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Adhesion Protein YadA of *Yersinia* Species Mediates Binding of Bacteria to Fibronectin

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The interaction between fibronectin and *Yersinia* strains was studied. Wild-type *Y. enterocolitica* strains expressing the virulence-plasmid-encoded adhesion protein YadA adhered strongly to fibronectin-coated coverslips, while their plasmid-cured variants expressed weaker binding. The cloned *yadA* gene of *Y. enterocolitica* or *Y. pseudotuberculosis* conferred fibronectin-binding ability both to *Escherichia coli* and to *Y. pseudotuberculosis* strains lacking the YadA protein. The YadA protein did not mediate binding to isolated fragments of fibronectin or to soluble fibronectin.

Virulence of the three pathogenic *Yersinia* species, *Y. enterocolitica*, *Y. pseudotuberculosis*, and *Y. pestis*, is dependent on a common 42- to 47-MDa plasmid (pYV) (1). However, chromosomal genes are also needed for full virulence (7). Adhesion of yersiniae onto different substrates has been shown to depend mainly on four gene products, i.e., chromosomally encoded invasin, Ail, and fimbriae and the pYV-encoded outer membrane protein YadA (formerly known as Yop1 or OMP1). Invasin mediates binding to eukaryotic cell surface structures of the integrin superfamily, fibronectin receptors (10), and this is a prerequisite for bacterial invasion into the cells. Ail binds also to epithelial cells but promotes entry of bacteria into only a few cell lines (18). Proteins encoded by pYV are expressed at 37°C in calcium-deficient conditions, with the exception of YadA, which is produced at 37°C independently of calcium (25). YadA forms a fibrillar matrix covering the bacterium (12). This protein is associated with several virulence-related and adherence-associated functions such as binding to soluble collagen types I, II, and IV (4), adhesion to HEp-2 and HeLa cells (8) and to intestinal brush border vesicles and mucus (19, 20), resistance to complement-mediated killing by serum (17), and inhibition of the anti-invasive effect of interferon (3). Expression of YadA is also associated with increased surface hydrophobicity (16), autoagglutination (26), and agglutination of guinea pig erythrocytes (11). The adherence-associated functions of YadA may be due to hydrophobic interactions, as no specific receptors have been detected.

Fibronectin is a large adhesive protein which is found in soluble (plasma) and insoluble (tissue-associated) forms. Since its ability to bind staphylococci was reported in 1978 (14), both gram-positive and -negative bacteria have been shown to interact with fibronectin (22). In the present study, the role of the virulence plasmid in the interactions between yersiniae and fibronectin was studied. We provide evidence that virulence plasmid-encoded protein YadA is associated with the binding of *Y. enterocolitica* and *Y. pseudotuberculosis* to fibronectin.

Bacterial strains and culture conditions. The bacterial strains studied are presented in Table 1. The wild-type *Y. enterocolitica* O:3 and *Y. enterocolitica* O:9 strains were our own isolates from patients with clinical yersiniosis. Their isogenic plasmid-cured variants were obtained as previously described (29, 30). Wild-type *Y. enterocolitica* O:8 and its pYV-negative variant were obtained from Hans Wolf-Watz, Umeå, Sweden. Transformation of pYMS1, pYMS3, and pYMS4 into YpIII was done as described previously (3). Single colonies of bacteria from lactose agar plates were grown overnight in brain heart infusion at room temperature. After the culture was pelleted, the bacteria were inoculated in a calcium-deprived medium (9) consisting of salt medium supplemented with 1% tryptone (Difco Laboratories, Detroit, Mich.), 0.5% yeast extract (Oxoid Ltd., Basingstoke, United Kingdom), and 0.2% glucose. For the strains carrying the pYMS plasmids, ampicillin (25 μg/ml) was added to cultures. The cultures were grown on a shaker at 37°C for 3 h. Bacteria were washed at 4°C twice with phosphate-buffered saline (PBS). The number of bacteria was measured by using the most probable number method (13).

Assay for bacterial adherence to fibronectin. Fibronectin was purified from outdated human citrated plasma by affinity chromatography on Sepharose-gelatin as described previously (5, 32). Purified fibronectin and fibronectin fragments were iodinated by the chloramide-T method (6). The labelled proteins were isolated from the free isotope by gel filtration on a Sepharose G-25 column first washed with PBS supplemented with 1% bovine serum albumin (BSA) and then equilibrated with PBS. The cathepsin G fragments of fibronectin were isolated as described by Vartio (31). Glass coverslips with ground edges were coated with fibronectin and BSA, and assay of adherence to insoluble fibronectin was carried out as previously described (15). Different concentrations of bacterial suspension were incubated on coverslips coated with fibronectin for 2 h at 4°C. All the experiments were performed in duplicates. After incubation, the coverslips were washed twice with PBS and once with water. The number of attached bacteria was determined by counting the microbes in photographs of Gram-stained coverslips taken through a microscope. Four microscope fields per sample were photographed. For measurement of binding of soluble fibronectin to bacteria, labelled fibronectin or fibronectin fragments were incubated with 10^7 to 10^9 bacteria for 1.5 h at 4°C in 0.05% (vol/vol) Tween 20. After incubation the bacteria were pelleted by centrifugation and washed with

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TABLE 1. Bacterial strains studied

Bacterial strain	Serotype	Source or reference
Y. enterocolitica		
YeHo	O:3	Tertti et al. (30)
YeSa	O:3	Tertti et al. (30)
6471/76	O:3	Skurnik (24)
W-W 8081	O:8	Portnoy et al. (21)
YeRu	O:9	Skurnik (24)
Y. pseudotuberculosis		
YpIII/pIB1	III	Bölin et al. (2)
YpIII/pYMS 1	III	This work
YpIII/pYMS 2	III	Bukholm et al. (3)
YpIII/pYMS 3	III	This work
YpIII/pYMS 4	III	This work
E. coli PM191		
PM191/pYMS 1		Skurnik and Wolf-Watz (27)
PM191/pYMS 2		Skurnik and Wolf-Watz (27)
PM191/pYMS 3		Skurnik and Wolf-Watz (27)
PM191/pYMS 4		Skurnik and Wolf-Watz (27)

the incubation buffer. The radioactivity associated with the bacteria was quantitated in a gamma scintillation counter.

Role of the virulence plasmid in binding of wild-type *Y. enterocolitica* strains to immobilized fibronectin. The pYV^+ wild-type strain of *Y. enterocolitica* O:3 YeHo isolated from a human yersiniosis patient bound to immobilized fibronectin more strongly than to BSA (Fig. 1). The result, analyzed by grouping the numbers of bacteria incubated according to logarithms of ten (10^7, 10^8, and 10^9), is statistically significant in Student's *t* test (Table 2). The difference varied in subsequent experiments and tended to be highest (five- to eightfold) when 5×10^7 to 1×10^9 bacteria were applied on the coated surfaces. Use of a bacterial concentration higher than 10^9/ml increased the nonspecific adherence strongly. The plasmid-bearing strain bound more effectively to fibronectin than did its plasmid-cured (pYV^-) isogenic variant. Testing of four other strains of *Y. enterocolitica*, two serotype O:3, one O:8, and one O:9, gave similar adherence results (Fig. 2). The virulence plasmid did not affect binding of soluble fibronectin to *Y. enterocolitica* O:3 YeHo, which was very low (data not shown). Binding of this strain to fibronectin fragments was also studied. *Y. enterocolitica* O:3 YeHo did not bind to any of the three fragments at the level seen for the whole molecule (Fig. 3).

TABLE 2. Adherence of *Y. enterocolitica* O:3 (YeHo) to fibronectin and BSA

Y. enterocolitica O:3 strain	No. of bacteria			No. of expts
	Added	Fn binding	BSA binding	
$YeHo/pYV^+$	10^9	430 ± 280	34 ± 51	9
	10^8	430 ± 240	60 ± 94	12
	10^7	200 ± 140	20 ± 22	12
$YeHo/pYV^-$	10^9	68 ± 68	200 ± 250	4
	10^8	38 ± 34	68 ± 48	4
	10^7	13 ± 10	40 ± 39	6

Role of YadA in bacterial binding to fibronectin. The role of the YadA protein in bacterial attachment to fibronectin-coated surfaces was studied with various constructs of *Y. pseudotuberculosis* and *Escherichia coli*. In subsequent experiments *Y. pseudotuberculosis* YpIII/pYMS2, YpIII/pYMS3, and YpIII/pYMS4, which contained the intact *yadA* gene, showed strong binding to solid-phase fibronectin (Fig. 4). Strain YpIII/pYMS1, containing the nonexpressable mutated *yadA* gene of *Y. pestis*, and the plasmid-free strain YpIII showed clearly reduced binding. Usually the difference between numbers of adhered bacteria varied between five- and ninefold, but in some experiments it was impossible to demonstrate any difference. The same constructs in *E. coli* behaved in an analogous way. The parental strain PM191 and PM191/pYMS1, the latter containing the *yadA* gene of *Y. pestis*, adhered generally very poorly on fibronec-

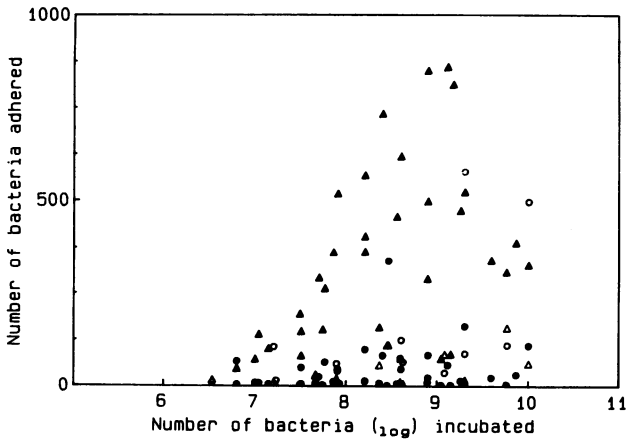

FIG. 1. Adherence of *Y. enterocolitica* O:3 (YeHo) to glass coverslips coated with fibronectin and BSA. For experimental details, see the text. Symbols: ▲, *Y. enterocolitica* O:3 (YeHo), pYV^+, binding to fibronectin; △, *Y. enterocolitica* O:3 (YeHo), pYV^-, binding to fibronectin; ●, *Y. enterocolitica* (YeHo), pYV^+, binding to BSA; ○, *Y. enterocolitica* (YeHo), pYV^-, binding to BSA.

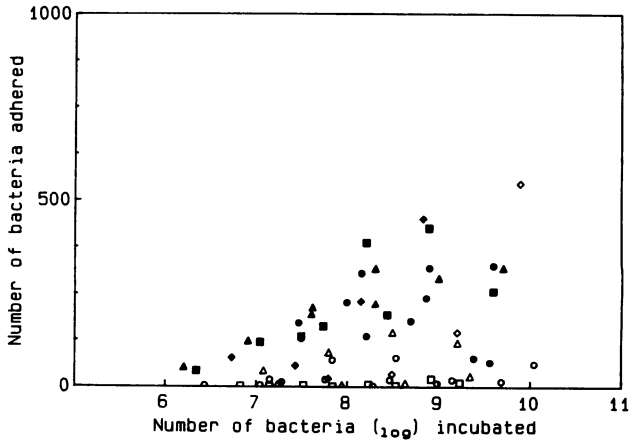

FIG. 2. Adherence of *Y. enterocolitica* O:3 (YeSa and 6471/76), *Y. enterocolitica* O:8 (W-W), and *Y. enterocolitica* O:9 (YeRu) to glass coverslips coated with fibronectin. For experimental details, see the text. Symbols: ●, *Y. enterocolitica* O:3 (YeSa), pYV^+; ○, *Y. enterocolitica* O:3 (YeSa), pYV^-; ▲, *Y. enterocolitica* O:3 (6471/76), pYV^+; △, *Y. enterocolitica* O:3 (6471/76), pYV^-; ◆, *Y. enterocolitica* O:8 (W-W), pYV^+; ◇, *Y. enterocolitica* O:8 (W-W), pYV^-; ■, *Y. enterocolitica* O:9 (YeRu), pYV^+; □, *Y. enterocolitica* O:9 (YeRu), pYV^-.

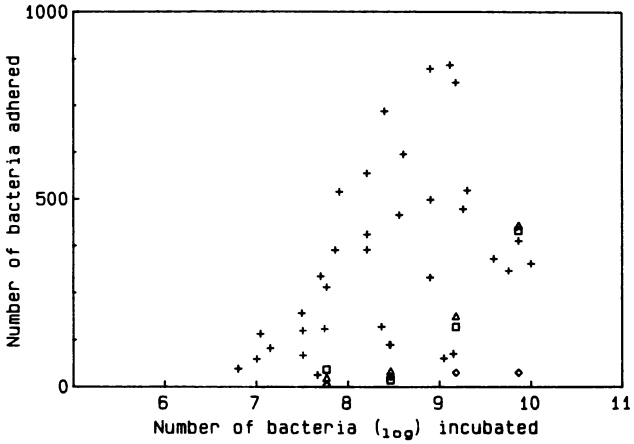

FIG. 3. Binding of *Y. enterocolitica* O:3 (YeHo) to glass coverslips coated with fibronectin fragments. For experimental details, see the text. Symbols: △, 30,000-molecular-weight fragment (30K fragment) of fibronectin; □, 40K fragment of fibronectin; ◇, 120K/140K fragment of fibronectin; +, fibronectin (same as ▲ in Fig. 1: YeHo, pYV$^+$, binding to fibronectin.

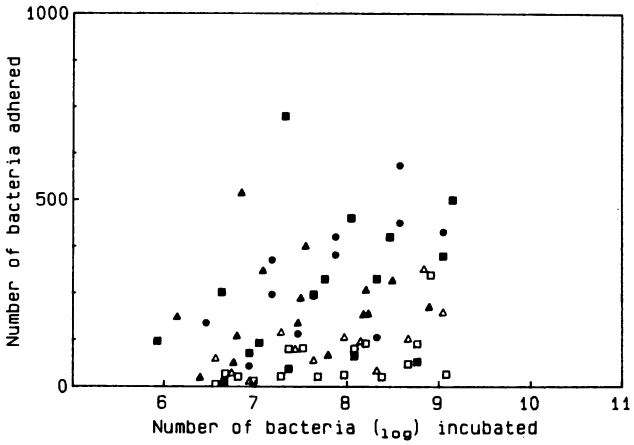

FIG. 5. Binding of *E. coli* PM191 strains to glass coverslips coated with fibronectin. For experimental details, see the text. Symbols: □, pYMS 1; ▲, pYMS 2; ■, pYMS 3; ●, pYMS 4; △, *E. coli* PM191.

tin-coated surfaces, whereas the ones containing the expressable *yadA* gene showed increased adherence (Fig. 5).

Discussion. The role of YadA in the pathogenesis of yersiniosis is not fully understood (23). The yersiniae have their reservoir in mammals, but they can probably survive very well in nature. When an infection is established, the bacterium must adapt itself to very different conditions when moving by ingestion of contaminated food from the environment to host tissues. On the basis of its adhesive properties, the YadA protein might help the bacterium to colonize the gut surface.

Our results indicate that the YadA protein interacts with insoluble fibronectin. Wild-type strains of *Y. enterocolitica* carrying the virulence plasmid bound more strongly to solid-phase fibronectin than did the plasmidless variants. Further, *Y. pseudotuberculosis* and *E. coli* clones constitutively expressing the YadA protein had this capability to adhere on fibronectin. The nature of the YadA-associated binding of yersiniae to fibronectin is not known. The finding that soluble fibronectin did not bind to yersiniae may have two implications. Either YadA binds to some conformation-dependent determinants expressed on insoluble fibronectin but hidden in soluble fibronectin, or the binding is due to hydrophobic interaction between the bacterium and insoluble fibronectin. The latter may be more probable considering the binding of YadA-bearing yersiniae to collagens, in which no specific inhibitable receptor was found (4). Likewise, Pærregaard et al. (19) demonstrated that because of hydrophobicity provided to yersiniae by the virulence plasmid, the bacteria tend to bind not only to intestinal tissues but also to polystyrene. Furthermore, this notion is also supported by the fact noted in the present study that the pYV$^+$- and YadA-expressing bacteria did not bind to fibronectin fragments.

In addition to the YadA-mediated binding, type 3 fimbria-mediated binding of *Y. enterocolitica* and *Y. intermedia* strains, which is a chromosomally encoded property, has recently been reported (28). Its role in yersinial virulence remains to be shown. YadA, in addition to its adherence functions, appears to cause resistance to complement-mediated killing by normal human serum (17). While the exact role of YadA in pathogenesis of human *Yersinia* infection is not yet fully established, it probably plays a role in several steps in the establishment of colonization. Whether YadA plays any role after the invasion process is not clear. Recently, it has been suggested by Pærregaard et al. (19) that the YadA protein works in concert with the chromosomally encoded proteins Inv and Ail for optimal expression of adhesion and invasion, YadA first helping the bacterium to get into closer contact with host cells and Inv and Ail then bringing about invasion.

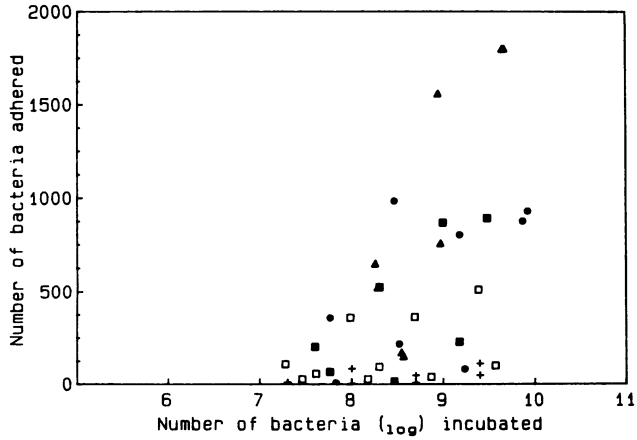

FIG. 4. Binding of *Y. pseudotuberculosis* (YpIII) strains to glass coverslips coated with fibronectin. For experimental details, see the text. Symbols: □, pYMS 1; ▲, pYMS 2; ■, pYMS 3; ●, pYMS 4; +, *Y. pseudotuberculosis* (YpIII/pIB1).

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