Abstract
Our work-group has isolated thermophilic spore-forming bacteria Bacillus smithii TBMI12 from human gut and supposed it to be a potential probiotic. An objective of the current research was to investigate the following hypotheses: 1) B. smithii spores are able to colonize intestinal tract of mice; 2) Mice colonized previously with B. TBMI12 spores will not be infected with pathogenic Salmonella Enteritidis (S. Enteritidis).
In total 25 mice were divided into three groups. The group A was intragastrically inoculated with one dosage of S. Enteritidis cells 10^6 CFU. The group B was treated daily for three days with B. TBMI12 spores 10^8 CFU and after that inoculated with one dosage of S. Enteritidis cells 10^6 CFU. The group C was inoculated intragastrically with B. TBMI12 spores 10^6 CFU on the 1st and the 8th day. Samples of faeces were taken maximally over 72 hours and plated on selective media to count B. TBMI12 or S. Enteritidis colonies. Whole A and B group were executed on the 18th day (group C: 30th day) and the samples from liver and spleen were plated on selective media too.

Introduction and Purpose
Traditionally antibiotics are used to protect animals against Salmonella infections. Unfortunately using antibiotics as feed supplements has turned many pathogens resistant to antibiotics. To avoid spreading of antibiotic resistant pathosis European Union have banned all antibiotics in animal feed since 2006 (EC, 2003). According to the above mentioned regulation vaccines, probiotics and competitive exclusion agents have achieved paramount importance in protection of animals against infections. Probiotic microbes are using different mechanisms to prevent establishment of potential pathogens. They may block mucosal receptor sites of host, secrete antimicrobial peptides, production of immunostimulative compounds. The aim of the current study was to test whether intragastric inoculation with B. TBMI12 spores may form stabile population of above mentioned bacteria in gastrointestinal tract of mice. The aim of the further experiment was to test whether intragastric inoculation with B. TBMI12 spores will not be infected with pathogenic Salmonella Enteritidis (S. Enteritidis). The experiment was conducted on the 1st day of the experiment the mice from groups A and B were inoculated with 1 dosage of B. TBMI12. During the experiment the number of bacteria fell about ten times. Only one mouse from group A died because of salmonellosis on the 12th day of the experiment. Other mice lived until termination of the experiment and were executed by cervical dislocation on the 17th day. Plate count of liver and spleen showed that 100% of group A mice and 40% of group B mice were infected with S. Enteritidis. Results are shown on the Figure 3. Conclusion
The current research showed that 1) Bacillus smithii TBMI12 spores will not be colonized in intestinal tract of mice. 2) 60% of mice colonized previously with Bacillus smithii TBMI12 spores will not be infected with pathogenic Salmonella Enteritidis. Therefore we suggest Bacillus smithii TBMI12 as a potential competitive exclusion agent against Salmonella Enteritidis.

References

Methods
Three-month-old, male, BALB/c mice were obtained from the Institute of Molecular and Cell Biology (University of Tartu, Estonia) and maintained in the vivarium of the above mentioned institute during the experiment.

Bacillus smithii TBMI12 was grown overnight at 30°C whereas only the

S. Enteritidis was cultured on solid XLD medium (Oxoid) overnight at 30°C. To prepare inocula for mice experiments S. Enteritidis was grown for six hours in Nutrient Broth (Difco) media at 30°C, aerobically with agitation 220 rpm. The culture of S. Enteritidis was suspended in sterile Nutrient Broth medium to achieve bacterial number 10^6 CFU per 0.5 ml.

The mice were intragastrically inoculated with 0.5 ml dosage using a sterile syringe blunt-ended tube. In total 25 mice were divided into three groups: A, B and C. The scheme of the experiment is described in the Table 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>Day</th>
<th>B. TBMI12 (10^8 CFU)</th>
<th>S. Enteritidis (10^6 CFU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1st</td>
<td>0.9 % NaCl</td>
<td>S. Enteritidis cells 10^6</td>
</tr>
<tr>
<td>B</td>
<td>1st</td>
<td>0.9 % NaCl</td>
<td>B. TBMI12 10^8 spores</td>
</tr>
<tr>
<td></td>
<td>2nd</td>
<td>0.9 % NaCl</td>
<td>B. TBMI12 10^6 spores</td>
</tr>
<tr>
<td></td>
<td>8th</td>
<td>0.9 % NaCl</td>
<td>B. TBMI12 10^6 spores</td>
</tr>
</tbody>
</table>

The aim of the experiments was to test whether intragastric inoculation with B. TBMI12 spores may form stabile population of above mentioned bacteria in gastrointestinal tract of mice. If this is possible and B. TBMI12 does not cause any harm to mice, another question rises: can B. TBMI12 spores colonized the group C mice only for a couple of days. However, unfortunately the number of bacteria fell about ten times in two weeks. 10% of the mice colonized with B. TBMI12 spores were infected with S. Enteritidis. Therefore we suggest Bacillus smithii TBMI12 spores as a potential competitive exclusion agent against Salmonella Enteritidis.