JOURNAL OF BACTERIOLOGY, Aug. 1982, p. 1006–1012
0021-9193/82/081006-07$02.00/0

Vol. 151, No. 2

Genetics of Hemolysin of *Escherichia coli*

SHEILA I. HULL,[1]* RICHARD A. HULL,[1] BARBARA H. MINSHEW,[2] AND STANLEY FALKOW[3]

Department of Microbiology and Immunology, Baylor College of Medicine, Texas Medical Center, Houston, Texas 77030,[1] Department of Clinical Microbiology, U.S. Public Health Service Hospital, Seattle, Washington 98114,[2] and Department of Medical Microbiology, Stanford University Medical School, Stanford, California 94305[3]

Received 9 November 1981/Accepted 5 April 1982

The expression of alpha-hemolysin is a property frequently associated with *Escherichia coli* extraintestinal infections. We have examined the genetic basis for hemolysin expression by an *E. coli* strain isolated from a human urinary tract infection. The genes necessary for hemolysin synthesis were found to be chromosomal and to map near the *ilv* gene cluster. Isogenic *hly*$^+$ and *hly* derivatives were also prepared and tested for virulence in the chicken embryo model system. Hemolysin was found to be necessary but not in itself sufficient for *E. coli* virulence in this in vitro model.

Hemolysins are bacterial products which mediate the lysis of erythrocytes. Expression of a diffusible (alpha) hemolysin by *Escherichia coli* was first described by Lovell and Rees (20); Smith (33) later confirmed these findings and demonstrated a cell-bound (beta) hemolysin. A third hemolysin (gamma) has been reported to be expressed by *E. coli* cells resistant to nalidixic acid (40).

The association of hemolysin expression with disease has been recognized for some time, but the role of hemolysin in the disease process remains unclear (5, 12, 13, 17, 33). Several model systems which demonstrate that hemolysin has potential as a virulence factor have been devised. Smith (33) showed that supernatant fluids from hemolytic *E. coli* isolates were lethal when administered intravenously to mice, rabbits, and guinea pigs but not when administered via the alimentary tract. Smith and Linggood found that the presence of an Hly plasmid in an *E. coli* strain usually increased its virulence for mice upon intraperitoneal injection but that the increase varied according to the plasmid introduced (37). Using a chicken embryo model developed by Powell and Finkelstein (26), Minshew et al. (23) reported that about 80% of strains virulent for chicken embryos are hemolytic, perhaps suggesting a causal relationship.

Alpha-hemolysins expressed by *E. coli* from a variety of sources are antigenically cross-reactive (33) and have similar, although not identical, physical properties. They are, in general, heat labile (4, 18, 27, 33, 38, 42), acidic (21, 27, 31) proteins (18, 27, 31, 42) with a molecular weight of 58,000 (10) to 120,000 (41). They require Ca^{2+} for full activity (28, 31, 39) and are most active at pHs 7 to 8 (31, 42).

The genetic basis for the expression of alpha-hemolysin by some strains has been determined. Smith and Gyles (34), Smith and Halls (35), Smith and Heller (32), Smith and Linggood (36), and others (16, 19) found that the alpha-hemolysin (Hly) determinant in most strains associated with diarrheal disease is self-transmissible or mobilizable by F. They concluded that Hly was often plasmid mediated. Many Hly plasmids have since been identified and found, with the exception of the region encoding hemolysin expression, to be genetically diverse (10, 11, 29; F. de la Cruz, D. Müller, J. M. Ortiz, and W. Goebel, Mol. Gen. Genet., in press). In a few other isolates of intestinal origin, the Hly determinant was not transferable and was therefore thought to be chromosomally encoded or to exist on a nonmobilizable plasmid (36). Minshew et al. (23) found that in most instances of strains from human extraintestinal infections, alpha-hemolysin expression did not appear to be plasmid mediated.

The purpose of this paper is to describe an attempt at genetic characterization of a chromosomally encoded Hly determinant in an *E. coli* strain isolated from a human urinary tract infection. During the course of the work, genetically defined Hly$^+$ and Hly$^-$ derivatives of the original wild-type *E. coli* and of *E. coli* K-12 were prepared. These strains were tested for virulence in the chicken embryo model system.

MATERIALS AND METHODS

Media. L broth containing (per liter) 5 g of yeast extract, 10 g of tryptone, 5 g of NaCl, and 0.1% glucose was adjusted to pH 7.0 with NaOH. L broth without glucose was solidified with 1.5% agar (Difco Laboratories) for plates. Blood agar plates were made with Blood Agar Base (BBL Microbiology Systems)

supplemented with 0.5% yeast extract–0.1% glucose–10 mM CaCl–5% defibrinated sheep blood. Minimal salts media and the concentrations of various growth supplements used have been described elsewhere (15). Buffered saline (pH 7) contained (per liter) 8.5 g of NaCl, 0.3 g of KH_2PO_4, and 1.22 g of $Na_2HPO_4 \cdot 7H_2O$.

Mutagenesis. Cells from 5 ml of an exponentially growing L broth culture were collected by centrifugation, washed once with 0.1 M sodium citrate (pH 5.0), and suspended in 0.1 M sodium citrate (pH 5.0) with 100 μg of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine per ml (1). After 15 min of incubation at 37°C, the cells were washed twice with buffered saline, diluted 10-fold into L broth, and incubated overnight at 37°C. The method used for nitrous acid mutagenesis was exactly as described by Miller (22). Auxotrophic mutants were identified after two cycles of cycloserine enrichment (7).

Isolation of Hfr derivatives. Strains HU865 and HU868 were identified among 36,000 *E. coli* J96 F' *kan* colonies screened for transfer as described by Curtiss and Stallions (9). HU895 was selected from *E. coli* J96 *aroB*::Tn*5* (F_{ts}::Tn*5* *lac*$^+$ *trp*$^+$) by growth on L agar plates with kanamycin (20 μg/ml) at 42°C. The transfer characteristics of this strain are consistent with the F having inserted stably at the *lac* homology rather than at the intended Tn*5* homology.

Mating conditions. Overnight cultures of the donors were diluted 10-fold in L broth and grown statically at 37°C to a concentration of about 2×10^8 cells per ml. Recipients were grown similarly but with aeration to about 4×10^8 cells per ml. A 5-ml amount of each was mixed and then filtered through a sterile, prewashed membrane filter (47-mm diameter, 0.45-μm-pore diameter; Millipore Corp.). The filter was then transferred aseptically to a fresh, prewarmed L agar plate (bacteria side up) and incubated at 37°C. At the end of the mating period, the filter was transferred to 10 ml of buffered saline and blended vigorously in a Vortex mixer for 15 s. Bacteria were collected, washed once with buffered saline, diluted, and spread on selective media containing 1 mg of streptomycin per ml.

Plasmid DNA isolation and electrophoresis. The method used for plasmid DNA purification and conditions for electrophoresis have been described previously (25).

Bacteria. Strain AB1133 (2) was obtained from our laboratory stocks. *E. coli* K-12 Hfr strains were obtained from the *E. coli* Genetic Stock Center (Yale University) and have been described by Low (21).

Chicken embryo virulence test. The method used was that described by Powell and Finkelstein (26). Bacteria were grown overnight at 37°C on L agar and suspended in sterile phosphate-buffered saline; dilutions into phosphate-buffered saline were made and plated onto L agar for viable counts. Embryonated hen eggs free of *Salmonella pullorum* and antibiotics were obtained from commercial suppliers and incubated in a humidified atmosphere at 37°C until they were 14 days old. After disinfection of the eggs with 95% ethanol, two small holes were made into each with an egg punch; one hole was punched into the air sac, and one was punched into the allantois in an area free of large blood vessels. The allantois of each egg was inoculated with 0.1 ml of a bacterial suspension or with sterile phosphate-buffered saline with a 26-gauge needle and a 1.0-ml tuberculin syringe. Holes were sealed with hot paraffin wax, and eggs were incubated in a humidified atmosphere at 37°C. Eggs were candled to determine viability at 24, 48, 72, and 96 h after inoculation. The allantois of each dead egg was sampled for purity of bacteria by removing, through the same hole used for inoculation, 0.01 ml of the allantois with a sterile capillary pipette. The allantoic fluid was streaked for single colonies onto blood agar. On day 4 after inoculation, live eggs were likewise sampled to confirm bacterial growth within each egg.

Liquid hemolysin assay. Overnight cultures were diluted into L broth containing 40 μg of thymidine per ml, 10 mM $CaCl_2$, and 0.02% glucose and grown with shaking for 2 h at 37°C (optical density at 600 nm, ~0.5). The cultures were then centrifuged, and the supernatants were collected and diluted twofold into Tris-buffered saline (pH 6.8) containing 10 mM $CaCl_2$. Defibrinated whole sheep blood was diluted 1:50 in Tris-buffered saline just before the experiment. The supernatant (0.5 ml) and 2% sheep erythrocytes (0.5 ml) were mixed gently in a glass tube (12 by 75 mm) and incubated for 1 h at 37°C.

RESULTS

Nonhemolytic mutants. An *E. coli* isolate (J96) from a human pyelonephritis infection was chosen for study. This strain produces a diffusible hemolysin easily detectable on sheep blood agar plates. In addition, *E. coli* J96 is colicin V positive and motile and exhibits both D-mannose-sensitive and D-mannose-resistant hemagglutination of erythrocytes. A series of mutations were introduced into this strain to facilitate subsequent genetic analysis (Fig. 1). The choice of auxotrophic requirements was based on the unique sites and well-spaced map positions in *E. coli* K-12 of the mutations (3).

Strain SH14 was treated with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine as described above, and mutants altered in hemolysin expression were identified on sheep blood agar plates. Mutants were found at a frequency of 14 out of 5,250. Several classes of hemolysin-negative (*hly*) derivatives were found. Class I mutants did not grow on MacConkey lactose agar containing bile salts but did grow on other nutrient media; members of this class were also defective in colicin expression. They are thought to be altered in some nonspecific membrane property and have not been studied further. Class II mutants produced no hemolysin but expressed colicin V normally and grew as well as the SH14 parent strain on MacConkey lactose agar. Class III mutants produced smaller zones of hemolysis on sheep blood agar than did strain SH14, expressed colicin V normally, and grew well on MacConkey lactose agar. Similar mutant classes were found among nonhemolytic Hly plasmid derivatives by Noegel et al. (24).

A nonhemolytic class II mutant (SH30) was chosen for further study. This strain was tested with a liquid hemolysin assay to measure levels of hemolysin relative to levels in the hemolytic

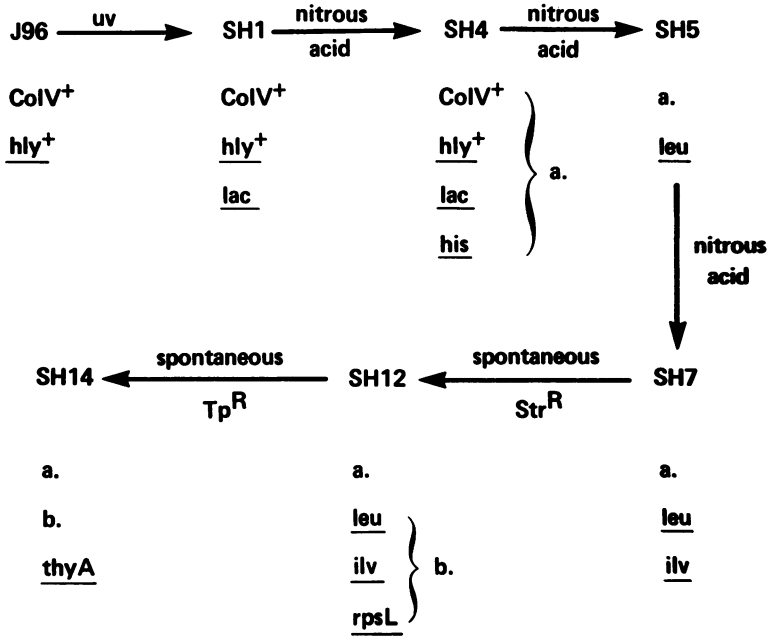

FIG. 1. Pedigree of *E. coli* J96 strain SH14.

parent strain. No hemolytic activity could be detected in undiluted culture supernatants of SH30, whereas a 1:16 dilution of J96 supernatants consistently produced unequivocal hemolysis within 60 min.

Genetic analysis. In initial experiments we tried to mobilize the *hly* gene(s) by introducing the sex plasmid F′ *kan* into *E. coli* J96 and then mating the resulting F^+ derivative with a nonhemolytic recipient. Plasmids which are incapable of self-transfer are often mobilized under these conditions. Of 900 kanamycin-resistant F^+ exconjugates tested, none were hly^+. These results suggested that hemolysin synthesis was not encoded on a plasmid mobilizable by F.

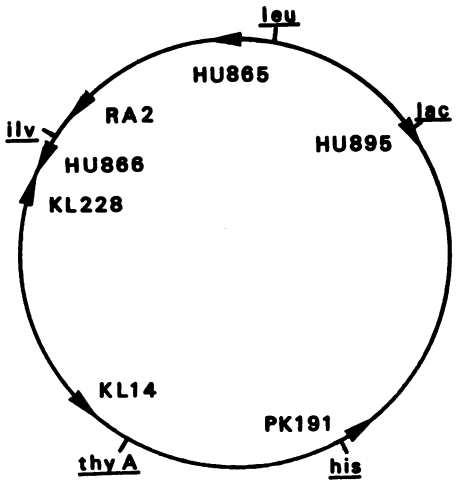

FIG. 2. Relative map positions of genetic markers and origins and directions of transfer of *E. coli* Hfr derivatives.

Linkage between Hly and a known chromosomal marker was shown by bacterial matings with prototrophic Hfr derivatives of *E. coli* J96 as donors and SH30 as the recipient. The appropriate origins and directions of transfer of the donors are shown in Fig. 2. The results of the matings are shown in Table 1. For all three donors, hly^+ recombinants were found at the highest frequency among ilv^+ recombinants. Moreover, when the hemolytic leu^+ recombinants from the mating with HU866 were tested for inheritance of *ilv*, 25 of 26 were ilv^+.

Matings were also done between SH14 and several different *E. coli* K-12 derivatives. Recombinants were selected for inheritance of one of the four auxotrophic markers in SH14 and then scored for loss of hemolysin expression. The Hfr strains used are depicted in Fig. 2. Crosses of this type show where genes adjacent to *hly* map in *E. coli* K-12. The results are shown in Table 2. Once again the *hly* genotype inherited from the donor was found most frequently among ilv^+ recombinants.

Based on the data in Tables 1 and 2, it is apparent that proximal unselected markers not linked to the selected distal marker are often inherited at reduced frequency (less than 50%),

TABLE 1. Isogenic matings with SH30[a]

Donor	Selected marker	No. of recombinants inheriting indicated unselected marker/total no. of recombinants tested				
		leu$^+$	*his*$^+$	*thy*$^+$	*ilv*$^+$	*hly*$^+$
HU865	*leu*$^+$		0/54	0/54	0/54	0/54
	his$^+$	3/50		0/50	0/50	0/50
	thy$^+$	3/50	0/50		0/50	0/50
HU866	*ilv*$^+$	0/54	0/54	0/54		17/54
	leu$^+$		0/40	0/40	29/40	26/40
HU895	*leu*$^+$		NT[c]	NT	NT	0/50
	ilv$^+$	6/47	NT	NT		12/47[b]

[a] Matings were conducted from 60 min at 37°C.
[b] 11/12 *ilv*$^+$ *hly*$^+$ strains were still *leu*.
[c] NT, Not tested.

as compared with the frequency expected in *E. coli* K-12 × *E. coli* K-12 matings. The molecular basis for this difference is unclear. Donor transfer from multiple origins is an unlikely explanation since the gradient of transfer of auxotrophic markers by the Hfr donors is consistent with transfer in one direction from a unique origin. For example, in a mating between HU865 and SH30, the frequency of prototrophic recombinants of each class was as follows: *leu*$^+$, 4×10^{-2}; *ilv*$^+$, 7.5×10^{-4}; and *thy*$^+$, 7.5×10^{-6}. Unusual inheritance has also been noted for intergenic matings between *E. coli* K-12 and *Shigella flexneri* (30) or for *E. coli* K-12 × *E. coli* K-12 matings when certain deletion mutations are present in the recipient strain (8). Although further analysis of this phenomenon and of the observed poor inheritance of distal genes unlinked to the selected marker is beyond the scope of this report, several plausible explanations have been presented by Curtiss in his review of bacterial conjugation (6) and by Falkow et al. (12).

One of the *E. coli* J96 donor strains, HU895, was also mated with the multiauxotrophic *E. coli* strain AB1133. Recombinants were selected for one of several amino acid markers and then scored for hemolysin production. Although mating efficiency between the wild-type donor strain and AB1133 was poor (ca. 10^{-6} recombinants per donor per 2-h mating), several AB1133 *hly*$^+$ derivatives were identified among *argE*$^+$ recombinants. These produced hemolysin at levels equivalent to those the *E. coli* J96 donor. The *argE* locus, at 88 min on the *E. coli* K-12 map, is 5 min from *ilv*; AB1133 is *ilv*$^+$.

The data presented above suggest that genes required for hemolysin production by the urinary tract strain *E. coli* J96 reside on the chromosome near the *ilv* gene cluster rather than on a plasmid. As further confirmation, we examined the plasmid complement of AB1133 and two AB1133 *hly*$^+$ recombinants to see if any plasmids had been transferred from the *E. coli* J96 donor. We also examined the plasmid complement of *E. coli* J96, of the auxotrophic and nonhemolytic mutant strains SH14 and SH30, and of the SH30 *hly*$^+$ recombinant HU887. The results shown in Fig. 3 indicate that *E. coli* J96 contains a single plasmid and that all *hly*$^+$ and *hly* derivatives of *E. coli* J96 retain this plasmid. AB1133 and AB1133 *hly*$^+$ were found to be plasmid free, indicating that the hemolytic recombinant did not receive a plasmid from the *E. coli* J96 donor. These results further suggest that hemolysin in this strain is chromosomally encoded.

Chicken embryo virulence. Previous work in this laboratory has shown that hemolytic *E. coli* derivatives are frequently virulent in the chicken embryo model system (23). It was therefore of interest to determine the contribution of hemolysin production to chicken embryo virulence with the various *hly*$^+$ and *hly* derivatives prepared during this study. Embryonated eggs (14 days old) were injected allantoically with a 0.1-ml

TABLE 2. Intergenic matings with SH14[a]

Donor	Selected marker	No. of recombinants inheriting indicated unselected marker/total number of recombinants tested		
		leu$^+$	*ilv*$^+$	*hly*
RA2	*leu*$^+$		NT	9/64
KL228	*thy*$^+$	NT[b]	0/48	0/48
KL14	*leu*$^+$		7/46	9/46
	ilv$^+$	0/48		18/48
PK191	*ilv*$^+$	0/48		21/48

[a] Matings were conducted for 120 min at 37°C.
[b] NT, Not tested.

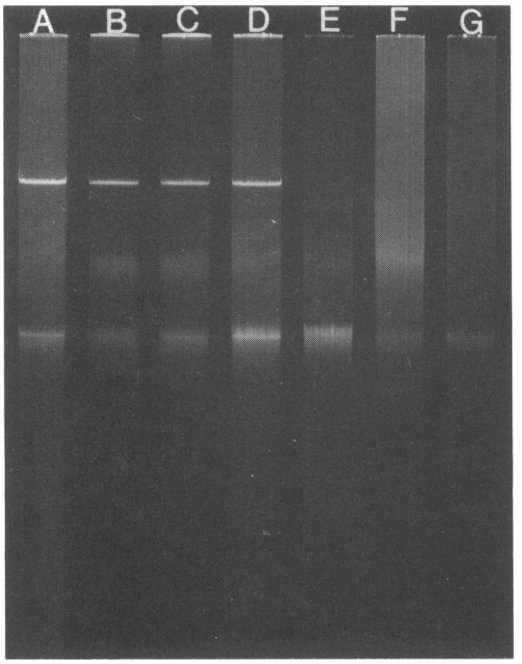

FIG. 3. Separation of DNA species from *E. coli* *hly*$^+$ and *hly* strains by electrophoresis through 0.7% agarose. Lane A, J96; lane B, SH14; lane C, SH30; lane D, HU887; lane E, AB1133; lane F, AB1133 *hly*$^+$ (isolate 1); and lane G, AB1133 *hly*$^+$ (isolate 2).

inoculum of bacteria suspended in phosphate-buffered saline (pH 7.0). Embryos were examined each day for 4 days after injection. The results of tests with several different strains are shown in Table 3. HU887 (*hly*$^+$) and HU888 (*hly*) are homogenic *thy*$^+$ *his*$^+$ *ilv*$^+$ *leu* recombinants of SH30 prepared by sequential matings with HU865 and HU866.

After 24 h, 80% or greater mortality was observed in those eggs inoculated with $<10^3$ colony-forming units (CFU) of the hemolytic derivatives J96, SH14, and HU887 per egg. In contrast, neither the nonhemolytic mutant strain SH30 nor the nonhemolytic recombinant HU888 affected embryo viability even when the inoculum was 10^9 CFU/ml. These results suggest that hemolysin is an important factor in determining the virulence of bacterial isolates in the chicken embryo test.

We also examined the laboratory strain AB1133 and two AB1133 recombinants (HU879 and HU880) produced by crossing AB1133 with the J96 donor strain HU895. When eggs were injected with $<10^9$ CFU no mortality was seen (data not shown); only when inocula of $>10^9$ CFU per egg were used did mortality result. These differences were not due to lack of growth; samples of allantoic fluid from live eggs at 96 h indicated that all strains had grown within the eggs. All strains tested except J96 were multiply auxotrophic, yet no differences in growth within eggs was observed.

DISCUSSION

Synthesis of alpha-hemolysin by clinical *E. coli* isolates has been shown in many instances to be plasmid directed. Other hemolytic *E. coli* isolates (especially those from human extraintestinal infections) wherein the Hly determinant is not transmissible and a chromosomal location for the gene(s) has been postulated have been described. We examined a hemolytic *E. coli* strain isolated from a human urinary tract infection and showed that the genes necessary for hemolysin expression are encoded on the chromosome in the region of the *ilv* gene cluster. Transfer of this region by conjugation from *E. coli* J96 to nonhemolytic strains frequently confers a fully hemolytic phenotype on the recipient. Concurrent transfer of plasmids was not detected.

Attempts at more precise mapping of *hly* were frustrated first by a high level of instability of Hfr derivatives of *E. coli* J96 along with the low efficiency of mating with *E. coli* K-12 recipients and later by our inability to propagate transducing phage P1 on the clinical isolate. Although P1 adsorbs to *E. coli* J96 and lysogens with P1 *cm* CLR100 could be prepared, the burst size upon thermal induction was prohibitively low (0.01 to 0.1).

As an alternate approach, we cloned the genes for hemolysin from *E. coli* J96 (16). Although it

TABLE 3. Virulence of hemolytic *E. coli* for chicken embryos

Strain	Hly	Inoculum (CFU per egg)[a]	Cumulative 96-h mortality (no. of dead eggs)[b]
J96	+	3.6×10^2	10
SH14	+	9×10^1	8
SH30	−	3×10^9	1
HU887	+	2×10^2	10
HU888	−	2×10^9	2
AB1133	−	3×10^9	2
HU879	−	4×10^9	4
HU880	+	3×10^9	5

[a] Although bacterial concentrations from 10^2 to 10^9 were tested for all strains, the results for only the lowest titers which resulted in at least 80% mortality are shown for J96, SH14, and HU887; for all other strains, the results for only the highest titers which could be injected are shown.

[b] A total of 10 eggs were tested with each strain.

is unlikely that the recombinant plasmids will provide us with an improved map position, they will yield information about DNA sequence relationships between the chromosomally encoded and plasmid-encoded hemolysins, perhaps revealing a common transposable element.

The chicken embryo model system was used to compare virulence of the essentially isogenic *hly*$^+$ and *hly* sets of strains prepared during the course of this study. We found that the hemolytic isolates from wild-type *E. coli* were virulent but that virulence was lost in the nonhemolytic mutant. Full virulence was restored in *hly*$^+$ recombinants of the mutants. However, when the ability to produce chromosomally encoded hemolysin was transferred from the wild-type isolate to the otherwise avirulent *E. coli* K-12 strain AB1133, the resulting AB1133 *hly*$^+$ recombinant was not virulent for chicken embryos. The few deaths seen in the eggs injected with very high titers of the nonhemolytic mutant strain SH30 and recombinant HU888 and of the AB1133 recombinants may be the result of endotoxic shock. A similar result was obtained by Minshew et al. (23) when an *E. coli* K-12 strain with a porcine Hly plasmid was tested in this model system. It would therefore seem that factors in addition to hemolysin production, such as surface properties, are necessary for full expression of virulence.

ACKNOWLEDGMENTS

Part of this work was performed at the University of Washington, Seattle, and was supported by Public Health Service grants AI 14740 and AI 18719-01 from the National Institute of Allergy and Infectious Diseases and by a grant (PCM 8015722) from the National Science Foundation. R.A.H. was a Fellow of the National Kidney Foundation. S.I.H. was supported by a Fellowship from the National Science Foundation. B.H.M. was a recipient of Public Health Service Research Career Development award AI 00290 from the National Institutes of Health. Work performed at Baylor College of Medicine was supported by Biomedical Research Support grant 9509 from the National Institutes of Health.

LITERATURE CITED

1. **Adelberg, E. A., M. Mandel, and G. C. Chen.** 1965. Optimal conditions for mutagenesis by N-methyl-N-nitro-N-nitrosoguanidine in *Escherichia coli* K-12. Biochem. Biophys. Res. Commun. **18**:788–795.
2. **Bachmann, B. J.** 1972. Pedigrees of some mutant strains of *Escherichia coli* K-12. Bacteriol. Rev. **36**:525–557.
3. **Bachmann, B. J., K. B. Low, and A. L. Taylor.** 1976. Recalibrated linkage map of *Escherichia coli* K-12. Bacteriol. Rev. **40**:116–167.
4. **Chaturvedi, U. C., A. Mathur, A. M. Khan, and R. M. L. Mehrotra.** 1969. Cytotoxicity of filtrates of hemolytic *Escherichia coli*. J. Med. Microbiol. **2**:211–218.
5. **Cooke, E. M., and S. P. Ewins.** 1975. Properties of strains of *Escherichia coli* isolated from a variety of sources. J. Med. Microbiol. **8**:107–111.
6. **Curtiss, R., III.** 1969. Bacterial conjugation. Annu. Rev. Microbiol. **23**:69–136.
7. **Curtiss, R., III, L. J. Charamella, C. M. Berg, and P. E. Harris.** 1965. Kinetic and genetic analysis of D-cycloserine inhibition and resistance in *Escherichia coli*. J. Bacteriol. **90**:1238–1250.
8. **Curtiss, R., III, L. J. Charamella, D. R. Stallions, and J. A. Mayes.** 1968. Parental functions during conjugation in *Escherichia coli* K-12. J. Bacteriol. **32**:320–348.
9. **Curtiss, R., III, and D. R. Stallions.** 1969. Probability of F integration and frequency of stable Hfr donors in F$^+$ populations of *Escherichia coli* K-12. Genetics **63**:27–38.
10. **de la Cruz, F., J. C. Zabala, and J. M. Ortiz.** 1971. Incompatibility among alpha-hemolytic plasmids studied after inactivation of the hemolysin gene by transposition of TN802. Plasmid **2**:507–519.
11. **Dudgeon, L. S., E. Wordley, and B. Bawtree.** 1921. On *Bacillus coli* infections of the urinary tract, especially in relation to hemolytic organisms. J. Hyg. **20**:137–164.
12. **Falkow, S., H. Schneider, L. S. Baron, and S. B. Formal.** 1963. Virulence of *Escherichia coli-Shigella* genetic hybrids for the guinea pig. J. Bacteriol. **86**:1251–1258.
13. **Fried, F. A., and R. J. Wong.** 1970. Etiology of pyelonephritis: significance of hemolytic *Escherichia coli*. J. Urol. **103**:718–721.
14. **Goebel, W., B. Royer-Pokora, W. Lindenmaier, and H. Bujard.** 1974. Plasmids controlling synthesis of hemolysin in *Escherichia coli*: molecular properties. J. Bacteriol. **118**:964–973.
15. **Hull, R., and E. E. M. Moody.** 1976. Isolation and genetic characterization of *Escherichia coli* K-12 mutations affecting bacteriophage T5 restriction by the ColIb plasmid. J. Bacteriol. **127**:229–236.
16. **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.
17. **Hull, R. A., S. I. Hull, B. H. Minshew, and S. Falkow.** 1980. Genetics of hemolysin of *Escherichia coli*, p. 1283–1285. *In* J. D. Nelson and C. Grass (ed.), Current Chemotherapy and Infectious Disease, American Society for Microbiology, Washington, D.C.
18. **Jorgensen, S. E., E. C. Short, Jr., H. J. Kurtz, H. K. Mussen, and G. K. Wu.** 1976. Studies on the origin of the alpha hemolysin produced by *Escherichia coli*. J. Med. Microbiol. **9**:173–189.
19. **Le Minor, S., and E. LeCoueffic.** 1975. Étude sur les hémolysines des Enterobacteriaceae. Ann. Microbiol. (Paris) **126**:313–332.
20. **Lovell, R., and T. A. Rees.** 1960. A filterable hemolysin from *Escherichia coli*. Nature (London) **188**:755–756.
21. **Low, K. B.** 1972. *Escherichia coli* K12 F-prime factors, old and new. Bacteriol. Rev. **36**:587–607.
22. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
23. **Minshew, B. H., J. Jorgensen, G. W. Counts, and S. Falkow.** 1978. Association of hemolysin production, hemagglutination of human erythrocytes, and virulence for chicken embryos of extraintestinal *Escherichia coli* isolates. Infect. Immun. **20**:50–54.
24. **Noegel, A., U. Rdest, W. Springer, and W. Goebel.** 1979. Plasmid cistrons controlling synthesis and excretion of the exotoxin alpha-hemolysin of *Escherichia coli*. Mol. Gen. Genet. **175**:343–350.
25. **Portnoy, D. A., S. L. Moseley, and S. Falkow.** 1981. Characterization of plasmids and plasmid-associated determinants of *Yersinia enterocolitica* pathogenesis. Infect. Immun. **31**:775–792.
26. **Powell, C. J., Jr., and R. A. Finkelstein.** 1966. Virulence of *Escherichia coli* strains for chick embryos. J. Bacteriol. **91**:1410–1417.
27. **Rennie, R. P., and J. P. Arbuthnott.** 1974. Partial characterization of *Escherichia coli* hemolysin. J. Med. Microbiol. **7**:179–188.
28. **Rennie, R. P., J. H. Freer, and J. P. Arbuthnott.** 1974. The kinetics of erythrocyte lysis by *Escherichia coli* haemolysin. J. Med. Microbiol. **7**:189–195.

29. **Royer-Pokora, B., and W. Goebel.** 1976. Plasmids controlling synthesis of hemolysin in *Escherichia coli*. II. Polynucleotide sequence relationships among hemolytic plasmids. Mol. Gen. Genet. **144**:177–183.
30. **Schneider, H., and S. Falkow.** 1964. Characterization of an Hfr strain of *Shigella flexneri*. J. Bacteriol. **88**:682–689.
31. **Short, E. C., Jr., and H. J. Kurtz.** 1971. Properties of the hemolytic activities of *Escherichia coli*. Infect. Immun. **3**:678–687.
32. **Smith, H., and E. D. Heller.** 1973. The activity of different transfer factors introduced into the same plasmid-containing strain of *Escherichia coli* K-12. J. Gen. Microbiol. **78**:89–99.
33. **Smith, H. W.** 1963. The haemolysins of *Escherichia coli*. J. Pathol. Bacteriol. **85**:197–211.
34. **Smith, H. W., and C. L. Gyles.** 1970. The relationship between different plasmids introduced by F into the same strain of *Escherichia coli* K-12. J. Gen. Microbiol. **62**:277–285.
35. **Smith, H. W., and S. Halls.** 1967. The transmissible nature of the genetic factor in *Escherichia coli* that controls haemolysin production. J. Gen. Microbiol. **47**:153–161.
36. **Smith, H. W., and M. A. Linggood.** 1970. Transfer factors in *Escherichia coli* with particular regard to their incidence in enteropathogenic strains. J. Gen. Microbiol. **62**:287–299.
37. **Smith, H. W., and M. A. Linggood.** 1971. Observations on the pathogenic properties of the K88, Hly and Ent plasmids of *Escherichia coli* with particular reference to porcine diarrhoea. J. Med. Microbiol. **4**:467–485.
38. **Snyder, I. S., and N. A. Koch.** 1966. Production and characteristics of hemolysin of *Escherichia coli*. J. Bacteriol. **91**:763–767.
39. **Snyder, I. S., and P. Zwadyk.** 1969. Some factors affecting production and assay of *Escherichia coli* haemolysins. J. Gen. Microbiol. **55**:139–143.
40. **Walton, J. R., and D. H. Smith.** 1969. New hemolysin (γ) produced by *Escherichia coli*. J. Bacteriol. **98**:304–305.
41. **Williams, P. H.** 1979. Determination of the molecular weight of *Escherichia coli* α-hemolysin. FEMS Microbiol. Lett. **5**:21–24.
42. **Zwadyk, P., Jr., and I. S. Snyder.** 1971. Purification and kinetic studies of the hemolysin from *Escherichia coli*. Can. J. Microbiol. **17**:741–745.