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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 (pHadj) 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 pHadj.

Broth co-culture in Brain Heart Infusion (BHI)
BHI broth was inoculated with C. difficile or C. perfringens and probiotic supernatant (48h, pHorig or pHadj). 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%.

Results

Table 1. Inhibition activity of probiotic supernatant against C. perfringens and C. difficile in agar well diffusion and broth co-culture assays. Probiotics outlined in yellow and blue showed inhibition against both clostridia. Probiotics 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.

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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