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Thème

Effect of some drugs on the viability of free and encapsulated probiotic Lactobacillus

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List of abbreviations

$CaCl_2$	Calcium Chloride
CFU	Colony Forming Unit
COX 1	Cyclooxygenase 1
COX 2	Cyclooxygenase 2
GIT	Gustro Intisinal truct
LAB	Lactic Acid Bacteria
M	Molarity
MIC	Minimal Inhibitory Concentration
MRS	De Man, Rogosa and Sharpe
N	Normality
NSAIDs	Non-Steroidal Anti Inflammatory Drugs
OD	Optical Density
Rpm	Revolution per Minute
V	Volume
ND	Not determine

I: Introduction

I. Introduction

The concept of orally taking mixtures of microorganisms for improved health is not new. Yogurt has long been thought to have health benefits. As early as 1908, **Metchnikoff**, put a scientific spin on the ingestion of microbes in stating that "ingested lactobacilli can displace toxin-producing bacteria, promoting health and prolonging life."**[Elmer, 2001]**.

Recently, it is reported that concomitant use of probiotic with medication may produce adverse or beneficial effects, which are determined by the specific substances and conditions of use **[Serna and Sanchez, 2011]**. The number of documented adverse interactions is matched by the number of documented beneficial interactions. Meanwhile, the most adverse interactions are pharmacokinetic, *i.e.*, the probiotics can metabolize the drug, resulting in sub-therapeutic or toxic plasma concentrations, the ability of probiotic to decrease toxicity or side effects of drugs, through diverse mechanisms is the most important beneficial effects **[Tursi *et al.*, 2004]**. Beneficial effects depend on the effect of drugs on the viability of the probiotic strains. Thus, it is important to determine drug-probiotic interactions to identify the probiotic that may improve the performance of medications.

Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used to relieve pain and fever but also typically cause gastrointestinal side effects such as mucosal injury. Several clinical studies had shown that probiotic is a developped strategy that minimizes adverse events of non-steroidal anti-inflammatory drugs **[Spiegel *et al.*, 2005]**.

The main purpose of our work was to investigate the effect of non-steroidal anti-inflammatory drugs, antibiotics and some commercial drugs on the growth of some probiotic *Lactobacillus* strains, in order to study the possible concomitant use of medicaments like ibuprofen and diclofenac and these probiotic strains.

I I: The review

II.1. Probiotic definition

Probiotic is generally defined as a live microorganism or a microbial mixture administered to beneficially affect the animal host by improving its microbial balance **[Elmer, 2001]**. According to the food and agriculture organization and the world health organization probiotic are defined as: "live microorganisms which, when administrated in adequate amounts as part of food, confers benefit to the host."**[Cremonini *et al.*, 2002; Gregor, 2006; Tiwari *et al.*, 2012]**. **Charteris *et al.* (1998)** defined probiotics as "microorganisms, which when ingested, may have a positive effect in the prevention and treatment of a specific pathological condition." Probiotic bacteria should be safe for consumption, reach the intestines alive in large numbers, provide specific health benefits to the host. These bacteria should maintain the balance of the intestinal flora by altering favorably the gut environment in such a manner that the growth of friendly beneficial bacteria are promoted and harmful disease causing organisms are inhibited **[Tiwari *et al.*, 2012]**.

Most current probiotics have been selected using the following features listed by **Fuller** in **1989**: **[Fuller, 1989; Sapathy and Schin, 2000; Prakash *et al.*, 2008]**.

- Genera of human origin
- The strain should be capable of exerting a beneficial effect on the host
- It should be non pathogenic and non toxic
- It should be present as viable cells, preferably in large number
- Stable against bile, acid, enzymes and oxygen
- Ability to adhere to intestinal mucosa
- Colonization potential in the human gastrointestinal tract
- Demonstrable efficacy and safety
- Production of antimicrobial substances

II.2. Probiotic bacteria

Probiotics are microorganisms that transfer a tiny number of congenital health benefits to the host and have numerous applications in food and medicine **[Tiwari *et al.*, 2012]**. **Table 01** summarized some examples of probiotic strains.

Lactic acid bacteria (LAB) are the most important probiotic microorganisms typically associated with the human gastrointestinal tract **[Mezaini *et al.*, 2009]**. They are mainly used as starter cultures and play an important role in food preservation **[Todorov and Dicks, 2005]**. They have a capacity to inhibit spoilage and pathogenic bacteria **[Saidi *et al.*, 2011]**, they also play an important role in microbiological stability and production of aroma compounds in various food products **[Todorov and Dicks, 2005]**. These bacteria are Gram-positive, rod-shaped, non-spore-forming, catalase-negative organisms that are devoid of cytochromes and are of non-aerobic habit but are aero-tolerant, fastidious, acid-tolerant and strictly fermentative; lactic acid is the major end-product of sugar fermentation. LAB are the most important group for industrial purposes, since their fermentative activity involves a notable preservative capacity as a result of the drop in pH and antimicrobial activity of their metabolites such as lactic and acetic acid, diacetyl or bacteriocin **[Saidi *et al.*, 2011]**.

Table 01: Strains considered for use as probiotic **[Leroy *et al.*, 2008; Guchte *et al.*, 2012]**.

Lactobacillus sp.	*Bifidobacterium* sp.	Other LAB	Other microorganisms
Lb. acidophilus *Lb. amylovorus* *Lb. brevis* *Lb. casei* *Lb. crispatus* *Lb. curvatus* *Lb. fermentum* *Lb. gasseri* *Lb. johnsonii* *Lb. rhamnosus* *Lb. plantarum*	*B. adolescentis* *B. animalis* subsp. *animalis* *B. animalis* subsp. *lactis* *B. bifidum* *B. breve* *B. longum*	*Enterococcus faecalis* *Enterococcus faecium* *Lactococcus lactis* *Leuconostoc mesenteroides* *Sporolactobacillus inulinus* *Streptococcus thermophilus*	*Bacillus cereus* *Bacillus subtilis* *Clostridium butyricum* *Escherichia coli* *Propionibacterium freudenreichii* *Saccharomyces boulardii*

A few of the commonly used probiotic lactic acid bacteria include lactobacilli. Lactobacilli, although not predominant enteric organisms, are present throughout the gastrointestinal tract of healthy humans and rodents. Several *Lactobacillus* strains, such as *Lb. plantarum* 299v (LP 299v), *Lb. acidophilus*, *Lb. fermentum*, and *Lb. rhamnosus* GG (L GG) can colonize the human gastrointestinal tract **[Bengmark, 2000]**.

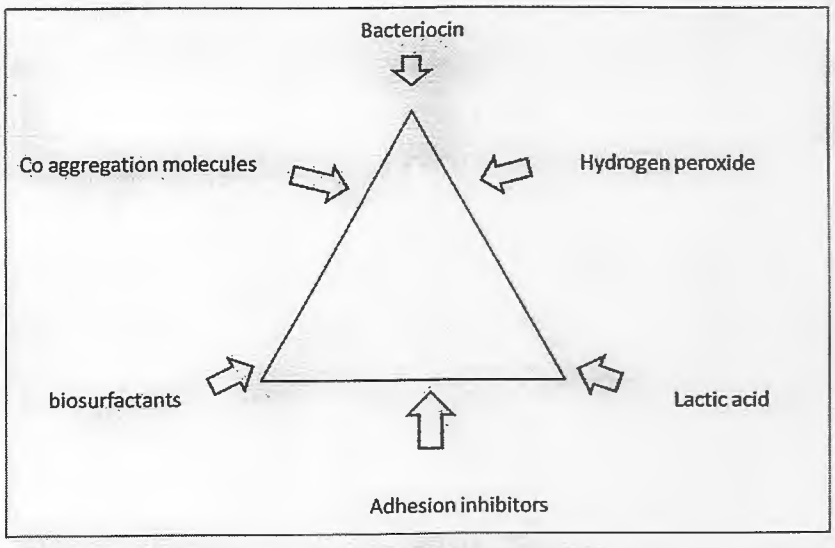

Figure 01: Metabolites of lactic acid bacteria **[Deegan *et al.*, 2006]**.

Several studies have shown the beneficial therapeutic effects of probiotic lactic acid bacteria. The main health benefits of LAB are: Enhancement of immunity against intestinal infections, prevention of diarrheal diseases, prevention of colon cancer, prevention of hypercholesterolaemia, improvement of lactose utilization, prevention of upper gastrointestinal tract diseases, stabilization of the gut mucosal barrier, synthesis of vitamins and increase in bioavailability of minerals and possible anti-carcinogenic activity **[Sapathy and Schin, 2000; Todorov *et al.*, 2011]**.

II.3. Probiotics in food

Because of their perceived health benefits, probiotics have been incorporated into a range of dairy products, including yoghurts, soft-, semi-hard and hard cheeses, ice cream, milk powders and frozen dairy desserts **[Kumar and Singh, 2007]**. Many probiotic non-dairy products have been developed. There is a wide range of probiotic *Lactobacillus* species that are technologically suitable for food applications than bifidobacteria. They are resistant to low pH, have native association with traditional fermented foods, and have adaptation to milk and other food substrates **[Mortazavian *et al.*, 2012]**.

II.4. Medical applications of probiotics

Improvement of lactose utilization

Lactose intolerant persons suffer from abdominal cramping, bloating, and diarrhea after ingesting lactose-containing foods. Some probiotics, such as *Lactobacillus,* contain β-galactosidase or lactase intracellularly so that ingestion of lactase-containing probiotics might be beneficial for lactose-intolerantindividuals, either consumed with food or taken separately as a supplement. Probiotics ingested assupplements would adhere to the intestinal lining and digest dietary lactose, thereby alleviating malabsorptive symptoms from excessive lactose **[Levri *et al.*, 2005]**.

Colon Cancer

Colon cancer is one of the leading causes of death; some probiotic bacterial strains have been suggested as having the potential to protect against colon cancer, through several mechanisms such as: alteration of the metabolic activities of the intestinal microflora, alteration of the physicochemical conditions in the colon, binding and degradation of potential carcinogens, **Thirabunyanon *et al.* (2009)** suggested that the probiotic strains *Enterococcus faecium*RM11 and *Lactobacillus fermentum* RM28 also triggered antiproliferation of colon cancer cells. This suggested that boths trains could be used as potential probiotics in functional food or for colon cancer biological products.

Acute Diarrhea

Probiotics are useful as treatment of acute infectious diarrhea in children. Different strains, including *Lactobacillus reuteri, Lb. rhamnosus* strain GG and *Lb. acidophilus*, have been tested in controlled clinical trials and were proven useful in reducing the severity and duration of diarrhea **[Guarner, 2009]**.

Table 2: Evidence-based indications of probiotics in gastroenterology **[Guarner, 2009]**.

Disorder	Product
Acute infectious diarrhea in children	*Lb. rhamnosus* GG
	Lb. reuteri ATTC 55730
	Lb. acidophilus + *B. infantis*
Acute infectious diarrhea in adults	*Enterococcus faecium* LAB SF68
Antibiotic associated diarrhea in children	*Lb. rhamnosus* GG
	Bacillus lactis Bb12 + *S. thermophilus*
Antibiotic associated diarrhea in adults	*Enterococcus faecium* LAB SF68
	Lb. rhamnosus GG
	Lb. casei DN-114 001in fermented milk with *Lb. bulgaricus* +*S. thermophilus*
	Lb. acidophilus CL1285 + *Lb. casei* Lbc80r
Nosocomial diarrhea in children	*Lb. rhamnosus* GG
	B. lactis BB12 + *S. thermophilus*
	B. lactis BB12
	Lb. reuteri ATTC 55730
C. difficile diarrhea in adults	*Lb. casei* DN-114 001 in fermented milk with *Lb. bulgaricus* + *S. thermophilus*
	Lb. acidophilus + *B. bifidum*

The use of probiotic to prevent infections of the urogenital and intestinal tracts

The presence and dominance of *Lactobacillus* in the vagina is associated with a reduced risk of bacterial vaginosis and urinary tract infections. The mechanisms appear to involve anti-adhesion factors, by-products such as hydrogen peroxide and bacteriocins lethal to pathogens, and perhaps immune modulation or signaling effects. The instillation of *Lactobacillus* GR-1 and B-54 or RC-14 strains into the vagina has been shown to reduce the risk of urinary tract infections, and improve the maintenance of a normal flora. Ingestion of these strains into the gut has also been shown to modify the vaginal flora to a more healthy state **[Reid and Burton, 2002]**.

These bacteria improve food digestion and the body's capacity for absorption and help to maintain the intestinal flora balance. As a result, they facilitate the digestion of food and the assimilation of nutrient **[Tiwari *et al*, 2012]**.

II.5. Mode of action

There is still much controversy as to how probiotics work, however the mechanisms represented in the following shirt are considered important:

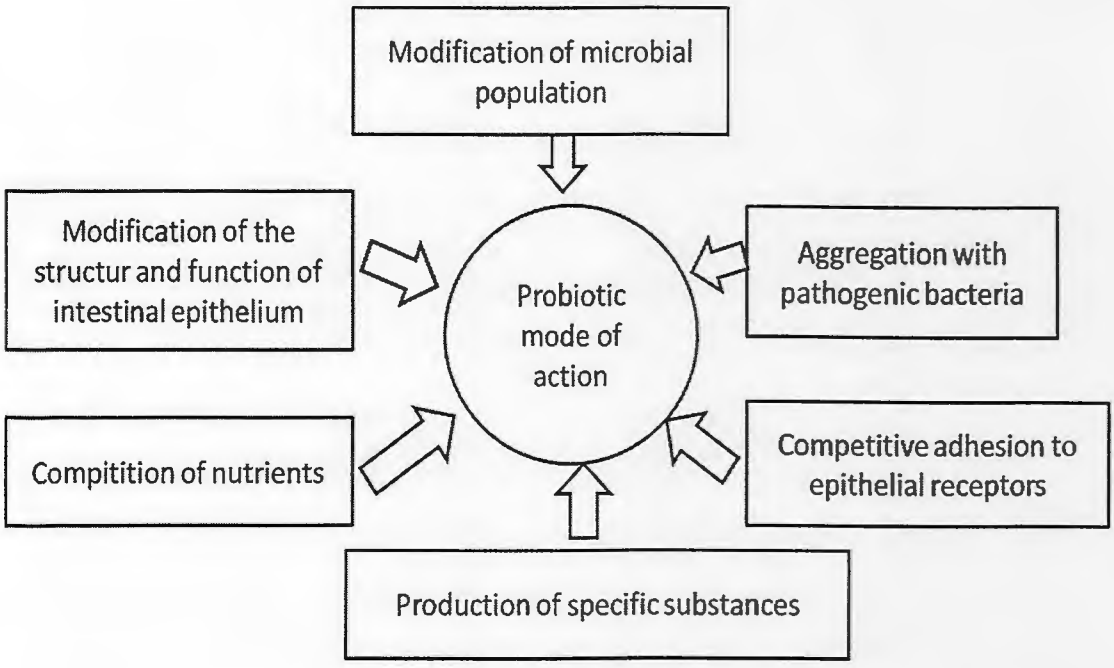

Figure 02: Probiotic mode of action **[Tiwari *et al.*, 2012]**.

II.5.1. Competitive adhesion to epithelial receptors

The ability of probiotics to adhere to intestinal cells is a desirable quality, as this is the first step in colonisation and may enable modification of the host immune system. A number of probiotics have been shown to strongly adhere to human cell lines, including *Lb. casei* GG, *Lb. acidophilus* LA1, *Lb. plantarum* and a variety of *Bifidobacteria* **[MacNaught and MacFie, 2001]**.

II.5.2. Competition for nutrients and production of antimicrobial substances

Probiotic strains further inhibit pathogenic organisms by competing for the limited substrates required for fermentation and by secreting antimicrobial products called bacteriocins **[Fuller, 1989]**. For example, *Lb. acidophilus* has been shown to produce two compounds, bacteriocin lactacin B and Acidolin. Lactacin B was shown to inhibit other *Lactobacilli* in *vitro*, whereas Acidolin inhibited enteropathogenic organisms **[Barefoot and Klaenhammer, 1984; Zamfir *et al.*, 1999]**. **Silva** and colleagues also demonstrated an inhibitory substance produced by *Lactobacillus* GG, with similar broad spectrum activity **[Silva and Jacobus 1987]**.

II.5.3. Immunity modulation

There is now good evidence that some strains of Lactobacilli and Bifidobacteria can influence immune function through a number of different pathways including effects on enterocytes, antigens presenting cells (including both circulatory T cells, and effector T and B cells) **[Tiwari *et al.*, 2012]**.

II.6. Non-steroidal anti-inflammatory drugs

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used for the treatment of pain, fever, and inflammation. The worldwide NSAID market for both occasional and chronic users has been conservatively estimated at over 60 million people and some NSAIDs (aspirin, naproxen, ibuprofen, diclofenac…etc) are among the most popular over the counter drugs **[Richy *et al.*, 2012]**.

Non-steroidal anti-inflammatory drugs as the name suggests, are drugs which suppress inflammation. Patients often call them pain-killers. But they do more than stop the pain they reduce the inflammation in arthritis. NSAIDs are used in all types of arthritis regardless of the etiology. Treatment with NSAIDs will quickly reduce the signs of inflammation which are pain, redness, and swelling, heat and loss functions. However, they do nothing to treat the cause of the arthritis or to prevent initial tissue damage or to modify the outcome of the chronic arthritis. NSAIDs act quickly and the effect wears out quickly when the drug is discontinued **[Hospital and Road, 1992]**.

Many of the NSAIDs can be purchased over the counter including acetylsalicylic acid, ibuprofen, naproxen, diclofenac and indomethacin. In 2001, naproxen, diclofenac and ibuprofen were amongst the top 25 pharmaceuticals **[Mehinto, 2009]**. The compound 2-[3-(2-methylpropyl)phenyl] propionic acid (shown in **Figure 03**), commercially available as ibuprofen (IBP), is widely used as an NSAID especially prescribed for the treatment of fever, migraine, muscle aches, arthritis and tooth aches. According to literature, several kilotons of IBP are produced worldwide each year **[Zheng *et al.*, 2011]**.

Diclofenac (2-[(2, 6-dichlorophenyl)amino]benzeneacetic acid) (shown in **Figure03**)is a non-steroidal anti-inflammatory drug used in human medical care as analgesic antiarthritic and antirheumatic compound **[Mehinto, 2009]**.

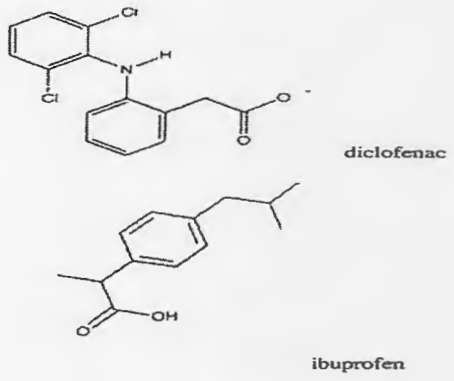

Figure 03: Molecular structure of diclofenac and ibuprofen **[Mehinto, 2009]**.

II.6.1. Adverse effects

NSAIDs represent a very effective class of drug, but their use is associated with a broad spectrum of untoward reactions, because all NSAIDs have possible adverse effects on all the major organ system (the liver, kidney, skin and gut). Although the upper gastrointestinal (GI) toxicity of NSAIDs is well documented. Generally the toxicities are dose related **[Richy *et al.*, 2012]**.

It has been shown that the pathogenesis of NSAID enteropathy is multifactorial, involving a combination of biochemical events, represented by inhibition of cyclooxygenase(COX) 1 and 2 and by topical effects of NSAIDs on enterocytes, all responsible for an alteration in mucosal integrity and the disruption in intercellular junctions, this results in an increased intestinal permeability, allowing mucosal exposure to a variety of luminal aggressors (bacteria, bile acids, etc.), with consequent inflammatory reactions and macroscopic alterations **[Montalto *et al.*, 2010]**.

Traditional non-selective NSAIDs, such as aspirin, ketoprofen, indomethacin and diclofenac, affect the expression of COX-1 and COX-2 present in the gastrointestinal (GI) membrane **[Radi and Khan, 2006]**. The suppression of COX-2 alleviates inflammation, whereas the simultaneous suppression of COX-1 hampers the prostaglandin production essential for mucin formation and a functional epithelial barrier in the GI tract **[Radi and Khan, 2006; Somasundaram, 2000]**. Thus GI adverse effects such as erosion and increased permeability are common during the long-term use of non-selective NSAIDs **[Radi and Khan, 2006; Laine *et al.*, 2006]**. Next-generation NSAIDs that selectively inhibit COX-2 are less prone to causing moderate GI side effects **[Laine *et al.*, 2006]**, although complicated side effects are as common among selective COX-2 inhibitor users as among traditional NSAID users **[Laine *et al.*, 2007; Laine *et al.*, 2010]**. Recent studies on the pathogenesis of NSAID-induced mucosal injury indicate that NSAIDs inhibit oxidative phosphorylation in epithelial cell mitochondria. The resulting mitochondrial dysfunction leads to disturbances in cellular energy metabolism and ion regulation, causing increased intestinal permeability and mucosal damage **[Uejima *et al.*, 1996]**.

II.6.2. Probiotic solution for NSAIDs complications

It has been suggested that intestinal micro-organisms are necessary for the development of NSAID-induced small bowel lesions, as 'germ-free' animals were found to be resistant to indomethacin injuries. It has also been shown that NSAID ingestion may disrupt the homeostasis of intestinal flora and may induce the overgrowth of Gram-negative and anaerobic bacteria species that are able to exacerbate the intestinal injury caused by NSAIDs.

Experimental models have shown that the stimulation of *Lactobacillus* colonization by means of lactulose ingestion promotes ulcer healing due to the consumption of NSAIDs, suggesting a protective effect by this bacterial species. Microflora alterations may be modulated by the administration of microorganisms such as *Bifidobacterium* or *Lactobacillus*, which may survive passage through the gastrointestinal tract and exert specific physiologic effects **[Gotteland *et al.*, 2001]**.

An intriguing alternative for protecting humans from NSAID-induced side effects is the parallel use of probiotics. Indeed, certain probiotic strains induce epithelial cell proliferation and mucus secretion, thus potentially beneficially affecting NSAID-induced adverse effects, and are capable of stabilizing distorted GI microbiota **[Kamil *et al.*, 2007]**. To date, a limited number of studies have investigated the potential protective effect of different probiotic supplements against NSAID-induced gastrointestinal damage with varying outcome measures. *In vitro* studies with *Lactobacillus casei* DN-114 001, and animal studies applying *Lb. casei* strain Shirota and a multi-strain mixture of human origin have yielded promising results **[Watanabe *et al.*, 2009]**. In clinical trials, *Lactobacillus rhamnosus* GG has been shown to reduce indomethacin-induced gastric permeability and the multi-strain supplement VSL#3 has been shown to alleviate inflammation caused by indomethacin. Moreover, *Lactobacillus acidophilus* NCFM and lactitol may protect against the GI microbiota alterations associated with NSAID use. Among elderly subjects regularly consuming NSAIDs. Taken in parallel with NSAIDs, probiotics are a promising complementary treatment for relieving NSAID-induced adverse effects **[Kamil *et al.*, 2007]**.

Gotteland and co-workers have successfully applied permeability probes to evaluate the protective effect of a probiotic in a clinical study. They supplemented live and heat-killed *Lactobacillus rhamnosus* GG cells to 16 human subjects consuming indomethacin. The intestinal permeability was assessed using gastric (sucrose) and small intestinal (lactulose/mannitol) permeability markers which showed a significant protective effect against increased gastric permeability with live *Lactobacillus rhamnosus* GG cells **[Gotteland *et al.*,2001]**.

II.7. Viability of probiotic bacteria and microencapsulation technology

Beneficial effects depend on the ability of the probiotic strains to maintain viability in the food during shelf-life and to survive the natural defenses of the host and multiply in the gastrointestinal tract **[Todorov *et al.*, 2011]**. Many factors such as acidity, oxygen content, and concentration of lactic and acetic acids affect the survival of probiotics in food and in the gastrointestinal tract of the host. Several methods have been used to enhance the viability of probiotics, including selection of resistant strains, stress adaptation, incorporation of micronutrients, and microencapsulation **[Rokka and Rantamäki, 2010]**.

- **Encapsulation as a tool for improving probiotic viability**

Encapsulation is the most developed method for cell immobilizing which involves a large number of processes that entrap an active material in mainly spherical particles in order to immobilize it, protect it, control its release and provide new physical properties or functions **[Ivanova *et al.*, 2002; Kotikalapudi, 2009]**. Immobilized cells have many advantages over free-cell cultures which include higher productivity as a result of high cell densities, long-term operational stability, improved control process, protection against contamination, and improvement of plasmid stability and improvement of the ability to separate and reuse cells **[Collins et al, 2009]**.

The materials used for encapsulation consist of a semi-permeable, spherical, thin and strong membrane surrounding a solid or liquid core, with a diameter varying from a few microns to 1 mm **[Collins *et al.*, 2009; Rokka and Rantamaki, 2010]**. The encapsulation is defined as the technology for packaging solids, liquid, or gaseous materials in capsules to protect the microencapsulated materials from the surrounding environment, or conversely to protect the environment from the active ingredient. While nutrients and products can migrate through the semi-permeable membrane of the capsule. Polysaccharides like alginate, gellan, *k*-carrageenan and starch are the most commonly used materials in microencapsulation of bifidobacteria and lactobacilli **[Patel *et al.*, 2008; Prakash *et al.*, 2008; Rokka and Rantamaki, 2010]**. Microencapsulation of probiotic cells has been recently used as an efficient method for improving the viability of probiotic bacteria in fermented milk drinks, fermented frozen dairy desserts, ice cream and juices, and simulated gastrointestinal tract **[Rosas-Ledesma *et al.*, 2011]**. Besides enhancing the viability of bacteria, microencapsulation facilitates handling of cells and allows a controlled dosage **[Rokka and Rantamaki, 2010]**.

Various researchers have studied the efficiency of encapsulation; the conventional encapsulation method, with sodium alginate in calcium chloride ($CaCl_2$), has been used to encapsulated *Lb. acidophilus* to protect this organism from the harsh acidic conditions in gastric fluid, studies have shown that calcium-alginate-immobilized cell cultures are better protected, shown by an increase in the survival of bacteria under different conditions, than the non-encapsulated state **[Kumar and Singh, 2007; Mokkaram *et al.*, 2009]**. Similar results were obtained with

Chandramouli *et al.* (2004) who found significant increase in viable numbers of *Lb. acidophilus* at pH 2.0 when encapsulated in alginate. Microencapsulated cells of *Lb. acidophilus* in alginate beads survived better after sequential incubation in simulated gastric and intestinal juices **[Chandramouli *et al.*, 2004]**. Higher survival was also reported when lactobacilli immobilized in alginate beads were incubated in simulated gastric fluid **[Sabikhi *et al.*, 2010]**.

III: Materials and Methods

III.1. Material

III.1.1. Bacterial strains and culture media

Lb. plantarum G1 strain used in this study has isolated from chicken gizzard and exhibited good probiotic properties **[Bouridane and Arid, 2011]**, *Lb. plantarum* F12 and *Lb. curvatus* G6 isolated from new born feces, which were characterized as bacteriocin producing strains **[Sifour *et al.*, 2012]**. All strains were maintained in glycerol 20% (v/v) at -20 °C.

Isolates were grown at 37°C in Man Rogosa Sharpe (MRS) broth (Biokar Diagnostics. France)(10g glucose, 10g beef extract, 5g yeast extract, 5g sodium acetate, 2g Bipotassic phosphate, 2g ammonium citrate, 0.2 magnesium sulfate, 0.05g manganese sulfate, 1ml tween 80, pH 6.5). To confirm the purity of the isolates each strain was individually streaked on MRS agar plates (MRS broth + 15g agar) and single colonies were isolated and tested for antimicrobial activity. Isolates were stored in growth medium supplemented with glycerol (1/1 v/v, 50 % final concentration).

MRS broth deficient in any carbon source was used as a basal medium specific for *Lactobacillus* according to **Morishita *et al.* (1981)** in this medium the carbon source was replaced by different NSAIDs and antibiotics with different concentration to study the ability of strains to use these compounds as the sole carbon source.

III.1.2. Antibiotics

Antibiotic-susceptible disks (Oxoid) were stored in sealed containers with a desiccant at 4°C. The antibiotics tested including amoxylin (25µg), erythromycin (15µg), streptomicin (10µg), sulfonamide (200µg), colestinsulfat(50µg), penicillin G (10µg), and tetracycline (30µg).

III.1.3. NSAIDs

Two non-steroidal anti-inflammatory drugs were used in this study, ibuprofen 2-[3-(2-methylpropyl) phenyl] propionic acid and diclofenac (2-[(2,6-dichlorophenyl) amino] benzeneacetic acid) were purchased from **"Shasun chemicals and drugs"(India)**.
Commercial drugs listed in Table 04 were purchased from a local pharmacy.

Reagents

- Sodium hydroxide (NaOH) 5N
- Hydrochloric acid (HCl) 1N
- Phosphate buffer
- Calcium chloride ($CaCl_2$) 0.05 M

Equipments

- Colony counter (FUNKE)
- Vortex (IKA)
- Centrifuge (HETTICH ZENTRIFUGEN)
- pH meter (Denver Instrument)
- Incubators (Memmert)
- Heat magnetic stirrer(Bunsen)
- Balance (Denver Instrument)
- Spectrophotometer (Shimadzu UV mini 1240)
- Millipore filters, pore size 0.22µm

III.2. Methods

II.2.1. Antimicrobial susceptibility testing

Standard procedures for antibiotic susceptibility testing of bacteria with clinical significance are well established, but currently there is no consensus for susceptibility testing of LAB.

In this work, strains were subjected to antibiotic susceptibility testing using the agar disc diffusion method on solid MRS medium with the use of 07different antibiotics. The strains were grown on MRS broth at 37°C for 24h. Then the inoculum was standardized as described by **Huys *et al.*, (2002)**. The optical density was adjusted to 0.1± 0.02 at 660 nm. Twenty milliliters of semisolid MRS agar that was cooled to 45°C were poured in a Petri dish and inoculated with standardized inoculums. The disks were placed on the top of the inoculated plate. After 24h incubation at 37 °C, inhibition zones around the discs were measured. Inhibition zones diameters were measured and results were interpreted as resistant (R), intermediate (I), or sensitive (S) in accordance with the method of **Charteris et *al.* (1998)**.

III.2.2. Susceptibility of the strains to drugs

The strains *Lb. curvatus* G6, and *Lb. plantarum* G1 were tested for their susceptibility to commercially provided drugs according to **Todorov et *al.* (2011)**. The commercially provided drugs were solubilized in distilled sterile water, then an overnight culture of each strain (*Lb. plantarum* G1 and *Lb. curvatus* G6) were cultured separately on MRS soft agar (1% agar) to achieve 10^6 cfu/ml. 10 µl of each drug was spotted on the surface of the agar, the plates were incubated at 37°C for 24 hours and tested for the presence of inhibition zones.

III.2.3. Minimal inhibitory concentration

The drugs presenting the inhibition zones larger than 2 mm were subjected to the determination of the minimal inhibition concentration, using serial two fold dilutions of the medicaments. For the test, 10 µl of each dilution were spotted onto the surface of the agar, previously imbedded with *Lb. plantarum* G1 and *Lb. curvatus* G6 separately. The plates were incubated for 24 h at 37°C and examined for inhibition zones. Those presenting inhibition zones above 2 mm in diameter were considered as positive.

III.2.4. Use of NSAIDs as the sole carbon source

Preparation of the inoculum

Ten ml of an overnight culture in MRS broth was centrifuged at 4000rpm×3min, the supernatant liquid was decanted and replaced by sterile distilled water, the cells were re-suspended and centrifuged again to eliminate the trace of glucose and again the pellet was suspended in 10ml of sterile distilled water.

Each NSAID was added aseptically after being sterilized by filtration through Milliporefilter (0.22µ) to the basal medium instead of glucose which was sterilized by autoclaving, the following concentrations were used 1mg/ml and 0.5mg/ml. These concentrations were selected based on the maximal solubility of drug. Basal medium without a carbon source was used as negative control and with the addition of 1% of glucose was used as a positive control. 50ml of each medium were placed in flasks and inoculated with 3% of cell suspension to give an initial absorbance of 0.05 ± 0.003 which is equivalent to a concentration of 10^7 cfu/ml according to the methodology of **Serna and Sánchez (2011)**. The flasks were incubated at 37°C and the growth was followed as the change in absorbance at 660nm. Measurement of absorbance has been

shown to be the suitable method for the study of the use of different carbon sources in basal media [**Serna and Sánchez, 2011**].

III.2.5. MRS agar disk diffusion method

In this method, serial dilutions of NSAIDs were prepared in suitable solvents, and sterile paper discs (6 mm) containing 10µl of each dilution (to obtain 500µg, 250µg, 125µg, 65µg, and 30µg per disc) were placed on MRS agar plate seeded with an overnight culture of the desired strain (10^6CFU/ml). Then the plates were incubated at 37 °C for 24 h. Growth inhibition was recorded by measuring the diameter of the zones (6 mm diameter of the disc included).

III.2.6. MRS macrobroth dilution method

The assay was realized in test tubes containing MRS broth and different concentrations of the NSAIDs. Two test tubes containing the broth without the NSAIDs were included in each test, one was inoculated with the strain (positive control) and the other was left uninoculated (negative control) as a check for smedia sterility. The minimal inhibitory concentration (MIC), defined as the lowest concentration of the antimicrobial compound that will inhibit the growth of the microorganism detected by a lack of turbidity at 660 nm matching the negative control after 24 h of incubation at 37°C.

III.2.7. Effect of NSAIDs on the free and microencapsulated cells

Cell microencapsulation

Lb. plantarum G1 and *Lb. curvatus* G6 were cultured in MRS broth at 37°C for 20h. Then the cells were harvested by centrifugation (4000g for 3 min) and washed twice with sterile normal saline and suspended in normal saline, the final OD was 1.6 at 660nm.

Probiotic organisms were microencapsulated using the method of **Ding and shah (2009)**. 45ml of 2% sodium alginate were prepared in a volumetric flask and was sterilized by autoclaving (121°C per 15min). The sterile alginate was mixed with 5 ml of the bacterial suspension and aseptically homogenized using a magnetic stirrer.

The resulting mixture was introduced into a sterile syringe (2.5ml), then a suspension was cast drop wise to the coagulation solution $CaCl_2$ (0.05M+0.1%tween 80) previously autoclaved and cooled, the beads formed were then left for one hour under gentle agitation to complete the ion exchange Na^+, Ca^{++} after that the calcium alginate beads were removed from the aqueous phase and washed twice with sterile distilled water **[Ding and Shah, 2009]**. Finally the beads were conserved on normal saline at 4°C for further utilization.

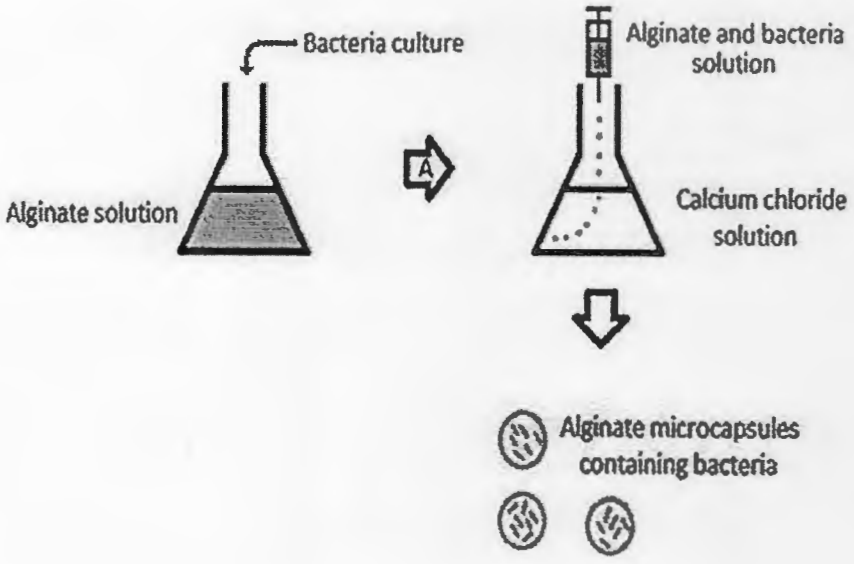

Figure 04: Bacterial encapsulation in alginate by extrusion **[Cook *et al*, 2012].**

Testing the effect of NSAIDs on free and microencapsulated cells in gastric pH

To test the viability of *Lb. plantarum* G1and *Lb. curvatus* G6 in gastric conditions with 0.5mg/ml of NSAIDs both free and microcapsules were inoculated into pH 2 MRS broth supplemented with ibuprofen and diclofenac to achieve a concentration of 0.5mg/ml, and incubated at 37°C for 5h. To disperse the cells serial dilution were performed before inoculated to MRS agar plates. 30 microliters of appropriate dilutions were taken for plates count at T_0 (0h), and T_1 (after 5h) on MRS agar.

For enumeration of microencapsulated strains, bacteria were released from the capsules by sequestering calcium ions with (0.5M) phosphate buffer at pH 7. To aid in their release the phosphate buffer and capsules were vortexed for 1min. Plates were incubated at 37°C for 24-48h. The effect of NSAIDs in pH 2 MRS broth was determined by comparing the final plates count after 5h with the initial plate count at T_0.

IV: Results and discussion

IV.1. Susceptibility of *lactobacillus* to antibiotics

Currently, there is a great concern that food born and commensal intestinal bacterial populations may serve as reservoirs of antibiotic resistance determinants **[Salyers *et al.*, 2004; Ammor *et al.*, 2007]**. Besides desirable technological properties, lactic acid bacteria (LAB) used in food systems as starters or as probiotics need to meet several safety criteria, among which is the absence of acquired antibiotic resistance genes **[Ammor *et al.*, 2007]**. In addition, the optimization of the use of probiotic lactobacilli in cases of gastrointestinal disorders requires the knowledge of their antibiotic resistance to reinforce the concomitant antibiotic therapy **[Belletti *et al.*, 2009]**. This is important because transferable acquired genes have already been characterized in strains of LAB **[Ammor *et al.*, 2007]**.

All tested strains of *Lactobacillus* seem to be sensitive to tetracycline (only *Lb. curvatus* G6 had an intermediate sensitivity). Susceptibility was widespread also against amoxicillin and erythromycin, and bacterial strains were more resistant to colistine sulfat and sulfonamide. In addition, results showed that penicillin G inhibited the growth of *Lb. plantarum* G1, however had no effect on the growth of *Lb. plantarum* F12, *Lb. curvatus* G6 and *Lb. plantarum* G1 showed an intermediate sensitive with Streptomycin but *Lb. plantarum* F12 was sensitive to streptomycin.

Table3: Sensitivity of each strain to different antibiotics

Antibiotic	***Lb. curvatus* G6 Diameter in mm**	***Lb. plantarum* F12 Diameter in mm**	***Lb. plantarum* G1 Diameter in mm**
Amoxycilin	**32 (S)**	**33 (S)**	**32 (S)**
Tetracycline	**9 (I)**	**21 (S)**	**22 (S)**
Streptomycin	**8 (I)**	**14 (S)**	**08 (I)**
Sulfonamide	**6 (R)**	**0 (R)**	**09 (I)**
Colistine sulfate	**7 (R)**	**0 (R)**	**0 (R)**
Erhythromycin	**28 (S)**	**28 (S)**	**28 (S)**
Pnicillin G	**9 (I)**	**0 (R)**	**13 (S)**

(I): intermediate, (S): sensible, (R): resistant.

It is well known that a high frequency of resistance to penicillin G of the species *Lb. plantarum* was observed in isolates from cheese. Furthermore, high percentages of penicillin G resistance have been observed among lactobacilli isolated from sausage, Nigerian fermented foods, and European probiotic products **[Belletti *et al.*; 2009]**. **Zarazaga *et al.* (1999)** found a high portion of their investigated *Lb. plantarum* to be resistant to penicillin. This is in accordance to our observations with the *Lb. plantarum* F12 strains, and in contrast to the result obtained with *Lb. plantarum* G1.

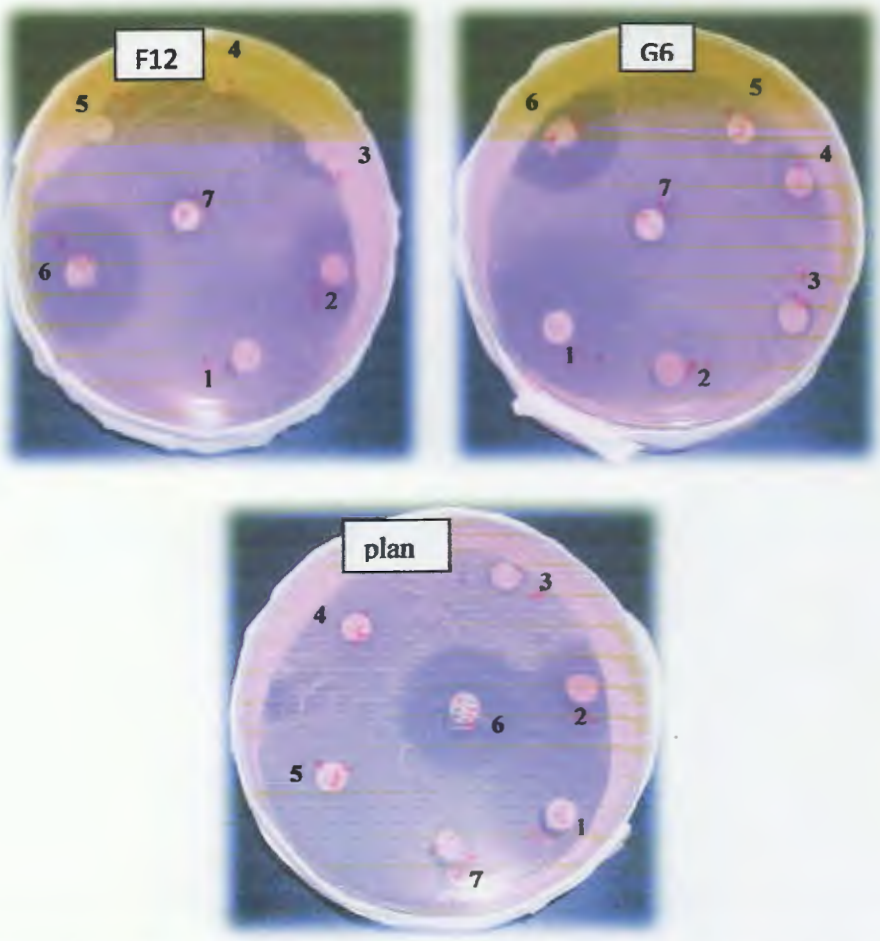

Figure 05: inhibition zones for the susceptibility of *Lactobacillus* strains to antibiotics. (1): amoxicillin, (2): tetracyclin, (3): streptomycin, (4): sulfonamide, (5): colistin sulfate, (6): erhythromycin, (7): penicillin G, (G6): *Lb. curvatus*, (plan): *Lb. plantarum* G1, (F12): *Lb. plantarum* F12.

Our results show that *Lb. plantarum* G1, *Lb. plantarum* F12 and *Lb. curvatus* G6 were sensitive to many antibiotics tested. We suggested then, that these strains don't represent hazard to be a reservoir for the transmission of antibiotic resistance.

IV.2. Susceptibility to commercially drugs

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 is thus important to determine the effect of medicaments on the growth of probiotic strains, especially if the product or foods which contain probiotic strains are considered as possible functional products **[Todorov *et al.*, 2009]**. The growth of both *Lb. plantarum* G1 and *Lb. curvatus* G6 was repressed in the presence of ibuprofen, cefalexine, loradine, and prednisolone. Furthermore, the growth of *Lb. curvatus* G6 was inhibited by N-acetylcysteine, cetirizine, carbocistiene and sulfamethoxazol/trimethoprime while *Lb. plantarum* G1 was inhibited by diclofenac sodic, domperidone and dechlorphenicamine-oraleate/parahydroxy-benzoate methyle as shown in **Table 04**.

Table 04: Susceptibility of *Lb. plantarum* G1 and *Lb. curvatus* G6 to medicaments.

Commercial name	Group of medicament	Active substance	Applied concentration	Zones Diameter (cm)	
				Lb.curvatus G6	*Lb. plantarum G7*
Algifan®	NSAID	Ibuprofen 20mg/ml	**20mg/ml**	**2.8**	**1.6**
Biofenac®	NSAID	Diclofenac sodic 100 mg	**16mg/ml**	**0**	**2.4**
Fluimucil®	Mucolitic agent	N-acetylcysteine 200 mg/ml	**200 mg/ml**	**3**	**0**
Aspegic®	Analgesic /antipyretic	N-acetylsalicylate DL-lysin 100mg/ml	**100mg/ml**	**0**	**0**
Dolic®	Analgesic /antipyretic	Paracetamol 500 mg	**100mg/ml**	**0**	**0**
Blopress®	Antihyper-tensive diuretic	Candesartancilantol 8 mg Hydrochlorothiazide 12.5 mg	**8mg/ml 12.5mg/ml**	**0**	**0**
Spasfon®		Phloroglucinoltrimethylphloroglucinol		**0**	**0**
Lexin®	Antibiotic(cefalosporin)	Cefalexine 250 mg	**25mg/ml**	**2.8**	**2.8**
Vastor®	Vasodilator	Timetazidine 20 mg/ml	**20mg/ml**	**0**	**0**
Zyrtec®	Anti-histaminic	Cetirizine 10 mg/ml	**10mg/ml**	**2.2**	**0**
Perydone®	Stimulator of the intestinal fonctions	Domperidone 1mg/ml	**1mg/ml**	**0**	**2.4**
Loradine®	Anti-histaminic	Loradine 100mg/ml	**100mg/ml**	**2**	**4**
Stopcolic®	gastrointestinald isorders	Purified sarjikshara 45mg/5ml	**9mg/ml**	**0**	**0**
Timonal®	Antispasmodic	Tiemonium 0.2%	**0.2%**	**0**	**0**
Augmentin®	Antibiotic (βlactam)	Amoxicilline 1g Acid clavulanique125 mg	**200mg/ml 25mg/ml**	**0**	**0**
Prednisolone®	Chorticoide	Prednisolone 20 mg/ml	**20mg/ml**	**2**	**1.4**
Debridat®	Gastrointestinal disorders	Trimebutine 0.787g/250 ml		**0**	**0**
NEO-codion®	Gastrointestinal disorders	Comphosulfonate of codeine 0.172 g/180 ml	**0.172g/180ml**	**0**	**0**
Histagan®	antihistaminac	Dichlorphenicamineoraleate 10 mg/100 ml methyl parahydroxy-benzoate0.12 g/100 ml	**0.1mg/ml 1.2mg/ml**	**0**	**2.2**
Rinastin®	Gastrointestinal disorders	Carbocysteine		**1.8**	**0**
Primazol®	Antibiotic	Sulfamethoxazol 200 mg Trimethoprime 40 mg	**200mg/ml 40mg/ml**	**3.4**	**0**

It was reported that growth of *Lb. plantarum*, ST414BZ and ST664BZ, *Lb. paracasei* ST242BZ and ST284BZ, *Lb. rhamnosus* ST461BZ and ST462BZ, and *Lb. pentosus* ST712BZ was inhibited by medicaments containing diclofenac and ibuprofen also, diclofenac and ibuprofen inhibited the growth of *Lb. lactis* ssp. *Lactis* HV219 **[Todorov *et al.*, 2009]**. When we compare our results with the reported results we can found a contradictory result for the sensitivity of *Lb. curvatus* G6 to diclofenac, and this may be dose-related. It is however, important to mention that the concentration of these substances is critical **[Todorov *et al.*, 2007]**.

The mechanism of the inhibitory effect against probiotic LAB and other GIT-related bacteria needs to be related to the chemical composition of drugs. A simple recommendation would be not to apply a drug presenting an inhibitory effect on the probiotic LAB at the same time, since the drug will have a negative effect on the probiotic cells, resulting in decreased viability. The application of drugs along with probiotic cultures needs to be reconsidered, regarding the possibility of a negative interaction **[Todorov *et al.*, 2011]**.

IV.3. Minimal inhibitory concentration

The interaction between medicaments and probiotic bacteria in the GIT depends on their concentration in this environment, so that the Minimal Inhibitory Concentration values play an important role for the proper evaluation of these interactions **[Todorov *et al.*, 2009]**. The minimal inhibitory concentrations of the medicaments tested in our study were shown in **Table 05**. Biofenac was taken as an example to illustrate the method followed for the determination of the minimal inhibitory concentration for each drug.

Results of the minimal inhibitory concentrations showed that both strains have a high sensitivity to cefalixine because when we use a concentration of 3.5 mg/ml we show a total inhibition, susceptibility of *Lactobacillus* to the cephalosporins was recorded by **Belletti *et al.* (2009)** during their study about antibiotic resistance of lactobacilli isolated from two Italian hard cheeses. *Lb. plantarum* G1 showed high sensitivity with domperidon, (Dechlorphenicamineoraleate/ methyl parahydroxy-benzoate) and loradine (with MICs 0.20, 0.05/0.60, 0.15 respectively) the last medicament (loradine) had also high effect against *Lb. curvatus* G6 (MIC=0.20 mg/ml). But *Lb. curvatus* G6 had a low sensitivity with N-acetylcysteine with a minimal inhibitory concentration of 66.6 mg/ml.

Table 05: Minimal inhibitory concentration for each drug

Commercial name	Active substance	MIC (mg/ml)	
		*Lb. curvatus*G6	*Lb. plantarum* G1
Algifan®	Ibuprofen 20 mg/ml	**5**	**5**
Biofenac®	Diclofenac sodic 100 mg	/	**4**
Fluimucil®	N-acetylcysteine 200 mg/ml	**66.6**	/
Aspegic®	N-acetylsalicylate DL-lysin 100 mg/ml	/	/
Dolic®	Paracetamol 500 mg	/	/
Blopress®	Candesartancilantol 8mg Hydrochlorothiazide 12.5 mg	/	/
Spasfon®	Phloroglucinol-trimethylphloroglucinol	/	/
Lexin®	Cefalexine 250 mg	**3.5** total inhibition	**3.5** total inhibition
Vastor®	Timetazidine 20 mg/ml	/	/
Zyrtec®	Cetirizine 10 mg/ml	**5**	/
Perydone®	Domperidone 1mg/ml	/	**0.20**
Loradine®	Loradine 100 mg/ml	**0.20**	**0.15**
Stopcolic®	Purified sarjikshara 45 mg/5ml	/	/
Timonal®	Tiemonium 0.2%	/	/
Augmentin®	Amoxicilline 1g Acid clavuolamique125 mg	/	/
Prednisolone®	Prednisolone 20 mg/ml	**4**	**2.5**
Debridat®	Trimebutine 0.787 g /250 ml	/	/
NEO-codion®	Comphosulfonate of codeine 0.172 g/180 ml	/	/
Histagan®	Dechlorphenicamineoraleate 10 mg/100 ml methyl parahydroxy-benzoate 0.12 g/100 ml	/	**0.05** **0.60**
Rinastin®	Carbocistiene	**ND**	/
Primazol®	Sulfamethoxazol 200 mg Trimethoprime 40 mg	**ND**	/

Table 06: inhibition zone diameter caused by the presence of different concentrations of biofenac with *Lb. plantarum.*

Strain / Dilutions	*Lb. plantarum* G1
16 mg/ml	2.4 cm
8mg/ml	1.5 cm
4mg/ml	1cm
2mg/ml	0 cm
1mg/ml	0 cm

IV.4. Use of NSAIDs as sole carbon source

Food does not considerably change the bioavailability of the NSAIDs. But this statement could not necessarily be true in the case of fermented foods, since they contain live microorganisms which could somehow interact with the NSAIDs. In the other hand, people who take these drugs, may combine NSAIDs and a probiotic treatment, to overcome some complications due to the consumption of NSAIDs, it is necessary that these probiotic don't affect medicament action. And because it have been reported that LAB are able to degrade some phenolic compounds, it is important to investigate whether NSAIDs could cause inhibition of these microorganisms or whether these microorganisms are able to use some of them as the sole carbon source **[Serna and Sanchez, 2011]**.

For this goal, we studied the ability of *Lb. plantarum* G1 and *Lb. curvatus* G6 to use NSAIDs as sole carbon source in a basal medium supplemented with different concentrations of ibuprofen and diclofenac (0.5 mg/ml and 1 mg/ml, respectively). The growth in these conditions was compared with the growth in a basal medium with 1% glucose as sole carbon source (positive control) and the growth in a basal medium without a carbon source (negative control).

The growth expressed as absorbance at 660 nm is presented in **Figure 06** for *Lb. plantarum* G1 and **Figure 07** for *Lb. curvatus* G6).

Before 24h, the observed results indicated that both *Lb. plantarum* G1 and *Lb. curvatus* G6 were unable to biodegrade and to use ibuprofen and diclofenac as sole carbon source. However, these compounds inhibited their growth. **Serna and Sanchez (2011)** obtained the same results with *Lb. casei* Shirota when they investigated the effect of different antibiotics and NSAIDs on the growth of *Lb. casei* Shirota. These results indicated that ibuprofen and diclofenac are not easy to biodegrade. But, near 24h, *Lb. curvatus* G6 with diclofenac showed an increase in growth up to that observed with the negative control, which allow us to think that *Lb. curvatus* G6 after this incubation period was adapted to the presence of diclofenac in the medium and it became able to develop in its presence.

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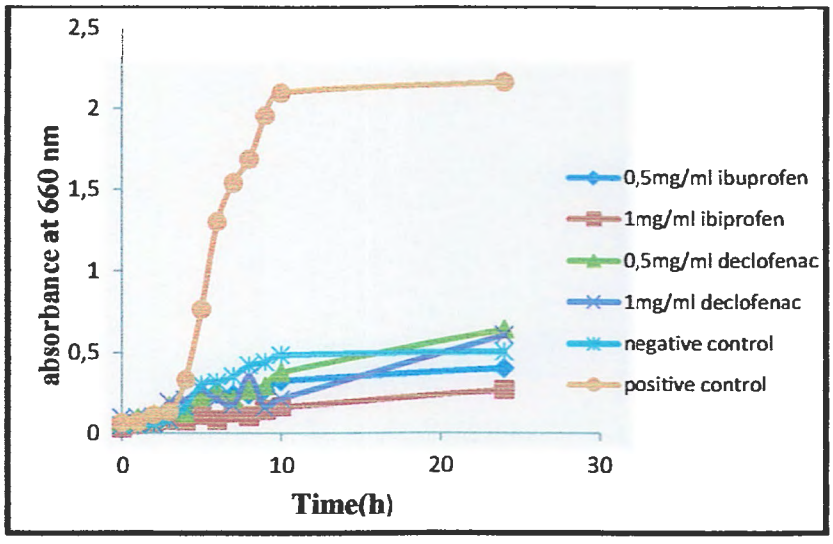

Figure 07: Effect of ibuprofen and diclofenac with different concentrations (0.5and 1mg/ml) on the growth of *Lactobaillus curvatus* G6

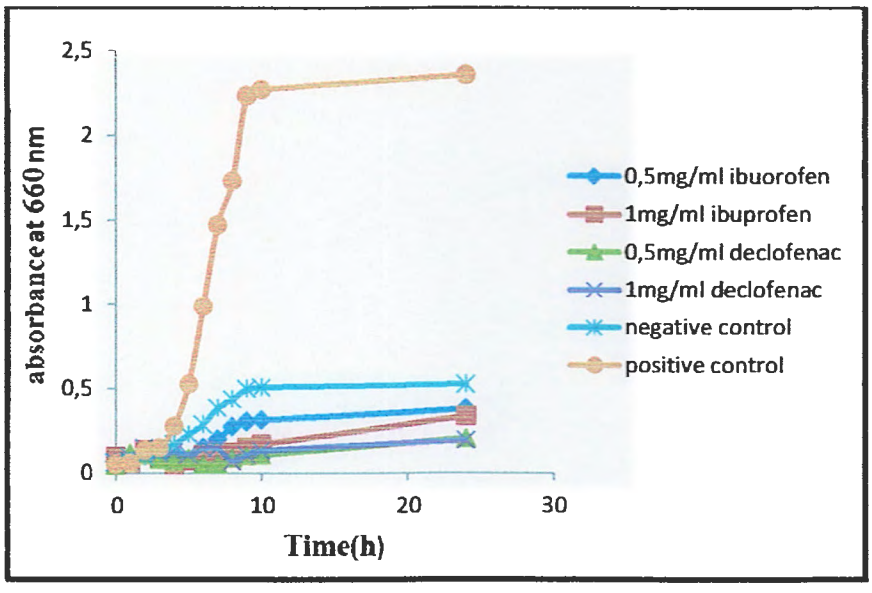

Figure 08: Effect of ibuprofen and diclofenac with different concentration (0.5 and 1mg/ml) on the growth of *Lactobacillus plantarum* G1

Based on the results obtained during our study, we can say that it is advisable to combine the administration of the medicaments (containing ibuprofen and diclofenac) and probiotic *Lb. plantarum* G1 and *Lb. curvatus* G6, while the probiotic should be administrated some hours after the administration of the drug, it is possible that the drug reduce the efficacy of the probiotic microorganisms. It is important to note that the reverse is not true: probiotics will not cause a

reduction in efficacy or effectiveness of the medicament because probiotic strains are unable to degrade both ibuprofen and diclofenac **[Serna and Sanshez, 2011]**.

IV.5. MRS agar disk diffusion method

In the case of the disks diffsion method, for *Lb. plantarum* G1, ibuprofen was inhibitory at all concentrations used as shown in **(Figure 09, I_p)**, the higher concentration disks (500µg and 250µg) showed halo with a diameter of 3 cm and 2,2 cm, respectively, whereas the smallest concentration disks (33µg) showed an inhibition zone with a diameter of 0,4cm. So, the ibuprofen MIC was lower than the smallest concentration disks used (<33µg). Diclofenac showed inhibition zones with an average diameter of 3,0 cm, 2,6 cm and 2,2 cm for the 500µg, 250µg and 125µg, respectively. We considered that 67µg is the miniml inhibitory concentration with an inhibion halo of 0,2 cm in diameter. Both ibuprofen and declofenac had not an inhibitory effect against *Lb. curvatus* G6 at the smallest concentration disk (33µg) and the MIC was equal to 67µg for the two medicaments.

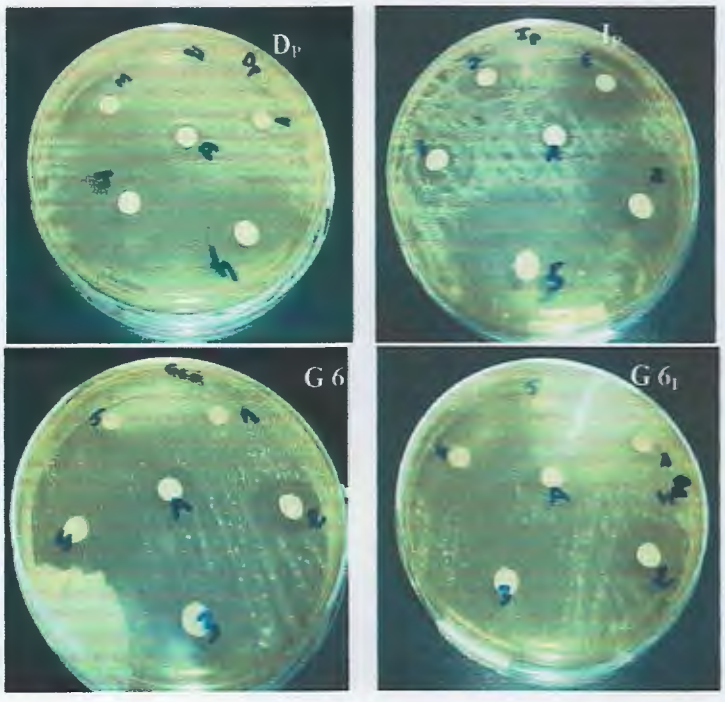

Figure 09: MRS disk diffusion method, (1): 33µg (2) : 67µg (3) : 125µg (4) :250µg (5) : 500µg (A) : acetone (I_p): *Lb. plantarum* G1 with ibuprofen (D_p): *Lb. plantarum* G1 with diclofenac ($G6_d$): *Lb. curvatus* G6 with diclofenac ($G6_i$): *Lb. curvatus* G6 with ibuprofen.

Based on these results we can conclud that both medicaments (ibuprofen and diclofenac) are strong growth inhibitors against both *Lb. plantarum* G1 and *Lb. curvatus* G6. Similar results were recorded by **Todorov *et al.* (2011)** which indicated that especially non-steroidal anti-inflammatory drugs that contain potassium diclofenac or ibuprofen arginine reduced the growth of *Lb. plantarum* **[Todorov *et al.*, 2011]**. In another study conducted by **Carvalho *et al.* (2009),** the growth of *Lb. casei* Shirota and *Lb. casei* LC01 were inhibited by diclofenac potassium and ibuprofen arginine.

Table 07: Diameter of inhibition zones in MRS agar for *Lb. plantarum* G1 and *Lb. curvatus* G6 in the presence of ibuprofen and diclofenac

Strains	Drugs µg	500µg	250µg	125µg	67µg	33µg
Lb. plantarum G1	Ibuprofen	3	2.2	1.4	1	0.4
	Diclofenac	3	2.6	2.2	0.2	0
Lb. curvatus G6	Ibuprofen	2.2	1.8	1.6	0.6	0
	Diclofenac	2.2	1.8	1.2	0.6	0

IV.6. MRS macrobroth dilution method

This method is an alternative to the agar disk diffusion method which could provide additional information on the possible inhibitory effect of NSAIDs. The diffusion method could have limitations such as the possible alteration of the physiological status of the cell due to internal accumulation of NSAIDs and the poor diffusion of these compounds in solid media **[Serna and Sanchez, 2011]**. For both organisms, the OD values increased with the decrease of medicament concentration as shown in the **Table 08**, ibuprofen inhibited the growth of both organisms only at high concentrations (500µg and 250µg), however, the results of this method showed that diclofenac had low effect against both strains comparatively with the effect of ibuprofen, when we used 500 µg of diclofenac we obtained an optical density of 2.082 for *Lb. plantarum* G1 and 1.486 for *Lb. curvatus* G6. But when we use the same concentration of ibuprofen we show a total inhibition (0.05 for *Lb. plantarum* G1 and 0.012 for *Lb. curvatus* G6).

Table 08: The effect of different concentrations of ibuprofen and diclofenac on the growth of *Lb. plantarum* G1and *Lb. curvatus* G6.

Strains	µg / Drugs	500µg	250µg	125µg	67µg	33µg	control
Lb. plantarum G1	Ibuprofen	0.05	0.366	2.300	2.327	2.332	2.480
	Diclofenac	2.082	2.133	2.242	2.299	2.347	
Lb. curvatus G6	Ibuprofen	0.012	0.318	2.227	2.158	2.200	2.298
	Diclofenac	1.486	2.075	2.027	2.028	2.030	

IV.4. The effect of NSAIDs on free and microencapsulated cells in gastric pH

The free and microencapsulated cultures of *Lb. plantarum* G1 were tested for survival in MRS broth supplemented with 0.5mg/ml ibuprofen and diclofenac under simulated gastric pH. The reduction rates of cell counts after 5h of incubation at pH 2.0 are listed in **Table 09**.

Table 09: The effect of NSAIDs on free and microencapsulated *Lb. plantarum* G1 in gastric pH.

Type of cells	Time (h)	Ibuprofen		Diclofenac	
		10^{-7} cfu	10^{-10} cfu	10^{-7}	10^{-10} cfu
Free cells	T_0	5.10^{12}	10^{15}	2.10^{11}	10^{15}
	T_1	1.10^{12}	5.10^{13}	/	2.10^{13}
	Reduction rate	13.31%	8.68%	/	11.33%
Encapsulated cells	T_0	2.10^{12}	2.10^{14}	4.10^{14}	2.10^{14}
	T1	4.10^{11}	2.10^{14}	/	4.10^{13}
	Reduction rate	5.26%	0%	/	4.31%

The results indicated that the survival of the probiotic microorganisms decreased in both cases (free and microencapsulated bacteria). In the case of free *Lb. plantarum* G1, the respective percentage of reduction was 13.31% and 11.33% in the presence of ibuprofen and diclofenac, respectively. For the encapsulated bacteria the reduction percentage of viable counts in ibuprofen and diclofenac was 5.26% and 4.31%, respectively. It is clear that the survival of microencapsulated cells was significantly higher than that of free cells after 5h of exposure to diclofenac and ibuprofen at gastric pH (2.0). Results indicated that microencapsulation of the cells had a protective effect.

Thus, we suggested that alginate microcapsules can enhance the survival of probiotic bacteria when exposed to drug at gastric pH. This results is in agreement with the published data that reported that microencapsulation of LAB has been showed many advantages for physical and chemical protection compared with free cells **[Doleyeres and Lacroix, 2005]**.

V. Conclusion

Our experimental study focused on the effect of some drugs (non steroidal anti-inflammatory drugs, antibiotics and commercially drugs) on the growth of some *Lactobacillus* probiotic strains (*Lb. curvatus* G6, *Lb. plantarum* G1, *Lb. plantarum* F12).

The antibiotic resistance profiles reported in this work showed that *Lb. plantarum* G1 can be ingested by patients treated with colistine sulfate, *Lb. curvatus* G6 by patients treated with sulfonamide, and colistine sulfate, *Lb. plantarum* F12 was resistant sulfonamide, colistine sulfate and penicillin G. The use of probiotic preparation during treatment usually helps to minimize the imbalance in the ecosystem of the intestinal biota, leading to diarrhea.

Lb. plantarum G1, and *Lb. curvatus* G6 showed a good resistance to several commercially available drugs such as **Aspegic®**, **Dolic®**, **Blopress®**, **Spasfon®**, **Vastor®**, **Timonal® Augmentin®**, **Debridat®**, **NEO-codion®**. As a result, they may be applied in combination with probiotics for therapeutic use, **Stopcolic®** and **Perydone®** can be used in combination only with *Lb. curvatus* G6, while *Lb. plantarum* G1 can be applied only with patient treated by **Fluimucil®**, **Rinastin®**, **Primazol®**. Other drugs inhibited the growth of both organisms, the MIC of each active drug was determined, for *Lb. curvatus* G6 they were equal to approximately 66.6, 5, 0.20, 4, for **Fluimucil®**, **Zyrtec®**, **Loradine®**, **Prednisolone®** respectively. In the case of *Lb. plantarum* G1 they were equal to 4, 0.2, 0.15, 2.5, for **Biofenac®**, **Perydone®**, **Loradine® Prednisolone®**. The MIC of **Algifan®** was equal to 5mg/ml for the both strains, **Lexin®** show a total inhibition with an MIC equal to 3.5mg/ml for the both strains.

Based on the obtained results, both *Lb. plantarum* G1 and *Lb. curvatus* G6 were not capable to biodegrade and to use ibuprofen and diclofenac as sole carbon source. However, it may be concluded that *Lb. curvatus* G6 after 24 h of incubation was adapted with the presence of diclofenac in the medium and it became able to grow in the presence of diclofenac.

Microencapsulation of *Lb. plantarum* G1 in calcium alginate beads can significantly improved the survival of this bacterium when exposed to drug at gastric pH. Consequently, this method can be used to overcome the inhibitory effect observed with free cells in similar conditions.

Finally, future interventional approaches should be carried out to improve efficiency of probiotics in the prevention and control of NSAIDs complications and side effect. In addition, further efforts must take into consideration the growing consumer interest in functional foods.

Table 07: Diameter of inhibition zones in MRS agar for *Lb. plantarum* G1 and *Lb. curvatus* G6 in the presence of ibuprofen and diclofenac

Strains	Drugs µg	500µg	250µg	125µg	67µg	33µg
Lb. plantarum G1	Ibuprofen	3	2.2	1.4	1	0.4
	Diclofenac	3	2.6	2.2	0.2	0
Lb. curvatus G6	Ibuprofen	2.2	1.8	1.6	0.6	0
	Diclofenac	2.2	1.8	1.2	0.6	0

IV.6. MRS macrobroth dilution method

This method is an alternative to the agar disk diffusion method which could provide additional information on the possible inhibitory effect of NSAIDs. The diffusion method could have limitations such as the possible alteration of the physiological status of the cell due to internal accumulation of NSAIDs and the poor diffusion of these compounds in solid media **[Serna and Sanchez, 2011]**. For both organisms, the OD values increased with the decrease of medicament concentration as shown in the **Table 08**, ibuprofen inhibited the growth of both organisms only at high concentrations (500µg and 250µg), however, the results of this method showed that diclofenac had low effect against both strains comparatively with the effect of ibuprofen, when we used 500 µg of diclofenac we obtained an optical density of 2.082 for *Lb. plantarum* G1 and 1.486 for *Lb. curvatus* G6. But when we use the same concentration of ibuprofen we show a total inhibition (0.05 for *Lb. plantarum* G1 and 0.012 for *Lb. curvatus* G6).

Table 08: The effect of different concentrations of ibuprofen and diclofenac on the growth of *Lb. plantarum* G1and *Lb. curvatus* G6.

Strains	µg / Drugs	500µg	250µg	125µg	67µg	33µg	control
Lb. plantarum G1	Ibuprofen	0.05	0.366	2.300	2.327	2.332	2.480
	Diclofenac	2.082	2.133	2.242	2.299	2.347	
Lb. curvatus G6	Ibuprofen	0.012	0.318	2.227	2.158	2.200	2.298
	Diclofenac	1.486	2.075	2.027	2.028	2.030	

IV.4. The effect of NSAIDs on free and microencapsulated cells in gastric pH

The free and microencapsulated cultures of *Lb. plantarum* G1 were tested for survival in MRS broth supplemented with 0.5mg/ml ibuprofen and diclofenac under simulated gastric pH. The reduction rates of cell counts after 5h of incubation at pH 2.0 are listed in **Table 09**.

Table 09: The effect of NSAIDs on free and microencapsulated *Lb. plantarum* G1 in gastric pH.

Type of cells	Time (h)	Ibuprofen		Diclofenac	
		10^{-7} cfu	10^{-10} cfu	10^{-7}	10^{-10} cfu
Free cells	T_0	5.10^{12}	10^{15}	2.10^{11}	10^{15}
	T_1	1.10^{12}	5.10^{13}	/	2.10^{13}
	Reduction rate	13.31%	8.68%	/	11.33%
Encapsulated cells	T_0	2.10^{12}	2.10^{14}	4.10^{14}	2.10^{14}
	T1	4.10^{11}	2.10^{14}	/	4.10^{13}
	Reduction rate	5.26%	0%	/	4.31%

The results indicated that the survival of the probiotic microorganisms decreased in both cases (free and microencapsulated bacteria). In the case of free *Lb. plantarum* G1, the respective percentage of reduction was 13.31% and 11.33% in the presence of ibuprofen and diclofenac, respectively. For the encapsulated bacteria the reduction percentage of viable counts in ibuprofen and diclofenac was 5.26% and 4.31%, respectively. It is clear that the survival of microencapsulated cells was significantly higher than that of free cells after 5h of exposure to diclofenac and ibuprofen at gastric pH (2.0). Results indicated that microencapsulation of the cells had a protective effect.

Thus, we suggested that alginate microcapsules can enhance the survival of probiotic bacteria when exposed to drug at gastric pH. This results is in agreement with the published data that reported that microencapsulation of LAB has been showed many advantages for physical and chemical protection compared with free cells **[Doleyeres and Lacroix, 2005]**.

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VI: References

VI. Referance

Ammor MS., Fl'orez AB. and Mayo B.; 2007; Antibiotic resistance in nonenterococcal lactic acid bacteria and bifidobacteria. Food Microbiol.; 24: 559-70.

Barefoot SF. and Klaenhammer TR.; 1984; Purification and characterisation of the *Lactobacillus acidophilus* bacteriocin lactacin B. Antimicrob. Agents Chemother. 26(3): 328 - 324.

Belletti N., Gatti M., Bottari B., Neviani E., Tabanelli G. and Gardini F.; 2009; Antibiotic resistance of Lactobacilli isolated from two Italian hard cheeses. J. Food Prot.; 72: 2162-2169.

Bengmark S.; 2000; Colonic food: pre- and probiotics. Am. J. Gastroenterol.; 95: 5-7.

Bouridan H. and Arid S.; 2012; Effet de la microencapsulation des bactéries lactiques par différentes matrices sur leurs aptitudes probiotiques et technologiques. Thèse de master; Université de Jijel ; Algérie.

Carvalho KG., Kruger MF., Furtado DN., Todorov SD. and Franco BDG.; 2009; Evaluation of the role of environmental factors in the human gastrointestinal tract on the behaviour of probiotic cultures of *Lactobacillus casei* Shirota and *Lactobacillus casei* LC01 by the use of a semi-dynamic *in vitro* model. Ann. Microbiol.; 59: 439-445.

Chandramouli V., Kailasapathy K., Peiri P. and Jones M.; 2004; An improved method of microencapsulation and its evaluation to protect *Lactobacillus* spp. in simulated gastric conditions. J. Microbiol. Methods; 56: 27-35.

Charteris P., Kelly P., Morelli L. and Collins JK.; 1997; Selective detection, enumeration and identification of potentially probiotic *Lactobacillus* and *Bifidobacterium* species in mixed bacterial populations. Int. J. Food Microbiol.; 35: 1-27.

Charteris WP., Kelly PM., Morelli L. and Collins JK.; 1998; Antibiotic susceptibility of potentially probiotic *Lactobacillus* species. J. Food Prot.; 61:1636-1643.

Collins JW., La Ragione RM., Woodwar MJd. and Searle LEJ; 2009; Application of probiotic and prebiotic in livestock In Prebiotics and Probiotics Science and Technology. Charalampopoulos D. and Rastall R A. (eds.); (1): 1123; Springer.

Cook MT., Tzortzis G., Charalampopoulos D. and Khutoryanskiy VV.; 2012; Microencapsulation of probiotics for gastrointestinal delivery. J. Controlled Release.; DOI: 10-16.

Cremonini F., Dicaro S., Nista E.C., Bartolozzi F., Campelli G. Gasbarrini G. and Gasbarrini A.; 2002; Meta-analysis the effect of probiotic administration on antibiotic associated diarrhea. Aliment. Pharmacol. Ther.; 16: 1461-1467.

Deegan LH., Cotter PD., Hil C. and Ross P.; 2006; Bacteriocin: biological tools for bio-preservation and shelf-life extension. Int. dairy J.; 16: 1058-1071.

Ding WK. and Shah NP.; 2009; An improved method of microencapsulation of probiotic bacteria for their stability in acidic and bile conditions during storage. Food Microbiol. Safety; 74: 2.

Doleyres Y. and Lacroix C.; 2005; Technologies with free and immobilised cells for probiotic bifidobacteria production and protection. Int. Dairy J.; 15: 973-988.

Elmer GW.; 2001; Probiotics: 'Living Drugs'. Am. J. Health. Syst. Pharm.; 58(12): 1101-1109.

Fuller R.; 1989; Probiotics in man and animals. J. Appl. Bacteriol.; 66: 365-378.

Gotteland M., Cruchet S. and Verbeke S.; 2001; Effect of *Lactobacillus* ingestion on the gastrointestinal mucosal barrier alterations induced by indometacin in humans. Aliment Pharmacol. Ther.; 15(1): 11-17.

Gregor R.; 2006; Safe and efficacious probiotics: what are they? ; Trends. Microb.; 14 (8): 348-352.

Guarner F.; 2009; Probiotics and chronic gastrointestinal disease. In Probiotics and Chronic Gastrointestinal Disease. Charalampopoulos D., Rastall RA. and Robert A. (eds.); (1): 949; Springer science; New-york.

Guchte M. V., Chaze T., Jan G. and Mistou M.; 2012; Properties of probiotic bacteria explored by proteomic approaches. Current Opinion in Microb.; 15: 1-9.

Hospital A. and Road A.; 1992; Non-steroidal anti-inflammatory drugs uses and complications. Singapore Med. J.; 33: 510-513.

Huys G., D'Haene K. and Swings J.; 2002; Influence of the culture medium on antibiotic susceptibility testing of food-associated lactic acid bacteria with the agar overlay disc diffusion method. Lett. Appl. Microbiol.; 34: 402-406.

IvanovaE., Chipeva V., Ivanova I., Dousset X. and Poncelet D.; 2002; Encapsulation of lactic acid bacteria in calcium alginate beads for bacteriocin production. J. Culture Col.; 3: 53-58.

Kamil R., Geier MS, Butler RN and Howarth GS; 2007; *Lactobacillus rhamnosus* GG exacerbates intestinal ulceration in a model of indomethacin-induced enteropathy. Dig Dis Sci.; 52(5): 1247-1252.

Kotikalapudi BL.; 2009; Characterization and encapsulation of probiotic bacteria using a pea-protein alginate matrix; Thesis of Master; University of Saskatchewan; Canada.

Kumar AA. and Singh H.; 2007; Recent advances in Microencapsulation of probiotics for industrial applications and targeted delivery. Trends Food Scien. Technol.; 18: 240-251.

Laine L., Curtis SP., Cryer B., Kaur A. and Cannon CP.; 2006; Systematic review: the lower gastrointestinal adverse effects of nonsteroidal anti-inflammatory drugs. Aliment Pharmacol. Ther.; 24(5): 751-767.

Laine L., Curtis SP., Cryer B., Kaur A. and Cannon CP.; 2007; Assessment of upper gastrointestinal safety of etoricoxib and diclofenac in patients with osteoarthritis and rheumatoid arthritis in the multinational etoricoxib and diclofenac arthritis long-term (MEDAL) programme: a randomised comparison. Lancet.; 369: 465-73.

Laine L., Curtis SP., Cryer B., Kaur A. and Cannon CP.; 2010; Risk factors for NSAID-associated upper GI clinical events in a long-term prospective study of 34701 arthritis patients. Aliment Pharmacol. Ther.; 32(10): 1240-1248.

Leroy F., FalonyG. and Vuyst L.; 2008; Latest Developments in Probiotics. In Meat Biotechnology; Toldera F. (ed.); 217-229; Springer; New-york.

Levri KM., Ketvertis K., Deramo M, Merenstein JH. and D'Amico F.; 2005; Do probiotics reduce adult lactose intolerance?. Applied Evidence; 54(7): 613-620.

MacNaught CE. and MacFie J.; 2001; Probiotics in clinical practice: a critical review of the evidence. Nutrition Res.; 21: 343-353.

Mehinto AC.; 2009; Impacts of the human pharmaceutical diclofenac in the aquatic environment. Thesis of Doctorate; University of Exeter; UK.

Mezaini A., Chihib N., Bouras AD., Naima N. and Hornez JP.; 2009; Antimicrobial activity of some lactic acid bacteria isolated from an Algerian dairy product. J. Environ. Public Health; DOI: 10.1155/2009/678495.

Mokarram RR., Mortazavi SA., Najafi H MB. and Shahidi F.; 2009; The influence of multi stage alginate coating on survivability of potential probiotic bacteria in simulated gastric and intestinal juice. Food Res. Int.; 42: 1040-1045.

Montalto M., Gallo A., Curigliano V., D'Onofrio F., Santoro L., Covino M., Dalvai S., Gasbarrini A. and Gasbarrini G.; 2010; Clinical trial: the effects of a probiotic mixture on non-steroidal anti-inflammatory drug enteropathy: a randomized, double blind, cross-over, placebo-controlled study. Aliment Pharmacol. Ther.; 32: 209-214.

Morishita T., Deguchi Y., Yajima M., Sakurai T. and Yura T.; 1981; Multiple nutritional requirements of Lactobacilli: genetic lesions affecting amino acid biosynthetic pathways. J. Bacteriol.; 148: 64-71.

Mortazavian AM., Mohammadi R. and Sohrabvandi S.; 2012; Delivery of probiotic microorganisms into gastrointestinal tract by food products; In New advances in the basic and clinical gastroenterology. Brzozonwski T. (ed.); 121-146; Intech; Croatia.

Patel D., Parekh T. and Rema S.; 2008; Bacteriocin production by free and immobilized *Lactococcus lactis.* Elec. J. Pharmacol. Therapy; 1: 39-43.

Prakash S. and Urbanska AM.; 2008; Colon-targeted delivery of live bacterial cell biotherapeutics including microencapsulated live bacterial cells. Biologics: Targets Therapy 2(3): 355-378.

Radi ZA. and Khan NK.; 2006; Effects of cyclooxygenase inhibition on the gastrointestinal trac. Exp. Toxicol. Pathol.; 58 (3): 163-173.

Reid G. and Burton J.; 2002; Use of *Lactobacillus* to prevent infection by pathogenic bacteria. Microbes and Infection; 4: 319-324.

Richy F., Bruyere O., Ethgen O., Rabenda V., Bouvenot G., Audran M., Herrero-Beaumont G, Moore A, Eliakim R, Haim M and Reginster JY.; 2012; Time dependent risk of gastrointestinal complications induced by non-steroidal anti-inflammatory drug use: a consensus statement using a meta-analytic approach. Ann. Rheum.; 63: 759-766.

Rokka S. and Rantamäki P.; 2010; Protecting probiotic bacteria by microencapsulation: challenges for industrial applications. Eur. Food Res. Technol.; 231: 1-12.

Rosas-Ledesma P., Leon-Rubio JM., Alarcon FJ., Morinigo MA. and Balebona MC.; 2012; Calcium alginate capsules for oral administration of fish probiotic bacteria: assessment of optimal conditions for encapsulation. Aquaculture Res.; 43: 106-116.

Sabikhi L., Babu R., Thompkinson DK. and Kapila S.; 2010; Resistance of microencapsulated *Lactobacillus acidophilus* LA1 to processing treatments and simulated gut conditions. Food Bioprocess Technol.; 3: 586-593.

Saidi N., hadadji M. and Guessas B.; 2011; Secreening of bacteriocin producing lactic acid bacteria isolated from west Algerian goat's milk. Global J. biotechnol. Biochm.; 6(3): 154-161.

Salyers AA., Gupta A. and Wang Y.; 2004; Human intestinal bacteria as reservoirs for antibiotics resistance genes. Trends Microbiol.; 12: 412-416.

Sapathy K. and Schin J.; 2000; Survival and therapeutic potential of probiotic organisms with reference to *Lactobacillus acidophilus* and *Bifidobacterium* spp; Immunol. Cell Biol.; 78: 80-88.

Serna A. and Sanchez H.; 2011; Effect of different antibiotics and non-steroidal Anti-inflammatory drugs on the growth of *Lactobacillus casei* shirota. Curr. Microbiol.; 62: 1028-1033.

Sifour M., Idoui T., Ouled Haddar H., Namous H. and Aissaoui S.; 2012; Production and caracterization of bacteriocin of *Lactobacillus plantarum f12* with inhibitory activity against *Listeria monocytogenes*. Elec. J. Scien. Tech.; 2(1): 55-61.

Silva M. and Jacobus NV.; 1987; Antimicrobial substance from a human *Lactobacillus* strain. Antimicrob. Agents Chemother.; 1231-1233.

Somasundaram S.; 2000; Uncoupling of intestinal mitochondrial oxidative phosphorylation and inhibition of cyclooxygenase are required for the development of NSAID-enteropathy in *the rat.* Aliment Pharmacol. Ther.; 14(5): 639-650.

Spiegel MR., Chiou CF. and Ofman J.; 2005; Minimizing complications from nonsteroidal antiinflammatory drugs: cost-effectiveness of competing strategies in varying risk groups. Arthritis Rheumatism; 53: 185-197.

Thirabunyanon M., Boonprasom P. and Niamsup P.; 2009; Probiotic potential of lactic acid bacteria isolated from fermented dairy milks on antiproliferation of colon cancer cells. Biotechnol. Lett.; 31: 571-576.

Tiwari G., Tiwari R., Pandey S. and Pandey P.; 2012; Promising future of probiotics for human health. Current Scenario. Chroni. Young Scien.; 3 (1): 17-27.

Todorov SD. and Discks LMT.; 2005; Production of bacteriocin ST33LD, produced by *Leuconostoc mesenteriodes* subsp. Mesenteriodes, as recorded in the presence of different medium component. World J. Microb. Biotech.; 21: 1585-1590.

Todorov SD., Botes M., Danova ST. and Dicks LMT.; 2007; Probiotic properties of *Lactococcus lactis* ssp. *lactis* HV219, isolated from human vaginal secretions. J. Appl. Microbiol.; 103: 629-639.

Todorov SD., Mollendorff JW., Moelich E., Muller N., Witthuhn RC. and Dicks LMT.; 2009; Evaluation of potential probiotic properties of *Enterococcus mundtii*, its survival in boza and *in situ* bacteriocin production. Food Technol. Biotechnol.; 47 (2): 178-191.

Todorov SD., Le-blanc JG. and Franco DGM.; 2011; Evaluation of the probiotic potential and effect of encapsulation on survival for *Lactobacillus plantarum* ST16Pa isolated from papya. World J. Microbial. Biotechnol.; DOI 10.1007/s11274-011-0895.

Tursi A., Brandimarte G., Giorgetti GM., Forti G., Modeo ME. and Gigliobianco A.; 2004; Low-dose balsalazide plus a high-potency probiotic preparation is more effective than balsalazide alone or mesalazine in the treatment of acute mild-to-moderate ulcerative colitis. Med. Sci. Monit.; 10 (11): 126-131.

Uejima M., Kinouchi T. and Kataoka K.; 1996; Role of intestinal bacteria in ileal ulcer formation in rats treated with a nonsteroidal antiinflammatory drug. Microbiol. Immunol.; 40(8): 553-560.

Watanabe T., Nishio H., Tanigawa T., Yamagami H., Okazaki H., Watanabe K., Tominaga K., Fujiwara Y., Oshitani N., Asahara T., Nomoto K., Higuchi K., Takeuchi K. and Arakawa T.; 2009; Probiotic *Lactobacillus casei* strain Shirota prevents indomethacin-induced small intestinal injury: involvement of lactic acid. Am. J. Physiol. Gastrointest Liver Physiol.; 297: 506-513.

Zamfir M. and Callewaert R.; 1999; Purification and characterisation of a bacteriocin produced by *Lactobacillus acidophilus* IBB 801. J. Appl. Microbiol.; 87(6): 923-931.

Zarazaga M., Sainz Y., Portillo A., Tenorio C., Ruiz-Larrara RF., Campo R., Baquero F. and Torres C.; 1999; In vitro activities of ketolide HMR3647, macrolides, and other antibiotics against *Lactobacillus*, Leuconostoc and Pediococcus isolates. Antimicrob. Agents Chemother.; 43: 3039-3041.

Zheng BG., Zheng Z., Zhang JB., Luo XZ., Wang JQ., Liu Q. and Wang LH.; 2011; Degradation of the emerging contaminant ibuprofen in aqueous solution by gamma irradiation. Desalination; 276: 379-385.

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Theme

Effect of drugs on the viability of free and encapsulated probiotic *Lactobacillus.*

Abstract

The aim of this study was to investigate whether ibuprofen and diclofenac (two common non-steroidal anti-inflammatory drugs) could inhibit the growth of two probiotic lactic acid bacteria, *Lb. plantarum* G1 and *Lb. curvatus* G6 or whether these probiotic strains are capable to use one of the NSAIDs as a sole carbon source. Moreover, the susceptibility of these strains to 21 commercially available drugs and 7 antibiotics was studied.

A wide sensitivity against ibuprofen and diclofenac was obtained with both strains, signifying that both strains were not capable to use ibuprofen and diclofenac as a sole carbon source. Furthermore, the growth of both strains was repressed in the presence of ibuprofen, cefalexine, loradine, and prednisolone with respective MIC's for *Lb. plantarum* G1 (5, <3.5, 0.15, 2.5 mg/ml) and for *Lb. curvatus* G6 (5, <3.5, 0.20, 4mg/ml).

In addition, microencapsulation in sodium alginate gave an improvement in viability of about 7 % and 8 % of *Lb. plantarum* G1cells when incubated in stomach-like conditions with Diclofenac and ibuprofen, respectively.

Keywords: NSAIDs, ibuprofen, diclofenac, probiotic strains, *Lb. plantarum* G1 and *Lb. curvatus* G6, microencapsulation

Résumé

L'objectif de ce travail est d'étudier si deux anti-inflammatoires non stéroïdien (AINS) l'ibuprofen et diclofenac sont capables d'inhiber la croissance de deux bactéries (*Lb. plantarum* G1 et *Lb. curvatus* G6) ou si ces souches probiotiques sont capable d'utiliser l'un des deux médicaments comme source unique de carbone. De plus, l'effet de 21 médicaments commercialisés et de 07 d'antibiotique sur la viabilité des Lactobacilles a été étudié.

Une sensibilité relativement importante aux deux AINS ibuprofen et decclofenac a été notée avec les deux souches, ce qui signifie que les deux souches sont incapable d'utiliser l' ibuprofen et diclofenac comme source unique de carbone. D'autre part, la croissance des deux souches a été inhibée en presence de l'ibuprofen, cefalexine, loradine, et prednisolone avec des CMIs respective pour *Lb. plantarum* G1égale à (5, <3.5, 0.15, 2.5 mg/ml) et pour *Lb. curvatus* G6 égale à (5, <3.5, 0.20, 4mg/ml).

La microencapsulation des cellules probiotiques de *Lb. plantarum* dans du gel d'alginate a abouti à une amélioration de la viabilité cellulaire des souches égale à 7.02 % et 8.05 dans des conditions similaires à celles de l'estomac en présence de l'ibiprofen et diclofenac, respectivement.

Mots clés : anti-inflammatoire non stéroïdien, ibuprofen, diclofenac, souches probiotiques, *Lb. plantarum* G1 et *Lb. curvatus* G6, microencapsulation.

الملخص

أجري هذا البحث بهدف دراسة قدرة مضادات الالتهاب اللاستيرويدية ibuprofen و diclofenac على تثبيط نمو *Lb. plantarum* G1 و *Lb. curvatus* G6 و قدرة البكتيريا البربيوتيكية على استخدام أحدها مصدرا وحيدا للكربون و أيضا دراسة حساسية هذه البكتيريا ل 21 دواء تجاري و لمضادات حيوية مختلفة. و أخيرا تم اختبار فعالية الكبسلة الدقيقة على حيوية الخلايا في ظروف الرقم الهيدروجيني المعدي و وجود 0.5ملغ/مل من الدواء.

أظهرت الدراسة حساسية كبيرة للبكتيريا المختبرة تجاه ibuprofen و diclofenac و هذا يوضح أن هذه البكتيريا غير قادرة على استخدام الدواء مصدرا وحيدا للكربون في حين نمو كل بكتيريا قد أعيق في وجود ibuprofen, cefalexine, loradine, prednisoloneمع اصغر تركيز مثبط ل*Lb. Plantarum* (5, <3.5, 0.2, ملغ/مل) و *Lb. curvatus* (5, <3.5, 0.2, 4ملغ/مل) على الترتيب. بالإضافة إلى ذلك أعطت الكبسلة الدقيقة نسبة تحسن في حيوية الخلايا تقدر ب 7.02% و8.05% في الرقم الهيدروجيني المعدي مع 0.5ملغ/مل من diclofenac و ibiprofen على التوالي.

الكلمات المفتاحية : مضادات الالتهاب اللاستيرويدية. diclofenac. Ibuprofen. البكتيريا البربيوتيكية . *Lb. curvatus* G6 . الكبسلة الدقيقة. *Lb.plantarum* G1.