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Influence of carp intestinal mucus molecular size and glycosylation on bacterial adhesion

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ABSTRACT: The first step of the pathogenesis of many infectious diseases is the colonisation of the mucosal surface by the pathogen. Bacterial colonisation of the mucosal surface is promoted by adherence to high molecular weight mucus glycoproteins. We examined the effect of carp intestinal mucus glycoproteins on the adhesion of different bacteria. The bacteria used were 3 strains of *Aeromonas hydrophila*, and *A. salmonicida*, *Edwardsiella tarda* and *Yersinia ruckeri*. All bacteria adhered to mucus, but at varying intensities. All tested bacteria adhered best to molecules of 670 to 2000 kDa in size, less to molecules larger than 2000 kDa and weakest to molecules of 30 to 670 kDa. In general, bacteria that showed a stronger adhesion to intestinal mucus were cytotoxic to cells *in vitro*, and bacteria that showed a weaker adhesion to intestinal mucus did not lead to alterations of monolayers of EPC-cells. Furthermore, the involvement of glycan side chains of the glycoproteins for bacterial adhesion was analysed for one *A. hydrophila* strain. After cleavage of terminal sugar residues by treatment of mucus glycoproteins with different glycosidases, binding of bacteria was modulated. When mannose was cleaved off, adhesion significantly increased. Blocking of glycan receptors by incubation of bacteria with different oligosaccharides had no clear effect on bacterial binding to mucus glycoproteins. Our results suggest that bacteria interact with carbohydrate side chains of mucus glycoproteins, and that the carbohydrates of the core region are involved in bacterial binding.

KEY WORDS: Adhesion · Mucus · Intestine · *Aeromonas hydrophila* · Common carp

INTRODUCTION

Mucus covers the intestinal epithelium of mammals and fishes and protects it against a wide range of noxes, such as solutes, enzymes, bacteria and chemicals within the lumen of the intestinal tract (Forstner et al. 1973). Mucus consists mainly of water and high molecular glycoproteins, called mucins. Both components are important in gel formation (Verdugo 1990, Strous & Dekker 1992, Bansil et al. 1995, Cone 1999, Perez-Vilar & Hill 1999). Secreted mucins are long fibrous molecules that consist of a peptide backbone coated with a complex array of oligosaccharides (Gum 1995, Cone 1999). The molecular weight of mucins ranges between 500 and 30 000 kDa. The peptide part of the mucin, the core protein or apomucin, can contain more than 13 000 amino acids (Roussel & Delmotte 2004). Apomucins are covered by hundreds of carbohydrate chains that are responsible for about 80% of the mucin molecular mass. Typical mucins from mammals contain fucose, galactose, N-acetylglucosamine, N-acetylgalactosamine and neuraminic acid (Roussel & Delmotte 2004).

The ability of certain bacteria to adhere to mucus components may facilitate the colonisation of mucosal epithelial surfaces by immobilising the bacteria in the mucus gel. Adherence of bacteria to mucus is therefore a characteristic that promotes bacterial colonisation of the mucosal surface. Although mucus glycoprotein binding by probiotic bacteria may be protective for the host, there is experimental evidence suggesting that a number of pathogens can adhere to mucus glycoproteins (Ascencio et al. 1998). Gut, gills and skin are possible sites of entry for fish bacterial pathogens (Chabrillon et al. 2004). In many infectious diseases, the first stage of pathogenesis is adherence of the pathogen to mucosal surfaces (Beachey 1981, Tse & Chadee 1991, Rinkinen et al. 2000) and colonisation of these surfaces (Abraham et al. 1999).

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In the present study, the role of mucus glycoproteins in bacterial adhesion as an initial step in the colonisation and infection process was examined in greater detail. The adhesion ability of several bacterial strains to crude mucus and to mucus molecules of different size fractions was assessed. We used the following bacteria, which have all been found in routine diagnostics: *Aeromonas hydrophila*, *A. salmonicida, Yersinia ruckeri* and *Edwardsiella tarda*. Three different strains of *A. hydrophila* were used because *A. hydrophila* can often be isolated from carp after disease outbreaks. *A. hydrophila* is found in both fresh and salt water (Inglis et al. 1993, Doukas et al. 1998, Cipriano 2001) and is also part of the normal intestinal microflora of healthy fish (Trust & Sparrow 1974). *A. salmonicida* and *Y. ruckeri* are common pathogens of salmonids and are less often isolated from carp. *E. tarda* is often isolated from diseased catfish, but has been found in diseased carp as well. Furthermore, for one of the *A. hydrophila* strains, we examined which carbohydrate structures of mucus glycoprotein are used for adhesion.

The aim of this study was to improve knowledge about the interactions between carp gut mucus and bacteria. This increased knowledge may make it possible to control the adhesion process by interfering with bacteria–mucus interactions (Chabrillon et al. 2004).

MATERIALS AND METHODS

Animals. Parasite and virus-free sibling common carp *Cyprinus carpio* L. (R8S8 × R3S8, Wageningen Agricultural University) were used for mucus isolation. Carp were raised and kept in filtered recirculated tap water. Approximately 4-yr-old carp (n = 8) with a mean body weight ± SE of 96.2 ± 12.4 g and a mean standard length ± SE of 14.6 ± 1.2 cm were used. The carp were placed in 100 l tanks with filtered tap water and were starved for 3 d before sampling to reduce faeces on intestinal mucus. For sampling, fish were killed by bath immersion with 500 mg l^{-1} tricaine methane sulphonate (Sigma) and subsequently dissected. The entire intestinal tract between pseudogaster and anus was removed.

Mucus isolation. Intestines (mean gut weight of 1.2 ± 0.3 g) were opened longitudinally and cut into small pieces of 3 to 4 mm. Subsequently, secreted luminal mucus was isolated with isolation medium as described previously (Enss et al. 1996, Neuhaus et al. 2007a). In brief, tissue pieces were incubated for 20 min in 100 ml isolation buffer containing protease inhibitors. The isolation buffer was collected and centrifuged for 30 min at 12 000 × *g*. Subsequently, the supernatant was collected and frozen at –20°C until further processing. All samples were concentrated by ultrafiltration (Amicon; exclusion limit 30 000 Da) to a final volume of 2 ml. The isolated and concentrated mucus was then separated by molecular size by means of downward gel filtration on a 34 × 0.9 cm Sepharose CL-4B column (Sigma; flow rate 5.2 ml h^{-1}, fraction size 1.3 ml, 40 fractions). The fractions were pooled into 3 fraction pools of mucus molecules with different molecular sizes (30–670 kDa, 670–2000 kDA, >2000 kDa). These fraction pools were used in the adhesion and binding site experiments.

Bacteria. *Aeromonas hydrophila* strain 38 was a gift from the Friedrich-Loeffler-Institute, Federal Research Institute for Animal Health, Germany. *A. hydrophila* strains 42 and 60 were a gift from the Department of Veterinary Pathobiology, Royal Veterinary and Agricultural University Copenhagen, Denmark. *A. salmonicida*, *Edwardsiella tarda* and *Yersinia ruckeri* were a gift from Lower-Saxony State Office for Consumer Protection and Food Safety. All bacteria were isolated from their preferred host species, which all showed clinical signs of disease.

Bacteria were routinely grown on blood agar and stored at 10^9 CFU ml^{-1} at –80°C in veal infusion medium. To remove all possible nutrients, bacteria were harvested by centrifugation at 10 500 × *g* for 10 min and by removing the supernatant. Bacteria were resuspended in physiological salt solution.

Labelling of live bacteria. For adhesion experiments, bacteria were stained with a green fluorescent nucleid acid stain (Syto 9, Invitrogen; 2 µl per 10^9 bacteria). After staining, bacteria were harvested by centrifugation at 10 500 × *g* for 10 min, and the supernatant was removed. Bacteria were resuspended in physiological salt solution. All used bacterial strains stained equally, and the fluorescence of labelled bacteria was stable for at least 12 d at room temperature, as confirmed by spectrophotometry (470 nm excitation, 520 nm emission; BMG). Bacteria were stained specifically, and bacterial movement was not negatively influenced by the staining or the presence of mucus, as confirmed microscopically.

Adhesion assay. Bacterium adhesion to mucus molecules was examined in microtitre plates (MaxiSorp, Nunc). Binding of mucus to the plate was confirmed by a lectin binding assay using ConA as described previously (Neuhaus et al. 2007b). Bacterial adhesion to mucus was assessed as described previously (Van der Marel et al. 2008). In brief, fluorescently labelled bacteria (25 µl of 10^9 CFU ml^{-1}) were added in triplicate to plates coated with mucus. Fluorescence was then measured (470 nm excitation, 520 nm emission). Bovine serum albumin (BSA; 1 mg l^{-1}) and pig gastric mucin (5 mg l^{-1}, Invitrogen) were used to examine whether the used bacteria showed adhesion ability. Sodium chloride was used as a blank to correct for autofluorescence.

Possible binding sites. The involvement of the following sugars in carp mucus on the binding ability of bacteria was examined: N-acetyl-β-glucosamine, galactose, D(+)-mannose, 2′-fucosyl-D-lactose and N-acetylneuraminosyl-D-lactose. These sugars were chosen because they are present in mammalian mucus (Forstner 1995) as well as in carp mucus (Neuhaus et al. 2007a).

In a first experiment, mucus fraction pools were incubated for 30 min with one of the following glycosidases: β-N-acetylglucosaminidase, β-galactosidase, α-mannosidase and neuraminidase (Sigma). Experimental conditions of each reaction are given in Table 1. After incubation with the enzyme, adhesion of *Aeromonas hydrophila* 42 was determined as described above. In a second experiment, *A. hydrophila* 42 was incubated for 1 h at room temperature with several oligosaccharides dissolved in sodium acetate buffer (pH 5.0; 0.2 M) in an attempt to block bacterial binding sites for these oligosaccharides. The following oligosaccharides were used: N-acetyllactosamine (1.5 mM), N-acetylneuraminosyl-D-lactose (0.05 mM), 2′-fucosyl-D-lactose (0.15 mM), D(+)-mannose (140 mM) and 4-O-β-D-galactopyranosyl (1.5 mM) (all chemicals from Sigma). After incubation, the adhesion of *A. hydrophila* 42 was determined as described above. Fluorescence data were corrected on the basis of carbohydrate content of the mucus fractions and gut weight (Neuhaus et al. 2007b). Carbohydrate content was determined by the periodic acid Schiff reaction (540 nm; Dubois et al. 1956, Mantle & Allen 1978).

***In vitro* cytotoxicity of bacterial strains.** Cytotoxicity of bacteria was analysed *in vitro* to recognise possible differences in the cytotoxicity of the bacterial strains used. Epithelioma papulosum cyprini (EPC)-cell monolayers were cultivated with standard methods. Monolayers were incubated with 10^7 CFU bacteria in 24-well tissue culture plates at 20°C. This temperature was chosen because all bacterial strains grew well at this temperature without risking overgrowth. The experiment was done twice with 3 replicates each time. After incubation for 2, 6 and 24 h, the cell layers on each plate were examined microscopically.

Statistics. Data were statistically analysed using a Kruskal-Wallis 1-way analysis of variance (ANOVA) on ranks, because the normality test failed. Differences between treatment groups were considered significant at a probability of error at $p < 0.05$ and as highly significantly at a probability of error at $p < 0.01$.

RESULTS

Adhesion of different bacterial strains

Adhesion ability differed between the 3 *Aeromonas hydrophila* strains (Fig. 1). *A. hydrophila* 38 adhered more strongly to mucus of all size fractions than *A. hydrophila* 42 and *A. hydrophila* 60. *A. hydrophila* 60 adhered most weakly to the mucus. Bacterial adhesion to the total mucus (molecules larger than 30 kDa) recorded as fluorescence intensity for *A. hydrophila* 38 was 13 times higher than for *A. hydrophila* 60 and 6 times higher than for *A. hydrophila* 42. These differences were highly significant.

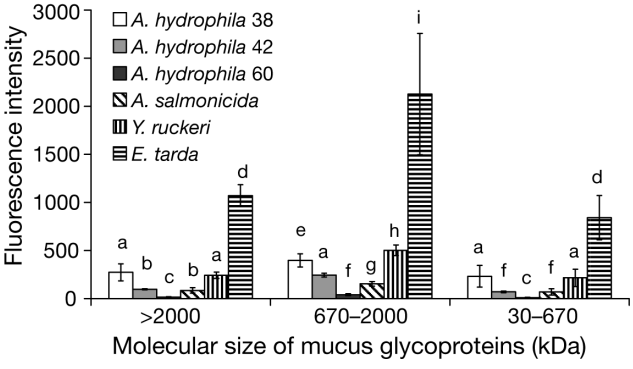

Fig. 1. Adhesion of the facultative pathogen *Aeromonas hydrophila* strains 38, 42 and 60 and the obligate pathogen bacteria *A. salmonicida*, *Edwardsiella tarda* and *Yersinia ruckeri* to intestinal mucus molecules from carp (n = 6). The adhesion of the bacteria to molecules of 3 different molecular size ranges was assessed. Measurements are given as mean values and SD of absorbency of fluorescently labelled bacteria. Statistically significant differences are marked by different letters

Table 1. Glycosidases applied to intestinal mucus of common carp

Glycosidase	Units ml^{-1} mucus	Buffer	pH	Temperature (°C) During reaction	Temperature (°C) To stop reaction
β-N-acetyl-glucosaminidase	0.25	PBS	7.3	25	37
β-galactosidase	0.25	PBS	7.3	37	45
α-mannosidase	0.05	Sodium acetate	4.5	25	37
Neuraminidase	0.2	Sodium acetate	5.0	37	45

The obligate-pathogenic bacterium *Edwardsiella tarda* adhered much more strongly to the intestinal mucus of carp than the *Aeromonas hydrophila* strains. *Yersinia ruckeri* showed an adhesion ability similar to that of *A. hydrophila* 38, and *A. salmonicida* showed an adhesion ability similar to that of *A. hydrophila* 42. The fluorescence intensity in preparations with *A. salmonicida* was 3 times lower than that of *Y. ruckeri* and 13 times lower than that of *E. tarda*. The differences between all 3 obligate pathogenic strains were highly significant. All examined bacterial strains showed the strongest adhesion to mucus molecules with a size of 670 to 2000 kDA and the weakest adhesion to molecules with a size of 30 to 670 kDa (Fig. 1).

Possible binding sites

Aeromonas hydrophila 42 was used to examine to which possible binding sites of mucus bacteria can adhere. *A. hydrophila* 42 adhered significantly more strongly to intestinal mucus after D(+)-mannose had been detached from the mucus (Fig. 2). Adherence increased slightly after incubation of the mucus with neuraminidase and decreased slightly after incubation with galactosidase. These results were not statistically significant (Fig. 2). Adherence of the bacterium was nearly unaffected by application of acetyl-glucosaminidase (Fig. 2).

Preincubation of *Aeromonas hydrophila* 42 with different oligosaccharides mainly led to a decreased adhesion ability of the bacteria to mucus glycoproteins, but none of the results were statistically significant (Fig. 3). Adherence of *A. hydrophila* 42 decreased slightly after incubation with N-acetyllactosamine, D(+)-mannose and 2-fucosyl-D-lactose. After incubation with N-acetyl-neuraminosyl-D-lactose and 4-O-β-D-galactopyranosyl, adherence of the bacteria was nearly unaffected (Fig. 3).

In vitro cytotoxicity of bacterial strains

When monolayers of EPC-cells were incubated with *Aeromonas hydrophila* 42, *A. hydrophila* 60, *A. salmonicida* or physiological salt solution, no changes in cell morphology or monolayer coherence were observed over a period of 24 h. EPC-monolayers incubated with *A. hydrophila* 38, *Edwardsiella tarda* or *Yersinia ruckeri* were still coherent after 2 h, but some EPC-cells were detached from the monolayer. After 6 h of incubation, the monolayer was destroyed, although most of the cells were viable. After 24 h, the EPC-monolayer had detached completely from the bottom of the culture vessel, and only a few viable cells could be recognised (Fig. 4).

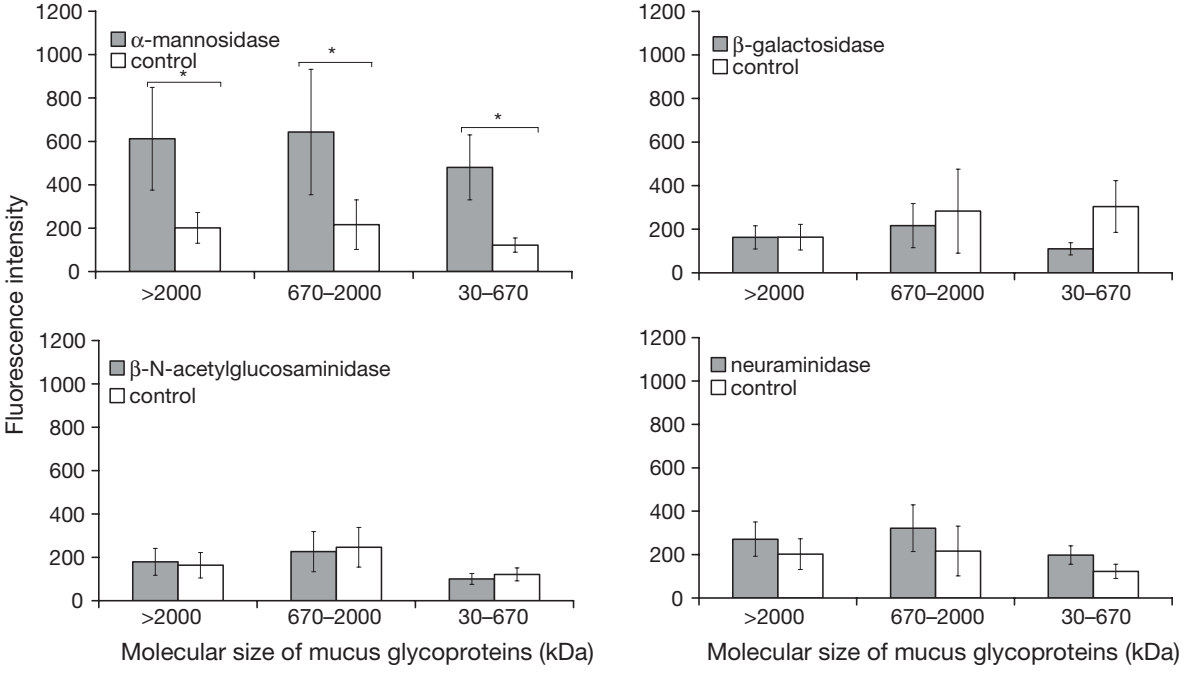

Fig. 2. *Aeromonas hydrophila.* Adhesion of *A. hydrophila* strain 42 bacteria to intestinal mucus molecules from carp (n = 6). After incubation of the mucus with the glycosidases α-mannosidase, β-galactosidase and β-N-acetylglucosaminidase and neuraminidase, adhesion to molecules of different molecular size ranges was assessed. Values are mean ± SD of absorbency measurements of fluorescently labelled bacteria. Significant differences are marked by * ($p < 0.05$)

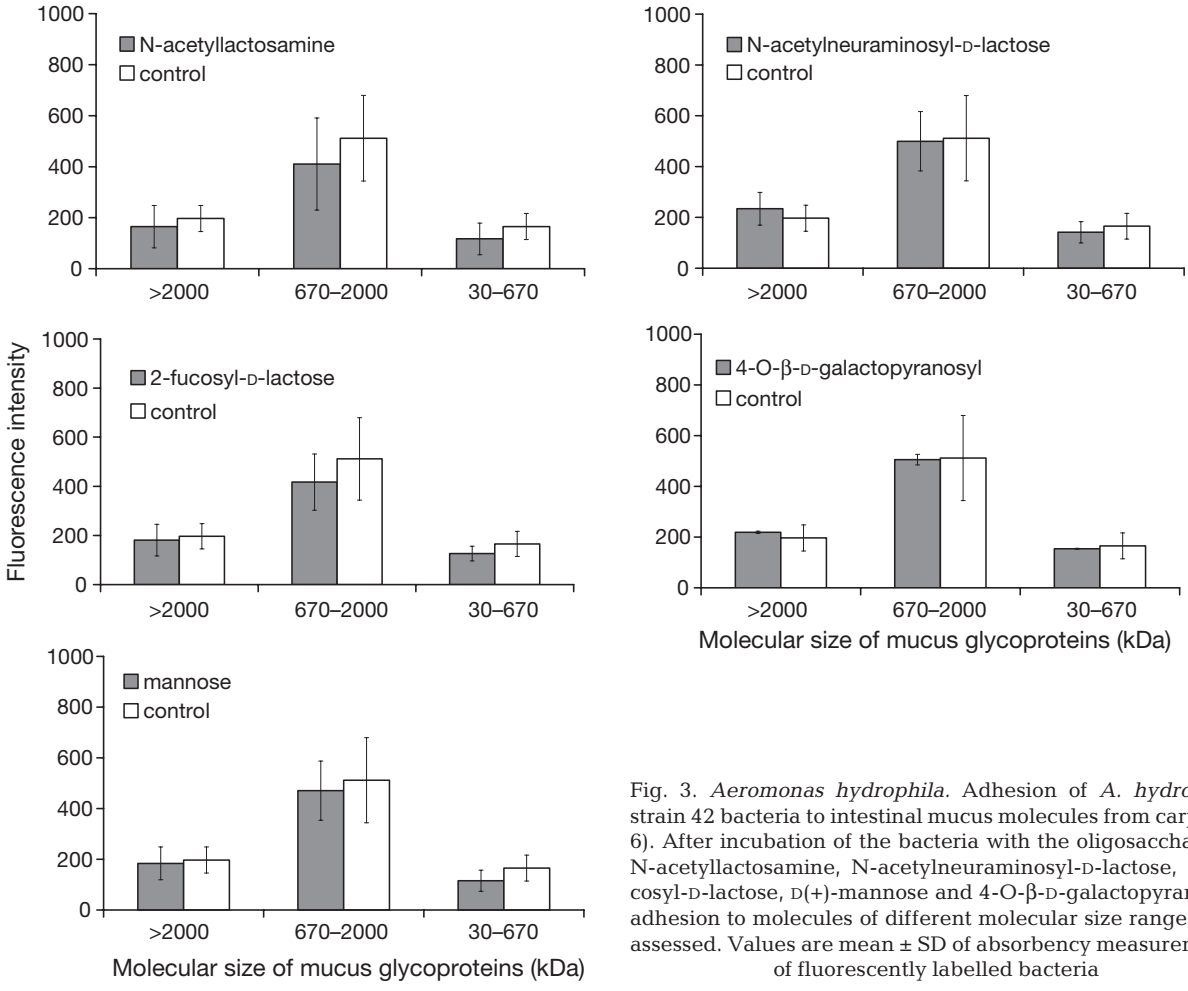

Fig. 3. *Aeromonas hydrophila.* Adhesion of *A. hydrophila* strain 42 bacteria to intestinal mucus molecules from carp (n = 6). After incubation of the bacteria with the oligosaccharides N-acetyllactosamine, N-acetylneuraminosyl-D-lactose, 2′-fucosyl-D-lactose, D(+)-mannose and 4-O-β-D-galactopyranosyl, adhesion to molecules of different molecular size ranges was assessed. Values are mean ± SD of absorbency measurements of fluorescently labelled bacteria

DISCUSSION

Adherence of pathogenic bacteria to specific receptors on mucosal surfaces is widely recognised as the important first step in the initiation of infectious diseases (Beachey 1981, Tse & Chadee 1991, Rinkinen et al. 2000). All bacteria used in this study showed the ability to adhere to intestinal mucus of undiseased fish. For some bacteria, adhesion to mucus is correlated with the virulence of different strains, for example in *Yersinia enterocolitica* (Mantle & Husar 1993, 1994), *Pseudomonas cepacia* (Sajjan et al. 1992) and *Aeromonas hydrophila* (Hazen et al. 1982). Significant differences in adhesion ability were observed between the *A. hydrophila* strains used in this study. Strains 60 and 38 showed the weakest and the strongest adhesion, respectively, to all mucus fractions. However, adhesion does not necessarily lead to an infection. Adhesion to intestinal mucosa is also among the main selection criteria for probiotic micro-organisms (Rinkinen et al. 2000, Vesterlund et al. 2005). After adhesion of bacteria to mucus, further steps are necessary to cause an infection. *A. hydrophila* 38 grew better than *A. hydrophila* 60 in mucus-supplemented media (Van der Marel et al. 2008). Also in the present study, the cytotoxicity of *A. hydrophila* 38 was higher than those of the 2 other *A. hydrophila* strains. These results led us to assume that *A. hydrophila* 38 is more virulent than both *A. hydrophila* 42 and 60. For some bacteria, e.g. *Neisseria* ssp. (Stephens et al. 1982), adhesion depends on the host. Thus, adhesion of *A. salmonicida, Edwardsiella tarda* and *Y. ruckeri* to carp mucus might not be as strong as to mucus of their preferred host species. A correlation between adhesion ability and pathogenicity or virulence can therefore not be drawn for bacteria, which have a different host. *A. salmonicida* has a lower adhesion ability because in contrast to all other examined bacterial strains, this bacterium is not motile. The need for bacterial motility to adhere to mucus and cells has been discussed by several authors. Some authors have stated that motility is necessary for adherence (Ormonde et al. 2000), whereas others have indicated that it is not (Nelson et al. 1990). The 3 bacterial strains that adhered best (*A. hydrophila* 38, *E.*

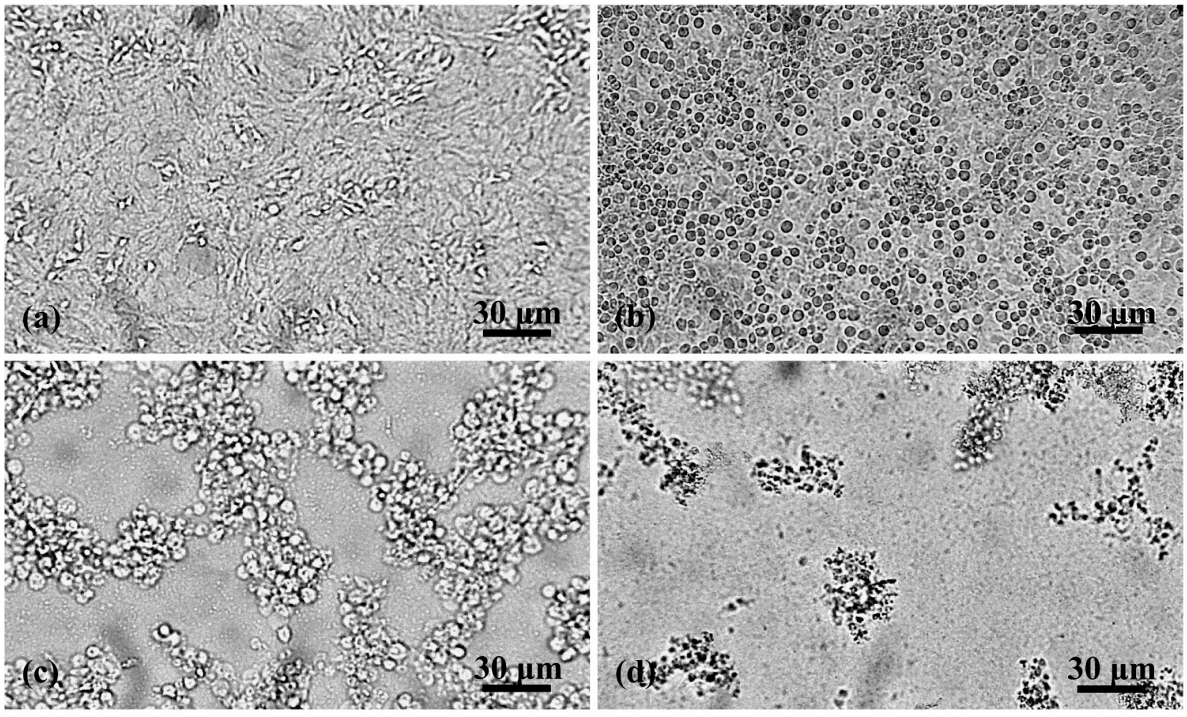

Fig. 4. EPC-cells after incubation with different *Aeromonas hydrophila* strains. (a) 24 h after incubation with 10^7 CFU *A. hydrophila* strain 60. The monolayer is coherent and resembles the control. (b) 2 h after incubation with 10^7 CFU *A. hydrophila* strain 38. Some EPC-cells are rounded and detached from the monolayer. (c) 6 h after incubation with 10^7 CFU *A. hydrophila* strain 38. The monolayer is destroyed, but there are still viable cells. (d) 24 h after incubation with 10^7 CFU *A. hydrophila* strain 38. The monolayer has detached completely from the bottom of the culture vessel and only a few viable cells can be seen

tarda und *Y. ruckeri*) also showed the highest cytotoxicity to epithelial cells.

If bacteria bind to mucus, they predominately bind to the carbohydrate side chains of the protein core. During the synthesis of mucus glycoproteins, the protein core is synthesised first, transported to the Golgi apparatus in the goblet cell and then glycosylated by glycosyltransferases. During the last step of glycosylation, terminal oligosaccharides are added to the carbohydrate side chains. As neuraminic acid and fucose are common terminal oligosaccharides, they are transferred to glycoproteins late during synthesis. Enhanced binding of bacteria to smaller mucus proteins suggests that these terminal oligosaccharides might interfere with bacterial binding.

All examined bacterial strains adhered significantly more strongly to mucus glycoproteins with a molecular size of 670 to 2000 kDa than to mucus glycoproteins of larger or smaller sizes. Therefore, the preference of the bacteria to adhere to mucus molecules of this size seems to be independent of adhesion ability and cytotoxicity. The presence of glycoproteins of this size in intestinal mucus can be explained by a release of glycoproteins with relatively low molecular weight that are not fully glycosylated (Enss et al. 1996) or by a degradation of the glycoproteins by the intestinal microflora (Hoskins et al. 1985). Bacteria may therefore be able to reach carbohydrates of the core region.

Adhesion to carbohydrate side chains of mucus glycoproteins has been found for several bacteria, e.g. *Aeromonas hydrophila*, *A. caviae*, *A. sobria* (Ascencio et al. 1998) and *Yersinia enterocolitica* (Mantle & Husar 1994). *A. hydrophila* 42 was used to examine the role of several common oligosaccharides of mucus glycoproteins on the adhesion ability of bacteria. *A. hydrophila* 42 was incubated with several oligosaccharides to block its receptors. Furthermore, oligosaccharides were cleaved off the mucus glycoproteins with glycosidases. Our results indicate that *A. hydrophila* 42 adheres to carbohydrates. Only minor differences in binding ability were observed between untreated bacteria and bacteria preincubated with oligosaccharides. After enzymatic treatment of the mucus, adhesion was unchanged for some oligosaccharides, but changed for others.

Mantle & Husar (1994) questioned whether the terminal oligosaccharide fucose is generally involved in bacterial adhesion. However, results from our study indicate that fucose may play a role in the adhesion of *Aeromonas hydrophila* 42 to mucus, as bacterial ad-

hesion decreased slightly after incubation of *A. hydrophila* 42 with 2′-fucosyl-D-lactose.

Little is known about the role of N-acetyllactosamine in mucus glycoproteins as a binding site for bacteria. We observed a slight decrease in adhesion ability of *Aeromonas hydrophila* 42 after incubation with N-acetyllactosamine to mucus.

Galactose is also assumed to be involved in bacterial adhesion to mucus (Sanford et al. 1989, Mantle & Husar 1993, 1994) as well as to cells (Thomas & Brooks 2004). After incubation of *Aeromonas hydrophila* 42 with 4-O-β-D-galactopyranosyl, no changes in adhesion to mucus were observed. However, bacterial adhesion was better (though not significantly) after cleaving off β-galactose. This indicates that some forms of galactose might also be involved in adhesion of *A. hydrophila* 42.

Mannose appears to be an important receptor for some bacteria (Mantle & Husar 1994). A decreased adhesion ability of *Aeromonas hydrophila* 42 was observed after incubation with D(+)-mannose. However, the adhesion ability increased significantly after mannose was enzymatically cleaved off the mucus glycoproteins. Mannose is a large molecule (Strous & Dekker 1992) and could therefore be a sterical obstacle for bacteria to adhere to carbohydrates of the core region or the protein core of mucus glycoproteins. It can be assumed that carbohydrates of the core regions of the sugar side chains are uncovered after elimination of D(+)-mannose. Therefore, *A. hydrophila* 42 seems to adhere to those carbohydrates of the core region. The role of mannose in bacterial adhesion to mucus is thus questionable.

The terminal oligosaccharide neuraminic acid in mucus from rabbits did not act as a binding site for *Yersinia enterocolitica* (Mantle & Husar 1994). Adhesion ability of *Aeromonas hydrophila* 42 did not change after incubation with N-acetylneuraminosyl-D-lactose. Therefore, N-acetylneuraminosyl-D-lactose does not seem to be an important receptor for adhesion. After treatment with neuraminidase, which cleaves off neuraminic acid from the glycans, adhesion increased slightly. Thus, neuraminic acid seems to reduce bacterial adhesion.

In the present study, bacteria adhered better after enzymatic cleavage with α-mannosidase and neuraminidase, but bound worse after cleavage with β-galactosidase. This suggests that β-galactose, which is a component of the core regions of mucus glycoproteins, is involved in adhesion of *Aeromonas hydrophila* 42. However, the elimination of oligosaccharide side chains has also been found to increase bacterial adhesion by enhancing the availability of the core protein of mucus glycoproteins (rather than the carbohydrate side chain) and thereby promoting bacterial binding by allowing greater interactions between the organism and the mucus protein core (Mantle & Husar 1994).

Adherence of bacteria can be inhibited by administration of specific oligosaccharides or enzymes that remove these oligosaccharides, as was shown by successful studies in the 1970s with mice (e.g. Aronson et al. 1979). An inhibited adherence might be useful in disease treatment. Especially in fish, treatment with antibiotics is often not successful because of the resistance of fish pathogenic bacteria to various antibiotics (Inglis et al. 1993). Administration of specific oligosaccharides or enzymes could be used in prophylaxis against bacterial infections. For instance, administration of polysaccharides increased the survival rate of tilapia and grass carp after experimental infection with *Aeromonas hydrophila* (Wang & Wang 1997). There seems to be no risk of developing resistances of bacterial strains against oligosaccharides (Bavington & Page 2005). Application of a carbohydrate mixture would be necessary to prevent infections because bacteria have numerous binding sites (Abraham et al. 1999).

In summary, the results of our study indicate that bacteria attach to special carbohydrate components of mucus glycoproteins and seem to interact with the core regions of the glycoproteins. Such interactions may protect the host by preventing the bacteria from attaching to the mucosal epithelium, but may also benefit the pathogen by providing it with a means of anchoring to the gut wall. The clearance of the bacteria from the intestinal tract could thereby be delayed and their ability to colonise the gut could be improved.

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