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Kučan, Martina; Gobin, Ivana; Markov, Ksenija; Jurčić Momčilović, Diana; Frece, Jadranka

Original scientific paper

Testing the adhesion and colonization ability of *Lactobacillus plantarum* strain S1 to the mice intestinal epithelium

Martina **KUČAN**[1*], Ivana **GOBIN**[1], Ksenija **MARKOV**[2], Diana **JURČIĆ MOMČILOVIĆ**[1], Jadranka **FRECE**[2]

ABSTRACT

Intestinal diseases are often the consequence of a myriad factors which disturb the complex ecosystem of the gastrointestinal system. For that reason, great attention is dedicated to the use of lactic acid bacteria as probiotics. When choosing the strains for probiotic use, one of the important criteria is the ability of adhesion and binding to the intestinal epithelium. Therefore, the aim of this study was to examine the possibility of adhesion and colonization of *Lactobacillus plantarum* strain S1 to gastrointestinal system of mice. At the same time the influence of *L. plantarum* S1 on composition of intestinal microflora of mice was examined. To test the *in vitro* adhesion properties, bacteria were added to freshly prepared tissue of the BALB/c mice small intestine. Mice were fed with *L. plantarum* strain S1 for 5 consecutive days. The result showed that *L. plantarum* strain S1 have good adhesion ability, *in vitro* and *in vivo*. The examined strain of *L. plantarum* successfully colonize the gastrointestinal system of mice and it showed a positive effect on the intestinal microflora, reducing the number of enterobacteria and clostridia.

In conclusion, *L. plantarum* strain S1 shows good properties of adhesion and colonization of the gastrointestinal system of mice and for that reasons could be used as a probiotic strain.

Key words: intestinal microflora, lactic acid bacteria, adhesion, colonization

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[1] University of Rijeka
School of Medicine
Department of Microbiology and Parasitology
Braće Branchetta 20
51000 Rijeka, Croatia

[2] University of Zagreb
Faculty of Food Technology and Biotechnology
Laboratory for General Microbiology and Food Microbiology
Pierottijeva 6
10000 Zagreb, Croatia

[*] *Corresponding author*
Martina Kučan
Braće Branchetta 20
51 000 Rijeka, Croatia
Phone: 0038551651145
Fax: 0038551651177
Mobile phone: 00385917824364
E-mail: martina.kucan@gmail.com

INTRODUCTION

Lactic acid bacteria (LAB) are gram-positive bacteria that ferment carbohydrates and higher alcohols in lactic acid. [1]. LAB are part of the natural microflora of the gastrointestinal system of humans and animals and rarely showed pathogenic properties [2, 3]. Among LAB many *Lactobacillus* strains have been characterised as probiotics. Probiotics are live microorganisms thought to be beneficial to the host organism [4]. The recent definition of probiotics says that these are live microorganisms which, consumed in high numbers (less than 10^9 CFU per day), express health effects beyond their usual well-known nutritional value [5, 6].

The long tradition of using LAB with no adverse effects on human health, has provided a GRAS status (Generally regarded as Safe) by U.S. FDA (United States Food and Drug Administration) or QPS status (Qualified presumption of Safety) by European legislation Union [4, 6].

They provide a beneficial effect on health without interference with the gastrointestinal microbial flora [5]. Characteristic of LAB is the formation different compounds and organic acids, hydrogen peroxide, diacetyl, CO_2, and bacteriocins, which secrete into the environment in which they grow [7, 8, 9]. These substances prevent the growth of undesirable microbial populations to humans [7].

To achieve the desired effect on health probiotic LAB must be able to survive and colonize the gastrointestinal (GI) system of the host during enough long period. The main barrier to survival of potential probiotic strains in the GI system are the low pH of the gaster, lysozyme, bile salts and digestive enzymes such as pepsin and pancreatic enzymes. Bacterial strains for probiotic use should satisfy many criteria: general, technical and functional criteria. Selection of potential probiotic strains is based on *in vitro* studies as a prerequisite to determine a probiotic properties *in vivo* [4, 10].

The aim of this study was to examine the probiotic properties of *L. plantarum* strain S1 *in vitro* and *in vivo*. Also, the abillity of adhesion and colonization of the GI system of mice was examined.

MATERIALS AND METHODS

Bacteria

The potentially probiotic properties of *L. plantarum* strain S1, isolated from whey were tested. This strain of *L. plantarum* was obtained from the Laboratory of Microbiology and Food Microbiology, Faculty of Food Technology and Biotehnology, University of Zagreb. Bacteria were stored in MRS (Man-Rogosa-Sharpe) medium containing 30 % (v/v) glycerol at -70 °C.

Mice

Eight to twelve weeks old BALB/c mice were used. Animals were obtained from the Central Animal Facility of the Medical Faculty, Universi-

ty of Rijeka. Each experimental group consisted of three mice, housed in cage. Mice had continual access to water and were fed ad libitum. All experimental procedures were carried out according to the standards set in "The International Guiding Principles for Biomedical Reaserch Involving Animals" from "The Council of International Organisations of Medical Science".

Mice feeding and feces sampling

Bacterial cells were centrifuged at 3500 rpmi for 5 minutes, washed three times and resuspended in sterile 0.8 % NaCl to final concetration of $1x10^{11}$ CFU/ml. The number of bacteria was determined spectrophotometrically. Mice were fed with 100 μL of this bacterial suspension.

Survival of *L. plantarum* strain S1, during transit trought GI system, was determined in one gram dry weight faceal samples, which were individually colleted 2 h after first feeding. Faceal samples were homogenized in 1 mL sterile 0.8 % NaCl and serially diluted before plating on selective media: VRBG agar (Violet Red Bile Glucose) for *Enterobacteriaceae* counts, MRS agar for Total LAB count and Sulphite agar for sulphite-reducing clostridia counts.

In vitro adhesion test

The fresh tissue of the small intestine of healthy BALB/c mice were used. Tissue samples were kept 30 min in phosphate buffer (pH = 7.2), at 4 °C. After washing three times in phosphate buffer, tissue samples were added in the prepared bacterial suspension (~10^8 CFU/ml) and incubated at 37 °C for 30 min. Next, the tissue was fixed in 10 % formalin, dehydrated through graded ethnol (70 % to 95 % to 100 %), embedded in paraffin, and cutted at a sections of 5 μm. Sections were stained by Brawn and Brenn staining. Gram-positive bacteria are colored blue, Gram-negative red or pink, core of tissue cells are red, and other tissue elements are yellow.

In vivo adhesion test and survival in GI system

Mice were fed *per os* with *L. plantarum* strain S1 with a daily dose of $1x10^{11}$ CFU/100 μL bacterial suspension in 0.8 % NaCl. The control group was fed with 100 μL sterile 0.8 % NaCl. After five days feeding procedure ended, and on the day 5 and day 21 adhesion and colonisation ability was determined in homogenates of small and large intestine of BALB/c mice. First time point was chosen to test whether *L. plantarum* strain S1 is able to survive, adhere and colonize the GI system of mice. The second time point was chosen in order to investigate whether *L. plantarum* S1 retained in the intestine of mice and if it has their impact on intestinal microflora.

Bacterial staining in intestine tissue

Fifth day after first feeding the small intestine tissue was fixed in 10 % formalin, dehydrated through graded ethanol, embedded in paraffin, and cutted at a sections of 5 μm. Sections were stained by Brawn and Brenn

staining. Gram-positive bacteria are colored blue, Gram-negative red or pink, core of tissue cells are red, and other tissue elements are yellow.

Statistical analysis

All data points in each experiment were obtained in duplicate, and statistical calculation were made in GraphPad Prism version 4.

RESULTS AND DISCUSSION

When selecting strains of lactic acid bacteria for probiotic use one of the most important criteria is the ability to bind to the intestinal epithelium. Complex interactions involved in adhesion were not yet fully clarified. The adhesion to the surface of intestinal epithelial cells is prerequisite for the colonization of probiotic bacteria in the GI system. This feature allows long-term effects of probiotic bacteria on intestinal microflora and the host immune system [4]. To test the ability of adhesion of *L. plantarum* strain S1, bacteria was added into freshly prepared tissue of the small intestine. The results show that *L. plantarum* strain S1 successfully adhere to intestinal epithelial cells of mice *in vitro.* (**Figure 1**). Perdigo et al. showed that adhesion to epithelial cells and mucus mediates colonisation of the GI system by lactobacilli and may be prerequisite for competitive exclusion of enteropathogenic bacteria and immunomodulation of the host [11].

To detemined if the examined strain passed and survived through the GI system, two hours after feeding, in feces the total number of lactic acid bacteria (TLAB) was determined. In the feces of mice fed with *L. plantarum* strain S1 more lactic acid bacteria than the control group of mice was detected (**Figure 2**).

If a bacterial population is constantly present in the intestine, without periodic oral intake, it is considered that it colonized GI system. For this reason, *in vivo* tests in the GI system of BALB/c mice have been made.

Mice were fed five days in a row with *L. plantarum* strain S1 and on 5th and 21st day after first feeding TLAB, E and SRC in small and large intestine was determined.

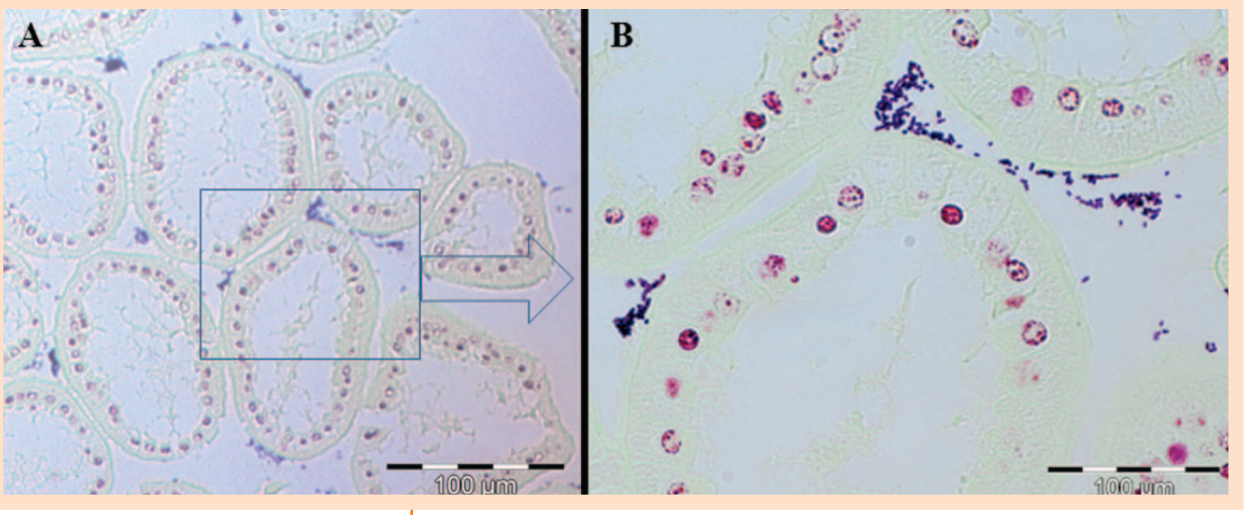

Figure 1.
Adhesion of *L. plantarum* strain S1 to the intestinal epithelium of BALB/c mice *in vitro*. Sections were stained by Brawn and Brenn staining and were analyzed under (A) 200x and (B) 600x magnification. Bacteria cells were stained blue.

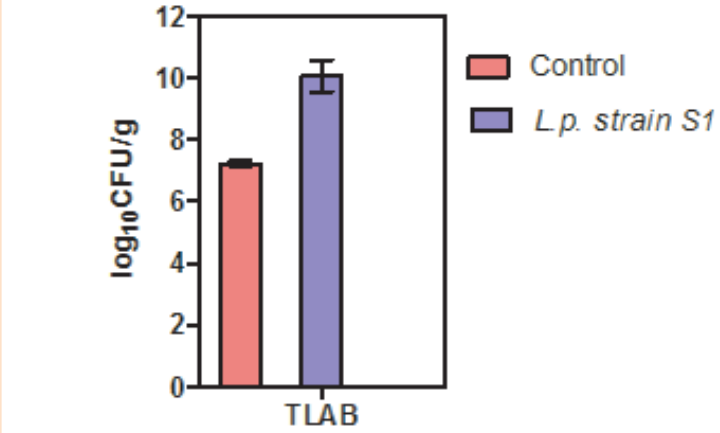

Figure 2.
The number of total lactic bacteria (TLAB) in the mice feces 2 hours after first feeding with *L. plantarum* strain S1. Control mice were fed with sterile saline. TLAB were detected on MRS-agar. Values are mean ± standard deviations of results from two separate experiments.

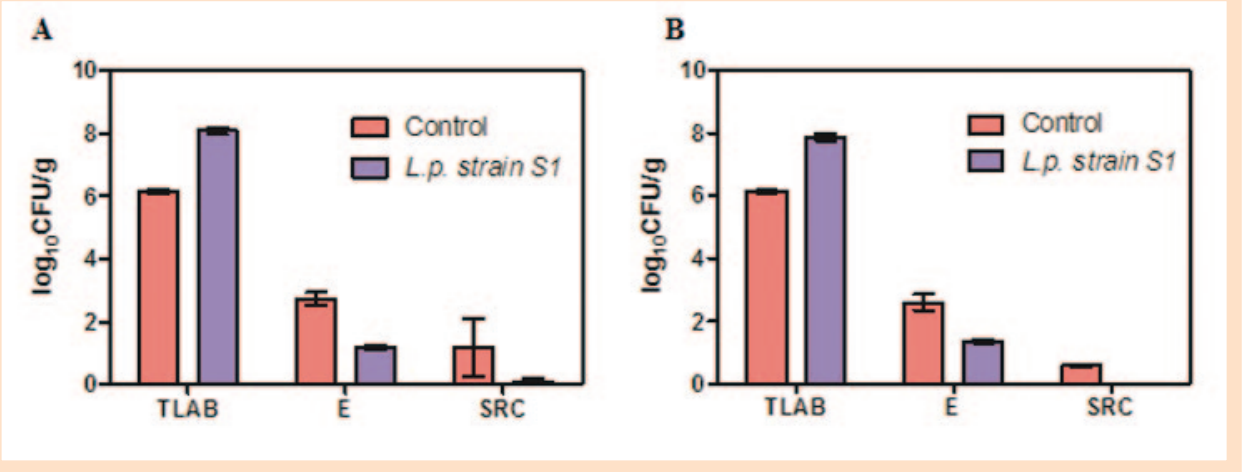

Figure 3.
The number of bacteria in the small intestine 5th day (A) and 21th day (B) after first feeding with *L. plantarum* strain S1. Control mice were fed with sterile saline. Total lactic acid bacteria (TLAB) on MRS-agar; *Enterobacteriaceae* (E) on Violet red bile glucose agar; sulphite-reducing clostridia (SRC) on Sulfite agar were detected. Values are mean ± standard deviations of results from two separate experiments.

The results show that in mice, fed with *L. plantarum* strain S1, was the higher number of lactic acid bacteria, compared to the control group. Also noticeable was the reduced number of enterobacteria and sulphite-reducing clostridia in the same group of mice (**Figure 3**). This results suggested that the tested strain successfully colonize the GI system of mice. Microscopic preparation intestine of mice showing clusters of blue stained bacilli in the intestinal content and on the surface epithelium (**Figure 4**).

It is still not fully explained the mechanism of probiotic effect. Possible mechanisms through which lactic acid bacteria have a protective or therapeutic effects are: competition for nutrients, competition for binding sites in the gastrointestinal system, production of antibacterial substances, modifications of metabolic processes in the gastrointestinal system and immune modulation [1, 3, 9].

Our results showed that *L. plantarum* strain S1 successfully survive the conditions in the GI system of mice and has the ability of adhesion and colonization of the colon and small intestine. Also, the tested strain has positive effect on the microbial flora because it reduces the presence of potentially adverse intestinal microflora populations.

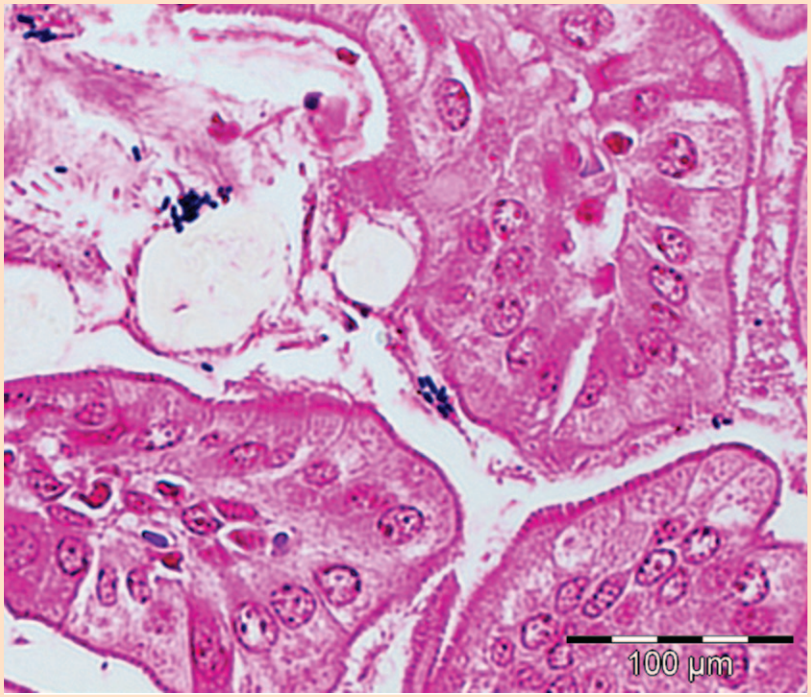

Figure 4. Representative pictures of *L. plantarum* strain S1 in the small intestine of BALB/c mice 5 days after first feeding. Slides were stained by Brawn and Brenn staining and were analyzed under 600x magnification. Clusters of blue stained bacilli in the intestinal content and on the surface epithelium were stain blue (arrow).

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