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 pH7.4 (pHspp) the other aliquot was left at original pH (pHo).

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

Broth co-culture in Brain Heart Infusion (BHI) BHI broth was inoculated with C. difficile or C. perfringens and probiotic supernatant (48h, pHspp or pHo). 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 of at least 50%.

Evaluation of growth characteristics
Growth was compared visually. Growth was compared between growth in standard MRS broth, pHspp and pHo, and MRS broth 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)
○ Supernatant from all timepoints (growth phases) was inhibitory
○ Supernatant at pHspp and pHo was inhibitory
○ Inhibition of pHspp or pHo was greater indicating presence of an additional antibiotic compound other than organic acids
○ 10/12 strains inhibited C. difficile (Tab. 1)
○ Only supernatant with pHspp was inhibitory indicating inhibition due to organic acids
○ 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 pHspp

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 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-vivo and in-vitro studies.

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Table 1 Inhibition activity of probiotic supernatants against C. perfringens and C. difficile in agar well diffusion and broth co-culture assays. Proteins outlined in yellow and blue showed inhibition against both clostridia. Proteins outlined in blue showed inhibition against both clostridia in both assays. Fields outlined in green show inhibitory potential in the respective experiment against the respective clostridial strains.

Table 2 Growth characteristics of commercial probiotics againstClostridia in standard Man-Rogosa-Sharpe (MRS) broth, MRS broth adjusted to pH2 and pH4, and MRS broth with 0.5% or 0.3% bile added. The numbers are given as a percentage of growth compared to growth in normal MRS broth.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Trade Name</th>
<th>pH2</th>
<th>pH4</th>
<th>Bile 0.3%</th>
<th>Bile 0.15%</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. plantarum BG112</td>
<td>100</td>
<td>81</td>
<td>1</td>
<td>85</td>
<td>95</td>
</tr>
<tr>
<td>L. rhamnosus LRH19</td>
<td>100</td>
<td>40</td>
<td>0</td>
<td>63</td>
<td>83</td>
</tr>
<tr>
<td>L. plantarum LPAL</td>
<td>100</td>
<td>60</td>
<td>1</td>
<td>90</td>
<td>99</td>
</tr>
<tr>
<td>L. rhamnosus SP1</td>
<td>100</td>
<td>75</td>
<td>0</td>
<td>71</td>
<td>81</td>
</tr>
<tr>
<td>B. animalis spp lactis BLC1</td>
<td>100</td>
<td>77</td>
<td>0</td>
<td>92</td>
<td>96</td>
</tr>
</tbody>
</table>

Growth in % compared to MRS

Declaration of Conflict of Interest: One of the co-authors (P Dedenroth) is employed by the Clerici-Sacco Group (Cadorago, 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.

Figure 2 Inhibition of C. perfringens by cell-free supernatants at pHspp and pHo, obtained from 5 animal lactis LBC1 and unsuccessful inhibition by C. perfringens strain 12h, 24h, 36h, 48h. Probiotic supernatant obtained after respective incubation time. Control well: sterile MRS broth adjusted to pH3.9 or standard MRS broth (pHo).