BIOCHEMICAL PROPERTIES AND VIABILITY OF TWO THERMOPHILIC LACTIC ACID BACTERIA IN A SINGLE CHAMBER GASTROINTESTINAL TRACT SIMULATOR – GIS1: PARTIAL CHARACTERIZATIONS

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Abstract

The viability of the thermophilic probiotic bacteria Lactobacillus plantarum BS1 and BS3 was determined in a in vitro unicameral system (www.gissystems.ro). The bacterial cells were isolated by centrifugation at 5000× g for 10 min, washed three times and freeze-dried in a Martin Christ Alpha 1-2 LD plus freeze dryer. The unicameral system GIS1 consists of 1 Duran bottle with removable screw cap, of 1,000-mL capacity, made of borosilicate glass. The Duran bottle screw cap has 4 entry points. The operating conditions for in vitro simulation depend mainly on pH values for each segment of the colon. Results proved that the two thermophilic strains had a good survival capacity (CFU/mL) after the gastric and small intestine transit. The synthesis of exopolysaccharides and viscosity, as major biochemical parameters, were determined. The exopolysaccharides quantity decreased during the GIS1 transit, generally not directly correlated to viscosity. It results that viscosity increase and viability loss in the GIS1 system was proportional, especially in the small intestine. It was also observed that the viscosity increased with the stationary time spent in each simulated segment. The current model is one of the most significant in vitro simulation systems, due to the correlation between physiologica, and microbiological effects, with a good biochemical stability during in vitro simulation.

Key words: exopolysaccharides, *Lactobacillus*, probiotic, GIS1 system

INTRODUCTION

In the dairy industry, several thermophilic lactic acid bacteria strains are used, especially *Streptococcus thermophilus*, *Lactobacillus lactis*, *Lactobacillus helveticus*, *Lactobacillus casei* and *Lactobacillus plantarum*. The processing technology implies establishing strain viability, the influence of gastrointestinal digestion on lactic acid bacteria strains from the final product and different biochemical parameters [1].

A modern method is the use of static or dynamic *in vitro* stimulators to characterize newly isolated strains. By means of *in vitro* studies but also combined with other *ex vivo* results one may demonstrate a series of features which might be useful when administering a classical medication. First of all, we talk about demonstrating a colonization and multiplication capacity in the intestinal environment. These effects on microbiota will determine an inhibition of pathogen strains and will form a barrier against other infections [2].

Simultaneous with the multiplication of these strains, a synthesis of various organic acids takes place, whereby the most important is the lactic acid, which will lead to a decrease of the value of intestinal pH. This environment is one the main methods to inhibit pathogen strains. The synthesis capacity of certain peptides with antimicrobial effect is associated to this mechanism [3]. The selected strains, with physiological properties demonstrated *in vitro*, are administered by means of lyophilized supplements or by the consumption of products based on fermented milk. Although effects are specific to each person, depending on the physiological and morphological structure of each individual, the

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consumption of these products determined the achievement of very good results. One noticed direct effects on constipation and on elimination of intestinal disturbances caused by the excessive administration of antibiotics [4]. The aim of the paper was the determination of viability of the thermophilic probiotic bacteria *Lactobacillus plantarum* BS1 and BS3 in an *in vitro* unicameral system.The results were correlated with the synthesis of lactic acid and exopolysaccharides but also with the viscosity value at the level of each simulated segment.

MATERIAL AND METHOD

Chemical. All chemicals and reagents were analytical grade and were purchased from the Sigma Aldrich.

Strains and cultivation conditions. A *Lactobacillus plantarum* BS1 and *Lactobacillus plantarum* BS3 strains were obtained from the collection of the Faculty of Biotechnology, Bucharest, Romania. The strains were kept in Nalgene cryotubes, in 20% glycerol, at -80^{0}C. The inoculum was obtained through cultivation under stirring at 100 rpm, during 48 hours, in a MRS medium, at 37^{0}C [5]. The each biomass were obtained in the same conditions. The bacterial cells were obtained after centrifugation at 5.000 rpm for 10 min and washed three times with NaCl 0.9%. The isolated biomass was freeze-dried in a Christ-Alpha 1-2 LD plus freeze dryer (Martin Christ Gefriertrocknungsanlagen GmbH) [6].

In vitro simulation systrain of the human gastrointestinal conditions. The operation conditions and a detailed description of the system (GIS1 *In Vitro* System – www.gissystems.ro) were detailed in a previous study [7]. The samples collected every 60 minutes were analyzed with ColonyQuant (Schuett-biotec GmbH, Germany), with the corresponding software, after MRS insemination. Survival capacity (Cs) was calculated according to Vamanu et al., 2012.

Determination of biochemical parameters. The quantity of exopolysaccharides was determined by ethanol precipitation [8]. Viscosity (cst) was measured on an Oswald cinematic viscometer (Sibata, Kyoto, Japan) (Frengova et al., 2002; Curtis et al., 2000) [9, 10].

Statistical analysis All the parameters were assessed in triplicate, and the results were expressed as mean ±SD values of three observations [7].

RESULTS AND DISCUSSIONS

The first barrier of the strains which may be used to produce probiotics of human or veterinary use is the pH [7]. Therefore, for a multiple use, a pH between 2 and 3 was necessary. It resulted that strain BS1 displayed a higher resistance, especially during long periods of exposure to these conditions (Table 1). After an hour, the viability differences (Cs) between the two strains were significant. For BS1, the difference was of approximately 5.70%, while for BS3 of 25%. The dramatic decrease of the viability during the exposure to the gastric transit was considered the result of molecular features which currently are not fully understood [11].

When arriving in the small intestine, the loss of viability had a dramatic effect on the BS3 strain, because the Cs value went under 0.5. This was considered the moment when the tested strain lost half of its viability [7]. During the last two hours of remaining at the level of the small intestine, the BS3 viability went under 10%, which might be considered the equivalent of a complete loss of this strain's viability. The behavior was similar with that of the probiotic strain *Lactobacillus acidophilus* LA1, which displayed an average viability of 20% at the end of the stay period at this level [7], results obtained *in vitro* with the same systrain. In exchange, the BS1 strain kept a higher viability, Cs – 0.66, which indicated microbial stability in the presence of the bile salts and pancreatin. This behavior was also met at the strain *Enterococcus faecium* VL47, under the same conditions prior mentioned, where even a multiplication at this level was determined [7].

The partial physical-chemical analysis did not demonstrate a direct correlation between

viscosity and the polysaccharide synthesis, which demonstrated a dependency of the microbial strain. Although belonging to the same species, the differences were significant whereby a direct relation between viability (the Cs value) and the polysaccharide synthesis was noticed. This was valid until the middle of the transit through the small intestine, when due to the enzymatic stress a polysaccharide synthesis took place, but also a strong decrease or a limitation of the number of viable cells (Table 1). In exchange, the decrease of the viscosity in the end stage of the intestinal transit is also confirmed by a series of prior studies conducted on the strains *Lactobacillus acidophilus* and *Lactobacillus casei* [12].

Table 1 Evolution of the viability and variation of the analyzed biochemical parameters

Time (h)	Survival Capacity (C_S)		Viscosity (cP)		Exopolysaccharides (mg%)	
	BS1	BS3	BS1	BS3	BS1	BS3
0	1.00±0.01	1.00±0.10	0.16±0.01	0.40±0.01	0.0±0.00	0.001±0.000
1	0.88±0.01	0.88±0.01	0.34±0.00	0.76±0.01	0.007±0.001	0.012±0.000
2	0.83±0.00	0.66±0.00	0.80±0.00	0.76±0.01	0.015±0.001	0.012±0.000
3	0.83±0.03	0.40±0.00	0.30±0.00	0.68±0.01	0.035±0.001	0.034±0.001
4	0.77±0.03	0.30±0.01	0.42±0.05	0.50±0.00	0.05±0.000	0.052±0.01
5	0.72±0.00	0.20±0.00	0.76±0.03	0.48±0.01	0.05±0.000	0.09±0.00
6	0.66±0.01	0.06±0.01	0.68±0.05	0.40±0.01	0.07±0.001	0.09±0.001
7	0.66±0.01	0.04±0.01	0.68±0.00	0.40±0.01	0.1±0.000	0.092±0.001

The use of probiotic strains increased significantly during the last decades, both for human use and for maintaining the health conditions of animals in a farm. The regulated administration demonstrated in both cases a balancing of microbiota. Because the food quality decreased, a change was noticed in what the balance between the species of microorganisms is concerned, which is corrected by using probiotic strains with features similar to strain BS1. By *in vitro* studies, it determines a series of features which might influence the intestinal homeostasis. At least strain BS1 may be used both for human use but also as supplement in zooculture [13].

By its high viability, following the gastrointestinal transit, the strain BS1 displayed initial features which may support administration to humans and animals (pigs) as prophylactic agent. It may be used as part of the formula of some products useful to eliminate the effects caused by various pathologies of animal origin [14]. This analogy is possible due to the morphological and physiological similarities of the two digestive systrains. Currently, the pig is used often for *in vivo* tests having as main subject researches in the nutrition and biomedical fields [15].

CONCLUSIONS

As a conclusion, the strains of *L. plantarum* are often used in probiotics due to the omnipresence in the animal and human tract [16]. The BS1 strain displayed the highest number of viable cells following the gastrointestinal transit. This feature enables the perspective of a superior capacity to adhere to the colon epithelium. In order to check and validate these tests, subsequent *in vivo* tests are necessary to determine (from faecal matter) the persistence capacity at this level.

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