

Stability of Selected Lactobacilli in the Conditions Simulating Those in the Gastrointestinal Tract

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Abstract

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The cell survival in the digestive tract is one of the main criteria required for the probiotics. The aim of this study was to evaluate the stability of the selected lactobacilli (*Lactobacillus acidophilus* CCDM 151; *L. casei* CCDM 198; *L. rhamnosus* CCDM 150, and *L. fermentum* ST 68) in conditions simulating those in the gastrointestinal tract as compared to the commercial probiotic strain *Lactobacillus casei* LAFTI L-26. The growth of lactobacilli decreased both after 2 h and 4 h incubation in MRS media with increasing concentration of bile salt but all lactobacilli had the ability to adapt in the environment of bile salt. Great differences in viability were detected between the isolated cells in the stomach simulating conditions. *L. casei* LAFTI L-26 and *L. acidophilus* CCDM 151 were most stable, *L. rhamnosus* CCDM 150 did not survive under these conditions. Milk revealed a strong protective influence on the viability of all lactobacilli in the stomach simulating conditions. The conditions existing in the small intestine did not influence the cell viability. Differences in autoaggregation were also observed.

Keywords: probiotic; *Lactobacillus*; bile tolerance; pH tolerance; autoaggregation

Lactic acid bacteria have been traditionally used as starter cultures in dairy industry. They contribute to the food digestibility, preservation and to the texture and sensory profiles of the final products. During last decades, they have been also intensively studied for their health benefits as probiotics. Probiotic bacteria are defined as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host. Most probiotics are bacteria similar to those naturally found in people's guts, especially in those of breastfed infants. A wide variety of species and genera could be probiotic, however, the most important and commercially used are lactobacilli, which belong to the group of lactic

acid bacteria, and bifidobacteria (SHAH 2007; REID 2008).

The influence on human health of microorganisms present in fermented dairy products has been studied since the turn of the 19th and 20th centuries when their positive impact was described by the Russian scientist Ilja Mecnikov. The next wave of interest appeared in the 50's, since then the term "probiotics" has been coined.

Probiotic microorganisms have the ability to influence the host's immunity system (prevention and therapy of contagious diseases, treatment of the gastrointestinal tract inflammation, suppression of cancer cells) (PERDIGÓN *et al.* 2001). They have a direct effect on other microorgan-

isms namely commensal microbes or pathogens (prevention and treatment of infections, renewal of the intestinal microbial balance, prevention against travel diarrhoea and diarrhoea caused by antibiotics use) (O'FLAHERTY & KLAENHAMMER 2010). The effect based on metabolic products operation (toxin inactivation and the detoxification of host and food ingredients in the colon) was also proved (OELSCHLAEGER 2010). Antimicrobial treatment consists in the production of substances (such as lactic and acetic acids, hydrogen peroxide, bacteriocins) that act bacteriostatically or bactericidally (FERNÁNDEZ *et al.* 2003).

To be classified as probiotic, a microorganism has to fulfil numerous criteria: starting with safety (strain origin, without pathogen impact or without transferable genes of antibiotic resistance), followed by functional features (survival in digestive tract, health benefit for the host) as well as technological ones (technological adequacy, storage stability) (SAARELA *et al.* 2000). The importance of these factors differs and depends also on their final use. The most studied criteria deal with the survival of the specific microorganisms in the gastrointestinal tract and with health benefits. Some media or carriers which form protective environment for probiotics can help their survival and tolerance of the conditions inside the gastrointestinal tract. Dairy products which are today regularly enriched with probiotic strains are among the best examples of such media.

New bacterial strains fulfilling the above mentioned criteria are continuously sought. The aim of this study was to evaluate the stability of selected lactobacilli in the conditions simulating those in gastrointestinal tract.

MATERIAL AND METHODS

Microorganisms. *Lactobacillus casei* LAFTIL26 – commercial probiotic strain (DSM Food Specialites, Heerlen, the Netherland); *L. acidophilus* CCDM 151; *L. casei* CCDM 198; *L. rhamnosus* CCDM 150 (Collection of Dairy Microorganisms, Laktoflora[®], Prague, Czech Republic); *L. fermentum* ST 68 – isolate from Edam type cheese (Department of Dairy and Fat Technology, Institute of Chemical Technology Prague, Czech Republic).

Cultivation and determination of lactobacilli count. The strains were cultivated at 37°C in CO₂ atmosphere (4%) for 48 h and counted as colony

forming units (CFU/ml) on MRS agar (Merck, Darmstadt, Germany), pH 5.6.

Tolerance towards bile salt. For the determination of the tolerance towards bile salt (BURNS *et al.* 2008), MRS broth (pH 6.6) with 0; 0.3 and 1.0% (w/v) of natrium salt of tauroglycocholic acid (Merck) was used. These media were inoculated with fresh culture (1% v/v) and in intervals of 2, 4, 7, 24 and 48 h, the absorbance at 560 nm was measured. The results were analysed as percentual growth compared to the control sample (0% of bile salt). The determination was performed three times and the results were statistically analysed using the Dean-Dixon test (RORABACHER 1991).

Tolerance towards the conditions in gastrointestinal tract for lactobacilli isolated from MRS broth. The method for the testing of tolerance towards the conditions simulating those in the gastrointestinal tract was adapted according to GUGLIELMOTTI *et al.* (2007) and BOTES *et al.* (2008). An overnight culture in MRS broth (pH 5.6, at 37°C in CO₂ atmosphere (4% v/v)) were centrifuged (8690 g, 12 min, 5°C), washed in buffer, centrifuged once again, and the isolated cells were transferred into a solution simulating the stomach conditions (50 ml HCl, pH 2, 0.5% w/v NaCl, 0.3% w/v pepsin; Sigma-Aldrich, St. Louis, USA). The cells were incubated at 37°C in CO₂ atmosphere (4% v/v) with occasional stirring. In the intervals of 0, 2, and 3 h, the counts were determined on MRS agar by the plate method. After 3 h of cultivation, pH was adjusted to 6.8 ± 0.2 (by 10% w/v NaOH) and Ox bile (0.3% w/v; Merck) and pancreatin (0.1% w/v; Sigma-Aldrich) were added for the simulation of the conditions existing in ileum. The incubation continued under the same conditions, the number of cells (CFU/ml) was determined in the intervals of 2, 3, and 4 h after the environmental change.

Tolerance towards the conditions in gastrointestinal tract for lactobacilli cultivated in milk. Lactobacilli strains were cultivated in sterile reconstituted skim milk (overnight cultivation, 37°C in CO₂ atmosphere (4% v/v)). The number of cells was detected (MRS agar) and 5 ml of fermented milk were transferred to the solution simulating the stomach conditions. Furthermore, the determination was carried out identically as in the previous case of lactobacilli cultivated in MRS broth. All experiments were replicated twice.

Autoaggregation. The autoaggregation of the lactobacilli tested was measured by the method of KOS *et al.* (2003).

RESULTS AND DISCUSSION

The concentration of bile salts in the digestive tract changes, and therefore the concentrations of 0, 0.3, and 1.0% (w/v) of sodium salt of tauroglycocholic acid were chosen for testing the resistance to bile salt. The results were expressed as % against the value found in the medium without bile salt (control culture).

Figures 1 and 2 show that the resistance of lactobacilli decreased both after 2 h and 4 h incubation with increasing concentration of bile salt. In this experiment however, the cultivation period was extended up to 48 hours. It was found that all lactobacilli tested had the ability to adapt in the environment of bile salt. In some cases, the

same growth was reached after this period as in the control culture without the bile salt addition. This adaptability was the least in the case of *L. casei* LAFTI L26, where after 24 h cultivation at 1.0% (w/v) bile salt, only 40% growth was detected compared to the control. In contrast, the other strains reached about 75% growth as compared to the control sample. A number of scientific papers (MAINVILLE *et al.* 2005; BURNS *et al.* 2008) have examined the effects of different bile salts concentrations on growth inhibition. The authors usually examines just 3–4 h exposure to bile salts. Comparable results for the 24 h incubation period were found only in the study of VINDEROLA and REINHEIMER (2003). NORIEGA *et al.* (2004) confirmed the adaptability of bifidobacteria to

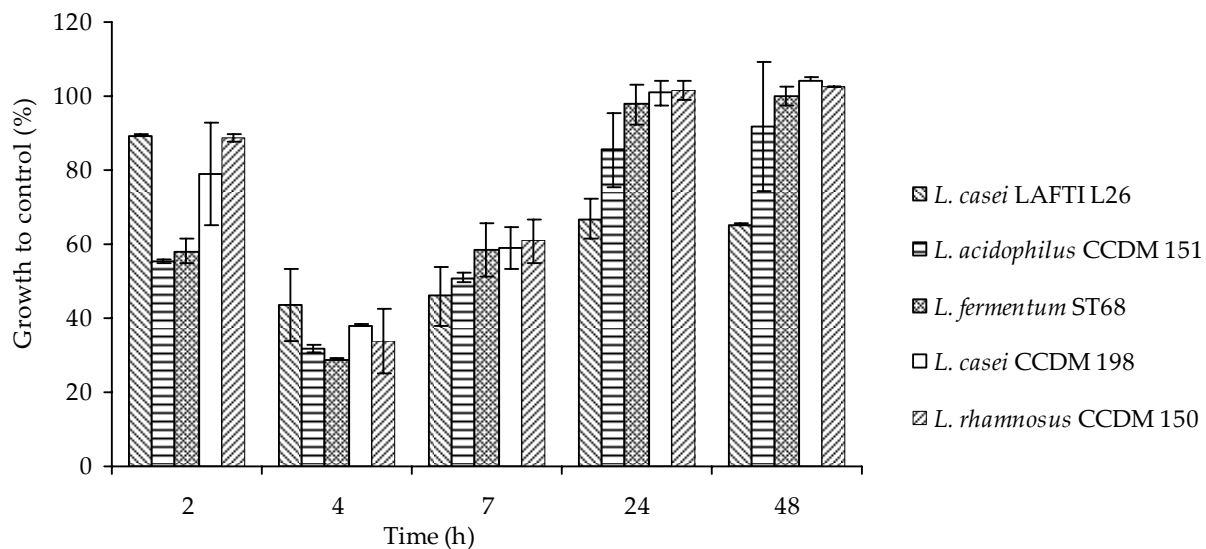


Figure 1. Growth of tested lactobacilli in MRS broth with 0.3% (w/v) bile salt compared to control culture

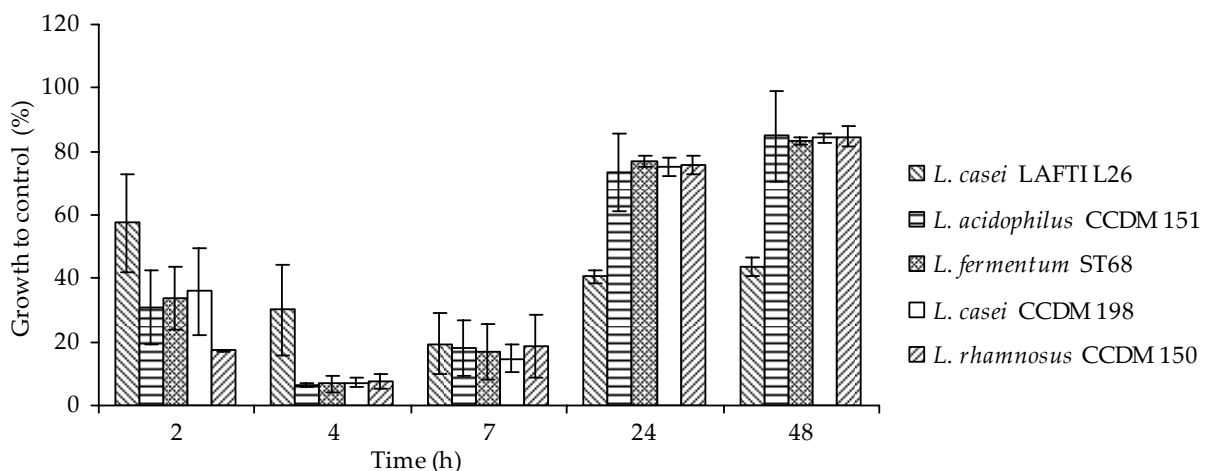


Figure 2. Growth of tested lactobacilli in MRS broth with 1.0% (w/v) bile salt compared to control culture

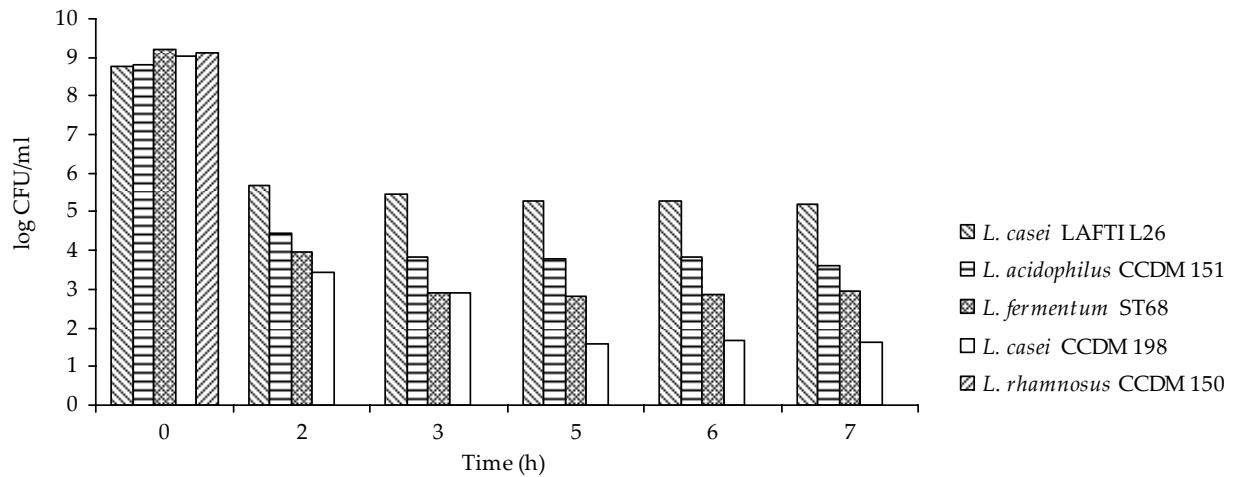


Figure 3. Stability of isolated lactobacilli cells in the conditions simulating gastrointestinal tract (stomach 0–3 h, pH 2; 0.3% w/v pepsin, small intestine 3–7 h, pH 6.8; 0.1% w/v pancreatin)

high concentrations of bile salts during long term cultivation. Prolonged exposure to bile salts gives a more accurate picture concerning the survival of microorganisms in the upper part of the small intestine, where a permanent influence of bile salts exists. It can also be expected that in the other parts of the tract the normal growth is restored.

The survival of microorganisms under the conditions encountered during the passage through the digestive tract was also tested. The survival of the digestive tract conditions is crucial for the later effects of probiotics in the gut. These conditions are different in different parts of the digestive tract. In the stomach, probiotics are exposed to very low pH, salts, and enzymes such as pepsin and lysozyme. The effects of these enzymes must play a role in affecting the survival of organisms, including probiotics (VIZOSO PINTO *et al.* 2006).

The environments of the duodenum and small intestine represent a shift into neutral to slightly alkaline pH and the presence of bile salts, i.e. the access to other stress conditions.

The tested strains showed a very different viability at low pH (Figure 3). *L. rhamnosus* CCDM 150 was not able to survive the conditions simulating those in the stomach. Compared to the commercial strain *L. casei* LAFTI L26, *L. acidophilus* CCDM 151 was the most stable in these conditions. Conditions existing in the small intestine did not influence significantly the viability of the strains. The similar trend was observed in the work of BOTES *et al.* (2008) who studied 5 different lactobacilli. When these strains were transferred in the simulating conditions of duodeum and small intestine, an increase was even observed in the number of cells.

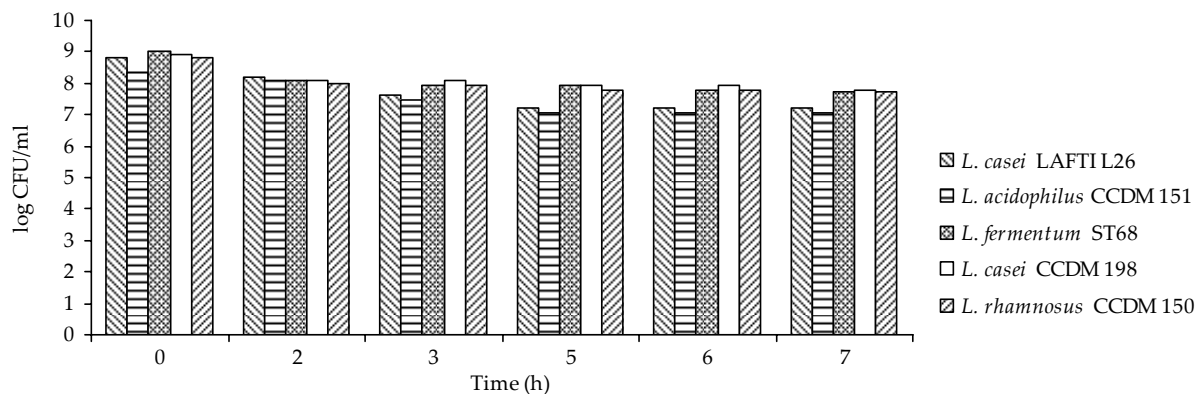


Figure 4. Stability of lactobacilli cells in milk in the conditions simulating gastrointestinal tract (stomach 0–3 h, pH 2; 0.3% w/v pepsin, small intestine 3–7 h, pH 6.8; 0.1% w/v pancreatin)

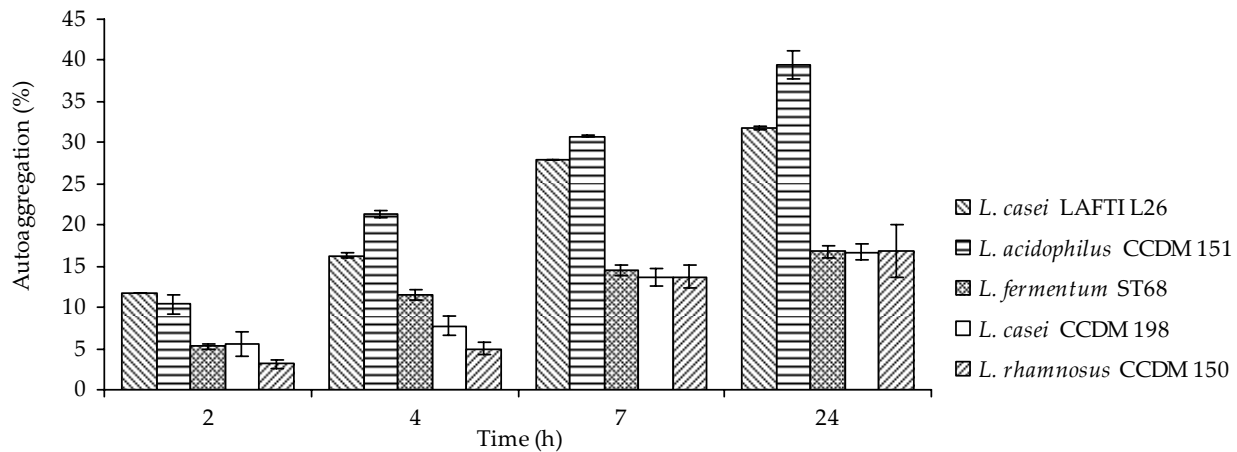


Figure 5. Autoaggregation of tested lactobacilli

Different results were obtained when the cells in the environment of fermented milk were transferred to the conditions of the stomach followed by the conditions of the small intestine (Figure 4). The decrease in the number of cells was only by 1–2 log cycles during the first 3 h of the experiment compared to 3–6 log cycles for the isolated cells. These results show that milk exerted a strong protective effect against the stress conditions of the digestive tract. The protective effect of milk on the survival of the *L. delbrueckii* strains in the environment simulating gastric juice was also confirmed by GUGLIELMOTTI *et al.* (2007). After the addition of re-skimmed milk (20% (v/v)) to the solution simulating gastric juice and 90 min incubation, no significant decrease was observed in the number of viable cells.

Autoaggregation has been correlated with adhesion which is known to be a necessary presumption for the colonisation of the gastrointestinal tract (Kos *et al.* 2003). Autoaggregation ability and also surface hydrophobicity determination can be used for preliminary screening to identify the potentially adherent cells (DEL RE *et al.* 2000; COLLADO *et al.* 2008). Figure 5 shows the autoaggregation of the tested lactobacilli during 24 h of incubation. The strain *L. acidophilus* CCDM 151 showed the highest value of autoaggregation, about 10% higher compared to the commercial probiotic strain *L. casei* LAFTI L-26.

To conclude our study it may be stated that the strain *L. acidophilus* CCDM 151 was found *in vitro* to possess desirable properties and is thus a good candidate for further investigation aimed at the evaluation of this application in dairy industry.

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