In vitro adhesion of lactic acid bacteria to canine small intestinal mucus

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Summary

Selected probiotic lactic acid bacteria have several documented health effects. For many of these health effects, adhesion to the intestinal mucosa is of primary importance. In the current study, the adhesive ability to canine small intestinal mucus of four lactic acid bacteria intended for human use, two for animal use and two strains isolated from dogs was assessed. The strains for human use were specifically chosen because they have documented health effects and have been proven to be safe. One strain for human use, Lactobacillus rhamnosus (ATCC 53103), was found to adhere significantly better than all other strains. Pretreatment of the strains with canine jejunal chyme, to simulate digestion, dramatically reduced the adhesion of all strains tested. However, three of the strains intended for human use were still adhering better than the strains from animal origin. The results show that probiotic strains from human origin and intended for human use also adhere to canine intestinal mucus. This warrants further investigation of these strains for use in dogs.

Introduction

Probiotics can be defined as microbial cell preparations or components of microbial cells that have a beneficial effect on the health and well-being of the host (Salminen et al. 1999). Most probiotics in use are lactic acid bacteria (LAB); that is Gram-positive bacteria which produce lactic acid as their main fermentation product. Studies in humans have shown several documented health effects associated with the use of selected probiotic strains (Ouwehand et al. 1999a). Although many studies have been performed in animals, this has mainly been limited to rats and mice. Feeding of LAB because of their beneficial effects has also been advocated for canine use, but few studies concerning probiotics have been performed with dogs. Trends towards increased digestibility of protein and an increased production of lactate and reduced pH were observed in the ileum (BIOURGE et al. 1998; ZENTEK et al. 1998),. In the present study, the adhesion of eight LAB to canine small intestinal mucus and their adhesive ability after pretreatment with jejunal chyme, to simulate digestion were investigated. Adhesion to the intestinal mucosa is one of the main selection criteria for probiotic micro-organisms (Ouwehand et al. 1999a). It is considered important for transient colonization (Alander et al. 1999), antagonism against pathogens (Coconnier et al. 1993), modulation of the immune-system (Schiffrin et al. 1997; O’Halloran et al. 1998) and enhanced healing of the damaged gastric mucosa (Elliott et al. 1998). The LAB tested, represent three probiotic Lactobacillus strains and one Bifidobacterium strain, all four of which were intended for human use, two Enterococcus strains for animal use and
two *Lactobacillus* strains isolated from dogs. The strains for human use were chosen because they have the greatest amount of documentation concerning health effects (Salminen et al. 1998a) and have been shown to be safe (Salminen et al. 1998b).

### Materials and methods

#### Animals

Permanent nipple valves for intestinal access were placed to six healthy beagles (five males, one female) in the mid-jejunum, using the method described elsewhere (Wilsson-Rahmberg and Jonsson 1997). Operations were performed 10 months prior to this study, and no alterations due to the valve were noticed in the dogs’ health or gastrointestinal function. At the time of the experiment, the dogs were 2 years of age. The dogs were fed a commercial balanced dog food (Baron Complete; Raisio Feed Ltd, Raisio, Finland). The main ingredients were cereal, meat, chicken, animal derivatives and oils; and the composition was: protein 22%, fibre 2.3%, fat 12%, ash 6.5%, Ca 1.1% and P 0.9%.

A sample of approximately 8 ml of jejunal chyme was obtained via the valve 2 h post-prandial and frozen immediately at −70°C.

#### Micro-organisms and growth conditions

The strains used and their culture conditions are listed in Table 1. The bacteria were grown from stocks stored at −75°C in 40% glycerol (1% inoculum). To the medium (1 ml), 10 µl of tritiated thymidine ([methyl-1.2–3H]thymidine, 120 Ci/mmol) was added to metabolically radiolabel the bacteria. After growth, the bacteria were harvested by centrifugation (2000 × g) and washed twice with phosphate-buffered saline (PBS; pH 7.2; 10 mM phosphate) and resuspended in PBS. The absorbance was adjusted to 0.25 ± 0.02 in order to standardize the number of bacteria (10^7–10^8 colony-forming units/ml) before use in the adhesion assay (see below).

In order to simulate digestion and study its effects on adhesion, bacteria were resuspended in clear supernatant from jejunal chyme (see above), a mixture of equal volumes from six dogs. After incubation for 1 h at 37°C, the bacteria were washed in PBS and used in the *in vitro* adhesion assay as described below.
Mucus preparation

Mucus was prepared from canine jejunal chyme essentially as described earlier (KIRJAVAINEN et al. 1998; OUWEHAND et al. 1999b). In short, jejunal chyme was centrifuged at 12,000 × g to remove particulate matter. Mucus was precipitated from the clear supernatants by dual ethanol precipitation and freeze dried. Equal amounts of mucus from each dog were pooled and a stock suspension of 5 mg/ml in N-2-hydroxy-ethylpiperazine-N’-2-ethane-sulfonic acid (HEPES)–Hanks buffer (HH; 10 mM HEPES; pH 7.4) was prepared and stored at −20°C until use.

Adhesion assay

The adhesion of the radioactively labelled bacteria to immobilized intestinal mucus was determined as described previously (KIRJAVAINEN et al. 1998; OUWEHAND et al. 1999b). In short, mucus stocks were thawed and centrifuged to remove any precipitate formed during storage and diluted in HH to a concentration of 0.5 mg/ml. The mucus was passively immobilized on polystyrene microtitre plate wells (Nunc Maxisorp, Roskilde, Denmark) by overnight incubation at 4°C. Excess mucus was removed by washing twice with HH. Radiolabelled bacteria were added to the wells and incubated for 1 h at 37°C. Non-bound bacteria were removed by washing twice and bound bacteria were released and lysed by incubation with 1% sodium dodecyl sulphate–0.1M NaOH for 1 h 60°C. Radioactivity was determined by liquid scintillation and the adhesion expressed as the percentage of radioactivity recovered after adhesion, relative to the radioactivity in the bacterial suspension added to the immobilized mucus.

Statistical analysis

The results from the adhesion experiments are expressed as the average of at least three independent experiments. Each experiment was performed with four parallels, to adjust for intra-experimental errors. A nonparametric Mann–Whitney U-test was used to evaluate the statistical difference (p < 0.05) in adhesion of each strain after pretreatment in comparison with the control. Analysis of variance with the Bonferroni/Dunn procedure was used to evaluate the statistical differences between the tested strains. All statistical analysis was performed with StatView® (Abacus, Berkeley, CA, USA).

Results

The adhesion to intestinal mucus of the tested strains was found to range from 0.5% (Lactobacillus casei strain Shirota) to 35% (Lactobacillus rhamnosus GG). Lactobacillus rhamnosus GG was found to adhere significantly better than all other tested strains (p < 0.0001). Bifidobacterium lactis Bb12 adhered 9.4% which was significantly different from all other tested strains (p < 0.05) with the exception of Enterococcus faecium (Tebo-bakt) and Lactobacillus johnsonii La1, (Fig. 1). Pre-treatment of the tested LAB with clear jejunal chyme significantly reduced the adhesion (p < 0.05) of L. rhamnosus GG, E. faecium (Biobak) and both canine Lactobacillus strains; Lactobacillus pentosus SK2A and Lactobacillus pentosus UK1A (Fig.1). Lactobacillus rhamnosus GG still exhibited the highest adhesion, but this was reduced to 7.8% and was not significantly different from B. lactis Bb12 at 4.8% adhesion. The adhesion of L. casei strain Shirota was, after pretreatment with jejunal chyme, not different from the tested Enterococcus strains and the lactobacilli isolated from dogs (p > 0.05).

Discussion

Adhesion to the intestinal mucosa is one of the main selection criteria for potential probiotic micro-organisms (OUWEHAND et al. 1999a) and was therefore the subject of the current
study. In the study, the adhesion to immobilized canine intestinal mucus of probiotics intended for human use (four strains) and animal use (two strains) and two *Lactobacillus* strains isolated from dogs were compared. The strains for human use were chosen since they have well-documented health effects (Salminen et al. 1998a) and have been shown to be safe (Salminen et al. 1998b).

The probiotics intended for human use were observed to bind to canine jejunal mucus in a similar manner to that observed earlier for human mucus (Kirjavainen et al. 1998; Ouwehand et al. 1999b, Tuomola et al. 1999). This suggests that the often-mentioned species specificity of probiotics (Casas et al. 1998) is not interfering with the *in vitro* adhesion to intestinal mucus of the tested strains. The probiotics intended for animal use – two *E. faecium* strains – exhibited a relatively low level of adhesion. Surprisingly, also both *Lactobacillus* strains isolated from dogs, showed a low level of adhesion. Probiotics, intended for human use, were observed to interact with immobilized intestinal mucus of dogs, this may provide a basis for studying the efficacy of these probiotics in dogs.

Exposure of the strains to jejunal chyme was observed to significantly reduce the adhesion of four of the tested strains, *L. rhamnosus* GG, *E. faecium* (Biobak), *L. pentosus* SK2A and *L. pentosus* UK1A. The other tested strains showed a trend (p < 0.15) towards reduced adhesion upon exposure to jejunal chyme. Which of the components in jejunal chyme: enzymes, mucus, bile, etc. are responsible for the observed reduction in adhesion remains to be determined. However, it has earlier been observed that pretreatment of *L. rhamnosus* GG with proteases reduces its adhesive abilities (Tuomola 1999), suggesting a possible role for proteolytic degradation in the observed effect. The observations also suggest that the adhesion observed *in vitro* may be quite different from *in vivo* after exposure to digestive juices. The selection criterion ‘adhesion’ for probiotics should therefore be further refined to include ‘adhesion after exposure to digestive factors’. It also indicates that the adhesive properties of probiotics should be tested after passage through an intestinal model or an
animal model. The jejunal chyme can in the future also be used to simulate passage through the small intestine in order to assess, in vitro, the survival of potential probiotic in this part of the gastrointestinal tract.

In conclusion, some probiotics, intended for human use, were found to interact well with immobilized canine intestinal mucus. This provides a basis for further study on the efficacy of these strains in dogs. The study also shows that canine jejunal chyme is a convenient source of intestinal mucus and can be used to assess the effects of digestion on probiotics in vitro.

Acknowledgements

Financial support was obtained from the Academy of Finland and by the National Technology Agency of Finland (Tekes). Ms. Pia Niemi is gratefully acknowledged for technical assistance and M.Sc. Efstathia Apostolou for identification of the canine Lactobacillus isolates.

References


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