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

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Publication date:
2012

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

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

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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/or 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 (pH something) the other aliquot was left at original pH (pH something). 

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

Broth co-culture in Brain Heart Infusion (BHI) broth
BHI broth was inoculated with C. difficile or C. perfringens and probiotic supernatant (48h, pH something or pH something). Clostridial growth was compared to growth of a control culture with Man-Rogosa-Sharpe (MRS) broth at pH 7.0 or pH 3.9 instead of supernatant using spectrophotometry. Inhibition was indicated by a reduction of growth at least 50%.

Results
Inhibition of C. difficile and C. perfringens

48 h diffusion assay (Fig. 1)
- 2/17 strains inhibited C. difficile (Tab. 1)
  o Supernatant from all timepoints (growth phases) was inhibitory
  o Supernatant at pH something and pH something was inhibitory
  o Inhibition of pH something was greater indicating presence of an additional antibiotic compound other than organic acids
- 10/12 strains inhibited C. difficile (Table 1)
  o Only supernatant with pH something 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 (Table 1)
- Inhibition was only seen with supernatant at pH something

Growth characteristics
- All strains grew aerobically except B. animalis lactis.
- None of the strains grew at pH 2.2, growth at pH 4.0 ranged between 40-95%.
- Growth ranged from 40-99% when bile was added
- 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.
- These 5 strains show growth characteristics suitable for probiotics and their possible use for control of clostridial disease will be further explored by in-vitro and in-vivo studies.

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Table 1: Growth characteristics of selected probiotic strains against C. perfringens and C. difficile in conventional and stationary phases

<table>
<thead>
<tr>
<th>Strain</th>
<th>pH 4</th>
<th>pH 2</th>
<th>Bile 0.3%</th>
<th>Bile 0.15%</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. plantarum</td>
<td>100</td>
<td>81</td>
<td>1</td>
<td>85</td>
</tr>
<tr>
<td>L. rhamnosus</td>
<td>100</td>
<td>60</td>
<td>1</td>
<td>70</td>
</tr>
<tr>
<td>L. plantarum</td>
<td>100</td>
<td>75</td>
<td>0</td>
<td>71</td>
</tr>
<tr>
<td>L. rhamnosus</td>
<td>100</td>
<td>77</td>
<td>0</td>
<td>72</td>
</tr>
</tbody>
</table>

*Table 1: Growth characteristics of selected probiotic strains against C. perfringens and C. difficile in conventional and stationary phases

*Fig. 1: Inhibition of C. perfringens by cell-free supernatants at pH something and pH something, obtained from a probiotic mixture BLC7 and unsuccessful inhibition by L. plantarum LbH.

*Figure 2: Broth growth inhibition assay (Fig. 1)