70) or of low hydrophobicity (% H: 0-35).

2.3.3. Saline aggregation test

This test was performed according to Ocaña et al. [32] without modifications. Twenty microliters of a phosphate buffered saline (PBS) cell suspension ($1x10^9$ CFU mL^{-1}) of *L. fermentum* UCO-979C strain or controls (*L. rhamnosus* GG or *L. johnsonii* La1) were mixed with 20 µL of 2 M ammonium sulfate on the surface of a glass slide, allowed to stand for two minutes before sealing the sample with a coverslip. Samples were analyzed using a light microscope to determine the saline aggregation status of the strains and classified as non-aggregative, medium aggregative and highly aggregative [32].

2.3.4. Glass adherence

L. fermentum UCO-979C strain or controls (*L. rhamnosus* GG or *L. johnsonii* La1) were grown as described below. Cells were collected by centrifugation and suspended in 20 mL MRS broth at a cellular density equivalent to McFarland Nº2 and incubated for 1 h. Sterile glass slides were introduced into the culture flask and incubated for 4 h, stained with crystal violet (0.1% w/v) for 5 min, washed and dried for 10 min at room temperature. Samples were observed under a light microscope (100 X objective lens). The strains were classified as non-adherent (less than 20 bacterial cells per field), mild adherent (20-50 bacterial cells per field), and strongly adherent (over 50 bacterial cells per field).

2.4. Innocuousness assays *L. fermentum* UCO-979C

2.4.1. Antibiotic susceptibility test

Susceptibility of *L. fermentum* UCO-979C and *L. rhamnosus* GG to the antibiotics amykacin, ampicillin, amoxicillin, cefotaxime, cefuroxime, ciprofloxacin, chlarytromycin, chloramphenicol, erithromycin, streptomycin, gentamycin, kanamycin, neomycin, levofloxacine, benzylpenicillin, rifampicin, sulphametoxazole/trimetoprim, tetracycline and vancomycin were determined by an agar diffusion test as described previously [33]. The criteria of Tang et al. [34] and Georgieva et al. [35] were used for classifying the strains as susceptible or resistant to each antibiotic.

2.4.2. Biogenic amines determination

Sample preparation was performed in 1 mL glass reaction vial. For that purpose, 100 µL of culture supernatant filtrate of *Lactobacillus* UCO-979C strain or *L. casei* Shirota were dansylated adding 400 µL of carbonate-bicarbonate buffer (pH 10), 300 µL of acetone and 200 µL of Dns-Cl solution. The mixture was vortex-mixed during 30 s and then incubated at 47ºC for 60 min. This solution was filtered through a 13 mm PVDF syringe mounted filter (0.45 µm) before HPLC injection. Chromatography was performed using a Waters HPLC system (Milford, USA) consisting of a 600 controller binary pump, a 717 plus autosampler, a 2475 multi-λ fluorescence detector, a 5CH column oven and a VWR international L-7614 online degasser (West Chester, Pennsylvania). Separation was carried out on a Waters C_{18} column YMC-Pack ODS-A (150 mm x 4.6 mm, 5 µm) set at 45ºC using methanol (A) and water (B) as mobile

phase. The flow rate was of 1.2 mL min^{-1} achieving a complete separation of all biogenic amines under study in 45 min. Detection was performed by fluorescence using 330 nm and 520 nm as excitation and emission wavelengths, respectively.

2.4.3. Haemolysis assay

The strain UCO-979C was assayed for detecting haemolytic activity on blood agar using Columbia agar base supplemented with 5% of blood from sheep, pig, and goat. The strain was incubated as described above and the results were expressed as positive for β-hemolysis when a clear zone surrounding the colonies was observed, γ-haemolysis producing strains if none hemolysis was observed surrounding the colonies or α-haemolysis producing strain when a green color was observed surrounding the colonies [8]. Thus, α and γ hemolytic strains are considered non-hemolytic ones.

2.5. *H. pylori* inhibition by *L. fermentum* UCO-979C

The method of Sgouras et al. [33] with minor modifications was used to reevaluate the anti-*H. pylori* 43504 activity [17], but two new reference strains (*H. pylori* J99 and G27) were also used. Agar plates containing 20 mL of Columbia agar (Oxoid, UK) supplemented with 5% defibrinated horse blood were inoculated with *H. pylori* (ATCC43504, J99 or G27 strain) using a swab. Wells of 6 mm in diameter were made and 50 µL of the *L. fermentum* UCO-979C inoculum at a cellular density of McFarland Nº2 were deposited per well. Plates were incubated at 37ºC for 72 h under microaerophillic conditions (Campygen, Oxoid, UK). In order to evaluate inhibition the criteria of Gaudana et al. [36] was followed, being the scorings: no inhibition (diameter of 1 mm or less), mild inhibition (diameters over 1 mm and up to 2 mm), strong inhibition (diameters over 2 mm and up to 5 mm) and very strong inhibition (diameters over 5 mm). The final values of the inhibition diameters were obtained by subtracting the well size (6 mm) to the inhibition zone measured. All experiments were performed in triplicate. MRS broth was used as negative control and *L. casei* Shirota, *L. rhamnosus* LGG and *L. johnsonii* La1 were used as positive controls.

2.5.1. Death curves

The kinetic of *H. pylori* ATCC43504 inhibition by *L. fermentum* UCO-979C was determined as follow: *H. pylori* 43504 was cultivated on Columbia agar supplemented with 10% of whole horse blood and incubated for 72 h at 37ºC under 10% CO_2. The colonies obtained were suspended in 3 mL of Brain-Heart Infusion (BHI) broth at McFarland Nº 2. One point eight milliliters of the suspension was mixed with 200 µL of a cell free supernatant of *L. fermentum* UCO-979C obtained by growing the strain for 24 h in MRS followed by filtration through a 0.22 µm diameters pore filter. Viable cell counts of *H. pylori* were determined at 0, 3, and 24 h (first experiment) and at 0, 0.5, 1, 1.5, 2, 4, 5, and 6 h (second experiment) after the challenge by removing 20 µL aliquots in triplicate at each sampling time and plating it in Columbia Agar supplemented with 10% horse blood. A killing curve with Amoxicillin was used as

control for bacterial death. Both sterile MRS broth and BHI broth were also used as control for measuring endogenous anti-*H. pylori* activity [37].

2.5.2. Urease inhibition assay

Urease inhibition assay was performed as described by Sgouras et al. [33] with minor modifications. One milliliters suspension of McFarland Nº2 inoculum of *H. pylori* ATCC43504 grew in BHI broth was mixed with 100 µL of a cell free supernatant obtained after filtering (using a 0.22 µm membrane) a 48 h culture of *Lactobacillus* UCO-979C strain. Amoxicillin (20 µg mL^{-1}) was used as positive control and Brain-Heart Infusion (BHI, Oxoid, UK) as negative control. Each suspension was incubated at 37ºC under 10% CO_2 atmosphere and samples of 150 µL were obtained after 0, 3 and 24 h of incubation. One hundred microliters were mixed with 900 µL of 20% urea in 10 mM phosphate buffer, pH 6.5 and 0.012% phenol red. The mixture was incubated for one more hour and the absorbance at 550 nm was recorded to establish the percentage of urease inhibition.

2.5.3 Adherence and protection assays with AGS cells

AGS cells, an adherent human gastric adenocarcinoma epithelial cell line, was used to further evaluate *L. fermentum* UCO-979C strain. The adherence of *H. pylori* to AGS cells was performed using the phenol red method according to Pastene et al. [27]. Briefly, AGS cells were cultured in T-75 flasks containing Roswell Park Memorial Institute medium (RPMI) 1640 medium supplemented with 10% Fetal Calf Serum (FCS) and 1% antibiotics solution (10 U/mL Penicillin, 10.000 µg/mL Streptomycin and 25 µg/mL Amphotericin B) for 4 d until more than 90% confluence was reached. AGS cells were trypsinized (0.25% Trypsin and 2.21 mM Ethylenediaminetetraacetic acid (EDTA) solution), counted using trypan with and a Neubauer chamber [28], and adjusted to a concentration of $5x10^3$ CFU mL^{-1}.

The potential protection provided by *L. fermentum* UCO-979C to AGS cells infected by *H. pylori* was assessed by adhesion assays. AGS cells were cultured in a 96-wells microplates and treated with the UCO-979C strain at different concentrations: 10^5, 10^6, 10^7, 10^8 or 10^9 CFU mL^{-1}. After 4 h, AGS cells were infected with *H. pylori* ATCC 43504 (McFarland Nº2). AGS cells infected only with strain ATCC 43504 were used as control. After 4 h of incubation, the supernatants were removed and conserved for cytokine analysis (-20ºC) and, phenol red indicator was added (0.003% phenol red, 2% urea, pH 6.8). Finally, microplates were read using a spectrophotometer at 570 nm wavelength.

2.5.4. Regulation of IL-8 production

In order to determine the possible anti-inflammatory effect of *L. fermentum* UCO-979C on AGS cells during *H. pylori* ATCC 43504 infection, the levels of IL-8 were measured using an enzyme-linked immunosorbent assay (ELISA). The cellular supernatants obtained in the experiments described in **2.5.3. section** were kept at -20ºC to be used for IL-8 production analysis. After supernatants were thawed, IL-8

was quantified using the DuoSet kit (R&D Systems) following the manufacturer's instructions.

2.6. Analysis of antimicrobial compounds synthetized by *L. fermentum* UCO-979C

2.6.1. L-lactic acid determination

L-lactic acid stock solution was prepared in ultra-pure water at a concentration of 1 mg/mL and chromatography was performed on HPTLC plates from Merck, coated with 0.2 mm of silica gel 60 F_{254}. A calibration curve with L-lactic acid between 3 to 15 µg/mL was used.

The samples of *L. fermentum* UCO-979C, *L. rhamnosus* and *L. casei* Shirota were only centrifuged at 15000 *g* for 5 min and the supernatant filtrated through a 0.45 µm pore diameter filter mounted in a syringe prior to be used in the assays. Samples (2-3 µL) and 3-15 µL standard of L-lactic acid were applied into the column by using an Automatic TLC Sampler (ATS4) from CAMAG (Muttenz, Switzerland).

Chromatography was performed using a mixture of diisopropyl ether:formic acid:water (80:15:5 v/v) as mobile phase. Then, plates were dried under a stream of warm air. Lactic acid was detected after a post-chromatographic derivatization with an ethanolic solution of bromophenol blue (0.5 mg mL^{-1}) and 0.1 aqueous NaOH is carefully added until the color of the solution changes to blue. Thereafter, plates were heated on a TLC plate heater (CAMAG) for 2 min at 100ºC. Scanning was performed using a TLC Scanner 3 (CAMAG) in visible absorption mode at 430 nm. All instruments were controlled via WinCats software 1.4.2 Planar Chromatography Manager (CAMAG).

2.6.2. Hydrogen peroxide (H_2O_2) production

The semi quantitative method described by Felten et al. [38] was used for determining H_2O_2 production. Briefly, the strains of lactobacilli were inoculated on MRS agar supplemented with 3, 3', 5, 5'-tetramethylbenzidine and H_2O_2, incubated during 48 h at 37ºC under 10% CO_2 and the color developed in colonies were recorded. A strain of *Enterococcus faecium* was used as a positive control.

The criterion applied for interpretation of the results, were: Negative synthesis (-), white colonies; Low synthesis (+), light blue colonies; Moderated synthesis (++), dark light blue colonies; and Strong synthesis (+++), the colonies show a blue color [38].

2.7. Colonization assays in Mongolian gerbils

Six females Mongolian gerbils (*Meriones unguiculatus*) (24-28 weeks old) were used divided in two groups of three animals each, one used as challenge group and the second as control. The gerbils were obtained from the closed colony kept at the Laboratory of Pharmacognosy (Universidad of Concepción, Chile). All experiments

were carried out in compliance with the Guide for Care and Use of Laboratory Animals and approved by the Ethical Committee of Animal Care at the University of Concepción, Chile and accordance with the U.K. Animals and associated guidelines, EU Directive.

During the days of study, the animals were kept in controlled environmental conditions with light dark cycles of 12 h, isolated in plastic cages in order to minimize contact with environmental particles. All animals were fed with balanced conventional diet and sterilized water *ad libitium*. The animals were fasted 20 h before received inoculation of probiotic bacteria *L. fermentum* UCO-979C. Labelling of *L. fermentum* UCO-979C was performed by suspending bacterial pellets in PBS buffer to a concentration of McFarland Nº4. Then 10 µL of Fluorescein isothiocyanate (FITC) was added and the suspension was incubated at 37ºC in dark for 1 h. Bacterial suspensions were centrifuged twice at 5000 rpm for 5 min in PBS. Animals were anesthetized by isoflurane inhalation (Baxter Forane 100%). Once animals were anesthetized, 1 mL of FITC-labeled *L. fermentum* UCO-979C (McFarland Nº4) was inoculated orally into de gerbil stomachs. The challenge group received 1 mL of the strain UCO-979C ($1.2x10^9$ CFU mL^{-1}) labelled with FITC. The control group received only 1 mL of PBS. Animals were euthanized after 10 h post inoculation, and their stomachs were excised and observed under fluorescence using the UVP i-Box Scientia Small Animal Imaging System (California, USA).

2.7.1 *L. fermentum* UCO-979C bacterial counts

For bacterial counts another group of six gerbils were used. The animals were managed and challenge as above but in this case the strain was not labelled with FITC. Pyloric antrum and corpus samples were aseptically obtained 14 d after the inoculation and the samples were homogenized with PBS, and 20 µL of macerated organs were taken and used for microdroplet technique on MRS agar. Samples were incubated at 37ºC for 24 h under microaerophilic conditions (10% CO_2) and bacterial counts were determined.

2.8. Statistical analysis

Experiments were performed in triplicate and results were expressed as mean ± standard deviation (SD). After verification of the normal distribution of data, 2-way ANOVA was used. Tukey's post hoc (for pair wise comparisons of the means) was used to test for differences between the groups. Differences were considered significant at $P < 0.05$.

3. Results

3.1. Physiological and functional properties of *L. fermentum* UCO-979C for its potential use as probiotic

Several physiological properties of *L. fermentum* UCO-979C related to its probiotic potential were evaluated including tolerance to acid and bile, hydrophobicity, saline

aggregation and *in vitro* adherence. Bile and pH tolerance tests showed that *L. fermentum* UCO-979C had a similar behavior when compared to control strains *L. casei* Shirota and *L. rhamnosus* GG. All the strains were susceptible to pH 2 since the acidic medium killed bacteria before one hour of incubation (data not shown). On the contrary, all the strain remain viable after 24 h of incubation at pH 3 (**Table 1**). A sudden decrease of two logarithms in the count of viable *L. fermentum* UCO-979C was consistently detected at the beginning of the exposure to pH 3. No statically significant differences ($P < 0.05$) were observed among the viable counts of the UCO-979C strain during the 24 h of pH 3 exposure. In addition, bile tolerance test indicated that all the strains were resistant to 2.0% of bile salts (**Table 2**).

L. fermentum UCO-979C showed both high hydrophobicity and positive saline aggregation (**Table 3**), indicating that this strain could have an increased capacity for cellular adherence. In fact, *L. fermentum* UCO-979C was highly adherent in the glass assay and to Caco-2 cells in culture, similarly to *L. rhamnosus* GG and *L. johnsonii* La1.

3.2. Safety assessment of *L. fermentum* UCO-979C for its potential use as probiotic

In order to evaluate safety of *L. fermentum* UCO-979C antibiotic resistance, hemolytic activity and production of biogenic amines were studied. The probiotic strain was susceptible to all of the 19 antibiotics assayed with the exception of benzylpenicillin (**Table 4**), suggesting that this strain does not harbor resistance plasmids. On the other hand, the commercial probiotic strain *L. rhamnosus* GG used as control showed resistance toward vancomycin, kanamycin, streptomycin and sulphametoxazole/trimethoprim (**Table 4**).

Our strain UCO-979C showed α-hemolysis in all the blood agar assayed which is indicative of a non β-hemolytic strain according to the criteria of Peres et al. [8].

As shown in **Table 5**, the total content of biogenic amines was 5.43 and 8.59 mg/L for *L. casei* Shirota and *L. fermentum* UCO-979C respectively. According the Bonferroni statistical test there were no significant differences ($P > 0.05$) between Shirota and UCO-979C strains. Interestingly, histamine that is the most important biogenic amine, was not detected in any of the strains.

3.3. Anti-*H. pylori* activity of *L. fermentum* UCO-979C

The UCO-979C strain showed the highest antibacterial activity against the three *H. pylori* strains used as indicators. Moreover, the antibacterial activity of *L. fermentum* UCO-979C was higher than the activity of the reference *L. rhamnosus* GG, *L. casei* Shirota and *L. johnsonii* La1 strains (**Table 6**). Inhibition kinetics studies further showed that *L. fermentum* UCO-979C had an antibacterial activity against *H. pylori* ATCC 43504 that was similar to amoxicillin, used as positive control (**Figure 1a**). Thus, in accordance with the bacterial death criteria of Pearson et al. [37], the effect of UCO-979C strain at 24 h can be considered as lethal for *H. pylori*. On the other

hand, after repeating this assay at shorter times and using the same Pearson criteria [37], it was possible to observe the death of *H. pylori* after 4 h of treatment with *L. fermentum* UCO-979C (**Figure 1b**).

Urease activity of *H. pylori* ATCC43504 showed a slight decrease in the presence of amoxicillin (**Figure 2**). This inhibition effect was more intense when the cell free supernatant obtained from a culture of *L. fermentum* UCO-979C was used, showing that a diffusible molecule should be present in this supernatant.

The ability of *L. fermentum* UCO-979C to inhibit *H. pylori* adhesion was evaluated in AGS cells. The results demonstrated that the UCO-979C strain is able to decrease *H. pylori* adhesion to AGS cells in a dose dependent manner (**Figure 3**). The lowest *H. pylori* adherence to AGS cells occurred when *L. fermentum* UCO-979C was used in a concentration of 10^{8-} or 10^{9} CFU mL^{-1} ($P < 0.05$) (**Figure 3**).

The capacity of *L. fermentum* UCO-979C to modulate IL-8 production in AGS cell was also evaluated. Treatment of gastric cells with the UCO-979C did not induce significant changes in the production of IL-8 (**Figure 4**). Challenge of AGS cells with *H. pylori* ATCC 43504 significantly increased the levels of IL-8 in agreement with the capacity of the pathogen to mount a robust inflammatory response in gastric cells. However, when AGS cells were colonized with *L. fermentum* UCO-979C previous to *H. pylori* challenge a significant decrease in IL-8 production was observed (**Figure 4**).

3.4. Analysis of the production of antimicrobial compounds by *L. fermentum* UCO-979C

In vitro analysis showed that lactic acid content in the growth medium of *L. fermentum* UCO-979C was 3.149 mg/mL and for *L. casei* Shirota was de 2.764 mg/mL, with no statistical differences among the strains studied. Similarly, both strains produced +++ H_2O_2 that according to the criteria of Felten et al. [38] is high, and no significant differences between the two strains was observed.

3.5. Colonization assays in *Mongolian gerbils*

Fluorescence studies demonstrated that *L. fermentum* UCO-979C strain adheres to the gastric mucosa of Mongolian gerbils (**Figure 5**). This adhesive capacity of UCO-979C strain was confirmed by the determination of bacterial counts. Animals were initially inoculated with 1.2 x 10^{9} CFU mL^{-1} of UCO-979C strain and 14 after we detected 2.5 x 10^{7} and 2.8 x 10^{7} CFU mL^{-1} in the pyloric antrum and corpus of the gerbil stomachs, respectively.

4. Discussion

In the group of pathogenic bacteria able to colonize and affect the human stomach, *H. pylori* is the most important microorganism. *H. pylori* selectively colonizes stomach epithelium, and in some individuals early infection and persistence of bacteria causes chronic gastric inflammation and tissue damage, changes that could progress to

severe diseases such as peptic ulcer, or gastric adenocarcinoma [38]. In addition to pathogenic bacteria, the combination of traditional analytical tools and culture independent molecular methods has shown the wide diversity of the bacterial microbial ecosystem of the stomach. This fact shows that although the extremely acidic environment of the stomach could theoretically prevent bacterial colonization, it does not mean that some bacteria have not been adapted to these extreme conditions, such as acidophilus bacteria that require or are able to live at low pH [39,40]. This fact implies that the microbiota of the gastric mucosa can be an interesting source of potential probiotic strains with the ability to prevent or reduce the colonization of pathogens such as *H. pylori*.

Interestingly, several studies were carried out on the effects of beneficial probiotic bacteria on *H. pylori*. Numerous *in vitro* studies demonstrating bacterial killing or inhibition were followed by preclinical and clinical studies that indicated only partial efficacy of probiotics against *H. pylori* when administered alone; but increase of efficacy and/or reduction of side effects when probiotics were administered together with the eradication treatment [19]. Moreover, few studies were performed with probiotics strains isolated from human stomach. In this paper we aimed to characterize a potential probiotic property of *L. fermentum* strain isolated previously from a human stomach.

In order to be considered a potential probiotic for human use, bacterial strains must comply with a series of characteristics grouped into two main categories: functional properties and innocuousness or safety. In fact, several studies have to be performed in order to determine that the candidate probiotic strain is not toxic and that it possesses the claimed properties [4,3,10]. Therefore, we studied several characteristic of *L. fermentum* UCO-979C in order to determine it safety and its functional capacities.

It was demonstrated here that the UCO-979C strain is resistant to one antibiotic: penicillin G that would be an undesirable property [23,41]. Nevertheless, penicillin resistance has been observed in diverse *Lactobacillus* strains and it is usually codified in the chromosome, suggesting a low probability of transferring it to other bacterial cells [42]. Additionally, the capacity of UCO-979C strain to produce biogenic amines was also evaluated. It was reported that the increase of biogenic amines in serum, histamine especially, is deleterious for human health. These amines are able to cause several detrimental effects including hypotension, hypertension, nausea, diarrhea, headache and, respiratory or skin problems [43,44,45]. It must be emphasized that *L. fermentum* UCO-979C did not produced detectable levels of histamine. These results clearly indicate that *L. fermentum* UCO-979C could be used as a safe probiotic microorganism.

Tolerance to acid and bile salts are desirable properties in bacterial species to be considered as human probiotics due to the stress conditions found in the gastrointestinal tract of humans. In this regard, probiotic microorganisms thought to colonize the stomach region should be tolerant to pH 3 [6,40] or, at least, tolerant to pH 4 which is the pH of the gastric mucus layer described. New species belonging to

the genus *Lactobacillus* sp. isolated from human stomach mucosa have been described (*Lactobacillus gastricus*, *Lactobacillus antri*, *Lactobacillus kalixensis* and *Lactobacillus ultunensis*) showing high resistance to acidic conditions allowing them to persist in this rigorous environment [46,47]. As shown here, *L. fermentum* UCO-979C strain survived at pH 3 for 24 h and it had a similar behavior to that observed for other probiotics strain such as *L. rhamnosus* GG with respect to its tolerance to pH and bile salts. Moreover, the *in vivo* experiments in Mongolian gerbils performed in this work clearly demonstrated the capacity of the UCO-979 strain to colonize and survive in the gastric mucosa.

We demonstrated that *L. fermentum* UCO-979C has several characteristics making it an excellent candidate as a probiotic to prevent infection by *H. pylori*. The UCO-979C strain inhibits bacterial pathogen growth, reduce *H. pylori* urease activity, decrease pathogen adhesion to gastric cells and beneficially regulate inflammatory response. There are several reports describing probiotic strains with anti-*H. pylori* activity through the production of antimicrobial compounds such as bacteriocins, autolysins and organic acids [7,48]. In this regard, inhibition of *H. pylori* by the production of lactic acid has been reported in *L. salivarius*, *L. acidophilus*, *L. rhamnosus* and *L. casei* strain Shirota [49], in both *in vitro* and animal studies. Lactic acid, in addition to its antimicrobial effect by the decrease of pH, is able to inhibit the urease enzyme of *H. pylori*.

L. fermentum UCO-979C was able to reduce colonization of *H. pylori* and decrease the production of the inflammatory chemokine IL-8 in human gastric epithelial cells. Human immune response has an important role in the development of serious diseases after *H. pylori* infection since increased pro-inflammatory cytokine expressions has been found in the gastric mucosa of infected patients [50]. Regulation of *H. pylori*-induced inflammation has been claimed to play key important roles in the prevention of chronic gastric damage and cancer. In fact, *H. pylori* infection can activate NF-$_k$B in gastric epithelium cells and subsequently up-regulate IL-8 gene transcription that greatly contribute to disease evolution. On the other hand, some probiotics, including lactobacilli, have previously been shown to decrease inflammatory markers in *H. pylori* infection models both *in vitro* and *in vivo* [51,52]. Here, we showed that *L. fermentum* UCO-979C was able to induce a significant reduction of IL-8 production in *H. pylori*-infected cells, indicating its potential as a beneficial immunomodulator. More detailed studies about *L. fermentum* UCO-979C immunomodulatory properties would allow us to include this strain in the group of bacteria known as immunobiotics [53,54].

In conclusion, the results presented here indicate that *L. fermentum* UCO-979C, is a good candidate to be used as human probiotic for gastric protection against *H. pylori*, since this strain is able to tolerate the stress conditions of this habitat in addition to its capacity to produce antimicrobial compounds and to beneficially modulate inflammatory response. In this regard, it should be noted that antimicrobial and immunomodulatory activities are independent properties. In fact, it was reported that some probiotic bacteria have a positive effect on *H. pylori*-associated inflammation without clearing the infection [55,56]. Then, *L. fermentum* UCO-979C, that harbor

both properties, has a high probability of operating as an effective anti-*Helicobacte*r probiotic.

Financialsupport

This study was financially supported by project INNOVA CHILE 14IDL2-29744 and INNOVA BIO BIO 12.139-IN.IEM.

Conflict of interest

The authors declared that they have no conflict of interest in the present publication.

References

[1] Sullivan A, Nord CE. Probiotics and gastrointestinal diseases. J Intern Med 2005;257:78-92. http://dx.doi.org/10.1111/j.1365-2796.2004.01410.x
[2] Williams NT. Probiotics. Am J Health-Syst Pharm 2010;67:449-58. http://dx.doi.org/10.2146/ajhp090168
[3] Mizock BA. Probiotics. Dis Mon 2015;61:259-90. http://dx.doi.org/10.1016/j.disamonth.2015.03.011
[4] Lee Y, Salminen S. Handbook of probiotics and prebiotics, 2th ed. New Jersey, USA; Johns Wiley & Sons: 2009, pp 14-15. http://dx.doi.org/10.1002/9780470432624
[5] Nuraida L. A review: Health promoting lactic acid bacteria in traditional Indonesian fermented foods. Food Science and Human Wellness 2015;4:47-55. http://dx.doi.org/10.1016/j.fshw.2015.06.001
[6] Vitor J, Vale F. Alternative therapies for *Helicobacter pylori*: Probiotics and phytomedicine. FEMS Immunol Med Microbiol 2015;63:153-64. http://dx.doi.org/10.1111/j.1574-695X.2011.00865.x
[7] Butel MJ. Probiotics, gut microbiota and health. Med Mal Infect 2014;44:1-8. http://dx.doi.org/10.1016/j.medmal.2013.10.002
[8] Peres CM, Alves M, Hernandez-Mendoza A, Moreira E, Silva S, Bronze MR, et al. Novel isolates of lactobacilli from fermented Portuguese olive as potential probiotics. LWT-Food Sci Technol 2014;59:234-46. http://dx.doi.org/10.1016/j.lwt.2014.03.003
[9] Zhou JS, Pillidge CJ, Gopal PK, Gill HS. Antibiotic susceptibility profiles of new probiotic *Lactobacillus* and *Bifidobacterium* strains. Int J Food Microbiol 2005;98:211-7. http://dx.doi.org/10.1016/j.ijfoodmicro.2004.05.011
[10] Patel PJ, Singh SK, Panaich S, Cardozo L. The aging gut and the role of prebiotics, probiotics, and synbiotics: A review. Journal of Clinical Gerontology and Geriatrics 2014;5:3-6. http://dx.doi.org/10.1016/j.jcgg.2013.08.003
[11] Coppola R, Succi M, Tremonte P, Reale A, Salzano G, Sorrentino E. Antibiotic susceptibility of *Lactobacillus rhamnosus* strains isolated from Parmigiano Reggiano cheese. Lait 2005;85:193-204. http://dx.doi.org/10.1051/lait:2005007
[12] Ancin-Azpilicueta C, González-Marco A, Jiménez-Moreno N. Current knowledge about the presence of amines in wine. Crit Rev Food Sci Nutr 2008;48:257-75. http://dx.doi.org/10.1080/10408390701289441

[13] Naila A, Flint S, Fletcher G, Bremer P, Meerdink G. Control of biogenic amines in food existing and emerging approaches. J Food Sci 2010;75:139-50. http://dx.doi.org/10.1111/j.1750-3841.2010.01774.x
[14] Cardona-Gálvez M, González-Domínguez A. Intoxicación histamínica o escombriosis en pescados. Alimentaria. 2005;365:68-73.
[15] Ljungh A, Wadstrom T. Lactic acid bacteria as probiotics. Curr Issuers Intestinal Microbiol 2010;7:73-90.
[16] Gotteland M, Brunser O, Cruchet S. Systematic review: Are probiotics useful in controlling gastric colonization by *Helicobacter pylori*?. Aliment Pharmacol Ther 2006;23:1077-86. http://dx.doi.org/10.1111/j.1365-2036.2006.02868.x
[17] Garcia A, Henríquez P, Retamal C, Pineda S. Probiotic properties of *Lactobacillus* spp isolated from gastric biopsies of *Helicobacter pylori* infected and non-infected individuals. Rev Med Chile 2009;137:369-76. http://dx.doi.org/10.4067/S0034-98872009000300007
[18] García A, Sáez K, Delgado C, González CL. Low co-existence rates of *Lactobacillus* spp. and *Helicobacter pylori* detected in gastric biopsies from patients with gastrointestinal symptoms. Rev Esp Enferm Dig 2012;104:473-8.
[19] Ruggiero P. Use of probiotics in the fight against *Helicobacter pylori*. World J Gastrointest Pathophysiol 2005;5:384-91. http://dx.doi.org/10.4291/wjgp.v5.i4.384
[20] Figueroa G, Acuna R, Troncoso M, Portell D, Valenzuela J. *Helicobacter pylori* infection in Chile. Clin Infect Dis 1997;25:983-9.
[21] Figueroa G, Troncoso M, Toledo M, Faúndez G, Acuña R. Prevalence of serum antibodies to *Helicobacter pylori* and gastric diseases in Chile. J Med Microbiol 2002;51:300-4. http://dx.doi.org/10.1099/0022-1317-51-4-300
[22] Ferreccio C, Rollán A, Harris PR, Serrano C, Gederlini A, Margozzini P, et al. Gastric cancer is related to early *Helicobacter pylori* infection in a high prevalence country. Cancer Epidemiol Biomarkers Prev 2007;16:662-7. http://dx.doi.org/10.1158/1055-9965.EPI-06-0514
[23] Sachs G, Scott DR. *Helicobacter pylori*: Eradication or preservation. F1000 Med Rep 2012;4:1-5. http://dx.doi.org/10.3410/M4-7
[24] Malfertheirner P, Megraud F, O'Morain C, Atherton JA, Axon A, Bazzoli F, et al. Management of *Helicobacter pylori* infection the Maastricht IV/ Florence Consensus report. Gut 2012;61:646-64. http://dx.doi.org/10.1136/gutjnl-2012-302084
[25] Camargo C, García A, Riquelme W, Otero C, Camargo CA, Hernandez-García T, et al. The Problem of *Helicobacter pylori* resistance to antibiotics: A systematic review in Latin America. Am J Gastroenterol 2014;109:485-95. http://dx.doi.org/10.1038/ajg.2014.24
[26] Walter J, Tannock G, Tilsala A, Rodtong R, Loach DM, Munro K. Detection and identification of gastrointestinal *Lactobacillus* species by using denaturing gradient gel electrophoresis and species-specific PCR primers. Appl Environ Microbiol 2000;66:297-303. http://dx.doi.org/10.1128/AEM.66.1.297-303.2000
[27] Pastene E, Parada V, Avello M, Ruiz A, García A. Catechin-based procyanidins from *Peumus boldus* mol. Aqueous extract inhibit *Helicobacter pylori* urease and adherence to adenocarcinoma gastric cells. Phytother Res 2014;28:1637-45. http://dx.doi.org/10.1002/ptr.5176

[28] Karlyshev A, Villena J, Gonzalez C, Albarracin L, Barros J. Draft Genome Sequence of a probiotic strain, *Lactobacillus fermentum* UCO-979C. Genome Announc 2015;3:e01439-15. http://dx.doi.org/10.1128/genomeA.01439-15
[29] Kaushik J, Kumar A, Duary RK, Mohanty AK, Grover S, Batish VK. Functional and probiotic attributes of an indigenous isolate of *Lactobacillus plantarum*. Plos One 2009;4:1-11. http://dx.doi.org/10.1371/journal.pone.0008099
[30] Rivera J. Microbial ecology of the digestive tract in neonatal stage. Rev Mex Pediatr 2010;69:257-60.
[31] Rosenberg M, Judes H, Weiss E. Cell surface hydrophobicity of dental plaque microorganisms *in situ*. Infect Immun 1983;42: 831-4.
[32] Ocaña VS, Bru E, De Ruiz Hogaldo AP, Nader-Macias ME. Surface characteristics of lactobacilli isolated from human vagina. J Gen Appl Microbiol 1999;45:203-12. http://dx.doi.org/10.2323/jgam.45.203
[33] Sgouras D, Maragkoudakis P, Petraki K, Martinez P. *In vitro* and *in vivo* inhibition of *Helicobacter pylori* by *Lactobacillus casei* strain Shirota. Appl Environ Microbiol 2004;70:518-26. http://dx.doi.org/10.1128/AEM.70.1.518-526.2004
[34] Tang H, Yuan J, Xie C, Wei H. Antibiotic susceptibility of strains in Chinese medical probiotic products. J Med Coll PLA 2007;22:149-52. http://dx.doi.org/10.1016/S1000-1948(07)60032-X
[35] Georgieva R, Iliev IN, Chipeva Va, Dimitonova SP, Samelis J, Danova ST. Identification and *in vitro* characterization of *Lactobacillus plantarum* strains from artisanal Bulgarian white brined cheeses. J Basic Microbiol 2008;48:234-44. http://dx.doi.org/10.1002/jobm.200700355
[36] Gaudana S, Dhanani A, Bagchi T. Probiotic attributes of *Lactobacillus* strains isolated from food and of human origin. Br J Nutr 2010;103:1620-8. http://dx.doi.org/10.1017/S0007114509993643
[37] Pearson R, Steigbigel R, Davis H, Chapman S. Method for reliable determination of minimal lethal antibiotic concentrations. Antimicrob Agents Chemother 2008;18:699-708. http://dx.doi.org/10.1128/AAC.18.5.699
[38] Felten A, Barreau C, Bizet C, Lagrange PH, Philippon A. *Lactobacillus* species identification, H_2O_2 production, and antibiotic resistance and correlation with human clinical status. J Clin Microbiol 1999;370:729-33.
[39] Nardone G. Compare D. The human gastric microbiota: Is it time to rethink the pathogenesis of stomach diseases?. United European Gastroenterol J 2015;3:255-60. http://dx.doi.org/10.1177/2050640614566846
[40] Sanhueza E, Paredes-Osses E, González C, García A. Effect of pH in the survival of *Lactobacillus salivarius* strain UCO_979C wild type and the pH acid acclimated variant. Electron J Biotechnol 2015;18:343-6. http://dx.doi.org/10.1016/j.ejbt.2015.06.005
[41] Hummel A, Hertel C, Holzapfel W, Franz C. Antibiotic resistances of starter and probiotics strains of lactic acid bacteria. Appl Environ Microbiol 2007;73:730-9. http://dx.doi.org/10.1128/AEM.02105-06
[42] Pérez R, Pulido N, Ben R, Lucas H, Martínez Cañamero M, Gálvez A. Resistance to antimicrobial agents in lactobacilli isolated from caper fermentations. Antonie Van Leeuwenhoek 2005;88:277-81. http://dx.doi.org/10.1007/s10482-005-6964-2

[43] Romero R, Gazquez D, Bagur MG, Sanchez-Viñas M. Optimization of chromatographic parameters for the determination of biogenic amines in wines by reversed-phase high-performance liquid chromatography. J Chromatogr 2010;871:75-83. http://dx.doi.org/10.1016/S0021-9673(99)00946-2

[44] Santos MH. Biogenic amines: Their importance in foods. Int J Food Microbiol 1996;29:213-31. http://dx.doi.org/10.1016/0168-1605(95)00032-1

[45] Ten Brink B, Damink C, Joosten HM, Huis J. Occurrence and formation of biologically active amines in foods. Int J Food Microbiol 1990;11:73-84. http://dx.doi.org/10.1016/0168-1605(90)90040-C

[46] Schreiber S, Konradt M, Groll C, Scheid A, Hanauer G, Werling HO, et al. The spatial orientation of *Helicobacter pylori* in the gastric mucus. Proc Natl Acad Sci USA 2004;101:5024-9. http://dx.doi.org/10.1073/pnas.0308386101

[47] Roos S, Engstrand L, Jonsson H. *Lactobacillus gastricus* sp. nov., *Lactobacillus antri* sp. nov., *Lactobacillus kalixensis* sp. nov., and *Lactobacillus ultunensis* sp. nov., isolated from human stomach mucosa. Int J Syst Evol Microbiol 2005;55:77-82. http://dx.doi.org/10.1099/ijs.0.63083-0

[48] Klaenhammer TR. Genetics of bacteriocins produced by lactic acid bacteria. FEMS Microbiol Rev 1993;12:39-85. http://dx.doi.org/10.1111/j.1574-6976.1993.tb00012.x

[49] Ryan KA, O'Hara AM, Van Pijkeren JP, Douillard FP, O´Toole PW. *Lactobacillus salivarius* modulates cytokine induction and virulence factor gene expression in *Helicobacter pylori*. J Med Microbiol 2009;58:996-1005. http://dx.doi.org/10.1099/jmm.0.009407-0

[50] Doger F, Meteoglu I, Ozkara E, Erkul Z, Okyay P, Yukselen V. Expression of NF-$_k$B in *Helicobacter pylori* infection. Dig Dis Sci 2006;51:2306-9. http://dx.doi.org/10.1007/s10620-006-9352-5

[51] Johnson K, Mitchell D, Avitzur Y, Galindo E, Jones NL, Sherman PM. Probiotics reduce bacterial colonization and gastric inflammation in *Helicobacter pylori*-infected mice. Dig Dis Sci 2004;49:1095-102. http://dx.doi.org/10.1023/B:DDAS.0000037794.02040.c2

[52] Tamura A, Kumai H, Nakamichi N, Sugiyama T, Deguchi R, Takagi A, et al. Suppression of *Helicobacter pylori*-induced interleukin-8 production and within the gastric mucosa by a live *Lactobacillus* strain. J Gastroenterol Hepatol 2006;21:1399-406. http://dx.doi.org/10.1111/j.1440-1746.2006.04318.x

[53] Kitazawa H, Villena J. Modulation of respiratory TLR3-anti-viral response by probiotic microorganisms: Lessons learned from *Lactobacillus rhamnosus* CRL1505. Front Immunol 2014;5:1-15. http://dx.doi.org/10.3389/fimmu.2014.00201

[54] Villena J, Kitazawa H. Modulation of intestinal TLR4-Inflammatory signaling pathways by probiotic microorganisms: Lessons learned from *Lactobacillus jensenii* TL2937. Front Immunol 2014;4:1-12. http://dx.doi.org/10.3389/fimmu.2013.00512

[55] Ryan K, Jayaraman T, Daly P, Canchaya C, Curran S, Fang F. Isolation of lactobacilli with probiotic properties from the human stomach. Lett Appl Microbiol 2008;47:269-74. http://dx.doi.org/10.1111/j.1472-765X.2008.02416.x

[56] Amara AA, Shibl A. Role of Probiotics in health improvement, infection control and disease treatment and management. Saudi Pharm J 2013;23:107-14. http://dx.doi.org/10.1016/j.jsps.2013.07.001

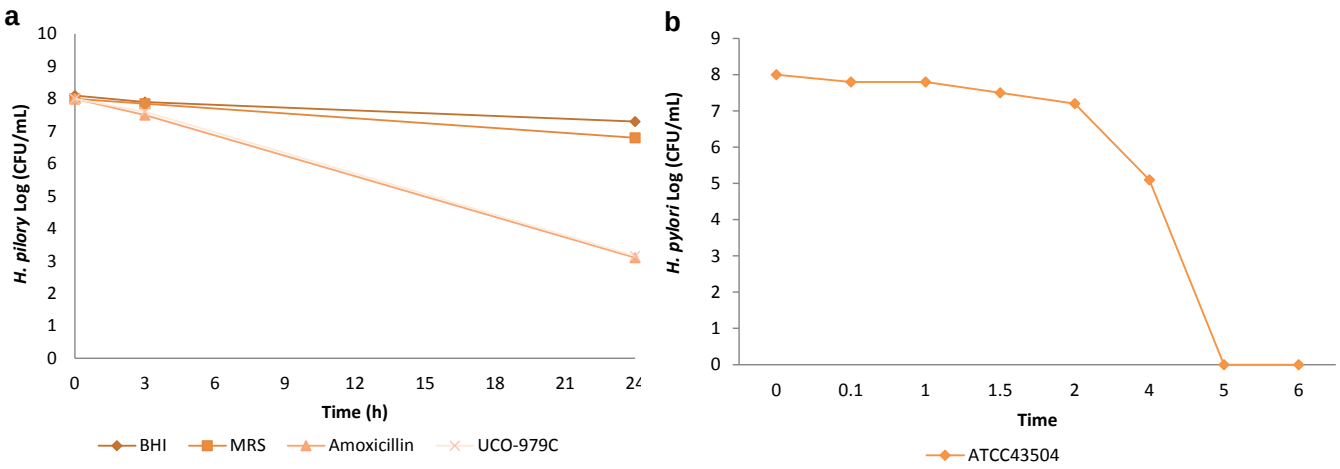

Fig. 1. Inhibition kinetics of *Lactobacillus fermentum* UCO-979C strain over *Helicobacter pylori* ATCC 43504. (a) Samples taken at 3 different times from 0 to 24 h. (b) Samples taken at 6 different times from 0 to 6 h.

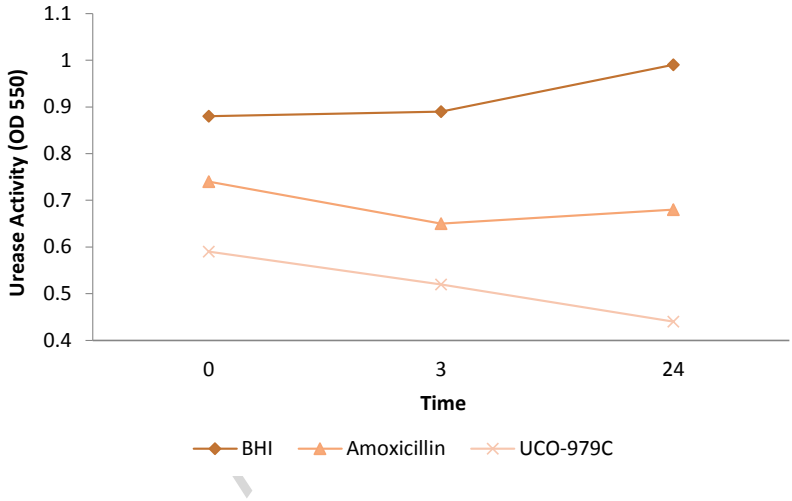

Fig. 2. Inhibitory activity of *Lactobacillus fermentum* UCO-979C strain over the activity of *Helicobacter pylori* urease. Samples taken at times 0, 3 and 24 h.

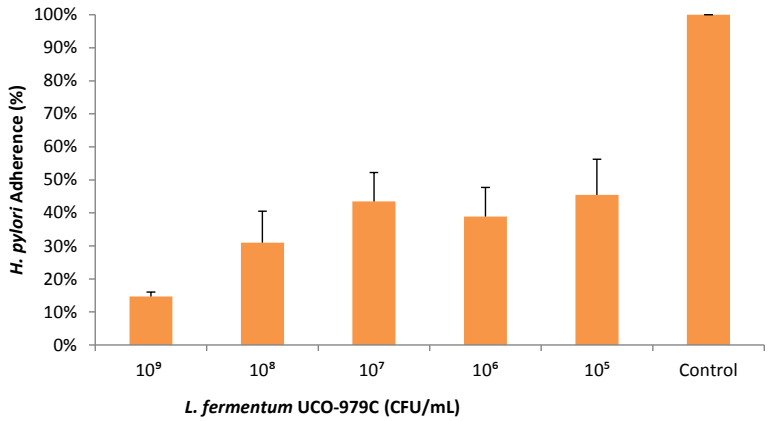

Fig. 3. Percentage of *H. pylori* (ATCC 43504) adherence on AGS cells previously colonized by *L. fermentum* UCO-979C (preventive treatment) strain at different bacterial concentrations.

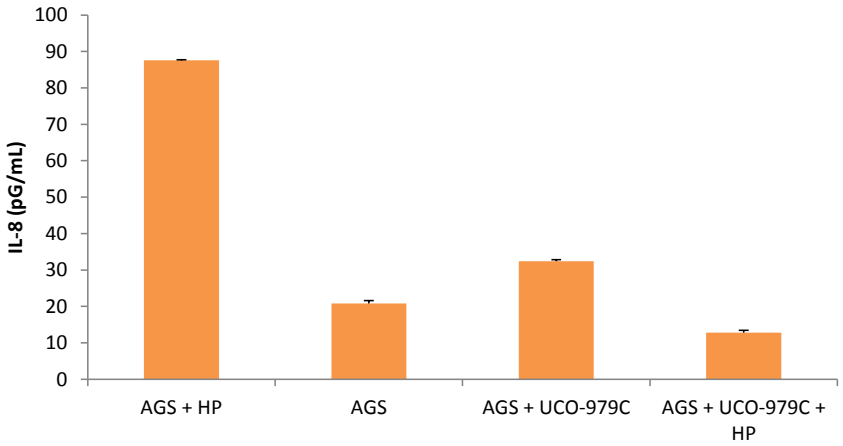

Fig. 4. Determination of IL-8 levels produced by AGS cells infected with *H. pylori* ATCC 43504 or/and colonized with *L. fermentum* UCO-979C and non infected (control), using an ELISA assay.

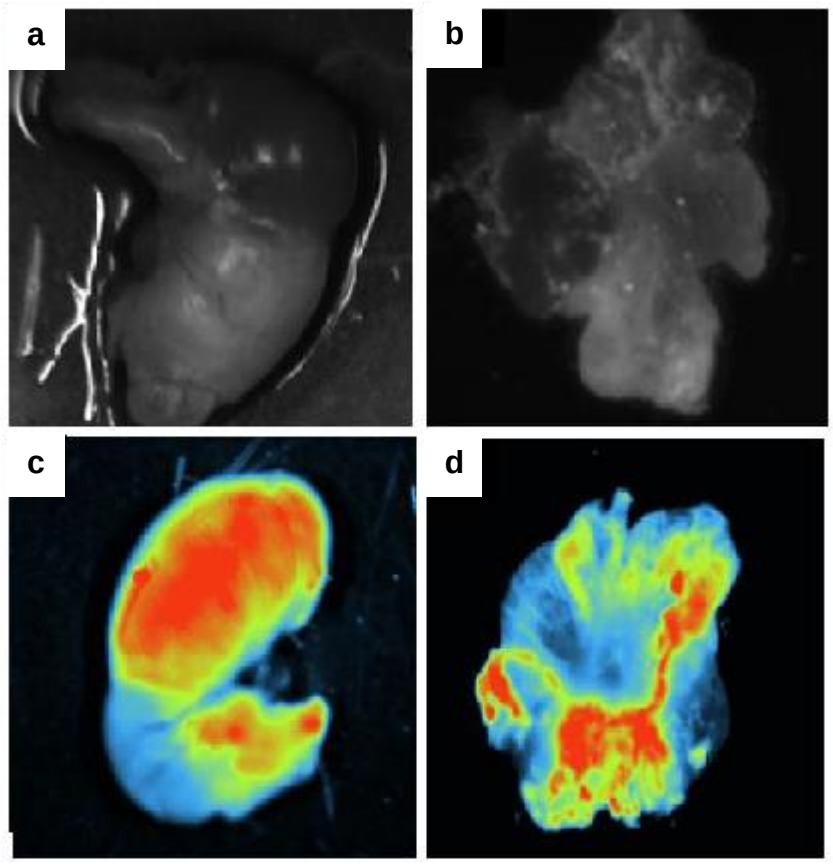

Fig. 5. Adherence of *L. fermentum* UCO-979C strain to Mongolian gerbil stomachs detected by fluorescence. (a) and (b): control stomach; (c) and (d): fluorescence in the stomach caused by the adherence of the UCO-979C strain.

Table 1. *Lactobacillus* strains tolerance to pH 3.0.

Strain	Viable counts (CFU/mL) at pH 3.0					
	Before exposure	**0 h**	**1 h**	**2 h**	**3 h**	**24 h**
L. fermentum UCO-979C	1.5×10^8	8.0×10^6	8.3×10^6	5.4×10^{6a}	4.4×10^6	4.5×10^6
L. casei Shirota	1.0×10^8	7.2×10^7	6.2×10^7	7.1×10^{7a}	9.9×10^7	1.0×10^8
L. rhamnosus GG	1.9×10^6	2.4×10^6	2.7×10^6	2.9×10^{6b}	2.6×10^6	2.1×10^6

Different superscripts (a and b) indicate significant differences ($P < 0.05$). For details, see **Material and Methods**.

Table 2. *Lactobacillus* strains tolerance to 2.0% bile salts.

Strain	Viable counts (CFU/mL)				
	Before exposure	**0 h**	**1 h**	**2 h**	**24 h**
L. fermentum UCO-979C	2.9×10^{6a}	6.4×10^{6a}	4.2×10^{6a}	4.2×10^{6a}	2.6×10^{7b}
L. casei Shirota	4.1×10^{6}	4.9×10^{6}	6.1×10^{6}	6.3×10^{6}	3.8×10^{6}
L. rhamnosus GG	3.1×10^{6}	5.9×10^{6}	3.9×10^{6}	7.1×10^{6}	3.0×10^{7}

Different superscripts (a and b) indicate significant differences ($P < 0.05$). For details, see **Material and Methods**.

Table 3. Physiological properties of *Lactobacillus* strains.

Assay	*L. fermentum* UCO-979C	*L. johnsonii* La1	*L. rhamnosus* GG
Hydrophobicity	strong	strong	moderate
Saline aggregative test	positive	negative	negative
Glass adherence	++	++	++

Table 4. Antibiotic susceptibility profile of *Lactobacillus* strains.

Antibiotic	**(µg)**	**Strains**			
		***L. fermentum* UCO-979C**		***L. rhamnosus* GG**	
Amikacin	30	S	S	-	MS
Amoxicillin	10	-	SS	-	MS
Ampicillin	10	-	SS	-	S
Cefotaxime	30	-	SS	-	SS
Cefuroxime	30	-	S	-	S
Ciprofloxacine	5	S	-	S	SS
Clarithromycin	5	-	S	-	SS
Chloramphenicol	30	S	SS	S	S
Erythromycin	15	-	S	S	SS
Streptomycin	10	S	S	R	MS
Gentamicin	10	S	S	S	S
Kanamycin	30	-	S	-	R
Levofloxacin	5	-	S	-	SS
Neomycin	30	-	S	-	S
Penicillin G	10	R	MS	S	S
Rifampicin	5	-	S	-	SS
Sulfatrimetoprim	25	S	S	R	R
Tetracycline	30	S	SS	S	SS
Vancomycin	30	-	MS	R	R

C1: Criterion1, Tang et al. [34]; C2: Criterion 2, Georgieva et al. [35]; MS: Moderately susceptible; S: Susceptible; SS: Strongly susceptible; R: Resistant.

Table 5. Biogenic amines content in MRS broth.

Biogenic amines (mg L^{-1})	**Strains**	
	***L. fermentum* UCO-979C**	***L. casei* Shirota**
2-Phenylethylamine	2.70 ± 0.16	1.07 ± 0.05
Putrescine	2.34 ± 0.29	1.56 ± 0.19
Cadaverine	1.44 ± 0.07	1.74 ± 0.11
Histamine	ND	ND
Tyramine	0.44 ± 0.04	0.53 ± 0.12
Spermidine	TR	TR
Spermine	1.68 ± 0.49	0.53 ± 0.01
TOTAL	8.59	5.43

ND: Not detected, TR: Traces (not quantifiable).

Table 6. Antimicrobial activity spectrum exhibited by various lactobacilli on *Helicobacter pylori* strains.

***Lactobacillus* strains**	***Helicobacter pylori* strains**					
	ATCC43504		**J99**		**G27**	
L. rhamnosus GG	2.3 ± 0.05*	++	2 ± 0.15	+	3 ± 0.12	++
L. casei Shirota	3.8 ± 0.05	++	3.5 ± 0.05	++	4 ± 0.1	++
L. johnsonii La1	2.7 ± 0.05	++	2.5 ± 0,15	++	3 ± 0.05	++
L. fermentum UCO-979C	5.1 ± 0.05	+++	5 ± 0.15	++	6 ± 0.1	+++

The inhibition zones 1 mm, 2 mm, 2-5 mm and more than 5 mm were classified as strains of no (+/-), mild (+), strong (++) and very strong (+++) inhibition, respectively (Gaudana et al. criterion [36]). *Results are expressed as mean ± standard deviation of three independent biological replicates.