Diversity of murine microbiota and its role in investigating probiotic effects of lactic acid bacteria

dr. Biljana Hacin

National Veterinary Institute, Veterinary Faculty
Agenda

- Murine microbiota
- Animal models
- Probiotics

- Effect of *L. gasseri* K7 on mice infected with *E. coli* O157:H7

- Effect of probiotic strains *Lactobacillus fermentum* L930BB and *Bifidobacterium animalis* subsp. *animalis* IM386 on DSS colitis in mice
Murine microbiota

• Mice and other mammals normally harbour an extensive bacterial flora not only in the large intestine, but also in the stomach and small intestine ($10 \times 10^{14}$)

• Lactobacilli and anaerobic streptococci become established immediately after birth and persist in large numbers in the stomach, small and large intestine

• In contrast, the anaerobic bacilli of the bacteroides group become established only after the 16th day, multiply only in the large intestine but persist in this organ in very large numbers

• Other bacterial species become established at different periods after birth

• In general, the populations of enterobacilli and enterococci decrease precipitously after having reached a maximum level shortly after the beginning of colonization

R. W. Schaedler, 1965
Murine microbiota

- Host’s genotype affects bacterial community of GM

- GM of inbred mice are more similar between animals than are those of outbred mice

- Viable counts of total bacterial load have revealed large differences in the cecal microbiota from different vendors

- No indication that IVC as a housing system results in intercage variation

- Exposure to different environmental factors, caretakers, deviations in treatments of food, water and bedding may influence the composition of GM

M.R.Hufeldt, Comparative Medicine, 2010
Standardization

- Host-genetics
- Diet
- Weight
- Age
- Gender
- Environment
- Caging history
- Hygene conditions
Mouse models

- Gnotobiotic mice
- SPF mice
- Altered Schaedler flora
- Streptomycin treated
- Protein-calorie malnutrition
- Germ-free
- Conventional
- *Citrobacter rodentium*
- DSS-colitis
- TNBS-colitis
Mouse models

- C57BL/6J, C57BL/10sn and B10A mice resistant to *L. monocytogenes* infection
- A/J, BALB/c, CBA/J, C3H/He, DBA/1 and DBA/2 sensitive to infection
- Sydney strain of *H. pylori* - high levels of colonization achieved consistently in C57BL/6 mice
- BALB/c, DBA/2 and C3H/He colonized in lower numbers
Probiotics

- Live microorganisms which, when administered in adequate amounts, confer a **health benefit on the host** (FAO/WHO, 2012)
- Host specific
- Status GRAS
- Safe (antibiotic resistance, mucin degradation)
- Bile and acid tolerance
- Antimicrobial activity (antimicrobial substances-bacteriocins, competition with pathogens)
- Adhesion and at least transient colonization
- Stimulation of the immune system
Effect of *L. gasseri*K7 on mice infected with *E. coli*O157:H7

<table>
<thead>
<tr>
<th>Study duration</th>
<th>day1</th>
<th>day7</th>
<th>day8</th>
<th>day14</th>
<th>day15</th>
<th>day21</th>
<th>day22</th>
<th>day28</th>
<th>Blood sampling</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adaptation phase</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment phase</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Washout period</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**A**
- Control
  - *n* = 10
  - Saline

**B**
- *E. coli*O157:H7
  - *n* = 10
  - Saline

**C**
- *L. gasseri*K7
  - *n* = 10
  - *L. gasseri*K7

**D**
- Co-incubation
  - *n* = 10
  - *L. gasseri*K7

**E**
- Pre-incubation
  - *n* = 10
  - *E. coli*O157:H7

Hematology
- *n* = 51
- White blood cell count

Immunochemistry
- *n* = 5
- Blood cell transcrption

Effect of *L. gasseri* K7 on mice infected with *E. coli* O157:H7

- Coliform microorganisms
Effect of *L. gasseri* K7 on mice infected with *E. coli* O157:H7

• **LAB**
Effect of *L. gasseri* K7 on mice infected with *E. coli* O157:H7

- K7

![Bar chart showing the effect of *L. gasseri* K7 on mice infected with *E. coli* O157:H7 over a period of 28 days. The chart displays the log CFU/g counts for different groups (B, C, D) across 0, 8, 15, 22, and 28 days.](image)
Effect of *L. gasseri* K7 on mice infected with *E. coli* O157:H7

- K7 survives the passage through the gastrointestinal tract and at least **transiently colonizes the intestines** of C57BL/6J mice

- Daily application of relatively high doses of K7 (1,2 x 10⁹ CFU) for 14 days had **no adverse effects on the animals** - the strain is safe

- K7 has an effect on the **immune system of the host** – increase in IgA-positive cells in ileal mucosa

- Application of K7 and/or *E. coli* O157:H7 had **no effect** on the population of lactic acid bacteria and coliform microorganisms
Effect of *L. gasser*K7 on mice infected with *E. coli*O157:H7

- A single dose of *E. coli* O157:H7 (5 x 10⁸ CFU) **did not induce clinical signs** in C57BL76J mice, but its effect was seen in:
  - increase of **segmented** and **band neutrophils** in the blood
  - increase of **IgM-positive cells** in ileal mucosa
  - thinning of **the colon mucosa**

- Transcriptomic study showed that K7 **modulates the blood cell transcriptomic profile** induced by *E. coli* O157:H7
  - a profile mainly characterized by an activation of immune modulatory pathways

SDLŽ, 2014
Effect of probiotic strains *L. fermentum* L930BB and *B. animalis* subsp. *animalis* IM386 on DSS colitis in mice
Effect of probiotic strains *L. fermentum* L930BB and *B. animalis* subsp. *animalis* IM386 on DSS colitis in mice

Group A (Control)

![Graph showing log10 (CFU g⁻¹) over days for different media: VRB, BHI, MRS + Cys.](image)
Effect of probiotic strains *L. fermentum* L930BB and *B. animalis* subsp. *animalis* IM386 on DSS colitis in mice

Group B (DSS + Control)

![Graph showing bacterial growth over days](image)
Effect of probiotic strains *L. fermentum* L930BB and *B. animalis* subsp. *animalis* IM386 on DSS colitis in mice

Group C (Probiotics)

![Graph showing the effect of probiotics on log10 (CFU g⁻¹) over 20 days.](image)
Effect of probiotic strains *L. fermentum* L930BB and *B. animalis* subsp. *animalis* IM386 on DSS colitis in mice

Group D (DSS + Probiotics)
Effect of probiotic strains *L. fermentum* L930BB and *B. animalis* subsp. *animalis* IM386 on DSS colitis in mice

Group E (Probiotics prior to DSS)
Effect of probiotic strains *L. fermentum* L930BB and *B. animalis* subsp. *animalis* IM386 on DSS colitis in mice

Group F (DSS + CsA)

![Graph showing log10 (CFU g⁻¹) over days for different conditions: VRB, BHI, MRS + Cys]
Effect of probiotic strains *L. fermentum* L930BB and *B. animalis* subsp. *animalis* IM386 on DSS colitis in mice

Group G (CsA)

![Graph showing log10 (CFU g⁻¹) over days](chart.png)

- **VRB**
- **BHI**
- **MRS + Cys**

SDLŽ, 2014
The use of selected probiotic strains had no adversary effects on the colonic mucosa of healthy animals.

Group with DSS induced colitis receiving probiotics showed statistically lower leucocyte infiltration than group with induced colitis that did not receive the probiotic strains (DSS/water).
Effect of probiotic strains *L. fermentum* L930BB and *B. animalis* subsp. *animalis* IM386 on DSS colitis in mice

- Treatment with probiotics (when animals were treated with probiotics 7 days before colitis induction) **improved the body weight loss** of animals with induced colitis

- **Decreased extent of colonic lesions** and the **depth of inflammation in the colon wall** was observed in this group of animals
Although variation in the microbiota of individual animals was observed, it had no statistically significant effect when taking into account a large enough group of animals.

LAB strains tested had no adverse effect on the animals:

- were capable of at least transiently colonizing the intestines
- amelliorated the effects of *E. coli* O157:H7 and chemically induced colitis

Strains are safe and are good candidates for probiotics.
Acknowledgements

• Institute of Dairy Science and Probiotics, Biotechnical Faculty

• Institute of Food Science, Agroscope, Berne, Switzerland

• Medical Experimental Centre, Institute of Pathology, Faculty of Medicine, Ljubljana

• National Veterinary Institute, Veterinary Faculty