

# The Viability of the *Lactobacillus Rhamnosus* IL4.2 Strain in Simulated Gastrointestinal Conditions

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## Abstract

The viability maintenance of *Lactobacillus rhamnosus* IL4.2 strain in gastrointestinal conditions represents one of the most important characteristics regarding its use for obtaining probiotic products. The tests were performed with a cell suspension kept in 0.5% NaCl. The influence of pepsin (3 g/l) at pH of 1.5, 2, 2.5 and 3, as well as of pancreatin (1 g/l) in the presence of bile salts (1.5, 2, 3 and 5 mg/ml) were determined. The influence of casein and mucin, in a concentration of 1 g/l, was also established in the aforementioned conditions. It was observed that mucin presented a longer viability maintenance, fact also confirmed by the calculation of the mathematical parameters of viability and mortality, when mucin was either used or not, especially in the case of gastric transit. The results proved that the tested strain maintained its viability even at pH between 1.8 - 2 and at an even higher concentration, of 2 mg/ml of bile salts, but up to two hours as of the exposure to the conditions of the simulated small intestinal juice. Such results were also confirmed by the cumulated effect of the simulated gastric and small intestinal juice, the strain thus increasing its viability with an average of 10% in the presence of mucin.

**Keywords:** casein, ColonyQuant, mucin, pancreatin, pepsin.

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## 1. Introduction

Probiotic bacteria which live in the intestinal tract are beneficial for human health. They decrease the lactose intolerance symptoms, increase the resistance of the intestine to diseases, inhibit the proliferation of cancerous cells, regulate the concentration of plasmatic cholesterol and stimulate the immune system [1].

The development of probiotic use in the diet is a current theme on the functional products market. The viability and stability of probiotics in gastrointestinal conditions, during the transit, the

resistance to antibiotics or the presence of other substances with antimicrobial effect have represented a significant challenge for all producers and researchers in the field. To be functional, probiotics must be viable and in sufficient number even after a longer period of time [2]. The synthesis of organic acids, antimicrobial peptides and polysaccharides, enhancing the installation of favorable microflora is added to all the above. The novelty in this field is the capacity of such strains to participate to the creation of biofilms, dependent upon the expolysaccharides synthesis in the intestine conditions [3, 4].

Lately, certain models to experiment *in vitro* the conditions in the human gastrointestinal tract were proposed. They allow the study of the lactic

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bacteria viability and of the influence of the products meant for balancing the disturbed intestinal microflora [5]. These simulation systems range from simple ones where the lactic bacteria is treated in solutions of acid medium and solutions of hepatic bile [5-7], to more complex systems that simulate the human gastrointestinal tract to study the probiotic lactic bacteria interactions within the intestinal microbial environment or determine the effect of probiotic lactic bacteria and synbiotic products in the human intestinal microbiota [5, 8, 9]. An intestinal human tract model that contained four chambers to simulate the stomach, duodenum, jejunum and ileum was proposed by Minekus [10] and Pacheco [5].

Thus, this research will determine the viability in the transit of the *Lactobacillus rhamnosus* IL4.2 strain through the stomach and the small intestine. The conditions at the gastric level were simulated by using pepsin, at various pH values ranging between 1.5 - 3.

The simulated pancreatic juice contained pancreatin and bile salts, in various concentrations, range 1.5 - 5. Furthermore, there was tested the influence of casein and mucin on viability, as protectors of probiotic cells. Finally, the combined effect of the gastric juice and of the simulated small intestine action was determined, and the mathematical parameters of cell viability and mortality were calculated.

## 2. Materials and methods

**Biological material.** The bacterial strain *Lactobacillus rhamnosus* IL4.2 was maintained in glycerol 20% (Collection of Faculty of Biotechnology, Bucharest), at -820C. The strain was revitalized by two successive cultures in MRS broth, at 370C. The experiments were performed in the Industrial Biotechnology Laboratory of the Department of Biotechnology, in the second half of 2010.

The gastric and small intestine juice were prepared according to the method described by Kos [11]. In case of simulated gastric juice (pepsin 3 g/l) there were used various pH values, of 1.5, 2, 2.5 and 3. The simulation of the small intestine juice (pancreatin 1 g/l) was made at various bile salts concentrations (1.5, 2, 3 and 5 mg/ml). The mucin and casein influence on the strain viability was determined in the gastric and small intestine juice. A concentration of 1 g/l in NaOH 0.5% was used

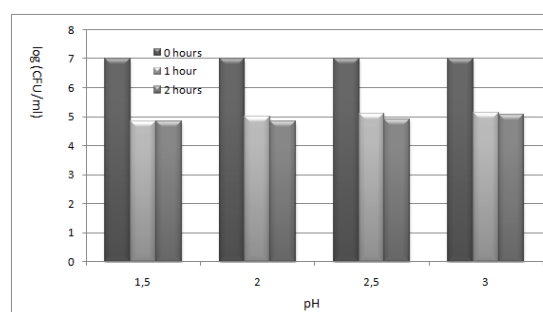
and the determination was performed according to the method described by Kos [11]. The cumulated effect of the simulated gastric and small intestine juice was determined at pH of 2 and a bile salts quantity of 3 mg/ml in the pancreatic juice. All tests were performed in Duran tubes, provided with silicone membrane meant for sampling. [3, 11-14]

Furthermore, the effect of trypsin, chymotrypsin and pronase on viability was determined separately for each enzyme. Thus, in a Duran tube, 1 ml of enzyme solution at a concentration of 1 mg/ml, 0.3 ml NaOH 0.5% and cell suspension of 0.2 ml were added. In two hours, the viability was determined in the presence of mucin and casein [4, 11, 12].

The viability and the mortality were determined at various pH values according to the method described by Kos [11], in the presence of pepsin and respectively of pancreatin, together with various concentrations of bile salts. The same mathematical indices were calculated as well in the presence of mucin and casein, according to the protection offered to the cell viability. The critical points were represented by the crossing between the viability and mortality curves [11, 12, 14, 15]. The viability was determined by insemination in double layer, in MRS broth, hourly. The plates were incubated for 48 hours at 370C and the results were read using the ColonyQuant and they were registered in the log (CFU/ml) [11, 12, 14, 16].

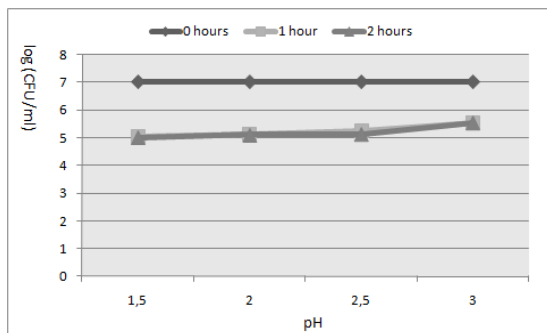
## 3. Results and discussion

The tested strain must have good viability in the conditions of gastric and intestinal transit, so as to be used as probiotic. The effect of the gastrointestinal transit commencing in the stomach is exercised by pepsin, at pH range 1.5 - 3. The dwell time at this level does not exceed 2 hours.

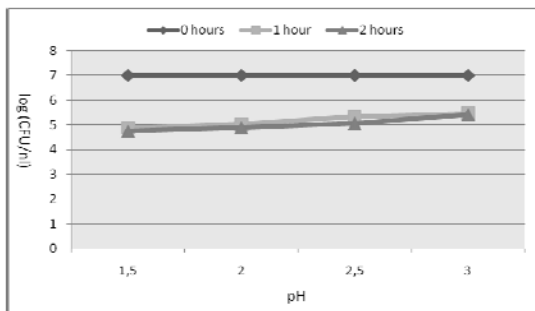


**Figure 1.** Viability of *Lactobacillus rhamnosus* IL4.2 strain at simulated gastric juice exposure

Therefore, Figure 1 represents the viability of the strain IL 4.2 at gastric level. It was noticed that viability was directly influenced by pH. At pH 1.5, the strain IL 4.2 had a viability loss of 30% as to the one registered for 0 hours of exposure. At pH exceeding the value of 2, the strain maintained its viability within an hour from the exposure to the simulated gastric fluid. Within two hours, as pH increased from 1.5 to 2, the viability also increased and it has not dropped below the one recorded for pH of 1.5. According to the presented data, it resulted that the strain was resistant to low pH, maintaining its high viability value, notwithstanding the value of the gastric pH.



**Figure 2.** Casein effect on the viability of *Lactobacillus rhamnosus* IL4.2 strain in case of exposure to simulated gastric juice

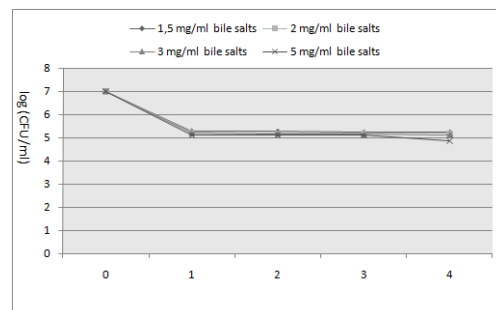


**Figure 3.** Mucin effect on the viability of *Lactobacillus rhamnosus* IL4.2 strain in case of exposure to simulated gastric juice

Mucin presented itself as a better protector than casein, in case of the viability of *Lactobacillus rhamnosus* IL4.2 strain, upon the action of the simulated gastric fluid. Although viability depended upon pH, it was higher than in case of its absence (Figure 2). In general, the viability values were higher by approximately 5%, at pH of 1.5, both for casein and for mucin (Figure 3). In exchange, at pH of 2, the viability value in the presence of mucin was by 1% higher than in the

presence of casein. At a value of 2.5 or 3 of pH, the viability was relatively constant, notwithstanding the presence of casein or mucin. The variations in favor of mucin, at values of 2.5 and 3 of pH, were of approximately 2 - 3%, at an exposure of one or two hours.

Before testing the viability, in case of exposure to the small intestine fluid, there was determined the influence of other enzymes on the *Lactobacillus rhamnosus* IL4.2 strains. Thus, it resulted that the viability was maintained further to the action of trypsin, pronase and chemotrypsin, in average of 6 log (CFU/ml) as to the viability of the strain without enzymes. Under the action of the three enzymes, a drop of approximately 10% resulted in a two-hour interval.

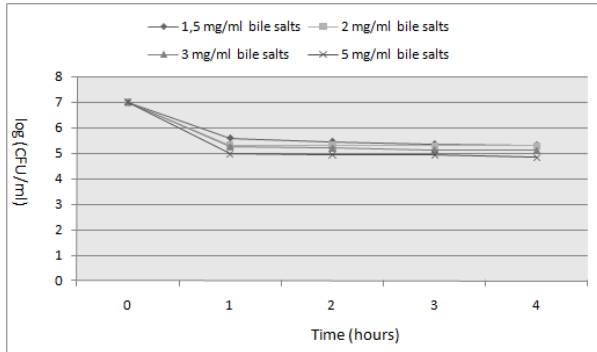


**Figure 4.** Viability of *Lactobacillus rhamnosus* IL4.2 strain in case of exposure to the simulated small intestine juice

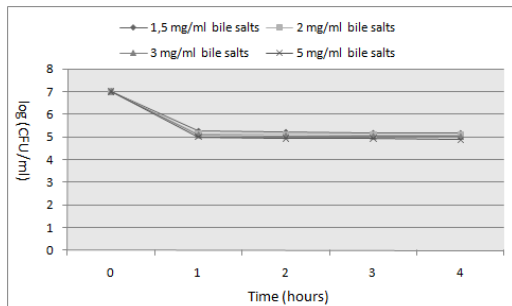
In case of direct exposure to the action of the simulated small intestine fluid, the presence of bile salts had the effect of viability decrease, firstly because of the exposure time and secondly due to the increase of the concentration thereof (Figure 4). A quantity of 2, 3 or 5 mg/ml bile salts caused, within an hour of exposure, a viability decrease of 26% on average. Viability stabilization occurred within two hours. A higher drop in viability was noticed for 5 mg/ml bile salts, within four hours of exposure. It resulted that once the dwell time in the presence of bile salts increased, the viability drop was small and constant, in general of 1 - 2%.

In case of small intestine fluid as well, there was determined the influence of casein and mucin. Both of them, but particularly mucin (Figure 6), had a protective effect on the viability of the probiotic strain, as opposed to the effect of pancreatin and bile salts. Although the difference was too small, the presence of casein (Figure 5) determined a higher increase in viability. The drop

was directly correlated to the increase of bile salts concentration and dwell time. Within two hours of exposure, notwithstanding the bile salt concentration, the viability dropped by 26% on average. In two more hours, the viability dropped by 5% more on average. In the presence of protecting agents, the viability did not drop below  $10^5$  CFU/ml, notwithstanding the concentration of bile salts or dwell time.

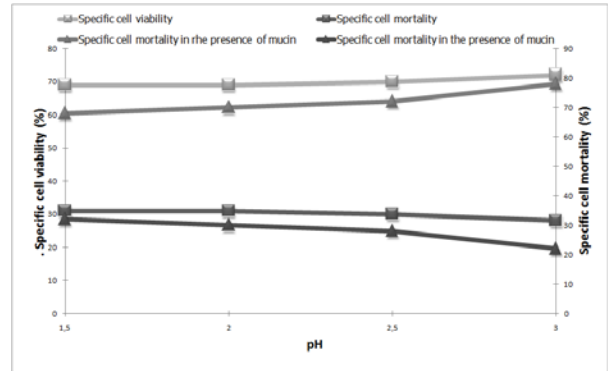


**Figure 5.** Casein effect on the viability of *Lactobacillus rhamnosus* IL4.2 strain in case of exposure to simulated small intestine juice

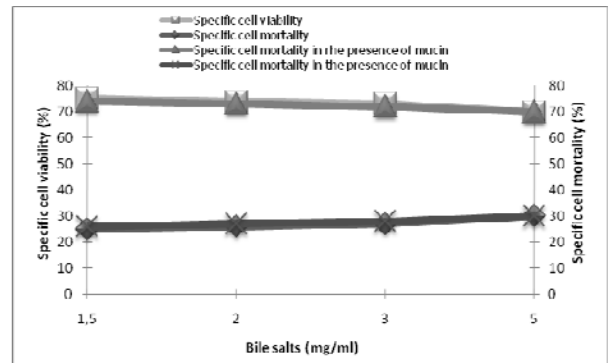


**Figure 6.** Mucin effect on the viability of *Lactobacillus rhamnosus* IL4.2 strain in case of exposure to simulated small intestine juice

The mathematical parameters of viability and mortality were determined at various pH values and in the presence of various bile salts concentrations. According to the previous data, it resulted that mucin was in general a better protector than casein. The mortality line, as well as the viability line, did not cross in the presence of mucin, resulting an appropriate protection at pH values below 2. Pursuant to the mathematical values, at pH of 2, the viability increased by 3% (Figure 7). According to the same figure, the *Lactobacillus rhamnosus* IL 4.2 strain had an appropriate viability at pH ranging between 1.5 - 2, pursuant to the literature data, of at least  $10^5$  CFU/ml for probiotics [11].



**Figure 7.** Specific cell mortality and viability of *Lactobacillus rhamnosus* IL4.2 strain in case of exposure to simulated gastric juice



**Figure 8.** Specific cell mortality and viability of *Lactobacillus rhamnosus* IL4.2 strain in case of exposure to simulated small intestine juice

The same trend was noticed in case of simulated small intestine fluid (Figure 8). The presence of mucin maintained cellular viability which is strengthened by the non-crossing of the viability and mortality graphs. In the lack of mucin, the strain was inhibited by the growth beyond 3 mg/ml of the bile salts concentration. Thus, at a bile salts concentration of 3 and 5 mg/ml, the presence of mucin did not determine an increase of the viability, which was maintained relatively constant.

The protector effect of mucin was noticeable in case of the cumulated action of gastric and small intestine juice on the viability of the IL 4.2 strain. The viability was directly influenced by mucin, although in case of gastric juice action, it was high, of more than 50%, at pH 2. In this situation, the presence of mucin increased the viability value by more than 10%. If the simulated small intestine juice acted on them as well, at a concentration of 2 - 3 mg/ml bile salts, the viability was kept at a percentage of 40%, when mucin was present.

These data are supported by the previous researches of Kos [11], Patel [17], Matijasic and Rogely [18]. The results also represent added data to the findings of Nasrollah [19], Homayony [20] and Trachoo [21].

Although mucin was a regular presence at the level of the gastric mucosa, it provided good protection for the lactic bacteria strains in case of direct administration. The effect of the mixture of mucin with various lactic bacteria freeze-dried strains merely determined an increase in viability, at the passage through the human gastrointestinal tract. This increase of the cell number at the stress exercised by pH 2 and a concentration of 2 - 3 mg/ml bile salts contribute to finding new strains of extremely resistant lactic bacteria. Although regularly a viability of approximately 20% is maintained, after such transit, finding strains and conditions able to double the viability is a significant aspect. The researches of Kos [11], Movsesyan [13] and Sumeri [22] are in support of this result, with no disagreement values.

#### 4. Conclusions

It was demonstrated that the *Lactobacillus rhamnosus* IL 4.2 strain was able to survive to the gastrointestinal transit. The presence of mucin as compared to casein determined a viability increase of approximately 20%. The conditions in which the strain had maximal sensitivity were determined, namely pH below 2 and bile salts concentration higher than 3 mg/ml, which was significant in order to be able to use the strain in clinical studies. Knowing the protector and the cumulated gastric and intestinal effect on strain viability rendered it more competitive when used to create new probiotic products.

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#### References

1. Ogueke, C. C., Owuamanam, C. I., Ihediohanma, N. C., Iwouno, J. O., Probiotics and Prebiotics: Unfolding Prospects for Better Human Health, Pakistan Journal of Nutrition, 2010, 9, 833-843
2. Luc De Vuyst, Technology Aspects Related to the Application of Functional Starter Cultures, Food Technol Biotechnol., 2000, 38, 105–112
3. Puangpronpitag, D., Niamsa, N., Sittiwet, C., Anti-Microbial Properties of Clove (*Eugenia caryophyllum* Bullock and Harrison) Aqueous Extract Against Food-Borne Pathogen Bacteria, International Journal of Pharmacology, 2009, 5, 281-284
4. Philip, K., Teoh, W. Y., Muniandy, S., Yaakob, H., Pathogenic bacteria predominate in the oral cavity of Malaysian subjects, Journal of Biological Sciences, 2009, 9, 438-444
5. Pacheco, K. C., del Toro, G. V., Martinez, F. R., Duran-Paramo, E., Viability of *Lactobacillus delbrueckii* under human gastrointestinal conditions simulated *in vitro*, Am J Agric Biol Sci., 2010, 5, 37-42
6. Fávoro-Trindade, C. S., Grosso, C. R. F., Microencapsulation of *L. acidophilus* (La-05) and *B. lactis* (Bb-12) and evaluation of their survival at the pH values of stomach and in bile, J Microencapsulation., 2002, 19, 485-494
7. Huang, Y., Adams, M., *In vitro* assessment of the upper gastrointestinal tolerance of potential probiotic dairy propionibacteria, Inter J Food Microbiol., 2004, 91, 253-260
8. De Boever, P., Deplancke, B., Verstraete, W., Fermentation by gut microbiota cultured in a simulator of the human intestinal microbial ecosystem is improved by supplementing a soygerm powder, J. Nutr., 2000, 130, 2599
9. Mainville, I., Arcand, Y., Farnworth, E. R., A dynamic model that simulates the human upper gastrointestinal tract for the study of probiotics, Inter J Food Microbiol., 2005, 99, 287-296
10. Minekus, M., Marteau, P., Havennar, R., Huis in't Veld, J., A multicompartamental dynamic computercontrolled model simulating the stomach and the small intestine, Alternatives Lab Anim., 1995, 23, 197-209
11. Kos, B., Suskovic, J., Goreta, J., Matosic, S., Effect of Protectors on the Viability of *Lactobacillus acidophilus* M92 in Simulated Gastrointestinal Conditions, Food Technology and Biotechnology, 2000, 38, 121–127
12. Sarahroodi, S., Arzi, A., Sawalha, A. F., Ashtazinehad, A., Antibiotics self-medication among Southern Iranian University students, International Journal of Pharmacology, 2010, 6, 48 - 52
13. Movsesyan, I., Ahabekyan, N., Bazukyan, I., Madoyan, R., Dalgalarroondo, M., Chobert, J., Popov, Y., Haertlé, T., Properties and survival under simulated gastrointestinal conditions of lactic acid bacteria isolated from armenian cheeses and matsuns, Biotechnology & Biotechnological Equipment, 2010, 24, 444-449
14. Vamanu, E., Vamanu, A., Viability of the *Lactobacillus rhamnosus* IL1 strain in simulated

- gastrointestinal conditions, International Journal of Pharmacology, 2010, 6, 732 - 737
15. Yateem, A., Balba, M. T., AL-Surrayai, T., AL-Mutairi, B., AL-Daher, R., Isolation of lactic acid bacteria with probiotic potential from camel milk, International Journal of Dairy Science, 2008, 3, 194-199
16. Otlés, S., Ozlem, C., Kefir: A probiotic dairy-composition, nutritional and therapeutic aspects, Pakistan Journal of Nutrition, 2003, 2, 54-59
17. Patel, P., Parekh, T., Subhash, R., Development of probiotic and synbiotic chocolate mousse: A functional food, Biotechnology, 2008, 7, 769-774
18. Matijasic, B.B., Rogelj, I., *Lactobacillus* K7 – A new candidate for a probiotic strain, Food Technol. Biotechnol., 2000, 38, 113–119
19. Nasrollah, V., 2009. Probiotic in Quail Nutrition: A Review. Int. J. Poult. Sci., 8:1218-1222
20. Homayony, A., A. Ehsani, M.R. Azizi, A. Razavi, S.H. and M.S. Yarmand, Growth and survival of some probiotic strains in simulated ice cream conditions. J. Applied Sci., 2008, 8: 379-382
21. Trachoo, N., P. Wechakama, A. Moongngarm and M. Suttajit, Stability of freeze-dried *Lactobacillus acidophilus* in banana, soybean and pearl barley powders, J. Boil. Sci., 2008, 8: 119-124
22. Sumeri, I., Arike, L., Stekolstiskova, J., Uusna, R., Adamberg, S., Adamberg, K., Paalme, T., Effect of stress pretreatment on survival of probiotic bacteria in gastrointestinal tract simulator, Appl. Microbiol. Biotechnol., 2010, 86:1925-31