INFECTION AND IMMUNITY, Nov. 1982, p. 739–744
0019-9567/82/110739-06$02.00/0

Vol. 38, No. 2

Cloning of Genes Determining the Production of Mannose-Resistant Fimbriae in a Uropathogenic Strain of *Escherichia coli* Belonging to Serogroup O6

STEVEN CLEGG

Department of Microbiology, University of Iowa, Iowa City, Iowa 52242

Received 15 April 1982/Accepted 7 July 1982

Chromosomal DNA from a uropathogenic strain of *Escherichia coli* was partially digested with the restriction enzyme *Eco*RI. The partial digests were ligated into a cosmid containing an ampicillin-resistant determinant and packaged into λ phage particles. An ampicillin-resistant transductant of *E. coli* HB101 was found to possess mannose-resistant hemagglutinating activity associated with a 50-kilobase-pair plasmid. Subcloning of the mannose-resistant fimbrial genes revealed that the genetic determinants were encoded by a 6.9-kilobase-pair DNA fragment of a recombinant plasmid. Chimeric plasmids smaller in size were unable to transform *E. coli* to fimbrial production. Physical maps of the recombinant plasmids were prepared showing restriction endonuclease sites within the inserted DNA fragments. The hemagglutinating activities of the wild-type strain and of the recombinant derivative were compared. Both strains agglutinated human erythrocytes in the presence of D-mannose to the same degree and also failed to produce fimbriae after incubation at 18°C. Also, both strains were agglutinated by antifimbrial serum at a high titer, whereas no such activity was observed when a strain of *E. coli* which did not possess a plasmid was used.

Strains of *Escherichia coli* associated with urinary tract infections are frequently found to possess fimbriae which mediate adherence to uroepithelial cells (16, 26) and agglutination of human erythrocytes in the presence of D-mannose (11, 18). These fimbriae, responsible for mannose-resistant (MR) hemagglutination (HA), are believed to contribute towards the virulence of uropathogenic *E. coli* by facilitating colonization of the urinary tract, leading to infection. Purified, cell-free fimbrial antigen has been shown to cause MR HA and to adhere to epithelial cells of the urinary tract (22).

Unlike the fimbrial antigens K88, K99, CFA/I, and CFA/II, which mediate adherence of enterotoxigenic *E. coli* to the gastrointestinal tracts of animals and humans (8, 10, 15, 23) and are genetically determined by plasmids, those of uropathogenic *E. coli* strains are chromosomally determined (13). Using a clinical isolate of *E. coli* (O4, K6), Hull and co-workers (13) constructed a recombinant plasmid encoding for MR fimbriae. Strains of *E. coli* possessing this plasmid were shown to agglutinate human erythrocytes and to be strongly fimbriate.

The use of recombinant DNA techniques should facilitate analysis of the control of expression of surface antigens associated with virulence in *E. coli*. This report describes the cloning of the chromosomal DNA region encoding for the synthesis of MR fimbriae from a urinary isolate of *E. coli* belonging to serogroup O6. Physical analysis of this DNA fragment is presented, and the expression of fimbriae by a strain of *E. coli* possessing hybrid plasmids is analyzed.

MATERIAL AND METHODS

Bacterial strains, culture conditions, and HA tests. *E. coli* IA2 (O6, H^-) is a clinical isolate obtained as a pure culture from a urine specimen of a female patient presenting with symptoms of an acute urinary tract infection. *E. coli* HB101 (2) was used in all transformation experiments and was rendered competent by the procedure of Morrison (21).

Unless otherwise stated, bacteria were grown in L broth or on Luria agar plates (20) for 18 to 24 h at 37°C. Media were supplemented with antibiotics at the following concentrations (in micrograms per milliliter): ampicillin, 100; chloramphenicol, 25 (200 for plasmid amplification); tetracycline, 20.

HA tests were performed as previously described with 3% (vol/vol) suspensions of human (group A) and guinea pig erythrocytes (9). To test for MR HA, the erythrocytes were suspended in phosphate-buffered saline containing 2% (wt/vol) D-mannose. The HA titer of suspensions was determined by the micro-HA procedure of Jones and Rutter (15).

Buffers and reagents. TE buffer consisted of 10 mM Tris (pH 8.0) and 1 mM EDTA. Electrophoretic analy-

ses of restriction endonuclease digests were performed in 0.8% agarose gels in 40 mM Tris–5 mM sodium acetate (pH 7.9)–1mM EDTA buffer. Endonuclease digests were performed under conditions recommended by the manufacturer, and ligations were done in ligase buffer containing 50 mM Tris-hydrochloride (pH 7.8), 10 mM $MgCl_2$, 20 mM dithiothreitol, 1 mM ATP, and 50 μg of bovine serum albumin per ml.

Restriction endonucleases used in this study were obtained from New England Biolabs and Bethesda Research Laboratories, Inc. T_4 DNA ligase was a product of New England Biolabs. Lysozyme and all antibiotics were purchased from Sigma Chemical Co.

Restriction endonuclease mapping. Restriction analyses of plasmids were performed with digestions of 0.5 μg of DNA in 25-μl volumes. After electrophoresis, the sizes of digested DNA fragments were determined by comparison with λ DNA digested with the restriction enzyme *Hin*dIII. The locations of restriction endonuclease sites were determined by analysis of single, double, or triple digestions of recombinant plasmids (25).

Preparation of DNA. Chromosomal DNA was prepared by the method of Stauffer et al. (25).

Plasmid DNA was purified by sodium dodecyl sulfate lysis of amplified cells (6), followed by ethidium bromide-cesium chloride equilibrium density gradient centrifugation (12, 24). Analysis of transformants for the presence of plasmid DNA was performed by isolating small quantities of DNA by the technique of Cameron and co-workers (3) as modified by Williams et al. (27). DNA was stored in TE buffer at 4°C.

Cosmid cloning technique. The packaging of cosmid DNA containing inserted chromosomal DNA fragments into λ phage particles was a modification of the procedure described by Meyerowitz et al. (19). The cosmid pMF7 was supplied by Michael G. Feiss and was constructed from plasmid pBR322 and λ DNA isolated from Charon strain 3A. The cosmid possesses an Amp^r determinant and the region encoding the *cos* cleavage site. *E. coli* IA2 chromosomal DNA was partially digested with the restriction enzyme *Eco*RI. The conditions of this partial digestion were adjusted so that the fragments of chromosomal DNA produced were approximately the size of λ DNA when the electrophoretic mobilities of the two DNA samples were compared by using a 0.2% agarose gel. Ligation of the partial digests (6.5-μg samples) to the *Eco*RI-digested cosmid (7.2 μg) was achieved by suspending the 95% ethanol-precipitated DNA in 60 μl of ligase buffer, adding 6 μl of 1-mg/ml T_4 DNA ligase, and incubating overnight at 14°C. In vitro λ packaging was performed with 4-μl volumes of ligation mixture, and bacteria possessing recombinant DNA molecules were selected by plating on Luria agar containing ampicillin.

Electron microscopy. Bacterial suspensions were washed in sterile distilled water, and 1 drop of suspension was placed on a carbon-coated copper grid. Excess fluid was removed with a piece of filter paper, and the bacteria were stained for 30 s with phosphotungstic acid. Bacteria were then examined with a JOEL transmission electron microscope.

RESULTS

Cloning of genetic determinants encoding for MR fimbriae. Chromosomal DNA of *E. coli* IA2 was partially digested with the restriction endonuclease *Eco*RI, and the fragments were ligated into the single *Eco*RI site of the Amp^r cosmid pMF7. The λ in vitro packaging protocol described above was used to obtain transductants possessing cosmid DNA with inserted fragments of IA2 DNA. Of 148 Amp^r transductants tested, one exhibited the HA reaction, and this clone was found to possess a plasmid (designated pIA1) consisting of approximately 50 kilobase pairs (kb). The plasmid from this strain transformed *E. coli* HB101 to Amp^r and HA activity with 100% cotransfer of the two properties. The size of pIA1 falls within the predicted range for the λ packaging procedure (DNA that is 75 to 105% of the size of native λ DNA is packaged by this method).

Subcloning of the fimbrial genes. Digestion of pIA1 with the restriction enzyme *Bam*HI revealed three recognition sites for the enzyme. To reduce the size of the initial 46-kb insert, pIA1 was digested with *Bam*HI, and the resulting fragments were ligated into the single *Bam*HI site of the cloning vehicle pACYC184 (4), resulting in insertional inactivation of the tetracycline resistance gene. The DNA was used to transform a non-hemagglutinating *E. coli* HB101 strain with selection for Cm^r Tc^s transformants which were subsequently tested for HA activity. One such transformant possessed a plasmid (pDC1) which had deleted two *Bam*HI fragments and had an estimated size of 16.3 kb. A physical restriction enzyme map of this plasmid is shown in Fig. 1. Next, plasmid pDC1 was digested with restriction endonuclease *Cla*I, and the 8.3-kb fragment (Fig. 1) was ligated into the *Cla*I site of a second pACYC184 plasmid molecule. The DNA was used to transform a nonfimbriate *E. coli* strain with selection for Cm^r transformants. One Cm^r Tc^s HA^+ transformant contained a plasmid, designated pDC5, which was 12.3 kb in size (Fig. 2) and which had lost 3.9 kb of inserted DNA of pDC1 (Fig. 1). Plasmid pDC5 contained a 300-base-pair repeat as a consequence of the subcloning procedure but remained as a stable plasmid in all transformants and throughout these studies. The stability of this plasmid is presumably due to the *rec*A phenotype of *E. coli* HB101.

Identification of the DNA fragment required for expression of fimbriae. Figure 3 shows the results of subcloning DNA fragments of pDC5 into the cloning vehicle pBR322 (1). Neither fragment 1 nor fragment 2 of the *Hin*dIII digest of pDC5 possesses sufficient information to produce functional fimbriae. Amp^r Tc^s Cm^s transformants containing plasmid A were detected by digestion of plasmid from these strains at the *Bgl*II site, and those containing plasmid B were detected by digestion at the *Sac*II site (plasmid

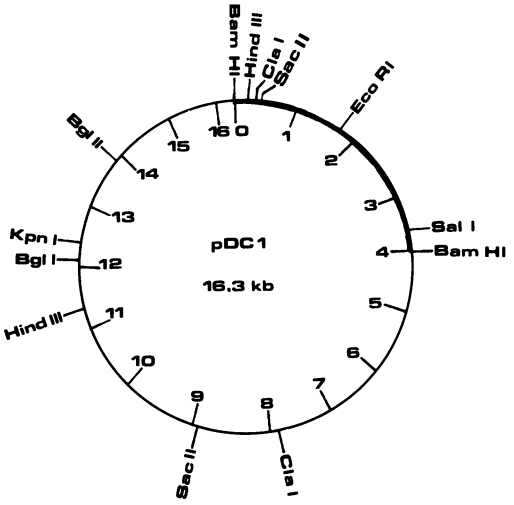

FIG. 1. Physical map of plasmid pDC1 showing restriction endonuclease sites. The plasmid was found to be 16.3 kb in size with an inserted DNA fragment of 12.3 kb. Thick line represents pACYC184 DNA.

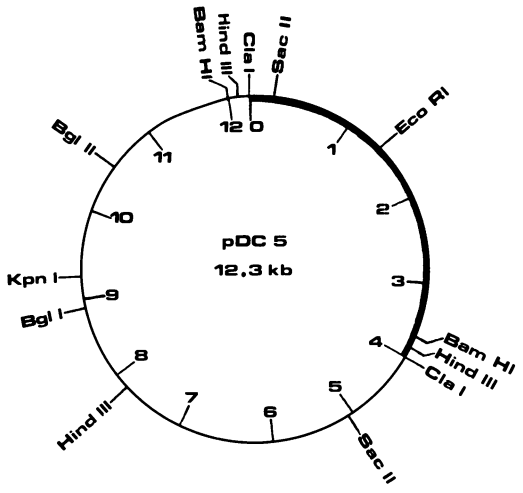

FIG. 2. Restriction enzyme map of pDC5 showing 8.3-kb inserted DNA fragment. This plasmid was derived from pDC1 by loss of the 4-kb *Cla*I *Bam*HI insert.

pDC5, from which fragments 1 and 2 were derived, possesses only one *Bgl*II and one *Sac*II site on the inserted DNA fragment [Fig. 2]). The absence of fimbriae on transformants possessing plasmid A or B was determined by negative HA activity and by electron microscopy.

Similarly, a *Bam*HI + *Bgl*II double digest of pDC5 with subsequent ligations of DNA frag-

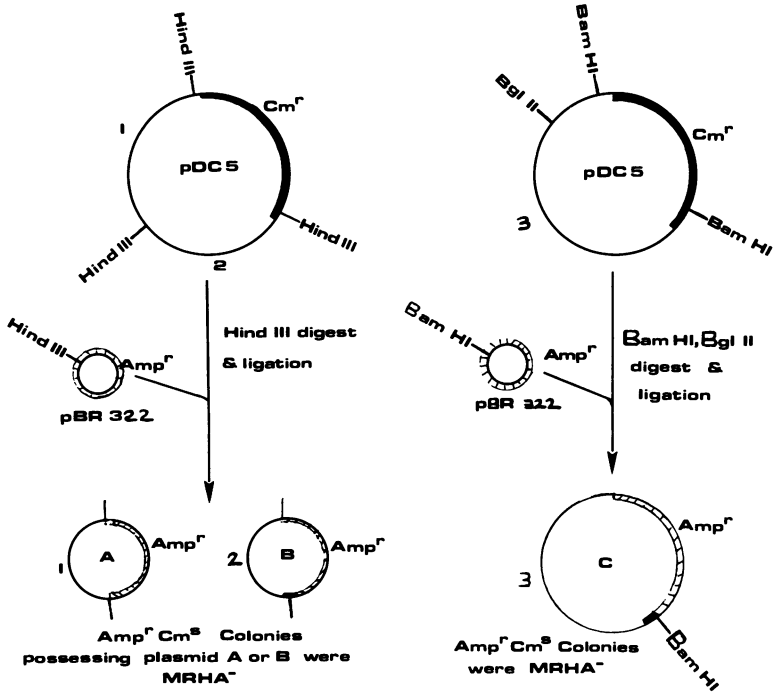

FIG. 3. Schematic diagram of the construction of plasmids which produce non-hemagglutinating, nonfimbriate transformants. Plasmids A, B, and C were derived from pDC5 and used to estimate the total size of inserted DNA necessary for fimbrial expression.

ment 3 into the *Bam*HI site of pBR322 resulted in Amp^r Tc^s Cm^s transformants which were nonfimbriate and non-hemagglutinating (Fig. 3). However, digestion of pDC5 by restriction enzyme *Sac*II and ligation of the resultant 7.4-kb fragment into the *Sac*II site of pACYC184 resulted in the formation of a plasmid designated pDC6 which could transform *E. coli* HB101 to HA activity.

Thus, the genetic elements determining the production of fimbriae in *E. coli* IA2 reside upon a DNA fragment which has a maximum size of 6.9 kb (4.5 megadaltons). This fragment spans the region from the *Sac*II site to the *Bam*HI site of pDC5 (Fig. 2).

Expression of fimbriae by an *E. coli* strain possessing a recombinant plasmid. *E. coli* SC802 was derived from strain HB101 by transformation with purified plasmid pDC5. Throughout these studies, strain HB101 never exhibited HA activity and was found to be nonfimbriate, whereas SC802 was strongly hemagglutinating and produced many fimbriae (Fig. 4). Table 1 shows the results of a comparison between the recombinant derivative SC802 and the wild-type *E. coli* strain IA2. Both strains were agglutinated

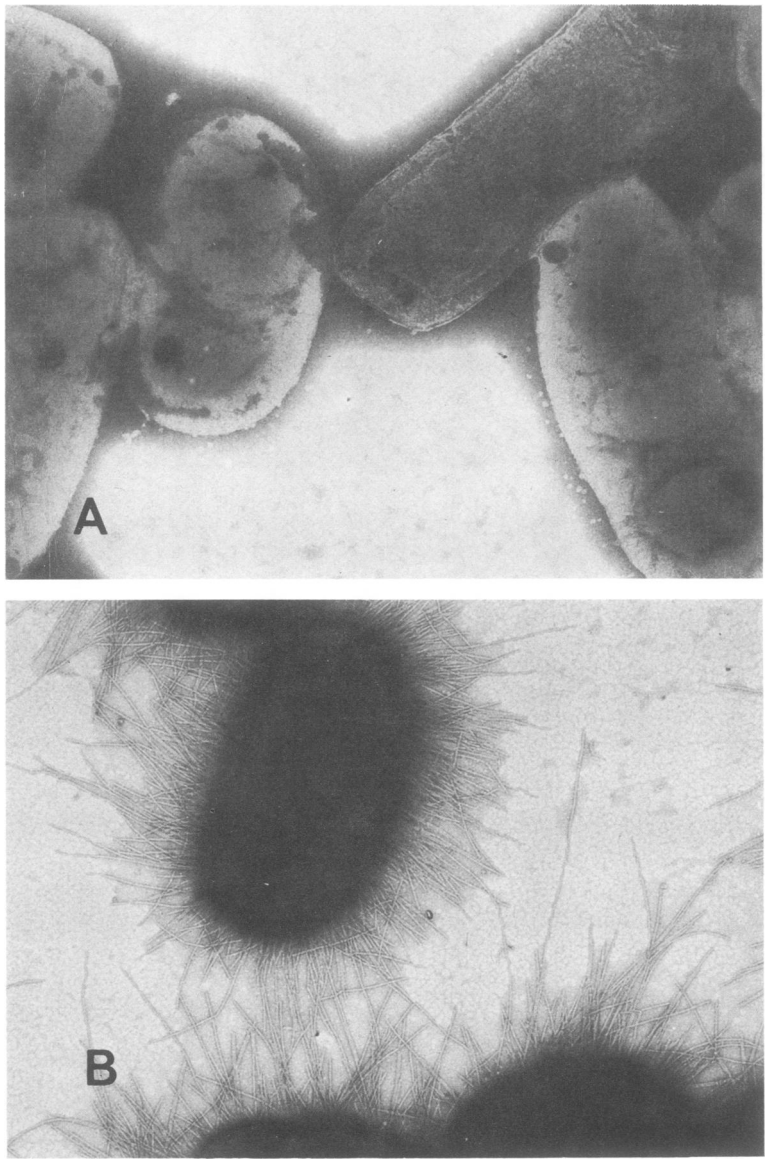

FIG. 4. Electron micrographs of *E. coli* HB101 before (A) and after (B) transformation with pDC5.

TABLE 1. Serological and HA properties of wild-type and plasmid-containing *E. coli* strains

Strain	Agglutination titer vs anti-fimbrial serum	MR HA titer[a]	MR HA at:	
			37°C	18°C
HB101	<4		−	−
SC802	320	1.73×10^8	+	−
IA2	256	1.07×10^8	+	−

[a] Number of bacteria per milliliter required to produce visible HA.

by IA2 antifimbrial serum which all nonfimbrial agglutinins had been removed by absorption with nonfimbrial phenotypes of IA2 produced by incubation at 18°C (5). In contrast, *E. coli* HB101 showed no reactivity with this immune serum. Also, no significant difference in the number of bacteria required to produce a visible HA reaction was observed between the wild-type strain and the recombinant derivative.

In common with most other strains of *E. coli* possessing fimbriae responsible for MR HA, *E. coli* IA2 does not produce fimbriae when grown at 18°C (7). Even though the gene(s) determining fimbrial production is carried in a multicopy plasmid vehicle, *E. coli* SC802 exhibited similar loss of HA activity at this low incubation temperature (Table 1). In both cases, the bacterial strains appeared to be nonfimbriate when examined by electron microscopy.

DISCUSSION

The objective of this study was to clone the genetic elements encoding for MR fimbriae from a clinical isolate of *E. coli*. Because these determinants have not been mapped on the *E. coli* chromosome, the in vitro λ packaging procedure was utilized for the construction of the initial recombinant plasmids. This method ensured that relatively large DNA fragments amounting to approximately 1% of the *E. coli* chromosome were selected for during incorporation of the DNA into the phage head. Further subcloning of DNA fragments by conventional procedures resulted in a smaller plasmid possessing the relevant genetic information. This fragment was 7.9 kb in size and possessed all of the genes necessary for fimbrial production and expression. Recently, Kehoe and co-workers have shown that the DNA encoding the plasmid-borne K88 fimbrial antigen consists of at least four cistrons (17). It is tempting to speculate that the number of distinct genes required for the expression of functional fimbriae in *E. coli* IA2 is also more than one and consists of an operon-like system. This is especially true since, in this study, small fragments of DNA were shown to not possess a full complement of genetic information for fimbrial expression. However, such a speculation would be premature since it has not been determined in the studies described in this paper whether nonessential sequences of DNA are contained on the plasmid pDC5. I propose to investigate this possibility by more accurately mapping the genes with the transposable element Tn*5*. Analysis of such insertional mutants (and deletion mutants) in a minicell-producing strain of *E. coli* (17) should provide more information. The results of this study strongly suggest that the structural gene encoding for the fimbrial protein is found on the recombinant plasmid pDC5. However, it is possible that the plasmid may be complementing a mutant accessory gene located on the *E. coli* HB101 chromosome. The minicell assay will also be useful in identifying the location of the structural gene on the recombinant plasmids.

Hull et al. (13) have described the cosmid cloning of genes encoding MR fimbriae from a urinary isolate of *E. coli* belonging to serogroup O4. Antiserum raised against the fimbriae of this urinary isolate did not react with the fimbriae produced by the recombinant strain *E. coli* SC802 (S. Normark, personal communication). Therefore, these two serological types of pili may represent the products of two distinct genes. Recent observations have shown that MR fimbrial antigens of urinary isolates are serologically diverse (5, 14) and, therefore, comparison of DNA homology between these two chromosomal DNA fragments should prove useful. Also, the observation that some pathogenic strains of *E. coli* produce a nonfimbrial hemagglutinin (7; V. L. Sheladia, D. J. Evans, Jr., and F. J. de la Cabada, Abstr. Annu. Meet. Am. Soc. Microbiol. 1982, B73, p. 30) suggests that a number of distinct genetic determinants for adherence factors may be found on the *E. coli* genome.

E. coli HB101 possessing a recombinant plasmid encoding for fimbrial synthesis and expression possessed HA activity identical to that of the wild-type strain. In neither strain was the MR HA reaction observed, nor were the strains found to be fimbriate when they were grown at 18°C. It is unclear from these studies whether the absence of HA represents control of the fimbrial genes at the molecular level or simply a change in the characteristics of the *E. coli* membrane at lower incubation temperatures.

A further characterization of plasmid pDC1 and its derivatives should be useful in understanding the mechanism by which the fimbrial genes are regulated. A physical map of the genetic determinants will facilitate isolation of pure DNA fragments, and these fragments will be used to perform DNA sequence analyses. Also, the use of in vitro transcription-translation studies with these DNA fragments will facilitate

analysis of the mechanism of fimbrial synthesis.

ACKNOWLEDGMENTS

I thank I. P. Crawford for his helpful comments and review of the manuscript. The assistance of W. Widner in the cosmid-cloning technique is greatly appreciated.

LITERATURE CITED

1. **Bolivar, F., R. L. Rodriguez, P. J. Greene, M. C. Betlach, H. L. Heyneker, H. W. Boyer, J. H. Crossa, and S. Falkow.** 1977. Construction and characterization of new cloning vehicles. II. A multipurpose cloning system. Gene **2**:95–113.
2. **Boyer, H. W., and D. Roulland-Dussoix.** 1976. A complementation analysis of the restriction and modification of DNA in *Escherichia coli*. J. Mol. Biol. **41**:459–472.
3. **Cameron, J. R., P. Philippsen, and R. W. Davis.** 1977. Analysis of chromosomal integration and deletions of yeast plasmids. Nucleic Acids Res. **4**:1429–1448.
4. **Chang, A. C. Y., and S. N. Cohen.** 1978. Construction and characterization of amplifiable multicopy DNA cloning vehicles derived from the P15A cryptic miniplasmid. J. Bacteriol. **134**:1141–1156.
5. **Clegg, S.** 1982. Serological heterogeneity among fimbrial antigens causing mannose-resistant hemagglutination by uropathogenic *Escherichia coli*. Infect. Immun. **35**:745–748.
6. **Clewell, D. B.** 1972. Nature of ColE1 plasmid replication in *Escherichia coli* in the presence of chloramphenicol. J. Bacteriol. **110**:667–676.
7. **Duguid, J. P., S. Clegg, and M. I. Wilson.** 1979. The fimbrial and non-fimbrial hemagglutinins of *Escherichia coli*. J. Med. Microbiol. **12**:213–227.
8. **Evans, D. G., and D. J. Evans, Jr.** 1978. New surface-associated heat-labile colonization factor antigen (CFA/II) produced by enterotoxigenic *Escherichia coli* of serogroups O6 and O8. Infect. Immun. **21**:638–647.
9. **Evans, D. G., D. J. Evans, Jr., S. Clegg, and J. A. Pauley.** 1979. Purification and characterization of the CFA/I antigen of enterotoxigenic *Escherichia coli*. Infect. Immun. **25**:738–748.
10. **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 *Escherichia coli* enterotoxigenic for humans. Infect. Immun. **12**:656–667.
11. **Green, C. P., and V. L. Thomas.** 1981. Hemagglutination of human type O erythrocytes, hemolysin production, and serogrouping of *Escherichia coli* isolates from patients with acute pyelonephritis, cystitis, and asymptomatic bacteriuria. Infect. Immun. **31**:309–315.
12. **Guerry, P., D. J. LeBlanc, and S. Falkow.** 1973. General method for the isolation of plasmid deoxyribonucleic acid. J. Bacteriol. **116**:1064–1066.
13. **Hull, R. A., R. E. Gill, P. Hsu, B. H. Minshew, and S. Falkow.** 1981. Construction and expression of recombinant plasmids encoding type 1 or D-mannose-resistant pili from a urinary tract infection *Escherichia coli* isolate. Infect. Immun. **33**:933–938.
14. **Jann, K., B. Jann, and G. Schridt.** 1981. SDS polyacrylamide gel electrophoresis and serological analysis of pili from *Escherichia coli* of different pathogenic origin. FEMS Microbiol. Lett. **11**:21–25.
15. **Jones, G. W., and J. M. Rutter.** 1974. The association of K88 with hemagglutinating activity in porcine strains of *Escherichia coli*. J. Gen. Microbiol. **84**:135–144.
16. **Källenius, G., R. Mölby, and J. Winberg.** 1980. In vitro adhesion of uropathogenic *Escherichia coli* to human periurethral cells. Infect. Immun. **28**:972–980.
17. **Kehoe, M., R. Sellwood, P. Shipley, and G. Dougan.** 1981. Genetic analysis of K88-mediated adhesion of enterotoxigenic *Escherichia coli*. Nature (London) **291**:122–126.
18. **Ljungh, A., A. Faris, and T. Wadström.** 1979. Hemagglutination by *Escherichia coli* in septicemia and urinary tract infections. J. Clin. Microbiol. **10**:477–481.
19. **Meyerowitz, E. M., G. M. Guild, L. S. Prestidye, and D. S. Hogness.** 1980. A new high capacity cosmid vector and its use. Gene **11**:271–282.
20. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
21. **Morrison, D. A.** 1977. Transformation in *Escherichia coli*: cryogenic preservation of competent cells. J. Bacteriol. **132**:349–351.
22. **Ørskov, I., F. Ørskov, and A. Birch-Andersen.** 1980. Comparison of *Escherichia coli* fimbrial antigen F7 with type 1 fimbriae. Infect. Immun. **27**:657–666.
23. **Ørskov, I., F. Ørskov, H. W. Smith, and W. J. Sojka.** 1975. The establishment of K99, a thermolabile, transmissible *Escherichia coli* K antigen, previously called "K_{co}", possessed by calf and lamb enteropathogenic strains. Acta Pathol. Microbiol. Scand. Sect. B **83**:31–36.
24. **Selker, E., K. Brown, and C. Yanofsky.** 1977. Mitomycin C-induced expression of *trp*A of *Salmonella typhimurium* inserted into the plasmid ColE1. J. Bacteriol. **129**:388–394.
25. **Stauffer, G. V., M. D. Plamann, and L. T. Stauffer.** 1981. Construction and expression of hybrid plasmids containing the *Escherichia coli glyA* gene. Gene **14**:63–72.
26. **Svanberg-Eden, C., L. Hagberg, L. A. Hanson, T. Korhenen, H. Leffler, and S. Olling.** 1981. Adhesion of *Escherichia coli* in urinary tract infection, p. 185. *In* K. Elliott, M. O'Conner, and J. Whelan (ed.), Adhesion and microorganism pathogenicity. CIBA Foundation Symposium 80, Pitman Medical, London.
27. **Williams, J. A., J. P. Yeggy, C. F. Field, and A. J. Markovetz.** 1979. Resistance plasmids in *Pseudomonas cepacia* 4G9. J. Bacteriol. **140**:1017–1022.