JOURNAL OF BACTERIOLOGY, Feb. 1981, p. 920-925
0021-9193/80/020920-06$02.00/0

Vol. 145, No. 2

Identification and Cloning of the Genetic Determinant That Encodes for the K88ac Adherence Antigen

PATRICIA L. SHIPLEY,[1]* GORDON DOUGAN,[2] AND STANLEY FALKOW[3]

Department of Microbiology, Virginia Commonwealth University, Richmond, Virginia 23298[1]; Department of Microbiology, Trinity College, University of Dublin, Dublin 2, Ireland[2]; and Department of Microbiology, University of Washington, Seattle, Washington 98195[3]

Strains of *Escherichia coli* capable of causing diarrhea in young pigs are often able to proliferate in the upper small intestine of the infected animal due to the presence of a specific surface antigen, K88. The genetic determinants for K88 antigen production and the ability to utilize the trisaccharide raffinose (Raf) are carried on a 50-megadalton plasmid. Recombinant deoxyribonucleic acid techniques were used to insert an 8.2-megadalton *Hin*dIII fragment carrying the K88ac gene(s) from the K88/Raf plasmid pPS100 into the vector pBR322. At least six polypeptides encoded by this fragment were expressed in minicells. These polypeptides ranged in size from 18,000 to 70,000 daltons. The K88ac antigenic subunit, which has an apparent molecular weight of 23,500, was identified by immunoprecipitation with staphylococcal protein A as the coprecipitant.

Diarrheal disease caused by enteropathogenic *Escherichia coli* is a frequent cause of death among young domestic animals. In addition to the production of one or both of two types of enterotoxin, enteropathogenic *E. coli* strains are characterized by the ability to proliferate in the upper small intestine of the infected animal (18, 25). Strains isolated from young pigs often possess a common proteinaceous surface antigen designated K88 (21, 28). The adhesive properties of this antigen allow K88-positive bacteria to adhere to the intestinal mucosa and thereby avoid the normal clearing mechanisms of the gut (13, 25). The structural gene for the K88 antigen, like those for the *E. coli* enterotoxins, is located on a transmissible plasmid (19, 25). The genetic determinants for the ability to utilize the trisaccharide raffinose (Raf) are also located on the K88 plasmid (26). At least three serological variants of the K88 antigen (K88ab, K88ac, and K88ad) can be distinguished (10, 22). In an earlier report, we characterized the plasmids which carry the K88ab and K88ac antigens (24). After transfer into *E. coli* K-12 hosts, two types of K88/Raf plasmids were found. The most common was a non-self-transmissible plasmid with a size of approximately 50 megadaltons (Mdal). Larger conjugative K88/Raf plasmids were found infrequently and probably represent a cointegrate between the smaller K88/Raf plasmid and a transfer factor plasmid. To characterize further the portion of the K88/Raf plasmids directly involved in antigen production, we sought to isolate and amplify the K88 determinant by using recombinant DNA technology. In this paper, we describe the cloning of a fragment carrying the K88ac determinant and the identification of polypeptides synthesized in minicells from this fragment. A preliminary report of part of this work has been presented previously (23).

MATERIALS AND METHODS

Bacterial strains and plasmids. *Escherichia coli* K-12 strain C600 (*thr leu thi lac supE44 tonA21*) served as the plasmid host strain. For minicell studies, plasmids were introduced into the minicell-producing *E. coli* K-12 strain DS410 (*thr leu thi lac gal mal xyl ara*) (6) by transformation (3). The K88/Raf plasmid pPS100 was isolated in this laboratory and has been described previously (24). The construction and properties of the RSF2124 cloning vector have been described by So et al. (27). Strains carrying the cloning vector plasmids pBR313 and pBR322 (1) were obtained from H. W. Boyer.

Isolation of plasmid DNA. Cleared lysates of plasmid-containing cells were prepared as described by Elwell et al. (7). Plasmid DNA was purified by isopycnic centrifugation in CsCl-ethidium bromide gradients as previously described (24). The procedure of Chilton et al. (2) was employed for the isolation of DNA fragments from agarose gels.

Agarose gel electrophoresis. Electrophoresis of DNA was carried out in vertical 0.7% agarose slab gels by the procedure of Meyers et al. (15).

Restriction endonuclease digestion and cloning. *Bam*HI restriction endonuclease was obtained from Miles Laboratories; *Hin*dIII and *Eco*RI were purchased from either New England Biolabs or Bethesda Research Laboratories. All other restriction enzymes were from Bethesda Research Laboratories. T4 DNA ligase was the generous gift of H. W. Boyer. Conditions for reactions employing commercially prepared enzymes were as described by the manufacturer. For restriction mapping studies, reaction volumes were 30 μl. In double-digestion studies, the first enzyme was

inactivated by heat (60°C for 10 min), and reaction conditions were adjusted appropriately before adding the second enzyme. Fragment molecular weights were determined by comparison with the known molecular weights of the *Eco*RI and *Hin*dIII fragments of bacteriophage lambda or the *Hin*dIII fragments of bacteriophage ϕX174. Ligation reactions contained 1 to 5 μg of DNA and 2 to 10 U of ligase and were carried out in a buffer containing 30 mM Tris, pH 8, 10 mM $MgCl_2$, 0.4 mM ATP, 500 μg of bovine serum albumin per ml, 0.2 mM EDTA, and 2 mM dithiothreitol. The reactions were incubated for 12 to 72 h at 12°C. The ligation reaction mix was used directly to transform *E. coli* C600 with selection for the ampicillin resistance marker of the cloning vehicle. Transformation was carried out by the procedure of Cohen et al. (3). Hybrid plasmids carrying an insertion in the *Hin*dIII site of pBR313 or pBR322 were identified by a reduction or loss of tetracycline resistance. Insertions into the *Eco*RI site of RSF2124 were detected by loss of colicinogenicity. K88ac-positive clones were identified by slide agglutination with rabbit anti-K88ac antiserum (24).

Isolation and labeling of minicells. Minicells were isolated by the procedure of Hallewell and Sherratt (11). Approximately 10^8 minicells were suspended in 1 ml of labeling medium (5) and incubated at 37°C for 10 min. [^{35}S]methionine (20 μCi, >1,000 Ci/mmol, Amersham Corp.) was added for protein labeling, and incubation was continued for 45 min at 37°C with aeration. The minicells were pelleted, suspended in sample buffer (0.0625 M Tris, pH 6.8, 3% sodium dodecyl sulfate, 5% 2-mercaptoethanol, 0.1% bromophenol blue, 10% glycerol), and heated to 100°C for 30 min. Samples were stored at −20°C until needed. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography were carried out as described by Dougan et al. (4).

Precipitation of antibody-antigen complexes with *Staphylococcus aureus* protein A. The polypeptide specifying the K88ac antigen determinants was identified by precipitation of specific antigen-antibody complexes with protein A containing *S. aureus* (Cowen strain 1), using a modification of the procedure described by Kessler (14). Labeled minicells were lysed by sonication, and the membrane fragments were separated by centrifugation. The pelleted fraction was heated for 5 min at 60°C to release the K88 antigen (28), and the membrane fragments were repelleted. Rabbit anti-K88ac antiserum and formalinized *S. aureus* (Cowan strain 1) cells were mixed with the supernatant fraction from the heated membranes, and the mixture was centrifuged through 1 M sucrose in phosphate-buffered saline (pH 7.5) containing 1% Triton X-100 and 10 mM EDTA. The antigen-antibody-cell complex was suspended in final sample buffer (4) and electrophoresed on a sodium dodecyl sulfate-polyacrylamide slab gel.

RESULTS

Cloning of the genetic determinant of the K88ac antigen. In our initial attempts to isolate the K88ac genes by molecular cloning, we inserted *Eco*RI fragments from the K88/Raf plasmid pPS100 into the ampicillin-resistant (Ap^r) colicinogenic (ColE1$^+$) cloning vector RSF2124. *Eco*RI-cleaved pPS100 and RSF2124 DNAs were mixed at a molar ratio of 2:1. The fragments were ligated and used to transform competent *E. coli* K-12 C600 cells with selection for the ampicillin resistance marker of RSF2124. Insertions into the unique *Eco*RI site of RSF2124 resulted in the loss of colicinogenicity (ColE1$^-$). A large number of ampicillin-resistant, colicin-negative hybrid plasmids carrying *Eco*RI fragments from pPS100 were obtained, but no K88-positive strains were isolated. These findings suggested that an *Eco*RI cleavage site might lie in the K88ac structural gene or a gene involved in K88 expression. We therefore attempted to insert *Hin*dIII fragments of pPS100 into the 5.8-Mdal ampicillin- and tetracycline-resistant (Ap^r Tc^r) vector pBR313. Hybrid plasmids in which a fragment has been inserted into the pBR313 *Hin*dIII site maintain the ability to express ampicillin resistance but suffer a loss or reduction in tetracycline resistance. Two ampicillin-resistant, tetracycline-sensitive, K88-positive isolates were obtained when a ligated mixture of *Hin*dIII-cleaved pPS100 and pBR313 DNAs was used to transform *E. coli* K-12 C600. The size of the inserted fragment was determined by comparison with the known molecular weights of the *Eco*RI and *Hin*dIII fragments of bacteriophage lambda (data not shown). Both of the K88-positive isolates contained a hybrid plasmid in which an 8.2-Mdal *Hin*dIII fragment was inserted into the *Hin*dIII site of pBR313 (Fig. 1). One isolate was selected for further study and designated pPS001.

To facilitate later studies of the proteins synthesized in the minicells from the chimeric plasmid, we wished to insert the K88ac fragment into the smaller cloning vector pBR322 (2.9 Mdal). To this end, the 8.2-Mdal *Hin*dIII fragment from pPS001 was isolated from an agarose gel and ligated with *Hin*dIII-cleaved pBR322 vector DNA. Hybrid plasmids were recognized in the same manner as with pBR313 vector. The pBR322 K88-positive hybrid plasmid pPS002 was used in all subsequent restriction mapping and minicell experiments. A partial restriction map of pPS002 was constructed by double restriction endonuclease digestion. Figure 2 shows the relative positions in pPS002 of the cleavage sites of four restriction endonucleases.

The cloned K88 fragment was found to contain a single asymmetrical *Bam*HI cleavage site. Both pBR313 and pBR322 also have a single BamHI site approximately 0.2 Mdal from the *Hin*dIII site. Therefore, one possible orientation of the K88 fragment would yield two *Bam*HI fragments of similar size, whereas the other orientation would yield one large and one small

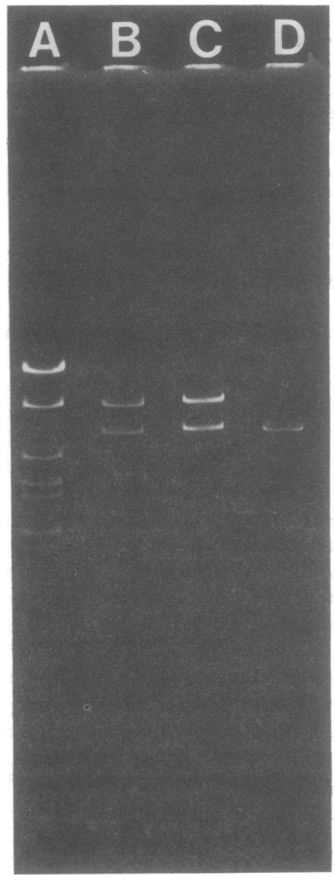

FIG. 1. *Agarose gel electrophoresis of HindIII digests of the K88/Raf parent plasmid, cloning vector, and K88-positive hybrid plasmids. (A) K88/Raf parent, pPS100; (B) and (C) K88-positive hybrid plasmids; (C) cloning vector, pBR313.*

fragment (Fig. 2). *Bam*HI cleavage of each of the K88-positive chimeric plasmids yielded two fragments of similar size, indicating that in each case the K88 fragment was oriented as shown in Fig. 2. Since hybrid plasmids with the K88 fragment in the opposite orientation have not been isolated, we cannot determine whether this orientation is required for expression of the K88ac antigen.

Polypeptides expressed by minicells carrying pPS002. Minicell-producing strains carrying the K88-positive hybrid plasmid pPS002 [strain DS410(pPS002)] or the cloning vector pBR322 [strain DS410(pBR322)] were isolated by transformation of strain DS410 with purified pPS002 or pBR322 DNA. Transformants were selected by ampicillin resistance, and the K88-positive phenotype of strain DS410(pPS002) was confirmed by slide agglutination. Minicells were isolated from each of these strains and incubated in the presence of [^{35}S]methionine to label plasmid-specific proteins. Figure 3 shows a comparison of the total Coomassie blue-stained proteins (columns C and D) and the [^{35}S]methionine-labeled plasmid-specific polypeptides (columns A and B) from minicells isolated from strain DS410(pPS002) and DS410(pBR322). At least six plasmid-specific polypeptides (indicated by arrowheads), not seen in strain DS410(pBR322) minicells, appeared in the minicells from the pPS002-containing strain. One of the polypeptides was present in a very large amount and could be distinguished in the Coomassie blue-stained total minicell protein (see legend to Fig. 3).

To identify the polypeptide which represents the K88ac antigenic subunit, we utilized the technique of immunoprecipitation with staphylococcal protein A as the coprecipitant. This protein has the special property of binding the Fc portion of the immunoglobulin G (IgG) antibody molecules. Specific antigen-antibody complexes between labeled minicell proteins and rabbit anti-K88ac antiserum were removed from solution by mixing with *S. aureus* cells carrying protein A. Labeled minicells from strain DS410(pPS002) were lysed, and membrane fragments were isolated by centrifugation. Since it has been shown that the K88 fimbriae can be released from cells by heating (28), the membrane fraction was treated at 60°C for 5 min to release the K88 fimbriae, and the membranes were repelleted. Antiserum and protein A-carrying *S. aureus* cells were mixed with the supernatant fraction from the heated membranes.

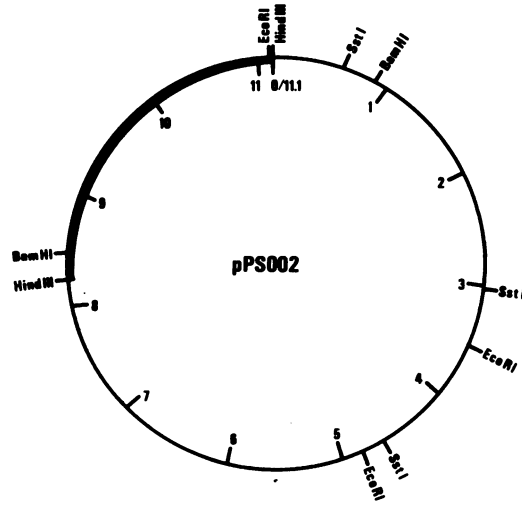

FIG. 2. *Restriction endonuclease cleavage map of pPS002. Numbered divisions represent megadaltons. The heavy line represents pBR322.*

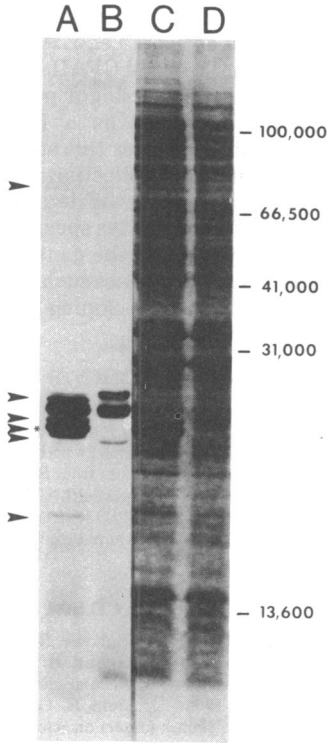

FIG. 3. *Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of minicell proteins from strain DS410(pBR322) and DS410(pPS002). Total minicell proteins were visualized by Coomassie blue staining (columns C and D), and plasmid-specific proteins were revealed by autoradiography (columns A and B). (A) DS410(pPS002) plasmid-specific proteins; (B) DS410(pBR322) plasmid-specific proteins; (C) DS410(pPS002) total minicell proteins; (D) DS410(pBR322) total minicell proteins. Arrowheads indicate proteins from pPS002 not seen in the minicells from the cloning vector strain. The protein marked by the asterisk can also be distinguished in the Coomassie blue-stained material. Numbers represent the approximate molecular weights of standard proteins.*

One polypeptide with a molecular weight of approximately 23,500 precipitated in large amount with the K88ac antiserum (Fig. 4, column A). We presume this to be the K88ac antigenic subunit. It is interesting to note that this is the polypeptide that was present in such large amounts that it could be distinguished in the unfractionated Coomassie blue-stained minicell protein preparations. Very little protein precipitated from the supernatant fraction from unheated membranes (Fig. 4, column B). Relatively small amounts of two larger polypeptides also appeared in the precipitated material. Densitometer tracings of the autoradiograph shown in Fig. 4 indicated that the 23,500-dalton band in the material that precipitated from the supernatant fraction after heating the membranes contained 42% as much radioactive material as the same band in the unheated membrane pellet, whereas the precipitated material in the two higher-molecular-weight bands was less than 10% of that in the corresponding bands from the membrane pellet.

DISCUSSION

The K88ac antigen gene was found by molecular cloning to lie on an 8.2-Mdal *Hin*dIII fragment from the K88/Raf plasmid pPS100. Studies with minicells indicated that at least six polypeptides ranging in size from 18,000 to 70,000 daltons were expressed by this fragment. The K88ac antigenic subunit was identified by immunoprecipitation and has an apparent molecular weight of 23,500. Thus, it is in the same molecular weight range as previously reported

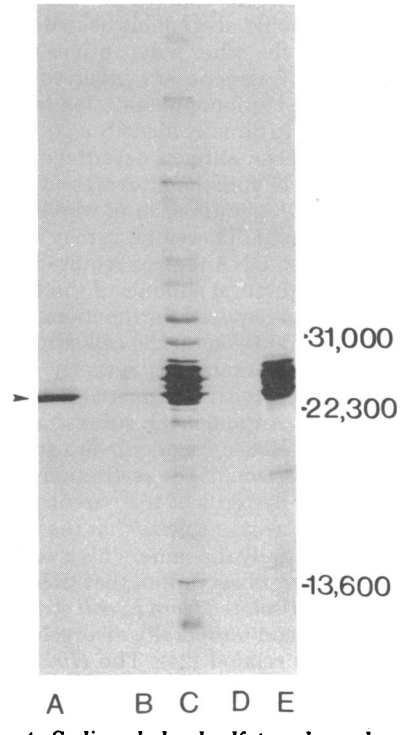

FIG. 4. *Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of pPS002-specific proteins after fractionation by immunoprecipitation. Proteins were visualized by autoradiography. (A) Immunoprecipitated material from heated sample; (B) immunoprecipitated material from unheated sample; (C) unheated membrane pellet (no immunoprecipitation); (D) antiserum and S. aureus alone; (E) unfractionated pPS002 minicell proteins. The arrowhead indicates the K88ac antigen protein. Numbers represent molecular weights of standard proteins.*

for purified K88 antigen isolated from whole bacterial cells (12, 16). Of the two polypeptides which were found in very small amounts in the immunoprecipitated material, the larger appears to be pBR322 specific, whereas the smaller is encoded for by the cloned fragment. The pBR322-specific polypeptide is probably the TEM beta-lactamase which is present in large amounts in the minicells and may be brought down nonspecifically during immunoprecipitation. The small amount of the other polypeptide appearing in the precipitated material, compared with the large amounts present in unfractionated minicells and membrane fragments, might also suggest a nonspecific interaction. However, it is also possible that this is a precursor of the K88 antigen subunit which interacts specifically with the antibody but is not effectively released from membrane fragments by heating. We hope to resolve this question by analysis of the proteins synthesized in minicells from deletion derivatives of the pPS002 plasmid. This analysis should also enable us to determine whether any of the other polypeptides specified by the cloned fragment are involved in K88 expression. The immunoprecipitation technique should allow us to directly identify a polypeptide carrying the K88ac antigen determinants even in the absence of functional expression on the cell surface (i.e., agglutination of whole cells by specific antiserum). Therefore, it may be possible to distinguish DNA regions required for synthesis of the structural subunit of the K88 fimbriae from these required for functional expression of the K88 antigen on the cell surface.

Mooi and co-workers (17) recently reported the cloning of a *Hin*dIII fragment encoding the K88ab antigen. Although the size estimates for the K88ab and K88ac fragments are somewhat different, comparison of the restriction endonuclease cleavage patterns of the parent plasmids and cloned fragments suggests that the two fragments are essentially the same. This is consistent with our earlier observation that K88ab and K88ac plasmids isolated from *E. coli* strains with geographically and temporally divergent origins are very highly related (24). The *Hin*dIII fragment that encodes the K88ac antigen contains two *Eco*RI cleavage sites, one of which is probably in a region essential for K88 expression since the antigen could not be cloned from pPS100 DNA digested to completion with *Eco*RI. Similarly, Mooi et al. (17) reported that two *Eco*RI fragments were necessary for K88ab expression.

Finally, it should be noted that enteropathogenic *E. coli* strains isolated from other young domestic animals or from humans rarely possess the K88 antigen, but often have an antigenically distinct but functionally and morphologically analogous surface antigen, i.e., K99 from calves or lambs (20) and CFAI and CFAII from humans (8, 9). We are currently using a portion of the cloned *Hin*dIII fragment as a hybridization probe to determine whether this structural and functional similarity is a reflection of nucleotide sequence homology among the determinants for antigens with different species specificities. Similarly, we plan to examine the gene products of other adherence antigen genes such as CFAI and CFAII through molecular cloning and minicell analysis.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grants AI10885 and AI15586 from the National Institute of Allergy and Infectious Disease, contract 223-73-7210 from the Food and Drug Administration, and grants from Smith, Kline and French, Inc., and Virginia Commonwealth University, P.L.S. received support from grant GM07187 from the National Institutes of Health, and G.D. was supported by a grant from Smith, Kline and French, Inc.

LITERATURE CITED

1. **Boyer, H. W., M. Betlach, F. Bolivar, R. L. Rodriguez, H. L. Heyneker, J. Shine, and H. M. Goodman.** 1977. The construction of molecular cloning vehicles, p. 9-20. *In* R. F. Beers, Jr., and E. G. Bassett (ed.), Recombinant molecules: impact on science and society. Raven Press, New York.
2. **Chilton, M.-D., M. H. Drummond, D. J. Merlo, D. Sciaky, A. L. Montoya, M. P. Gordon, and E. W. Nester.** 1977. Stable incorporation of plasmid DNA into higher plant cells: the molecular basis of Crown Gall tumorigenesis. Cell **11**:263-271.
3. **Cohen, S. N., A. C. Y. Chang, and L. Hsu.** 1972. Nonchromosomal antibiotic resistance in bacteria: genetic transformation of *E. coli* by R factor DNA. Proc. Natl. Acad. Sci. U.S.A. **69**:2110-2114.
4. **Dougan, G., M. Saul, A. Twigg, R. Gill, and D. Sherratt.** 1979. Polypeptides expressed in *Escherichia coli* K-12 minicells by transposition elements Tn*1* and Tn*3*. J. Bacteriol. **138**:48-54.
5. **Dougan, G., and D. J. Sherratt.** 1977. Changes in protein synthesis on mitomycin C induction of wild-type and mutant C10 DF13 plasmids. J. Bacteriol. **130**:846-851.
6. **Dougan, G., and D. J. Sherratt.** 1977. The transposon Tn1 as a probe for studying ColE1 structure and function. Mol. Gen. Genet. **151**:151-160.
7. **Elwell, L. P., J. deGraaff, D. Seibert, and S. Falkow.** 1975. Plasmid-linked ampicillin resistance in *Haemophilis influenzae* type b. Infect. Immun. **12**:404-410.
8. **Evans, D. G., and D. J. Evans, Jr.** 1978. New surface-associated heat-liable colonization factor antigen (CFA/II) produced by enterotoxigenic *Escherichia coli* of serogroups 06 and 08. Infect. Immun. **21**:638-647.
9. **Evans, D. G., R. P. Silver, D. J. Evans, Jr., D. G. Chase, and S. L. Gorbach.** 1975. Plasmid-controlled colonization factor associated with virulence in *E. coli* enterotoxigenic for humans. Infect. Immun. **12**:656-667.
10. **Guinee, P. A. M., and W. H. Jansen.** 1979. Behavior of *Escherichia coli* K antigens K88ab, K88ac, and K88ad in immunoelectrophoresis, double diffusion, and hemagglutination. Infect. Immun. **23**:700-705.
11. **Hallewell, R. A., and D. J. Sherratt.** 1976. Isolation and characterization of ColE2 plasmid mutants unable to kill colicin-sensitive cells. Mol. Gen. Genet. **146**:239-

245.

12. **Jones, G. W.** 1975. Adhesive properties of enteropathogenic bacteria, p. 137-142. *In* D. Schlessinger (ed.), Microbiology—1975. American Society for Microbiology, Washington, D.C.
13. **Jones, G. W., and J. M. Rutter.** 1972. Role of the K88 antigen in the pathogenesis of neonatal diarrhea caused by *Escherichia coli* in piglets. Infect. Immun. **6**:918–927.
14. **Kessler, S. W.** 1975. Rapid isolation of antigens from cells with a staphylococcal protein A-antibody adsorbent: parameters of the interaction of antibody-antigen complexes with protein A. J. Immunol. **115**:1617-1624.
15. **Meyers, J. A., D. Sanchez, L. P. Elwell, and S. Falkow.** 1976. Simple agarose gel electrophoretic method for the identification and characterization of plasmid deoxyribonucleic acid. J. Bacteriol. **127**:1529-1537.
16. **Mooi, F. R., and F. K. deGraaff.** 1979. Isolation and characterization of K88 antigens. FEMS Lett. **5**:17-20.
17. **Mooi, F. R., F. K. deGraaff, and J. D. A. vanEmbden.** 1979. Cloning, mapping and expression of the genetic determinant that encodes for the K88ab antigen. Nucleic Acids Res. **6**:849-865.
18. **Moon, H. W.** 1974. Pathogenesis of enteric disease caused by **E. coli.** Adv. Vet. Sci. Comp. Med. **18**:179-211.
19. **Orskov, I., and F. Orskov.** 1966. Episome-carried surface antigen K88 of *Escherichia coli.* I. Transmission of the determinant of the K88 antigen and influence on the transfer of chromosomal markers. J. Bacteriol. **91**: 69-75.
20. **Orskov, I., F. Orskov, H. W. Smith, and W. J. Sojka.** 1975. The establishment of K99, a thermolabile transmissible *E. coli* K antigen, previously called "Kco" possessed by calf and lamb enteropathogenic strains. Acta Pathol. Microbiol. Scand. Sect. B **83**:31-36.
21. **Orskov, I., F. Orskov, W. J. Sojka, and L. M. Leach.** 1961. Simultaneous occurrence of *E. coli* B and L antigens from diseased swine. Influence of cultivation temperature. Two new *E. coli* K antigens, K87 and K88. Acta Pathol. Microbiol. Scand. **53**:404-422.
22. **Orskov, I., F. Orskov, W. J. Sojka, and W. Wittig.** 1964. K antigens K88ab(L) and K88ac(L) in *E. coli:* a new O antigen 0147 and a new K antigen K89(B). Acta Pathol. Microbiol. Scand. **62**:439-447.
23. **Shipley, P. L., W. S. Dallas, G. Dougan, and S. Falkow.** 1979. Expression of plasmid genes in pathogenic bacteria, p. 176-180. *In* D. Schlessinger (ed.), Microbiology—1979. American Society for Microbiology, Washington, D.C.
24. **Shipley, P. L., C. L. Gyles, and S. Falkow.** 1978. Characterization of plasmids that encode for the K88 colonization antigen. Infect. Immun. **20**:559-566.
25. **Smith, H. W., and M. A. Linggood.** 1971. Observations on the pathogenic properties of the K88, Hly and Ent plasmids of *E. coli* with particular reference to porcine diarrhea. J. Med. Microbiol. **4**:467-485.
26. **Smith, H. W., and Z. Parsell.** 1975. Transmissible substrate-utilizing ability in enterobacteria. J. Gen. Microbiol. **87**:129-140.
27. **So, M., R. Gill, and S. Falkow.** 1976. The generation of a ColE1-Ap^r cloning vehicle which allows detection of inserted DNA. Mol. Gen. Genet. **142**:239-249.
28. **Stirm, S., F. Orskov, I. Orskov, and B. Mansa.** 1967. Episome-carried surface antigen K88 of *Escherichia coli.* II. Isolation and chemical analysis. J. Bacteriol. **93**:731-739.