INFECTION AND IMMUNITY, Jan. 1992, p. 25–30
0019-9567/92/010025-06$02.00/0
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Vol. 60, No. 1

Aggregation Substance of *Enterococcus faecalis* Mediates Adhesion to Cultured Renal Tubular Cells

B. KREFT,[1]† R. MARRE,[2] U. SCHRAMM,[3] AND R. WIRTH[4]*

Klinik für Innere Medizin,[1] Institut für Medizinische Mikrobiologie,[2] and Institut für Anatomie,[3] Medizinische Universität zu Lübeck, D-2400 Lübeck 1, and Institut für Genetik und Mikrobiologie, Ludwig-Maximilians-Universität München, Maria-Ward-Strasse 1a, D-8000 Munich 19,[4] Germany

Received 19 June 1991/Accepted 18 October 1991

The sex pheromone system of *Enterococcus faecalis* is a unique, highly efficient plasmid collection mechanism for this species. A crucial role in this system is played by an adhesin called aggregation substance which enables the cell-cell contact between donor and recipient strains. The existence of the amino acid motif Arg-Gly-Asp-Ser in the adhesin prompted us to look for a possible binding of *E. faecalis* cells expressing aggregation substance to eucaryotic cells. We were able to show that the adhesin mediated binding to cultured renal tubular cells (porcine cell line LLC-PK$_1$) via light microscopic, electron microscopic, and enzyme-linked immunosorbent assay-based studies. Synthesis of the adhesin was induced by some component(s) of serum. These data are interpreted to mean that aggregation substance is an adhesin mediating not only cell-cell contact between different *E. faecalis* strains but also binding of *E. faecalis* to eucaryotic cells, and therefore it might contribute to virulence.

The sex pheromone system of *Enterococcus faecalis*, first described in 1978 (2), can be viewed as a plasmid exchange mechanism for this species. Sex pheromones were identified as being small hydrophobic peptides consisting of seven or eight amino acids (17, 24) and are named after the corresponding plasmid whose conjugative transfer they enhance (3). Sex pheromone cAD1 (with c standing for clumping), e.g., induces the transfer of plasmid pAD1. In response to the secretion of sex pheromones by plasmid-free recipient strains of *E. faecalis* (or by strains not carrying a plasmid corresponding to a secreted sex pheromone), donor strains of this species produce a so-called aggregation substance. This adhesin was shown to be of a proteinaceous nature (26), was located on the surface of the producing cells (13), appeared as a hairlike structure (7), and was incorporated only into "old" cell wall (25). The adhesion of donor and recipient cells leads to a clumping reaction, easily visible by eye, and enables the conjugative transfer of the so-called sex pheromone plasmids. After transfer of the plasmid, the newly formed donor shuts off synthesis of the sex pheromone corresponding to the newly acquired plasmid; a transfer of the plasmid from donor into donor cells is thereby avoided. DNA sequencing of the structural gene for the pAD1-encoded aggregation substance revealed the presence of the amino acid motif Arg-Gly-Asp-Ser (5) which is also found, e.g., in fibronectin, where it mediates binding to eucaryotic cells via a class of eucaryotic receptors, the integrins (19).

No data exist as to a possible advantage of *E. faecalis* possessing this unique plasmid collection system or about possible additional functions of aggregation substance other than to mediate the clumping reaction between the bacterial cells. It was proposed, but not proven, that there might be an adhesive property of aggregation substance vis-à-vis eucaryotic cells (5). Thereby, aggregation substance could mediate or increase the binding to host cells (e.g., cells of the urinary tract) and therefore contribute to virulence. In this study, we demonstrate adhesive properties of (pAD1-encoded) aggregation substance of *E. faecalis* vis-à-vis cultured renal tubular cells (LLC-PK$_1$). We also present data which indicate that the synthesis of aggregation substance might be triggered by eucaryotic factors.

MATERIALS AND METHODS

Bacterial strains. Throughout this study *E. faecalis* OG1X strains (11) carrying various plasmids were used (Table 1). Growth was on brain heart infusion plates (Unipath-Oxoid, Wesel, Germany) at 37°C. For adhesion assays, cells grown overnight were washed once in phosphate-buffered saline (pH 7.2) and suspended in phosphate-buffered saline to give a final concentration of 10^8 CFU/ml.

Eucaryotic cell line. The eucaryotic cell line LLC-PK$_1$ (ATCC CL 101), which is derived from a pig (9), was used for this study, and cells were cultured as described previously (15). The culture medium (M199 [GIBCO] containing 5% fetal calf serum and 1% glutamine) was not supplemented with antibiotics. Cells were grown in 96-well flat-bottom plates (Falcon) at a density of 5×10^4 per well for 24 h until a confluent cell layer was obtained.

Adherence assay. The number of bacteria adhering to tubular cells was determined by an enzyme-linked immunosorbent assay (ELISA) as described recently (15), using a rabbit anti-*E. faecalis* antiserum and a second anti-rabbit antibody labelled with alkaline phosphatase. *p*-Nitrophenyl phosphate (Sigma) was used as substrate. The color reaction was allowed to develop for 30 min and was read at 405 nm with the aid of a microtiter plate reader (Behring EL 311). Each experiment consisted of at least 12 determinations in parallel and was done in triplicate.

For inhibition studies, peptide Arg-Gly-Asp-Ser (RGDS; Sigma) was dissolved in M199 and added to eucaryotic cells in a final concentration of 50 μg/ml. After 40 min, the cell line was washed twice with M199 (37°C) and then fixed with

* Corresponding author.
† Present address: Molecular Autoimmunity Section, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115.

TABLE 1. Bacterial strains used

Strain	Relevant phenotype	Reference
OG1X	Plasmid-free	11
OG1X:pAD1	Carries sex pheromone plasmid pAD1	10
OG1X:pAM721	Carries derivative of pAD1 with Tn*917* insertion in regulatory region of adhesin gene; clumps constitutively	10
OG1X:pAM944	Carries derivative of pAD1 with Tn*917* insertion in adhesin gene; no clumping, but secretion of truncated adhesin	4
OG1X:pAM944/pWMH6	Carries in addition to pAM944 a 6-kb *Hpa*II fragment of pAD1, which covers the adhesin gene in pWM401; clumping inducible by sex pheromone	18

glutaraldehyde (1.25% in water); thereafter, *E. faecalis* OG1X:pAM721 was added for the actual binding assay.

Stimulation of binding by sex pheromone. The sex pheromone cAD1 (titer of 64) was added to *E. faecalis* OG1X:pAD1 40 min before the bacterial cells were inoculated into the eucaryotic cell line. Because this hydrophobic peptide was dissolved in dimethyl sulfoxide, controls contained the same concentration of dimethyl sulfoxide.

Statistics. Statistical evaluation was performed by the Wilcoxon, Mann, and Whitney test, followed by the Nemenyi test, if significant differences were found (20). The level of significance was defined as $P < 0.05$.

Light microscopy. For light microscopic studies, renal tubular cells were cultured in tissue culture chamber slides (Lab-Tek, Nunc). A suspension of *E. faecalis* cells in M199 was added, and the mixture was incubated at 37°C for 1 h. After fixation with methanol, the slides were Giemsa stained.

Electron microscopy. For transmission electron microscopic studies, the renal tubular cells were grown in collagen-coated Transwell plates (Tecnomara). Bacterial cells in M199 were added to a confluent layer of eucaryotic cells, and incubation was continued for 3 h. Thereafter, bacterial and tubular cells were fixed for 30 min with 2.5% glutaraldehyde (in 0.1 M cacodylate buffer, pH 7.2), rinsed in 0.1 M cacodylate buffer, postfixed for 1 h with 2% OsO_4 (in 5% sucrose, pH 7), dehydrated in a graded series of ethanol, and embedded in araldite, using propylene oxide as an intermedium. Ultrathin sections were contrasted with 1% uranyl acetate and a solution of saturated lead citrate and examined with a Philips EM400 electron microscope.

Western immunoblotting. Western blotting was performed as described earlier (7). The antiserum used for detection is directed against the N-terminal half of aggregation substance, as described earlier (7, 25), and detects the mature 137-kDa adhesin and its main proteolytic product of 78 kDa.

RESULTS

Microscopic studies. Light microscopic studies demonstrated a distinct adherence of *E. faecalis* OG1X:pAM721 to renal tubular cells (Fig. 1). This bacterial strain harbors a derivative of the sex pheromone plasmid pAD1, constructed in the laboratory of, and kindly provided by, D. B. Clewell. The mutated plasmid carries a Tn*917* insertion in a regulatory region for the *asa1* gene (which codes for aggregation substance of plasmid pAD1 [5]), resulting in constitutive expression of the adhesin. This strain was also chosen for most of the other experiments because the constitutive expression leads to elevated levels of aggregation substance (8) and eliminates the need to induce the synthesis of the adhesin by addition of sex pheromones. We wanted to avoid the addition of sex pheromones to the kidney cell line, because it was shown earlier that sex pheromones might affect eucaryotic systems (21). The control strain *E. faecalis* OG1X, which harbors no plasmid, showed only a weak adherence to LLC-PK_1 cells (not shown).

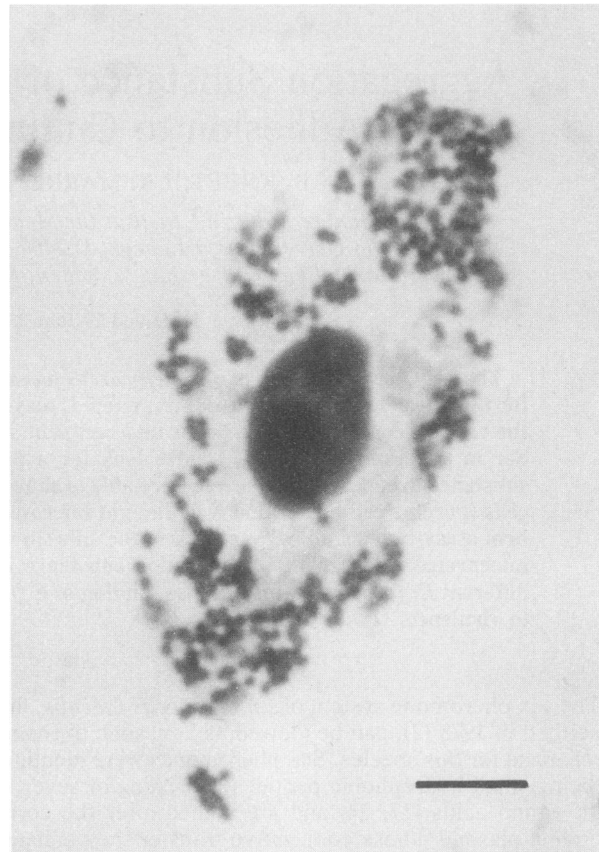

FIG. 1. Adherence of *E. faecalis* OG1X:pAM721 to cultured renal tubular cells (LLC-PK_1). After Giemsa staining, the preferential binding of the bacterial cells to the eucaryotic cell clearly can be seen by light microscopy. Bar, 10 μm. Though the adherence assays were done with confluently grown eucaryotic cells, this picture shows an isolated eucaryotic cell to clearly demonstrate the specific interaction of bacterial and eucaryotic cells.

Scanning and transmission electron microscopic studies demonstrated that the tubular cells grown on collagen-coated plates were polarized cells, clearly showing microvilli at the apical side of the cells. Again, *E. faecalis* OG1X:pAM721 was closely apposed to these cells, in most cases by interacting with the microvilli (Fig. 2). Very interestingly, this interaction between the eucaryotic cells and the bacteria was via hairlike structures protruding from the bacteria. These hairlike structures have been identified in earlier studies as aggregation substance (7, 25).

ELISA-based adherence studies. Quantitative studies on the adherence of *E. faecalis* to the kidney cell line were based on a system developed earlier to test for binding of

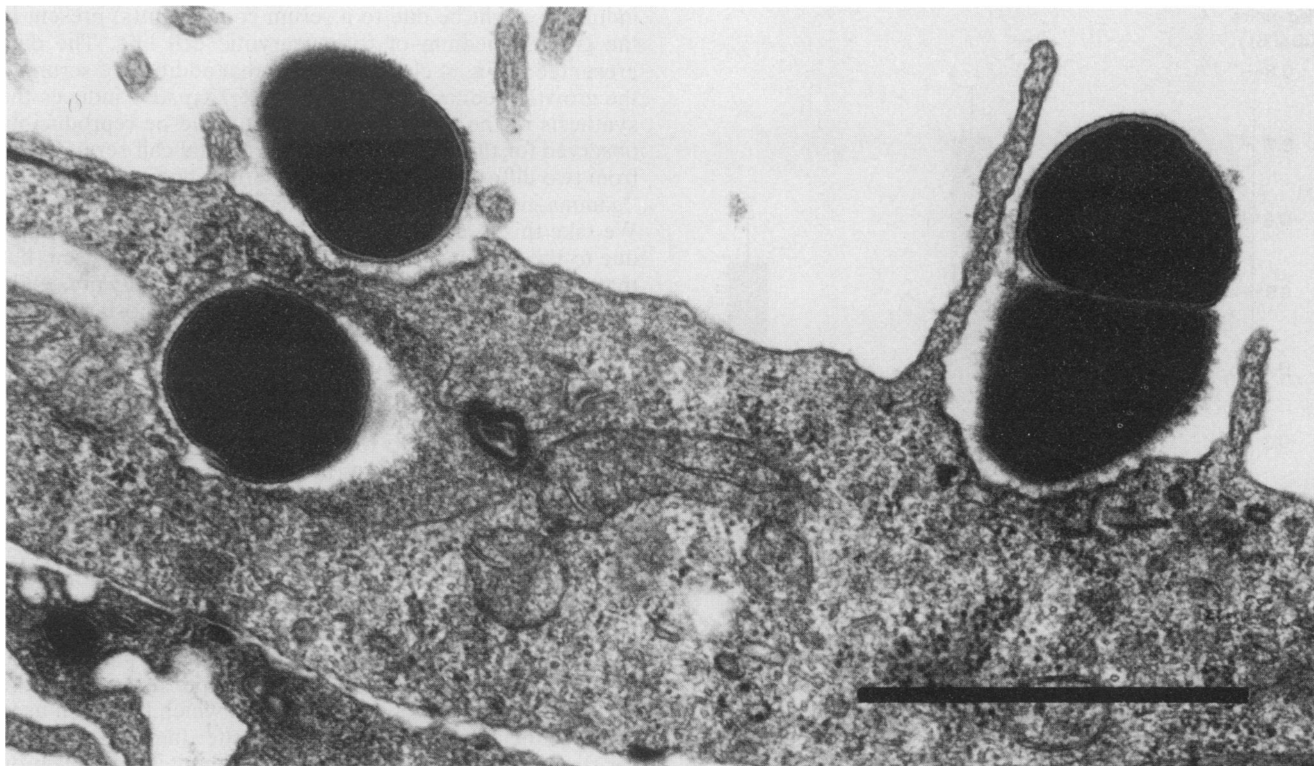

FIG. 2. Transmission electron microscopic demonstration of binding of *E. faecalis* OG1X:pAM721 to cultured renal tubular cells ($LLC-PK_1$). Preferential binding of *E. faecalis* via hairlike structures to the microvilli of tubular cells is evident. Bar, 1 μm.

Escherichia coli to $LLC-PK_1$ cells (15). The ELISA used an antiserum against whole, uninduced *E. faecalis* cells, kindly provided by G. M. Dunny. We obtained very similar results (data not shown) with an antiserum directed against purified aggregation substance which was used earlier for the characterization of aggregation substance (7, 25). As Fig. 3 demonstrates, only weak adherence of the plasmid-free control strain OG1X was observed. In contrast, *E. faecalis* OG1X:pAM721, which constitutively produces high amounts of aggregation substance, showed a strong adherence to the kidney cell line. Strain OG1X:pAD1 showed significant binding to the eucaryotic cell line compared with the plasmid-free strain; however, this binding was not as great as that of the constitutive adhesin-producing mutant OG1X:pAM721. As will be discussed below, the adherence of strain OG1X:pAD1 without induction by sex pheromone very probably is due to the initially unexpected induction of aggregation substance by some components of the eucaryotic cell line or medium or both. The results obtained for *E. faecalis* OG1X:pAM944 were initially somewhat surprising. pAM944 (provided by D. B. Clewell) is a pAD1 derivative which carries a Tn*917* insertion in the structural gene for aggregation substance and was isolated from a strain showing no clumping response. Data obtained in the laboratory of R. Wirth (18) demonstrated the following. The transposon is located in the last fourth of the structural gene, resulting in expression of a shortened version of the adhesin, which no longer possesses the C-terminal cell wall region and the membrane anchor. Most of the smaller form of the adhesin is thus secreted into the medium; nevertheless, a certain amount of the protein is still cell wall associated. It is therefore tempting to speculate that this shortened version of the adhesin still enables binding to the eucaryotic cell line. Unpublished data from our group indicate that a major domain responsible for the bacterium-bacterium clumping reaction is located in the N-terminal part of the adhesin; therefore, it is tempting to speculate that the C-terminal part of the protein contains the domain for interaction with eucaryotic cells. Furthermore, we constructed a high-copy-number plasmid which contains a 6-kb *Hpa*II restriction fragment of pAD1 covering the structural gene for aggregation substance. After transformation into OG1X:pAM944, the resulting strain, OG1X:pAM944/pWMH6, was shown to synthesize an increased amount of aggregation substance compared with OG1X:pAD1 (18). Indeed, we observed an increased binding of *E. faecalis* OG1X:pAM944/pWMH6 to the kidney cells in the adherence assay upon comparison to OG1X:pAD1.

The availability of a quantitative system for measuring the binding of *E. faecalis* to the tubular cells allowed us to look for binding specificity via addition of specific peptides. As expected, the binding could be manipulated in two ways. Addition of synthetic sex pheromone cAD1 to strain OG1X: pAD1 before the adherence assay induced synthesis of the adhesin (as seen by a beginning clumping reaction) and thereby increased the observed binding by some 20%. On the other hand, addition of the synthetic peptide Arg-Gly-Asp-Ser to the eucaryotic cells 40 min before the actual binding assay inhibited the binding by 34%, very probably by interfering with the binding to eucaryotic surface receptors of the integrin family.

Induction of aggregation substance by a eucaryotic component(s). Initially, it was not expected that *E. faecalis* OG1X: pAD1 would show significant binding in the adherence

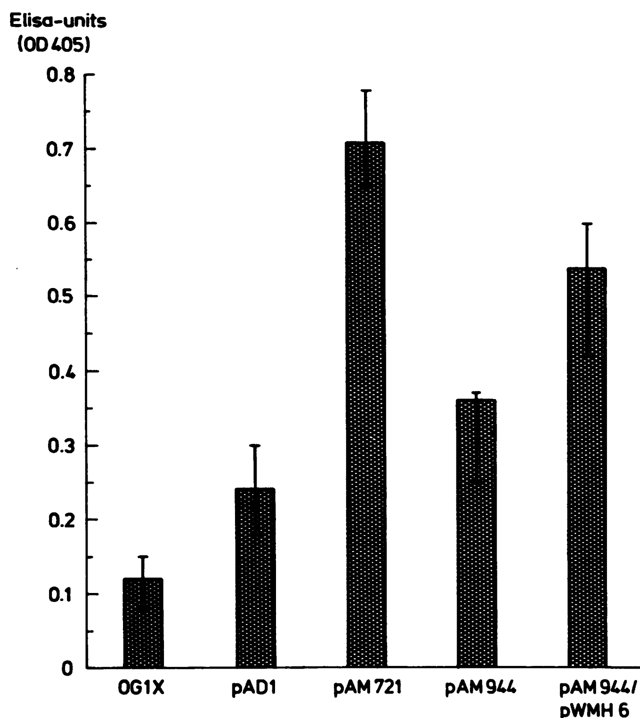

FIG. 3. Quantitative analysis of adherence of various *E. faecalis* strains to cultured and glutaraldehyde-fixed renal tubular cells by ELISA. The adherence of different *E. faecalis* strains (which were not induced for production of the adhesin by addition of sex pheromones) is shown in the 95% confidence limits as mean values. OG1X, plasmid-free strain; pAD1, OG1X carrying plasmid pAD1; pAM721, OG1X carrying plasmid pAM721; pAM944, OG1X carrying plasmid pAM944; pAM944/pWMH6, OG1X carrying plasmids pAM944 plus pWMH6. OD 405, optical density at 405 nm. See Table 1 and text for description of plasmids.

assay, because synthesis of the adhesin was not induced by addition of sex pheromone in the experiment presented in Fig. 3. The observed binding prompted us to look for a possible induction of the adhesin by a component(s) present in the adherence assay. It was found that the observed induction might be due to a serum component(s) present in the growth medium of the eucaryotic cell line. The data presented in Fig. 4 clearly indicate that addition of serum to the growth medium of *E. faecalis* OG1X:pAD1 induces the synthesis of the adhesin. This effect could be reproducibly observed for three different batches of fetal calf serum (each from two different suppliers), calf serum, bovine serum, and "gamma-globulin free" human serum (data not presented). We take this as evidence that the induction observed is not due to the presence of sex pheromone in the sera used, but that a heretofore unidentified component normally present in serum induces the formation of aggregation substance.

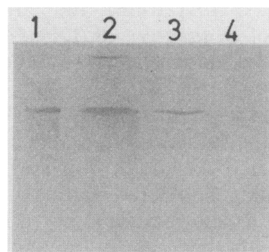

FIG. 4. Induction of synthesis of aggregation substance by a component(s) of serum. Shown is a Western blot that used antibodies which detect the 78- and 137-kDa forms of aggregation substance with cell extracts (lysozyme extraction as described in reference 5) of *E. faecalis* OG1X:pAM721 grown in Todd-Hewitt broth (lane 1); *E. faecalis* OG1X:pAD1 grown in Todd-Hewitt broth plus 50% fetal calf serum and induced by addition of synthetic sex pheromone (lane 2); *E. faecalis* OG1X:pAD1 grown in Todd-Hewitt broth plus 50% fetal calf serum without induction by sex pheromone (lane 3); *E. faecalis* OG1X:pAD1 grown in Todd-Hewitt broth without fetal calf serum and without synthetic sex pheromone (lane 4).

DISCUSSION

The assay system presented here is able to quantify the adherence of *E. faecalis* to a monolayer of tubular cells. The tubular cell line of a pig was used in this study as a model of nephropathogenicity since this cell line originates from the urinary tract, is well characterized (1, 9), and is available from the American Type Culture Collection. Primary human cell lines of tubular cells which had been used by others for nephropathogenicity studies (16) have, besides undoubted advantages, the disadvantage that they are not permanent, and also cell membrane receptor structures may vary in different cell lines. The glutaraldehyde pretreatment of the tubular cell monolayer, a fixation step which had been used also by other investigators (22), may alter the surface receptor structure of the cells; however, it inhibits internalization and apparently preserves the structures relevant for binding of *E. faecalis*. In addition, our light and electron microscopic studies, using live tubular cells, also demonstrated the adhesion of *E. faecalis*.

The adherence to tubular cells clearly correlated with the production of aggregation substance. Strain OG1X:pAM721 producing the highest amount of the adhesin gave the highest ELISA units. Also, induction of the adhesin in strain OG1X:pAD1 by sex pheromone cAD1 was shown to be associated with an increase in ELISA units. By the same token, strain OG1X, lacking the gene for aggregation substance, showed only a low-grade adherence. This is indicative of a role for aggregation substance not only in the procaryotic system but also in the attachment process between bacteria and tubular cells. The aggregation substance-mediated adherence very probably results from a specific interaction between the adhesin and the tubular cells. Another possible explanation would be that after unspecific binding of single *E. faecalis* cells to the tubular cell further enterococci might bind to these primary bacteria by means of the adhesin. A clumping response of *E. faecalis* on the tubular cells should thus be observed. The latter assumption, however, seems unlikely for three reasons. (i) In the case of OG1X:pAM721 (the self-clumping *E. faecalis* strain), no reclumping was observed after resuspension of the cells in M199 prior to the adhesion test used for the light microscopic studies. (ii) Our light and electron microscopic studies revealed an even distribution of single bacteria on the eucaryotic cells. (iii) An argument in favor of eucaryotic specificity of adherence is the observed inhibition by the oligopeptide Arg-Gly-Asp-Ser, an amino acid motif found in the enterococcal adhesin (5) and, e.g., in fibronectin, in which it mediates the binding to eucaryotic cells via a class of receptors, the integrins (19). As reported by Korhonen et al. (14), the α_6 subunit of integrins is expressed in adult human tubular epithelial cells. In addition, the β_1-integrin receptor for fibronectin can be found at the base of human tubular epithelial cells (12, 23). It

will be very interesting to learn about the identity of the eucaryotic receptor for aggregation substance. Up to now only the presence of the Arg-Gly-Asp-Ser motif in aggregation substance and the results of our peptide inhibition studies can be taken as circumstantial evidence of participation of integrins in the bacterial attachment process.

The inhibition of attachment of *E. faecalis* to the tubular cells by the integrin-specific peptide seems to be competitive because only a 20% reduction in binding was observed in experiments in which the bacteria and the peptide were added to the eucaryotic cells simultaneously. We take this observation as evidence of a stronger interaction of aggregation substance with the eucaryotic receptor than with the peptide used.

On the bases of the adherence ELISA values, the blockade of attachment by an integrin-specific oligopeptide, the increase of attachment by sex pheromone, and our microscopic studies, we conclude that aggregation substance mediates adherence between *E. faecalis* and tubular cells. In addition, we suggest that these findings also hold true for most of the other sex pheromone plasmid-encoded adhesins of *E. faecalis*, because these contain, with the exception of pAM373, a homologous gene for the adhesin (6); the respective proteins also show a close immunological relationship (8).

Although the gene for the bacterial adhesin is strictly regulated, the uninduced strain OG1X:pAD1 adhered better than the plasmid-free strain OG1X, which does not possess the adhesin gene. For the best-studied sex pheromone plasmid, pAD1, which also was used here, it was demonstrated that the adhesin is only expressed when the sex pheromone cAD1 is present. The data presented here indicate that there might exist also another factor(s) inducing the adhesin which is present during the adherence assay. The immunoblot data suggest an induction by serum; it should be noted, however, that during the actual adherence assay serum-free medium was used. Therefore, induction of the *E. faecalis* adhesin by a component(s) of the tubular cells also might be possible. It will be very interesting to learn about the nature of the serum component(s) inducing aggregation substance and whether it interacts with the same bacterial receptor as sex pheromones. It should be noted in this connection that sex pheromone-induced expression of aggregation substance takes 30 to 45 min, that serum-induced expression takes ca. 45 min, and that during the actual adherence assay (in which no serum is present) bacterial and eucaryotic cells are incubated together for 60 min.

The data presented here indicate that sex pheromone plasmid-encoded aggregation substance of *E. faecalis* can be viewed to have two functions. It mediates not only the cell-cell contact needed for conjugation via the sex pheromone system of the bacteria but also adhesion of the bacteria to a eucaryotic cell line. Therefore, aggregation substance might be a virulence factor.

ACKNOWLEDGMENTS

We thank D. B. Clewell for bacterial strains and synthetic sex pheromone and G. M. Dunny for an *E. faecalis* antiserum. The expert technical assistance of B. Karlberg, K. Fibelkorn, and A. Friesenegger is gratefully acknowledged, as are the helpful comments of the reviewer.

This work was supported in part by Deutsche Forschungsgemeinschaft grant Ma 864/3-2 to R.M. and B.K. and Deutsche Forschungsgemeinschaft grant B8 in the Sonderforschungsbereich 145 to R.W.

REFERENCES

1. **Chen, T. C., N. P. Curthoys, C. F. Lagenaur, and J. B. Puschkett.** 1989. Characterization of primary cell cultures derived from rat renal proximal tubules in vitro. Cell. Dev. Biol. **25:**714–722.
2. **Dunny, G. M., B. L. Brown, and D. B. Clewell.** 1978. Plasmid transfer in *Streptococcus faecalis*: evidence for a bacterial sex pheromone. Proc. Natl. Acad. Sci. USA **75:**3479–3483.
3. **Dunny, G. M., R. A. Craig, R. L. Carron, and D. B. Clewell.** 1979. Plasmid transfer in *Streptococcus faecalis*: production of multiple sex pheromones by recipients. Plasmid **2:**454–465.
4. **Ehrenfeld, E. E., and D. B. Clewell.** 1987. Transfer functions of the *Streptococcus faecalis* plasmid pAD1: organization of plasmid DNA encoding response to sex pheromone. J. Bacteriol. **169:**3473–3481.
5. **Galli, D., F. Lottspeich, and R. Wirth.** 1990. Sequence analysis of *Enterococcus faecalis* aggregation substance encoded by the sex pheromone plasmid pAD1. Mol. Microbiol. **4:**895–904.
6. **Galli, D., and R. Wirth.** 1991. Comparative analysis of *Enterococcus faecalis* sex pheromone plasmids identifies a single homologous DNA region which codes for aggregation substance. J. Bacteriol. **173:**3029–3033.
7. **Galli, D., R. Wirth, and G. Wanner.** 1989. Identification of aggregation substances of *Enterococcus faecalis* cells after induction by sex pheromones—an immunological and ultrastructural investigation. Arch. Microbiol. **151:**486–490.
8. **Hirt, H., et al.** Unpublished data.
9. **Hull, R. N., W. R. Cherry, and L. W. Weaver.** 1976. The origin and characteristics of a pig kidney cell strain, LLC-PK_1. In Vitro (Rockville) **12:**670–677.
10. **Ike, Y., and D. B. Clewell.** 1984. Genetic analysis of the pAD1 pheromone response in *Streptococcus faecalis*, using transposon Tn*917* as an insertional mutagen. J. Bacteriol. **158:**777–783.
11. **Ike, Y., R. A. Craig, B. A. White, Y. Yagi, and D. B. Clewell.** 1983. Modification of *Streptococcus faecalis* sex pheromones after acquisition of plasmid DNA. Proc. Natl. Acad. Sci. USA **80:**5369–5373.
12. **Kerjaschki, D., P. P. Ojha, M. Susani, R. Horvat, S. Binder, A. Hovorka, P. Hillemanns, and R. Pytela.** 1989. A β1-integrin receptor for fibronectin in human kidney glomeruli. Am. J. Pathol. **134:**481–489.
13. **Kessler, R. E., and Y. Yagi.** 1983. Identification and partial characterization of a pheromone-induced adhesive surface antigen of *Streptococcus faecalis*. J. Bacteriol. **155:**714–721.
14. **Korhonen, M., J. Ylänne, L. Laitinen, and I. Virtanen.** 1990. The α1-α6 subunits of integrins are characteristically expressed in distinct segments of developing and adult nephron. J. Cell Biol. **111:**1245–1254.
15. **Marre, R., B. Kreft, and J. Hacker.** 1990. Genetically engineered S and F1C fimbriae differ in their contribution to adherence of *Escherichia coli* to cultured renal tubular cells. Infect. Immun. **58:**3434–3437.
16. **Mobley, H. L. T., D. M. Green, A. L. Trifillis, D. E. Johnson, G. R. Chippendale, C. V. Lockatell, B. D. Jones, and J. W. Warren.** 1990. Pyelonephritogenic *Escherichia coli* and killing of cultured renal proximal tubular epithelial cells: role of hemolysin in some strains. Infect. Immun. **58:**1281–1289.
17. **Mori, M., H. Tanaka, Y. Sakagami, A. Isogai, M. Fujino, C. Kitada, B. A. White, F. Y. An, D. B. Clewell, and A. Suzuki.** 1986. Isolation and structure of the *Streptococcus faecalis* sex pheromone cAM373. FEBS Lett. **206:**69–72.
18. **Muscholl, A., et al.** Unpublished data.
19. **Ruoslahti, E., and M. D. Pierschbacher.** 1987. New perspectives in cell adhesion: RGD and integrins. Science **238:**491–497.
20. **Sachs, L.** 1984. Angewandte Statistik. Springer Verlag KG, Berlin.
21. **Sannomiya, P., R. A. Craig, D. B. Clewell, A. Suzuki, M. Fujino, G. O. Till, and W. A. Marasco.** 1990. Characterization of a class of nonformylated *Enterococcus faecalis*-derived neutrophil chemotactic peptides: the sex pheromones. Proc. Natl. Acad. Sci. USA **87:**66–70.
22. **Stanislawski, L., W. A. Simpson, D. Hasty, N. Sharon, E. H. Beachey, and I. Ofek.** 1985. Role of fibronectin in attachment of

Streptococcus pyogenes and *Escherichia coli* to human cell lines and isolated oral epithelial cells. Infect. Immun. **48**:257–259.

23. **Strooper, B., B. van der Schueren, M. Jaspers, M. Saison, M. Spaepen, F. van Leuven, H. van der Berghe, and J. Cassiman.** 1989. Distribution of the β1 subgroup of the integrins in human cells and tissues. J. Histochem. Cytochem. **37**:299–307.
24. **Suzuki, A., M. Mori, Y. Sakagami, A. Isogai, M. Fujino, C. Kitada, R. A. Craig, and D. B. Clewell.** 1984. Isolation and structure of bacterial sex pheromone, cPD1. Science **226**:849–851.
25. **Wanner, G., H. Formanek, D. Galli, and R. Wirth.** 1989. Localization of aggregation substances of *Enterococcus faecalis* after induction by sex pheromones—an ultrastructural comparison using immuno labelling, transmission and high resolution scanning electron microscopic techniques. Arch. Microbiol. **151**:491–497.
26. **Yagi, Y., R. E. Kessler, J. H. Shaw, D. E. Lopatin, F. An, and D. B. Clewell.** 1983. Plasmid content of *Streptococcus faecalis* strain 39-5 and identification of a pheromone (cPD1)-induced surface antigen. J. Gen. Microbiol. **129**:1207–1215.