In Vitro Qualitative Assessment of Tolerance to Simulated Gastric Juice, Bile, Fructose, Glucose and Lactose for Different Probiotic Bacteria

Maria-Cătălina MATEI LAȚIU1*, Victoria BUZA1, Flore CHIRILĂ2, Zsolt BOROS3, Călin LAȚIU4, Andrei Radu SZAKACS5 and Laura-Cristina ȘTEFĂNUT1

1 Faculty of Veterinary Medicine, Department of Animal Physiology, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania
2 Faculty of Veterinary Medicine, Department of Microbiology, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania
3 Faculty of Veterinary Medicine, Department of Parasitology, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania
4 Faculty of Animal Sciences, Department of Animal Physiology, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania
5 Faculty of Veterinary Medicine, Department of Animal Nutrition, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania

*Corresponding author: M.C. Matei Latiu e-mail: catalina.matei@usamvcluj.ro

SHORT COMMUNICATION

Abstract
Probiotics are live microorganisms that confer a health benefit to the host when administered in adequate amounts, with the main site of action at the intestinal level. In order to have a maximal effect, probiotic bacteria have to prove a high tolerance for the conditions present in the upper gastro-intestinal tract. The aim of the present study was to assess, from a qualitative point of view, the tolerance of different probiotic bacterial strains and combinations to simulated gastric juice, bile, fructose, lactose and glucose. Also, the influence of food on the bacterial growth after artificial digestion was analyzed. A bacteriologic assessment was realized for three different commercial probiotic products (Bacillus subtilis + Bacillus licheniformis + Pediococcus acidilactici; Enterococcus faecium; Bacillus subtilis + B. licheniformis + B.coagulans + B.indicus + B. clausii), simulating different conditions (artificial digestion method in the absence/presence of food, presence of bile, fructose, glucose, lactose). The obtained results suggested that the tolerance to simulated gastric juice is higher if the food is present in the moment of digestion, regardless of the probiotic bacterial strain or combination. Also, the tolerance to bile was different from one combination to another. The influence of fructose, lactose and glucose are not remarkable from a qualitative point of view. Moreover, the growth of different probiotic bacterial strains is influenced by the simulated gastro-intestinal conditions, sporulated bacteria presenting a slightly higher tolerance compared with non-sporulated strains.

Keywords: bacterial tolerance; gastro-intestinal tract; probiotics; simulated conditions.

INTRODUCTION
According to Food and Agriculture Organization/World Health Organization (FAO/WHO), probiotics are considered to be live microorganisms that are able to confer a health benefit to the host when administered in adequate amounts (FAO/WHO, 2001). From the large category of microorganisms, most types of probiotics are containing friendly bacteria (Georgieva et al., 2014). Also, a probiotic formula can be composed by a single bacterial strain, or a combination of several strains (Schmitz et Suchodolski, 2016). Lactobacilli and Bifidobacteria are the most common species of bacteria used in food supplements with a probiotic destination. Also, species from Enterococcus and
Bacillus genera are used (Saxelin, 2008; Georgieva et al., 2014). However, in order to have a probiotic effect, those bacteria have to prove a high tolerance to the gastro-intestinal conditions. As long as their main site of action is at the intestinal level, it is mandatory for the bacteria to contain different protection systems against the low pH of the stomach or against bile and digestive enzymes (Cotter and Hill, 2003). It is considered now that the tolerance to those harsh conditions became one of the most important criteria when selecting bacterial strains with a potential probiotic effect (Jensen et al., 2012). Lactic acid bacteria (LAB) are the most common type of microorganisms that are used as probiotics, being characterized by their ability to produce lactic acid. Moreover, LAB are commonly present in the gastro-intestinal tract (Marco et al., 2006; Jensen et al., 2012). Even if most of the probiotics are containing LAB, some bacteria from Bacillus genus are considered to have a higher probiotic potential, when compared to LAB. This opinion can be explained by the capacity of bacteria from Bacillus genus to form spores. This characteristic is able to enhance their viability in difficult conditions, such as low pH in the GIT (Schmitz et Suchodolski, 2016). The aim of the present study was to assess, from a qualitative point of view, the tolerance of different probiotic bacterial strains and combinations to simulated gastric juice, bile, fructose, lactose and glucose. Also, the influence of food on the bacterial growth after artificial digestion was analyzed.

MATERIALS AND METHODS

Probiotic bacteria strains

Three different commercial probiotic products were used, with the following bacteria combinations: Bacillus subtilis HU58+ B. licheniformis SL-307+ Pediococcus acidilactici; Enterococcus faecium SF68; B. subtilis H58+ B. licheniformis + B.coagulans 10B + B.indicus HU36+ B. clausii.

Artificial digestion

Simulated gastric juice was prepared using pepsin (1:10000 NF) (2g), heated water at 43oC (400 ml) and adjusting the pH to 2.0 using HCl 25%. Each probiotic combination was artificially digested in two ways: without food and with food added (Table 1), using the protocol for artificial digestion for direct detection of Trichinella infections (Gamble et al., 2000). The food used was represented by commercial kibbles for dogs, in an adapted quantity.

Medium and testing conditions

The model of bacteria sensibility to antibiotics and chemotherapeutic using the diffusion method in Mueller Hinton agar was utilized (Quinn et al., 1994). For the bile tolerance assessment, a round paper impregnated with bile (20 µl/10mm) was used. Fructose, glucose and lactose influence over bacterial growth was determined using three round papers with a diameter of 5 mm impregnated with 10 µl of fructose, 10 µl of glucose and 10 µl of lactose. Petri plates were incubated for 24h at 37oC then the inhibition diameter areas were measured.

<table>
<thead>
<tr>
<th>Sample composition</th>
<th>Sample code</th>
<th>Artificial digestion</th>
<th>Bile tolerance</th>
<th>Fructose, Glucose and Lactose influence</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacillus subtilis</strong></td>
<td>1.1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><strong>Bacillus licheniformis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pediococcus acidilactici</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Enterococcus faecium</strong></td>
<td>1.2</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td><strong>Bacillus subtilis</strong></td>
<td>1.3</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td><strong>Bacillus licheniformis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Bacillus coagulans</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Bacillus indicus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Bacillus licheniformis</strong></td>
<td>2.3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
RESULTS AND DISCUSSIONS

Bacterial tolerance to simulated gastric juice

The acidic pH of the gastric juice is able to influence the viability of the probiotic bacteria (Jensen et al., 2012). In the present study, the bacterial tolerance to simulated gastric juice was influenced by the acidic pH (2.0), for all the three samples (Figure 1 A, C, E). However, when the artificial digestion was performed in the presence of the food, the viability of the bacteria was higher (Figure 1 B, D, F).

A study conducted in 2019 shows that the viability of Enterococcus spp. is partially affected after maintaining a pH of 2 for one hour, while the contact between the acidic solution and the bacteria for 3 hours produced a total inhibition (Bagci et al., 2019). For bacterial species of the genus Bacillus it has been shown that some species can survive for 3 hours at pH 3 in a proportion of 90%, while other species only in a proportion of 60%. The authors explained this difference in tolerance in terms of the origin of Bacillus spp. (Penaloza-Vasquez et al., 2019). However, if the pH has a higher value, the tolerance of probiotic bacteria to the simulated conditions of the gastrointestinal
tract increases. It is considered that in the first 45 minutes after feeding, the gastric pH has values higher than 3. This modification is able to directly influence the survival rate of probiotic bacteria (Mainville et al., 2019). These observations can be explained by the fact that the addition of food changed the pH value and therefore created a less unfriendly environment for probiotic bacteria. Moreover, food has the ability to act as a buffer, increasing the pH value (Dressman et al., 1990). This observation is also supported by the present study where the presence of the food increased the viability of the bacteria.

**Bacterial tolerance to bile**

Probiotics main site of action is located at the intestinal level. Therefore, bacterial tolerance to bile is also an important factor in order to produce a maximal effect for the host (Jensen et al., 2012). For sample 1 and 3 a low bactericide action can be observed (lysis zone 1,5-2 mm around the round paper with liquid bile) (Figure 2 A, B, E, F). Similar results have been obtained by other researchers who evaluated the influence of bile on different species of *Bacillus* with probiotic potential. They demonstrated that a concentration of 5% bile allows the growth of only 60% of the tested Bacillus species (Bagci et al., 2019). Other studies claim that the same concentration allowed the growth of all *Bacillus* spp. isolated from the gastrointestinal tract (Nithya and Halami, 2013). Regarding the tolerance of *Enterococcus* spp. to bile, several studies suggest that probiotic bacteria belonging to this genus are able to survive in contact with bile components (Bagci et al., 2019; Pieniz et al., 2014; Saelim et al., 2012).

![Figure 2. Bacterial tolerance to liquid bile (20 µl/ 10 mm), cultivation on Mueller-Hinton agar, incubation 24h at 37 °C; A- sample 1 (face); B- sample 1 (back); C- sample 2 (face); D- sample 2 (back); E- sample 3 (face); F- sample 3 (back)](image_url)
Fructose, Glucose and Lactose influence over bacterial growth

The viability of probiotic bacteria in the GIT may also be influenced by the presence of a rich nutritional substrate. However, the addition of fructose, glucose and lactose had not a significant influence, from a qualitative point of view, over the bacterial growth (Figure 3 A, B, C).

Regarding the influence of different nutritional sources over the bacterial growth, a study conducted in 2016 demonstrated that the addition of different carbon sources as glucose and sucrose had the ability to enhance the development of the bacteria (Davoodi et al., 2016). However, the bacterial strains tested were represented by Lactobacillus strains. Because the mechanism of action is different for each bacterial genus, this observation available for Lactobacillus spp. cannot be extrapolated to other bacterial species like Enterococcus or Bacillus that were tested in the present study.

Figure 3. Fructose, Glucose and Lactose influence over bacterial growth - A- sample 1 (inside the dish - F- round paper impregnated with 10 μl of Fructose; G- round paper impregnated with 10μl of Glucose; L- round paper impregnated with 10 μl of Lactose); B- sample 2 (inside the dish- F- round paper impregnated with 10 μl of Fructose; G- round paper impregnated with 10μl of Glucose; L- round paper impregnated with 10 μl of Lactose); C- sample 3 ( inside the dish - F- round paper impregnated with 10 μl of Fructose; G- round paper impregnated with 10μl of Glucose; L- round paper impregnated with 10 μl of Lactose)

CONCLUSION

Regardless of the probiotic bacterial strain or combination, the obtained results suggested that the tolerance to simulated gastric juice is higher if the food is present in the moment of digestion. The acidic pH of the gastric juice is modified by the presence of the food, aspect that may explain the higher tolerance of the bacteria to the simulated gastric juice. Bile tolerance is different from one combination to another. For sample 1 and sample 3 a low bactericide action was noted with a lysis zone of 1,5-2 mm around the round paper with liquid bile. Both, sample 1 and sample 3 containsporulated bacteria that may improve their viability in the conditions of gastro-intestinal tract. The influence of fructose, lactose and glucose were not remarkable from a qualitative point of view.
Author Contributions:

Funding Source: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflicts of Interest
The authors declare that they do not have any conflict of interest.

REFERENCES