In-vitro growth characteristics of commercial probiotic strains and their potential for inhibition of Clostridium difficile and Clostridium perfringens

Schoster, A.; Permin, A.; Kokotovic, Branko; Dedenroth, P.; Guardabassi, L.

Publication date:
2012

Document Version
Publisher's PDF, also known as Version of record

Link back to DTU Orbit

Citation (APA):
IN-VITRO GROWTH CHARACTERISTICS OF COMMERCIAL PROBIOTIC STRAINS AND THEIR POTENTIAL FOR INHIBITION OF CLOSTRIDIUM DIFFICILE AND CLOSTRIDIUM PERFRINGENS

A Schoster1, B Kokotovic2, A Permin3, P Dedenroth4, L Guardabassi1.
1Faculty of Health Science, University of Copenhagen, Copenhagen, Denmark
2Technical University of Denmark, Copenhagen, Denmark
3The DHI group, Copenhagen, Denmark
4Clerici-Sacco Group, Cadorago, Italy

Background
C. difficile and C. perfringens are important animal pathogens. There are currently no preventative measures and therapeutics are limited to antibiotics. With antibiotic resistance on the rise new approaches are needed.

Probiotics have been evaluated in humans in relation to clostridial disease and results have been promising. There are no animal probiotics licensed for use against clostridial disease. Many strains that show promising activity can not be commercially produced as they are not technically robust. This could be overcome by using strains already in commercial production.

Objective and Hypothesis
1) To assess the ability of selected commercial probiotic strains to inhibit growth of C. difficile and C. perfringens in vitro
2) To evaluate their ability to grow in the presence of oxygen, acid and bile

Material and Methods
Seventeen probiotic strains were used (Table 1).

Inhibition of C. difficile and C. perfringens
The effect of a cell free probiotic culture supernatant on the growth of C. difficile ribotype 078 and C. perfringens Type C was assessed. Supernatant was harvested and sterilized after 12, 24, 36, 48, and 72 hours and six days. One aliquot was adjusted to pH 7.4 (pHlab) the other aliquot was left at original pH (pHorig). 

Agar well diffusion assay
The anti-clostridial activity was evaluated by agar well diffusion following addition of supernatant at pHorig or pHlab. 

Broth co-culture in Brain Heart Infusion (BHI)
BHI broth was inoculated with C. difficile or C. perfringens and probiotic supernatant (48h, pHorig or pHlab). Clostridial growth was compared to growth of a control culture with Man-Rogosa-Sharpe (MRS) broth at pH7.0 or pH3.9 instead of supernatant using spectrophotometry. Inhibition was indicated by a reduction of growth of at least 50%.

Evaluation of growth characteristics
Acidic and anaerobic growth of probiotics was assessed visually. Growth was compared between growth in standard MRS broth, MRS broth pH2.0 and pH4.0, and MRS supplemented with 0.15% and 0.3% bile spectrophotometrically.

Results
Inhibition of C. difficile and C. perfringens
Agar well diffusion assay (Fig. 1)
- 2/17 strains inhibited C. perfringens (Tab. 1)
  o Supernatant from all timepoints (growth phases) was inhibitory
  o Supernatant at pHorig and pHlab was inhibitory
  o Inhibition of pHorig was greater indicating presence of an additional antibiotic compound other than organic acids
- 10/12 strains inhibited C. difficile (Tab. 1)
  o Only supernatant with pHorig was inhibitory indicating inhibition due to organic acids
  o Only supernatant harvested after at least 36h of incubation inhibited C. difficile

Broth co-culture
- 5/17 probiotics inhibited C. perfringens and 10/17 inhibited C. difficile (Tab. 1)
  Inhibition was only seen with supernatant at pHorig

Growth characteristics
All strains grew aerobically except B. animalis lactis. None of the strains grew at pH2, growth at pH4 ranged between 40-95%

Growth parameters of selected probiotic strains that showed inhibitory potential against both clostridia are presented in Table 2.

Conclusions
- 5 strains (L. plantarum n=2), L. rhamnosus (n=2) and B. animalis lactis inhibit clostridial growth by a reduction of pH. This inhibitory effect is likely due to organic acid production during stationary growth phase
- 2 of the 5 strains (L. plantarum, B. animalis) produce an additional antibiotic compound that inhibits C. perfringens only. This compound is produced during the exponential phase and it’s activity is pH-independent.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Trade Name</th>
<th>C. difficile Agar Well Broth</th>
<th>C. perfringens Agar Well Broth</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. plantarum</td>
<td>BG112</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>L. rhamnosus</td>
<td>LRH19</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>L. plantarum</td>
<td>LP1</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>L. rhamnosus</td>
<td>SP1</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>B. animalis spp lactis</td>
<td>BC11</td>
<td>x</td>
<td>x</td>
</tr>
</tbody>
</table>

Table 2: Growth characteristics of probiotic strains against C. difficile and C. perfringens in standard MRS-Broth (MRS broth, MRS broth adjusted to pH2 and pH4, and MRS broth with 0.5% or 0.1% bile added). The numbers are given as percentage of growth compared to growth in standard MRS broth.

Contact Information
Angelia Schoster
E-mail: aschoster@life.ku.dk
University of Copenhagen
Department of Veterinary Disease Biology
Faculty of Health and Medical Sciences

Declarations of Conflict of Interest: One of the co-authors (P Dedenroth) is employed by the Clerici-Sacco Group (Cagliari, Italy), the company who supplied the probiotic strains for this study. The company did not make a financial contribution to this study and had no influence on study design or reporting of results.