Absence of host specificity for in vitro adhesion of probiotic lactic acid bacteria to intestinal mucus

Minna Rinkinen a,*, Elias Westermarck a, Seppo Salminen b, Arthur C. Ouwehand b

a Department of Clinical Veterinary Sciences, Faculty of Veterinary Medicine, University of Helsinki, P.O. Box 57, FIN-00014 Helsinki, Finland
b Functional Food Forum and Department of Biochemistry and Food Chemistry, Itäinen Pitkäkatu 4, University of Turku, FIN-20014 Turku, Finland

Received 12 February 2003; received in revised form 15 May 2003; accepted 15 May 2003

Abstract

Adhesion of probiotic lactic acid bacteria (LAB) has been reported to be host species specific. Host specificity is regarded as a desirable property for probiotic bacteria and therefore recommended as one of the selection criteria. However, previous studies have indicated that LAB originating from one host adhere well also to the mucus of other species. The aim of the study was to investigate the host specificity of LAB adhesion in human, canine, possum, bird and fish mucus in vitro. An in vitro mucus adhesion model was utilized in this study using immobilized mucus from faeces or intestinal material of these hosts. The results indicate that the adhesion trait was not host specific but rather was characteristic to LAB species. In conclusion, mucus adhesion properties are more dependent on the LAB strain than on the host. This suggests that animal models in probiotic adhesion assays may be more applicable to other species than thought earlier. Positive health effects facilitated by adherent probiotics in humans may also denote the possibility of similar outcome in other species and vice versa.

© 2003 Elsevier B.V. All rights reserved.

Keywords: Probiotics; Host specificity; Adhesion; Lactic acid bacteria; Mucus

1. Introduction

The ability to adhere to intestinal mucosa is considered an important selection criterion for lactic acid bacteria (LAB) intended for probiotic use (Ouwehand et al., 1999). For
beneficial health effects such as competitive exclusion of pathogens or immune regulation, an effective probiotic has to colonize gut mucosa at least temporarily (Salminen et al., 1998; Saarela et al., 2000). Adhesion of probiotic LAB has been reported to be species specific (Fuller, 1973; Barrow et al., 1980; Mäyrä-Mäkinen et al., 1983). Host specificity is regarded as a desirable property for probiotic bacteria and therefore recommended as one of the selection criteria (Salminen et al., 1998; Saarela et al., 2000). The importance of host species specificity has been debated, but no studies have focused on interspecies differences when it comes to adherence properties of probiotic LAB adhesion. However, many probiotics, especially products intended for animal use, are marketed for more than one species (Anon., 2002).

Previous studies have indicated that probiotic LAB originally isolated from humans also adhere to both canine (Rinkinen et al., 2000) and fish mucus (Nikoskelainen et al., 2001a,b) in vitro. Some dairy strains have also shown moderate to good adhesion to human cell lines (Tuomola et al., 1999). Rinkinen et al. (2000) observed that porcine Enterococcus faecium SF 273 also adhered in vitro to canine intestinal mucus. It has been shown that LAB populations in experimental animals (mice and rats) are not dependent on the host genetics but rather are influenced by environmental factors (Benno and Mitsuoka, 1986). Such findings underline the effect of intestinal microbiota for studies in probiotic trials and a similar competitive role for faecal bacteria has been reported in adherence studies using Caco-2 cells (de Waard et al., 2002; Haller et al., 2001). Thus, the specificity of adhesion properties should be further clarified prior to using the host specificity as a key for selection procedures.

The aim of the current study was to evaluate the adhesion of probiotic LAB of human, canine and dairy origin to intestinal mucus of various hosts and to compare this with earlier results from adhesion to human (Kirjavainen et al., 1998), canine (Rinkinen et al., 2000) and fish intestinal mucus (Nikoskelainen et al., 2001a,b). In vitro evaluation of LAB adhesion to intestinal mucus has proven to be a suitable model for studying probiotic adhesion (Kirjavainen et al., 1998); therefore this model was utilized also in this study.

2. Materials and methods

2.1. Intestinal mucus

For mucus isolation, faecal samples were collected from brush tail possum (Trichosurus vulpecula), emu (Dromaius novaehollandiae), ostrich (Struthio camelus) (three animals from each species all from Melbourne, Australia), and humans (10 individuals). Mucus was also isolated from jejunal chyme of six dogs (beagle, Helsinki University experimental animal colony) and from the intestines of a rainbow trout as described earlier (Kirjavainen et al., 1998; Ouwehand et al., 1999; Rinkinen et al., 2000; Nikoskelainen et al., 2001a,b). Briefly, mucus was isolated from faeces by the extraction and dual ethanol precipitation technique as described by Miller and Hoskins (1981). Mucus was separated from rainbow trout intestines by gently scraping the gut surface, particulate matter was removed by centrifugation at 13,000 x g. Canine jejunal chyme was collected as described earlier (Rinkinen et al., 2000) and centrifuged at 12,000 x g to remove particular matter. Mucus was
precipitated from clear supernatants with ethanol as described above and freeze-dried (Rinkinen et al., 2000).

Equal amounts of freeze-dried mucus from each individual of the same species were pooled and stock suspensions of 5 mg/ml in HEPES (N-2-hydroxy-ethylpiperazine-N'-2-ethanesulphonic acid)–Hanks buffer (HH; 10 mM HEPES; pH 7.4) were prepared. All mucus stock solutions were stored at −20°C until use.

2.2. Micro-organisms and growth conditions

LAB used for the current study were *Lactobacillus rhamnosus* GG (ATCC 53103), *L. johnsonii* L1, *L. casei* Shirota, *Bifidobacterium lactis* Bb12, *E. faecium* M74 (Lactiferm®) and *E. faecium* SF 68 (all human isolates), *L. bulgaricus* ATCC 11842 (dairy strain) *L. pentosus* UK1A (canine faecal isolate) and *L. pentosus* SK2A (canine jejunal isolate).

The bacteria were grown in MRS medium from stocks stored at −70°C in 40% glycerol (1% inoculum). To metabolically radiolabel the bacteria, 10 µl ml⁻¹ tritiated thymidine (methyl-1,2-³H-thymidine 120 Ci mmol⁻¹) was added to the medium. Bacteria were incubated over night in 37°C. After growth, the bacteria were harvested by centrifugation (2000 × g), washed twice with phosphate buffered saline (PBS; pH 7.2; 10 mM phosphate) and resuspended in PBS. Absorbance was adjusted to 0.5 ± 0.02 in order to standardize the number of bacteria (10⁷ to 10⁸ CFU ml⁻¹) before use in the adhesion assay (see below).

2.3. Adhesion assay

The adhesion of radioactively labelled bacteria to immobilized mucus was examined as described previously (Kirjavainen et al., 1998; Ouwehand et al., 1999). The adhesion is expressed as a percentage calculated by comparing the radioactivity of the bacterial suspension recovered after mucus incubation and adhesion, to the radioactivity of the suspension added to immobilized mucus.

2.4. Statistical analysis

All experiments are expressed as the average of at least three independent experiments with standard deviation. Each experiment was performed with four parallels to correct for intra-assay variation. A Wilcoxon signed rank test was used determine the significance (P < 0.05) of the difference in adhesion of the tested probiotic strains to mucus from the different hosts.

3. Results and discussion

We investigated the adhesion of LAB of human and canine origin to intestinal (dog, rainbow trout) or faecal (human, emu, ostrich, possum) mucus in vitro. Adhesion to intestinal mucus of the tested strains stated in Section 2 was found to range from 1.06% (the average adhesion of *L. casei* Shirota to possum mucus) to 42.1% (the average adhesion of *L. rhamnosus* GG to emu mucus). When compared to other LAB strains, *L. rhamnosus* GG
was found to adhere significantly better ($P < 0.05$) to the intestinal mucus of all tested animal species (33.4–42.1%), with the exception of mucus from rainbow trout (17.6%). L. casei Shirota was found to have the poorest adherence of tested LAB to all animal species included in this study (1.1–1.8%). In human mucus the adhesion of L. pentosus UK1A was lowest. The results are expressed in Fig. 1, where also the results from earlier works by Kirjavainen et al. (1998), Rinkinen et al. (2000) and Nikoskelainen et al. (2001a,b) have been collected for comparison. In general, no host specificity was observed in the present study, but there was a clear trend suggesting that the mucin adhesion of the tested LAB is dependent on the micro-organism (Fig. 1).

Lack of host species specificity in LAB adhesion observed in the present study supports the conclusions from earlier studies (Kirjavainen et al., 1998; Rinkinen et al., 2000; Nikoskelainen et al., 2001a,b). This suggests that mucus adhesion of certain LAB would be strain dependent rather than host dependent. This is in agreement with the earlier reports of LAB adhesion in different animals (Barrow et al., 1980; Mäyrä-Mäkinen et al., 1983). Barrow et al. (1980) observed that some lactobacilli from pigs, wild boar and chicken adhered to pig squamous epithelial cells in vitro. They also noted that many of the LAB did not adhere to pig epithelial cells, even when LAB were of porcine origin. Mäyrä-Mäkinen et al. (1983) found that adhesive L. fermentum strains isolated from calves also adhered to pig cells. It can be discussed whether the non-adhering strains would also have been non-adhering to their original host, which was not elucidated in the above mentioned reports. Hence the adhesion divergences noted in these reports could actually have been due to different, species or strain dependent adhesion factors of LAB involved in the study.
However, the papers did not systematically investigate host specificity as was done in the current study.

The adhesive ability of _L. rhamnosus_ GG was found to be superior to other LAB examined in humans (Tuomola et al., 1999; Kirjavainen et al., 1998) and in dogs (Rinkinen et al., 2000), but not in rainbow trout (Nikoskelainen et al., 2001a,b). Otherwise LAB adhesion percentages in rainbow trout and birds did not differ significantly from those observed in other species. Aquatic environment of cold-water fish differs markedly from living conditions of mammals and birds, and body temperature of birds is higher than that of mammals. In our study the adhesion was examined only at 37 °C, which does not simulate the natural circumstances of intestines of rainbow trout or birds. The effect of incubation temperature on LAB adhesion in birds and fish remains to be determined and should be further explored.

Some bacteria have well-described adhesion mechanisms, which are clearly species specific, such as _Escherichia coli_ K88 fimbriae specific to pigs (Jin and Zhao, 2000). On the other hand, the ability to bind to and colonize intestinal mucosa across the species boundaries is a well-known feature of many zoonotic pathogens: for example ubiquitous _Salmonella enterica_ serovars Typhimurium and Enteritis can infect a wide range of hosts (Uzzau et al., 2001). Many animal species are also known to serve as reservoirs for _Campylobacter jejuni_ (Nachamkin, 1997). Our findings and the available literature suggest that also the adhesion trait of beneficial LAB may be dictated more pronounced by the bacteria and intestinal microbiota rather than the host species itself.

Fuller (1973) and Barrow et al. (1980) along with Mäyrä-Mäkinen et al. (1983) examined LAB adhesion to epithelial cells. In our study adhesion to intestinal mucus was evaluated. Intestinal mucosa is the first contact surface for bacteria in gut. The ability to adhere to mucus in high level is of ecological importance to the bacteria when colonizing the gut mucosa (Mikelsaar et al., 1998). In vitro evaluation of the LAB adhesion to intestinal mucus has been proved to be a suitable model for studying the probiotic adhesion (Kirjavainen et al., 1998). Our results imply that the attachment of the studied LAB to mucus is not determined by specific receptors in host tissue. There may be some universal features in mucus common to different animal species (including man), enabling certain LAB to adhere to mucus better than others.

Species specificity is considered important for temporary colonization needed for the initiation of beneficial health effects, such as immunostimulation (Salminen et al., 1998). Host specificity was challenged earlier by Conway et al. (1987). They reported similar adhesion of lactobacilli to porcine and human epithelial cells and concluded that the adhesion was non-specific, suggesting the pig intestinal cells could be used in vitro to screen the adhesion properties of LAB aimed for human consumption. Our results support the use of animal models for probiotic studies, but further studies are needed to investigate whether determinants other than mucus adhesion are required to stimulate health effects. The results may also imply that probiotic strains isolated from humans may be beneficial for animal use, too (Nikoskelainen et al., 2001b). This may have important safety implications: strains shown to be safe for humans can be fed to livestock and pets without a potential safety concern for the consumer or owner. It is a subject of additional studies to investigate whether the highly binding LAB do also initiate similar immune effects in animals as in humans.

In conclusion, it appears that the mucus adhesion properties are more dependent on the LAB strain than on host animal, suggesting that mucus adhesion mechanisms may share
some universal features common to mammals, birds and fish. This may suggest that the animal models in probiotic adhesion assays may be more reliable than thought earlier. The documented positive health effects facilitated by adherent probiotics in humans may also denote the possibility of similar outcome in other species and vice versa.

Acknowledgements

Dr. Carolyn Haskard (Australian Water Quality Centre, Salisbury, South Australia) is thanked for providing faecal samples from brush tail possum, emu and ostrich and Ms. Riikka Laine for preparing the mucus from the samples. The fish mucus was kindly supplied by M.Sc. Sami Nikoskelainen (Department of Biochemistry and Food Chemistry, University of Turku). This study was supported by the Finnish Foundation of Veterinary Sciences and Hill’s Pet Nutrition, Inc.

References

Nikoskelainen, S., Ouwehand, A.C., Salminen, S., Bylund, G., 2001b. Protection of rainbow trout (Oncorhynchus mykiss) from furunculosis by Lactobacillus rhamnosus. Aquaculture 198, 229–236.