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Full Length Research Paper

The viability of the *Lactobacillus paracasei* IL2 and *Lactobacillus plantarum* IL3 strains in simulated gastrointestinal conditions

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The viability maintenance of *Lactobacillus paracasei* IL2 and *Lactobacillus plantarum* IL3 strains in gastrointestinal conditions represents one of the most important characteristics regarding their use for obtaining probiotic products. The tests were performed with a cell suspension kept in 0.5% NaCl. The influences of pepsin (3 g/l) at pH values of 1.5, 2, 2.5 and 3.0, 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, were also established in the aforementioned conditions. It was observed that casein presented a longer viability maintenance; a 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 strains maintained their viability even at pH between 1.8 and 2.0 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 strains thus increasing their viability with an average of 15% in the presence of casein.

Key words: Mucin, casein, ColonyQuant, pepsin, pancreatin.

INTRODUCTION

Probiotic bacteria which lives 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 (Ogueke et al., 2010).

During the last few years, special attention has been paid to the source of isolation of the probiotic lactic bacteria, their tolerance to the conditions of the stomach and small intestine and their capacity of adhering to the intestinal mucosa. Although lactobacilli have been isolated from all parts of the human gastrointestinal tract, the terminal ileum and colon seem to be the preferential sites of colonization of lactobacilli. Such criteria have been used to select probiotic lactic bacteria which have been and are still used for obtaining of nutraceutical products (Cheng et al., 2005). The results of the current research in the probiotic bacteria field indicate the fact that the strains used in the food products can survive in a viable state in simulated conditions of gastric and intestinal transit. Various levels of viability have been reported for different species of *Lactobacillus*. *In vitro* models can be used for the assessment of the strains viability tested in gastrointestinal conditions (Movsesyan et al., 2010).

If probiotic bacteria have to survive and be active in the digestive tract, they should be resistant to the defense mechanisms of the host (Manning and Gibson, 2004). At the level of the gastrointestinal tract, these include the

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physiological and physico-chemical processes. The physiological parameters comprise pH, concentrations of gastric and small intestinal enzymes, concentrations of bile salts and the kinetics of passage through stomach and intestine (Dunne et al., 2001). Unfortunately, most studies on probiotic actions ignore these facts and, as a result, data on the tolerance of probiotic strains are quite rare (Żyżelewicz et al., 2010).

The purpose of this research was represented by the establishment of the viability of *Lactobacillus paracasei* IL2 and *Lactobacillus plantarum* IL3 strains during their transit through the stomach and small intestine. The conditions at the gastric level were simulated by the use of pepsin, at various pH values between 1.5 and 3. The simulated pancreatic juice contained pancreatin and bile salts, in various concentrations, between 1.5 and 5. Moreover, the influences of casein and mucin, as protectors of the probiotic cells, on the viability were tested. Finally, the combined effect of the action of the simulated gastric and small intestine juice was determined and the mathematical parameters of the cell viability and mortality were calculated.

MATERIALS AND METHODS

Biological materials

The bacterial strains *L. paracasei* IL2 and *L. plantarum* IL3 were maintained in glycerol 20% (Collection of the Faculty of Biotechnology, Bucharest), at -82°C. The strain was revitalized by two successive cultures in MRS broth, at 37°C. 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 et al. (2000). In case of simulated gastric juice (pepsin 3 g/l), various pH values, of 1.5, 2, 2.5 and 3.0 were used. 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 influences on the strain viability in the gastric and small intestine juice were determined. A concentration of 1 g/liter in NaOH 0.5% was used and the determination was performed according to the method described by Kos et al. (2000). The cumulated effect of the simulated gastric and small intestine juice was determined at pH 2 and bile salts concentration of 3 mg/ml in the pancreatic juice. All tests were performed in Durham tubes, provided with silicone membrane meant for sampling (Kos et al., 2000; Sarahroodi et al., 2010; Puangpronpitag et al., 2009; Movsesyan et al., 2010; Vamanu and Vamanu, 2010).

Furthermore, the effects of trypsin, chymotrypsin, and pronase on viability were determined separately for each enzyme. Thus, in a Durham tube, 1 ml of enzyme solution at a concentration of 1 mg/ml, 0.3 ml NaOH 0.5% and 0.2 ml cell suspension were added. Within two hours, the viability was determined in the presence of mucin and casein (Kos et al., 2000; Sarahroodi et al., 2010; Philip et al., 2009).

The viability and mortality were determined at various pH values according to the method described by Kos et al. (2000), 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 (Kos et al., 2000; Sarahroodi et al., 2010; Yateem et al., 2008; Vamanu and Vamanu, 2010).

The viability was determined by insemination in double layer, in MRS broth, hourly. The plates were incubated for 48 h at 37°C and the results were read using the ColonyQuant equipment and they were registered as the log (CFU/ml) (Kos et al., 2000; Sarahroodi et al., 2010; Otles and Ozlem, 2003; Vamanu and Vamanu, 2010).

RESULTS AND DISCUSSION

The tested strains must have a good viability in order to be used as probiotic, since one of the greatest problems of these strains is their resistance in the conditions of the gastric and intestinal transit. The effect of the gastrointestinal transit, which begins in the stomach, was exercised by pepsin, at pH between 1.5 and 3. The stationary time at this level did not exceed 2 h. Thus, Figure 1 presents the viability of IL2 and IL3 strains at gastric level. The viability of the strains, especially that of IL2 strain, was directly influenced by the pH value. At pH of 1.5, the IL2 strain showed only 72% of the viability registered at 0 h of exposure, in comparison with 70% for IL3. The value of the viability was in such situation higher for IL3 in comparison with IL2. At pH higher than 2, the strains maintained their viability constant after one h of exposure to the simulated gastric juice. After two hours, as pH increased from 1.5 to 2, the viability also increased and remained constant after pH reached 2.5, being 77% of the initial one for IL2 and 72% of the initial one for IL3, respectively. The presented data proved that the strains were resistant to low pH, especially IL3, which maintained a higher viability value, irrespective of the values of gastric pH.

Casein was a better protector than mucin in the case of the viability of *L. paracasei* IL2 and *L. plantarum* IL3 strains, with respect to the action of the simulated gastric juice. The viability of the two strains depended on pH, but was not higher than in the absence of such substances (Figure 2). In general, the viability values were in average by 15% higher at pH of 1.5, both for casein, as well as for mucin, for the IL2 strain and by 2% higher for the IL3 strain (Figure 3). On the other hand, at pH of 2.0, the viability value in the case of casein presence in comparison with mucin was also approximately by 15% higher for IL2, while for the IL3 strain the viability was relatively similar. At pH values of 2.5 or 3.0, the viability maintained the same trend, irrespective of the presence of casein or mucin. The difference in favor of the presence of casein for strain IL2, at pH value of 2.5 and 3.0, was approximately 10%, for an exposure of one or two hours. The IL3 strain was an exception in this case as well, since at pH of 2.5 or 3.0 it maintained its viability constant, irrespective of the used protector.

Before testing the viability in case of exposure to the small intestinal juice, the influence of other enzymes on *L. paracasei* IL2 and *L. plantarum* IL3 strains was determined. The result was the relative maintenance of the viability under the action of trypsin, pronase and

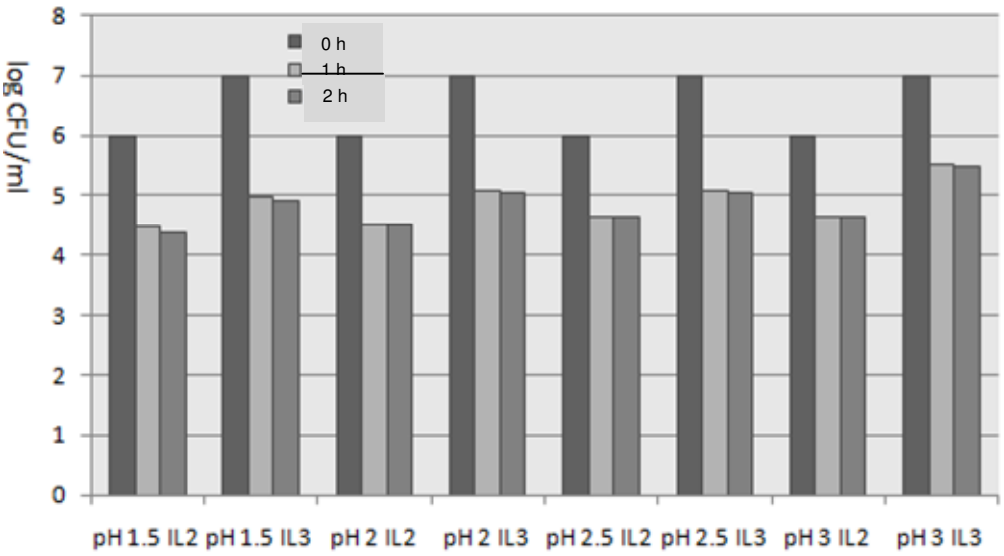

Figure 1. Viability of *Lactobacillus paracasei* IL2 and *Lactobacillus plantarum* IL3 strains at simulated gastric juice exposure

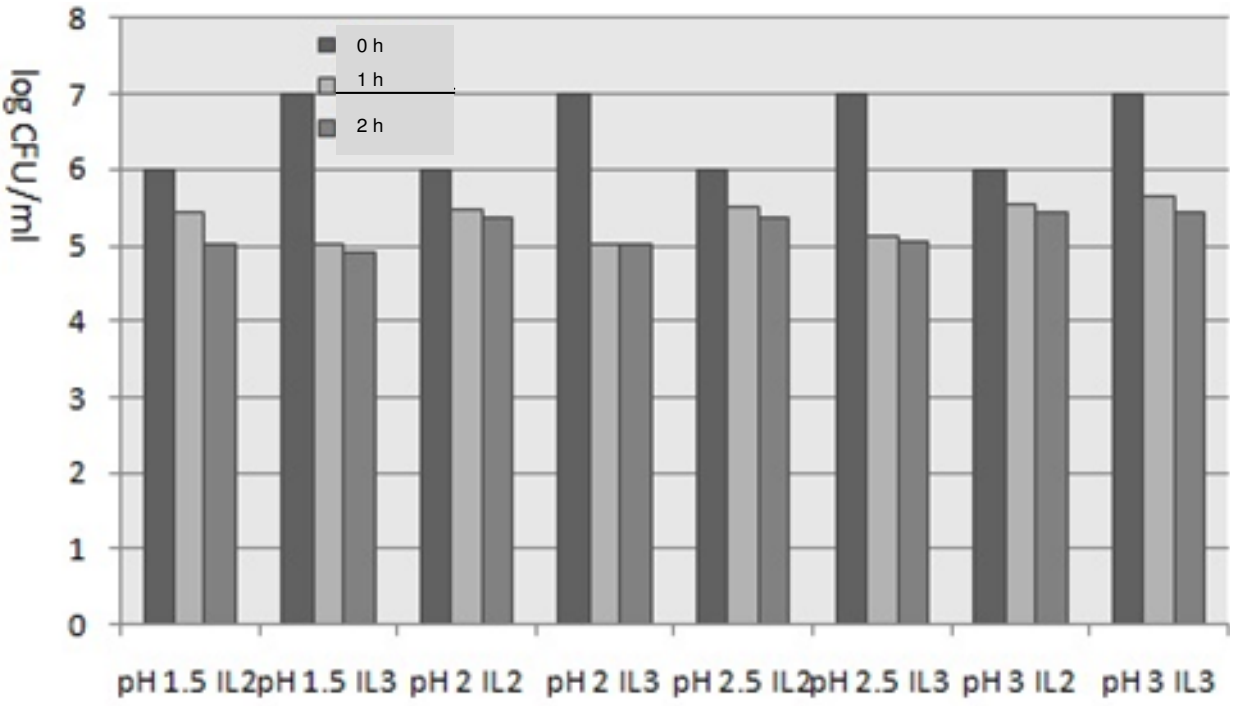

Figure 2. Casein effect on the viability of *Lactobacillus paracasei* IL2 and *Lactobacillus plantarum* IL3 strains in case of exposure to simulated gastric juice

chymotrypsin, namely a decrease of less than 6% was observed after two hours under the action of the three aforementioned enzymes for IL2 strain and a decrease of approximately 30% for IL3.

In the case of direct exposure to the simulated small intestinal juice, the presence of the bile salts had as effect the decrease of the viability, first of all due to the increase of their concentration (Figure 4). An increase of 3 or 5 mg/ml bile salts concentration determined, after two hours of exposure, a significant decrease of the viability, of 25% and respectively 28% for the IL2 strain. For IL3 strain, the presence of bile salts concentration of

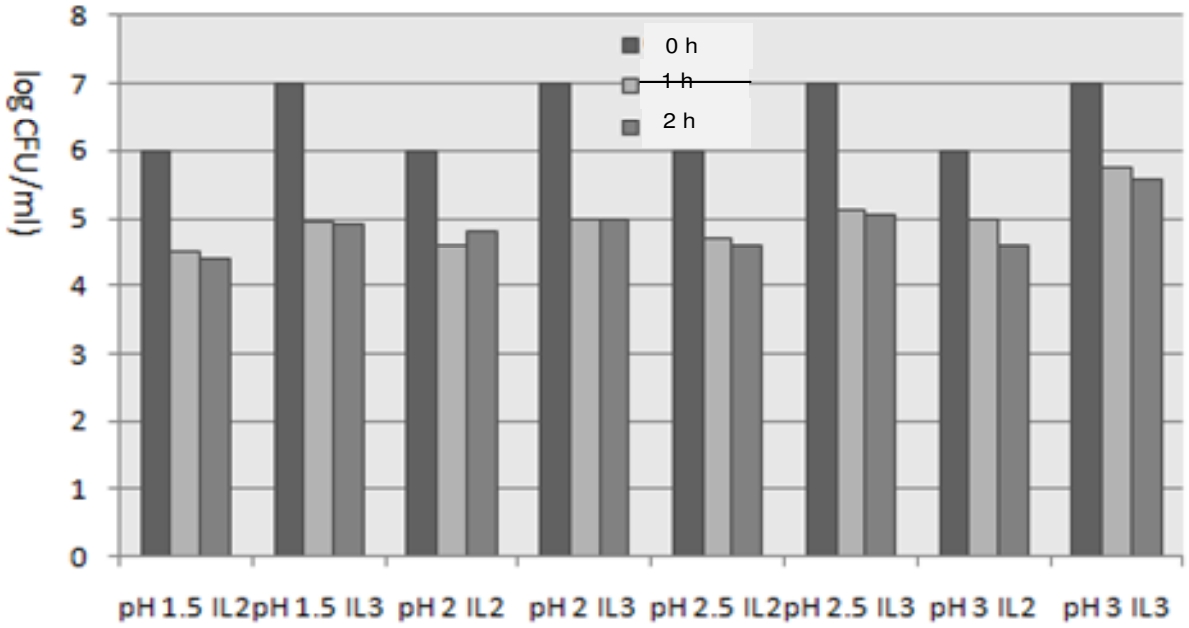

Figure 3. Mucin effect on the viability of *Lactobacillus paracasei* IL2 and *Lactobacillus plantarum* IL3 strains in case of exposure to simulated gastric juice

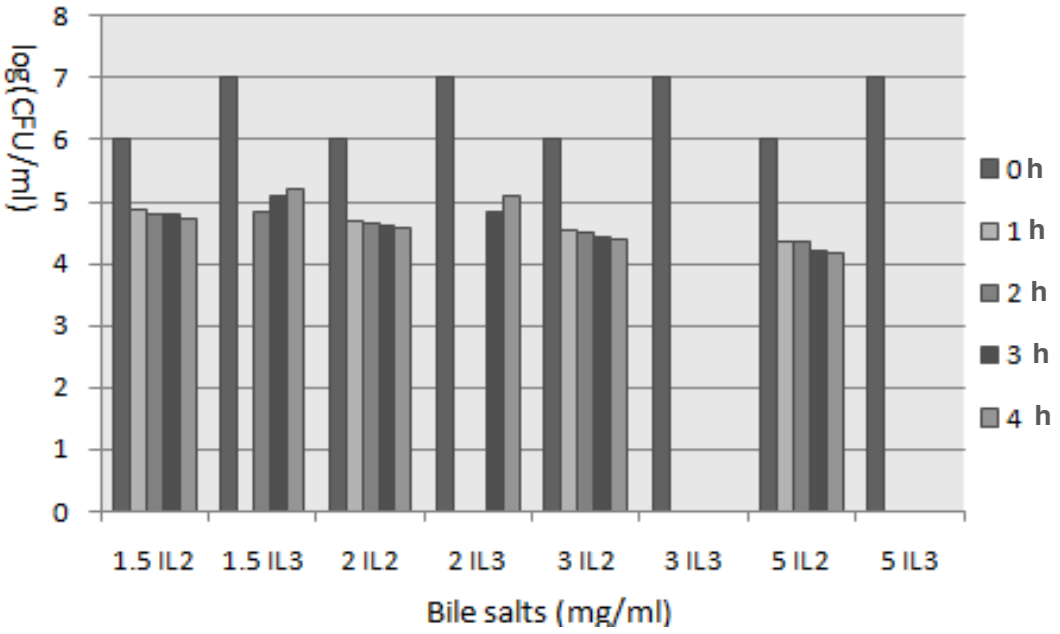

Figure 4. Viability of *Lactobacillus paracasei* IL2 and *Lactobacillus plantarum* IL3 strains in case of exposure to the simulated small intestine juice.

at least 3 mg/ml, determined an annulment of the cell viability. An important observation was that in the presence of no more than 2 mg/ml of bile salts, the viability did not drop below 10^5 CFU/ml. According to the used strain, the viability was directly influenced in a negative way once with the increase of the stationary time in the presence of bile salts. The doubling of the bile salts concentration determined after an exposure of 4 h, the decrease of the viability by no more than 10% for IL2 strain.

The influences of casein and mucin were also determined in the case of the simulated small intestinal juice. It was observed that these proteins, but mainly casein for IL2 strain (Figure 5), a protective effect upon

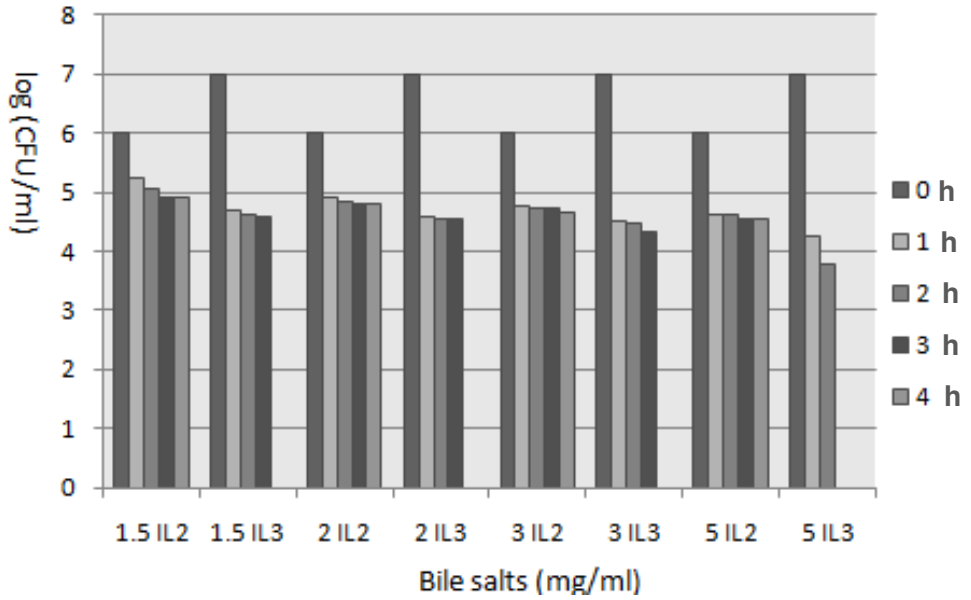

Figure 5. Casein effect on the viability of *Lactobacillus paracasei* IL2 and *Lactobacillus plantarum* IL3 strains in case of exposure to simulated small intestine juice.

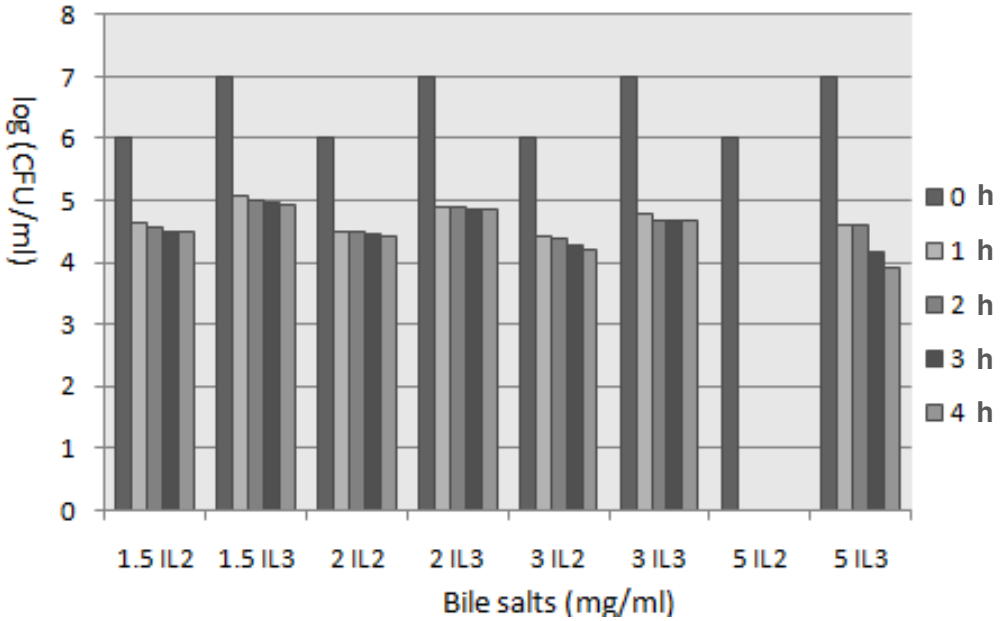

Figure 6. Mucin effect on the viability of *Lactobacillus paracasei* IL2 and *Lactobacillus plantarum* IL3 strains in case of exposure to simulated small intestine juice.

viability, unlike the effect of pancreatin and of bile salts. Although the difference was small, the presence of mucin (Figure 6) determined a higher decrease of viability, especially for IL2 strain. The decrease was directly correlated to the increase of the concentration of bile salts and the stationary time, with the strains having no viability whatsoever at a bile salts concentration of 5 mg/ml. After an exposure of two hours, irrespective of the concentration of bile salts, the viability of IL3 strain decreased on an average by 20%. After two more hours, the viability decreased on an average by 2%. In the presence of casein, the IL3 strain had no viability after four hours of exposure, irrespective of the bile salts concentration. At 5 mg/ml, the strain remained viable for

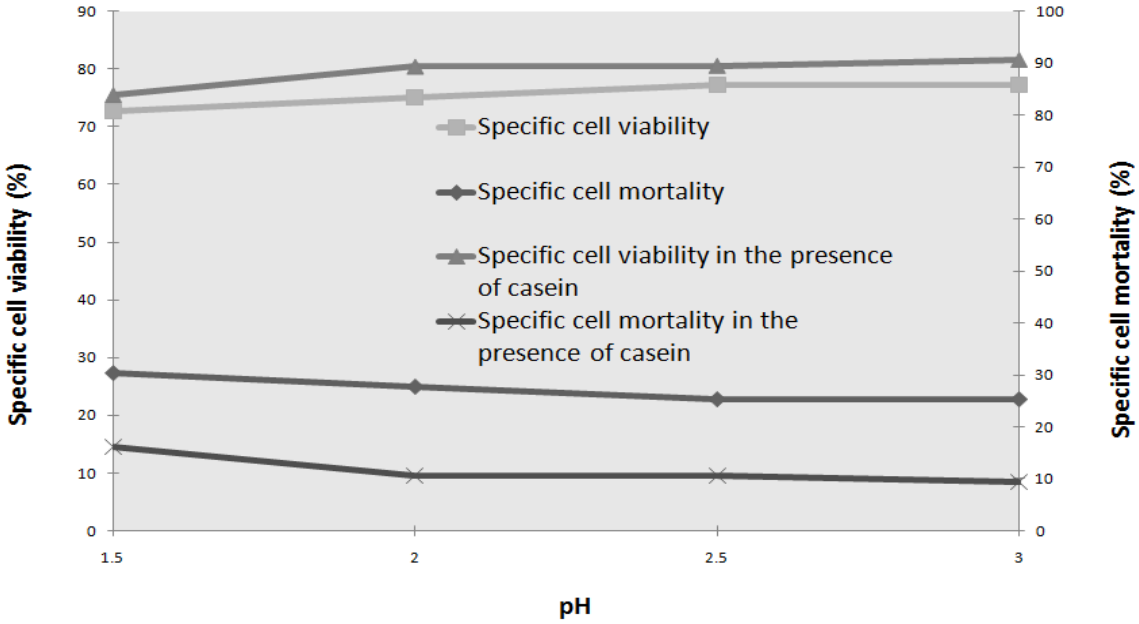

Figure 7. Specific cell mortality and viability of *Lactobacillus paracasei* IL2 strain in case of exposure to simulated gastric juice.

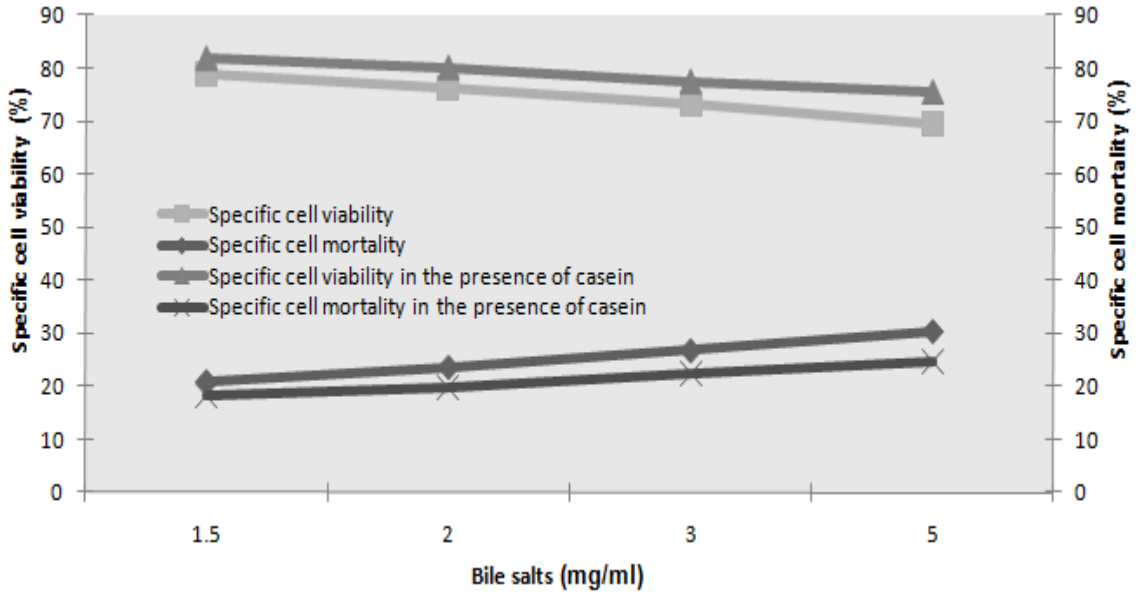

Figure 8. Specific cell mortality and viability of *Lactobacillus paracasei* IL2 strain in case of exposure to simulated small intestine juice.

no more than two hours.

The mathematical parameters of viability and mortality were determined at various pH levels and in the presence of different bile salts concentrations. From the aforementioned data, it resulted that casein was a better protector than mucin (Figure 7). An interesting observation was that the mortality line and the viability line, whether in the presence of casein or not, did not intersect for the two strains, and it resulted in an appropriate protection at low pH values at the gastric level (Figure 8). According to the mathematical calculations, the viability at pH 2 increased in the presence of casein by approximately 20% for IL2 and by 5% for IL3 strain (Figure 9). Out of the same figure it

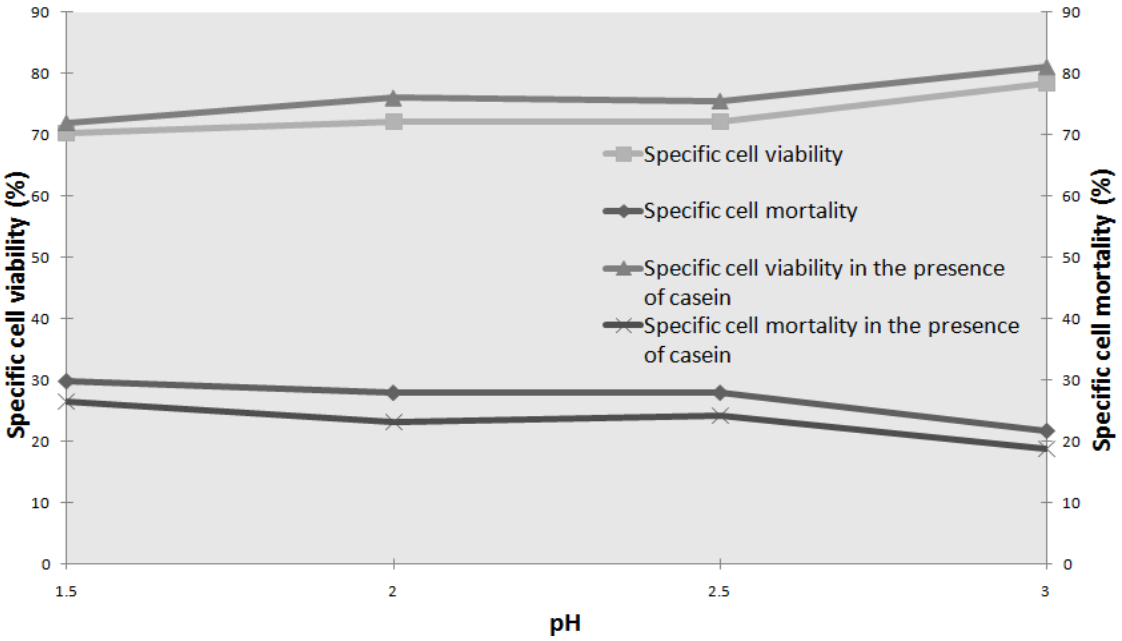

Figure 9. Specific cell mortality and viability of *Lactobacillus plantarum* IL3 strain in case of exposure to simulated gastric juice.

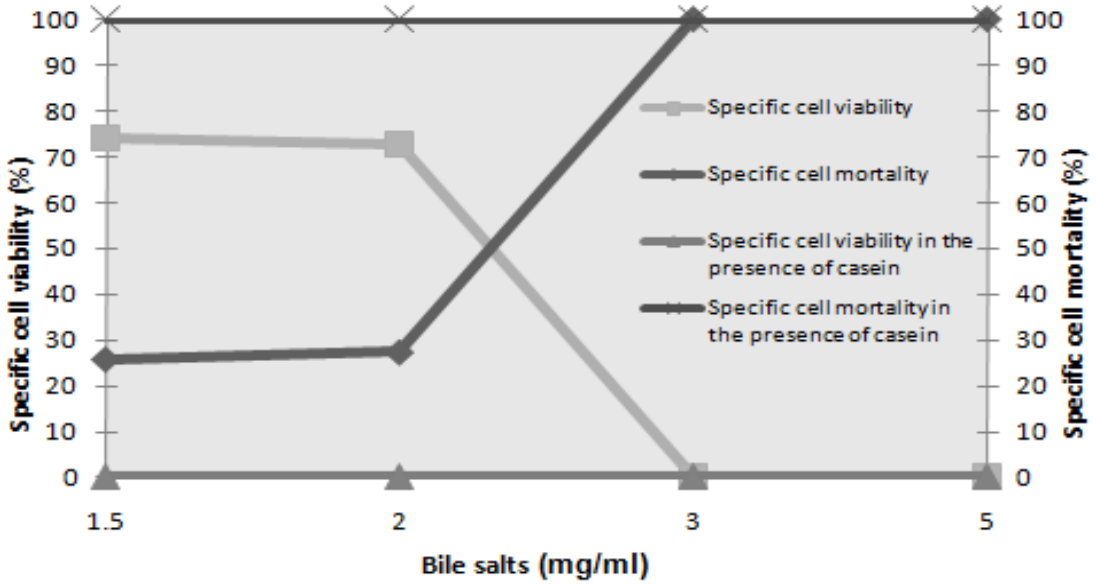

Figure 10. Specific cell mortality and viability of *Lactobacillus plantarum* IL3 strain in case of exposure to simulated small intestine juice.

resulted that the IL2 and IL3 strains had an appropriate viability at pH below 2, according to the literature data, of at least 10^5 CFU/ml for the probiotic bacteria (literature).

In the case of the simulated small intestinal juice, the behavior of IL2 strain was similar, since no critical point was observed and, thus, the strain maintained its viability. IL3 strain had a critical point in the presence of casein at pH of approximately 2. Unless the protector was present, the IL3 strain lost all its viability. The strain was strongly inhibited by bile salts concentration of approximately 2.5 mg/ml (Figure 10). Thus, at bile salts concentration of 5 mg/ml, the viability was present for only two hours. For the other concentrations, the viability was present for no more than three hours.

The protective effect of casein was also noticeable in the case of the cumulated action of the gastric and small intestinal juice upon the viability of IL2 and IL3 strains. The viability was directly influenced by casein, although in the case of the gastric juice action it was high, of approximately 75%, at the same pH value of 2, for IL2 strain and of 71% for IL3 strain, respectively. The presence of casein increased the value of the viability in this case by 15% for both strains. If the simulated small intestinal juice, containing bile salts concentration of 2 - 3 mg/ml, also acted upon them, the viability was no longer maintained, even in the presence of casein. These data are supported by the previous researches of Kos et al. (2000), Patel et al. (2008), Matijasic and Rogely (2000), Heidebach et al. *(*2010) and Vamanu and Vamanu (2010). The results also represent added data to the findings of Nasrollah et al. (2009), Homayony et al. (2008) and Trachoo et al. (2008).

Although, it is considered that it offers a great protection against the gastric juice and the presence of lactic bacteria strains implicitly, the effect of casein combination with freeze-dried strains of lactic bacteria did not determine a significant increase of the viability upon the passage through the compartments of the human gastrointestinal tract. For the tested strains, the number of viable cells, under the stress exercised by pH level of 2, was elevated, but, at a bile salts concentration of 2 - 3 mg/ml, they lost their viability after an exposure of two hours, although normally the maintenance of a viability of approximately 20% after such transit was mentioned. The researches of Kos et al. (2000), Sumeri et al. (2010), Vamanu and Vamanu (2010) and Movsesyan et al. (2010) are in support of this result, with no disagreement values.

Conclusions

It was proven that the *L. paracasei* IL2 and *L. plantarum* IL3 strains were capable of surviving in gastric conditions. The presence of casein, in comparison with mucin, determined a 15% viability increase. The conditions under which the strains became sensitive at pH lower than 2 were established. An exposure time of more than two hours, at bile salts concentration of more than 2 mg/ml, annuled the viability of the two tested strains. The knowledge of the protector and the cumulated gastric and intestinal effect upon the viability of the strains makes them more competitive when used for obtaining of new probiotic products.

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