INFECTION AND IMMUNITY, Sept. 1989, p. 2794–2798
0019-9567/89/092794-05$02.00/0

Vol. 57, No. 9

Expression in *Escherichia coli* K-12 of the 76,000-Dalton Iron-Regulated Outer Membrane Protein of *Shigella flexneri* Confers Sensitivity to Cloacin DF13 in the Absence of *Shigella* O Antigen

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Received 3 February 1989/Accepted 25 May 1989

One of the chromosomal segments associated with virulence in *Shigella flexneri* encodes the production of aerobactin and the synthesis of an iron-regulated 76-kilodalton outer membrane protein believed to be the ferric-aerobactin receptor. However, *S. flexneri* expressing this putative aerobactin receptor, which is slightly larger than that encoded by pColV, is insensitive to the killing action of cloacin DF13, a bacteriocin which binds to other aerobactin receptor proteins and kills the cells. In this paper we show that the conjugal transfer of DNA encoding the iron-regulated 76-kilodalton protein from *S. flexneri* to *Escherichia coli* K-12 conferred cloacin DF13 sensitivity on the recipients. However, *E. coli* K-12 which had also inherited genes specifying *Shigella* O-antigen biosynthesis remained cloacin insensitive. The data suggest that it is unwise to use cloacin DF13 sensitivity alone to screen transconjugants or clinical isolates for the expression of aerobactin receptor proteins.

The conjugal transfer of a plasmid and various chromosomal regions from *Shigella flexneri* into *Escherichia coli* K-12 results in a variety of *E. coli* K-12–*Shigella* hybrids, some of which contain the necessary determinants for *Shigella*-like pathogenicity (14). Analysis of these transconjugants has shown that three regions of the *Shigella* chromosome are associated with virulence phenotypes. The regions involved are those linked to the histidine locus (*his*) at 44 min, the arginine-mannitol region (*arg-mtl*) at 74 to 90 min, and the *purE* locus at 12 min (27). The presence of all three chromosomal regions, as well as a 140-megadalton plasmid, which is necessary for epithelial cell invasion, allows full expression of virulence in laboratory models (15, 27).

Previously, we showed that one of the chromosomal segments connected with virulence, the *arg-mtl* region, is associated with genes that code for the production of the hydroxamate siderophore aerobactin and for the synthesis of an iron-regulated 76,000-dalton (76-kilodalton [kDa]) outer membrane protein (11). Enteroinvasive strains of *E. coli* were also shown to produce aerobactin under conditions of iron restriction, as well as a 76-kDa outer membrane protein (11, 20). More recent work has shown that aerobactin production is not required for the intracellular growth of *S. flexneri* nor for mediating the early killing of infected cells (18, 22). However, aerobactin production does provide *S. flexneri* with a selective advantage and may operate as a virulence determinant at the stage of multiplication within tissues, when bacteria lie within the extracellular compartment of the intestinal villus (22). Aerobactin-mediated iron transport systems are known to play an important part in the virulence of *E. coli* strains which cause generalized extraintestinal infections in humans and animals and have been the subject of much interest (10).

The aerobactin receptor protein in *S. flexneri* and enteroinvasive *E. coli* is thought to be the iron-regulated 76-kDa outer membrane protein. However, this putative aerobactin receptor differs from that encoded by the ColV plasmid in being slightly larger (11, 20), the pColV-encoded receptor having a molecular weight of 74,000. Also, Payne et al. (24) reported that *S. flexneri* that expresses the putative aerobactin receptor was insensitive to the action of cloacin DF13. Cloacin DF13 is a bacteriocin that recognizes and binds to the aerobactin receptor in *Enterobacter cloacae* and to the 74-kDa aerobactin receptor protein of *E. coli*(pColV) (33). Whether the differences between the susceptibility of *S. flexneri* and *E. coli*(pColV) to cloacin action reflected differences in the binding of the bacteriocin to the receptor or differences in other steps in the killing process was not clarified. The possibility that the O-antigenic chains of lipopolysaccharide (LPS) could interfere with the lethal action of cloacin DF13 on certain smooth strains of *E. coli* was raised by Lafont et al. (17). In the present study, we characterized further the aerobactin-mediated iron uptake system of *S. flexneri*, paying particular attention to the iron-regulated 76-kDa protein. Results show that this protein does behave like the pColV aerobactin receptor and can bind cloacin DF13 when present on rough *E. coli* K-12–*S. flexneri* hybrids; however, it is masked by the O-antigenic chains of smooth hybrids.

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. Bacterial strains and plasmids used in this study are listed in Tables 1 and 2. Table 2 shows only examples of transconjugants from each mating series exhibiting differences in the phenotypic markers examined. The *E. coli* K-12–*S. flexneri* transconjugants shown in Table 2 were derived after donor transfer from *S. flexneri* 2a strain 256 to recipient *E. coli* K-12 strain 395-1. The parent strains and the procedures

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TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Genotype and relevant characteristic(s)	Source or reference
S. flexneri 2a 256	Donor of chromosomal DNA in *E. coli–Shigella* matings	Sansonetti et al. (27)
E. coli K-12		
395-1	Recipient strain in *E. coli*–Shigella matings	Sansonetti et al. (27)
F205	N3406(pJN73 [CloDF13::Tn*901*]) Ap^r	W. J. A. Krone
W3110	*supE42*	Bachmann (1)
KH576	W3110 *cir* Nal^r (pColV-K30)	K. Hardy
BZB1022	W3110 *cir*	Pugsley (25)
BZB1022(pABN1)	W3110 *cir*(pABN1)	This study
EN307	H1443(pEN7 *aerA iut*$^+$)	Gross et al. (13)
Enteroinvasive *E. coli*		
E11120/0	O124:H−	Griffiths et al. (11)
E15701/0	O164:H−	Griffiths et al. (11)
E12632/0	O124:H30	Griffiths et al. (11)
E12652/0	O164:H−	Griffiths et al. (11)
Plasmids		
pABN1	Ap^r, carries aerobactin biosynthesis and transport genes of pColV-K30	Bindereif and Neilands (2)
pEN7	Ap^r, carries aerobactin receptor gene of pColV-K30. Used to generate a 2-kilobase *iutA* DNA probe	Gross et al. (13)

used in matings have been described previously (27), as have all other transconjugants except 7687-1A, 7687-1B, and 7687-1C, in which selections were made for the transfer of Arg^+ Str^r, Mtl^+ Str^r, and Mtl^+, respectively.

The growth and storage of strains were as described previously (11). Iron-restricted bacteria were obtained by growing the organisms in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) containing one of the following: ovotransferrin (0.5 mg/ml) (11), Desferal (0.5 mM; CIBA-GEIGY Corp.), or ethylenediamine dihydroxyphenylacetic acid (EDDA, 0.004% [wt/vol]). For siderophore production assays, the bacteria were grown either in M56 medium (21) containing EDDA (0.004% [wt/vol]) or in the Tris-buffered medium of Simon and Tessman (28); EDDA was made iron-free by the method of Rogers (26). Specific amino acids, thiamine, and nicotinic acid were added as required (11). Ampicillin (100 μg/ml) was added to all media used for storing and growing plasmid-bearing strains of *E. coli*. L broth was used in place of Trypticase soy broth for determining bacteriocin sensitivity.

Electrophoresis of outer membrane proteins and LPS. Outer membrane proteins were isolated, prepared for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and separated as described before (12). Resolution of the iron-regulated outer membrane proteins was improved by running the electrophoresis for a longer time (11). After electrophoresis, the gels were either stained with Coomassie blue or silver (35) or used for immunoblotting. Immunoblotting was carried out as described by Stevenson and Griffiths (30). Goat anti-rabbit immunoglobulin G (Miles Laboratories, Inc., Elkhart, Ind.) was used as the second antibody.

LPS was isolated as described by Formal et al. (9), except that cultures were grown on Trypticase soy agar in place of meat extract agar. Proteinase K digestion of whole-cell and outer membrane preparations was carried out essentially as described by Chart et al. (5), except that the digestion was performed in SDS-PAGE sample buffer, with the samples being incubated for 10 min at 100°C prior to the addition of the enzyme. LPS was analyzed by SDS-PAGE and detected by silver staining as described previously (5, 31); electrophoresis was carried out at 50 mA.

Susceptibility to cloacin DF13. A crude preparation of cloacin DF13 was obtained from *E. coli* F205 (Table 1) by the method of de Graaf and Klaasen Boor (6); bacteriocin preparations were aliquoted and stored at −20°C. The killing activity of cloacin DF13, expressed in killing units ml^{-1}, was determined by using *E. coli* W3110(pColV-K30) as an indicator strain and was carried out essentially as described by de Graaf et al. (7); *E. coli* W3110 was used as a negative control.

Bacteriocin sensitivity of test strains grown at 37°C under iron-replete or iron-restricted conditions was determined by placing a 5-mm-diameter disk of Whatman 3 MM paper soaked in the cloacin DF13 preparation on a freshly seeded lawn of bacteria. Following overnight growth, any resultant zone of killing was noted and used as an indicator of the sensitivity of the strain to the bacteriocin. The sensitivity of strains to cloacin DF13 was further determined by adding a known number of killing units to bacteria grown to an optical density at 660 nm of about 0.2 at 37°C in 10 ml of L broth or L broth containing EDDA (0.004% [wt/vol]); cultures were contained in 50-ml Erlenmeyer sidearm flasks, and optical density readings were taken at 15-min intervals.

Estimation of siderophore production. Aerobactin production was assayed as described by Griffiths et al. (11).

Preparation of DNA probe and colony hybridization. Plasmid pEN7 DNA was purified by the Sephacryl S-1000 column gel filtration method of Bywater et al. (3). Plasmid DNA was then digested with restriction endonuclease *Bgl*II (Anglian Biotechnology Ltd., Colchester, United Kingdom), and a 2-kilobase *iutA* DNA fragment was separated and purified by the low-melting-point agarose gel elution method of Wieslander (34). The *iutA* gene (iron uptake transport) of pColV-K30 encodes the 74-kDa outer membrane receptor protein for ferric aerobactin. The *iutA* DNA probe was radiolabeled by the random priming technique of Feinberg and Vogelstein (8). Bacterial cells to be probed were grown at 37°C on hybridization filter disks placed on L-agar plates. Cellular DNA was denatured and bound to the hybridization filter (GeneScreen; Dupont (UK) Ltd., Stevenage, Hertfordshire, England) according to instructions of the manufacturer. Prehybridization and hybridization of filters was carried out by the method of Singh and Jones (29).

Preparation of antiserum against the 74-kDa aerobactin receptor protein. The antigen used was prepared from outer membranes of *E. coli* BZB102(pABNI) grown in Trypticase soy broth containing Desferal (0.5 mM). Benzamidine (Sigma Chemical Co. Ltd.) at a final concentration of 10 mM was added to all the reagents used in the isolation of the outer membranes (16). The 74-kDa protein was isolated by preparative SDS-PAGE essentially as described by Chart and Griffiths (4). Membranes were solubilized by heating at 66°C for 30 min in sample buffer (at the indicated final concentrations) (Tris hydrochloride buffer [0.06 M, pH 6.8],

TABLE 2. *E. coli* K-12–*S. flexneri* transconjugants: *S. flexneri* loci inherited and relevant characteristics[a]

Strain[b]	*arg*	*rha*	*tnaA*	Clo	76K	*iut*	Aer	*mtl*	*his*	LPS
443-2-1E	−	−	−	I	+	+	+	+	+	+
7306-1-2	−	−	+	I	+	+	+	+	+	+
7306-1-8E	−	+	+	I	+	+	+	+	+	+
7185-2-2	+	+	+	I	+	+	+	+	+	+
7300-1-5	+	+	+	I	+	+	+	+	+	+
7687-1A-2	+	−	−	I	−	−	−	−	−	−
7687-1A-3	+	+	−	I	−	−	−	−	−	−
7687-1A-6	+	+	+	S	+	+	−	−	−	−
7687-1A-15	+	+	+	S	+	+	−	+	−	−
7687-1A-7	+	+	+	S	+	+	+	+	−	−
7312-1-18	+	+	+	S	+	+	+	+	−	−
7687-1C-1	−	+	+	S	+	+	+	+	−	−
7687-1C-3	−	−	+	S	+	+	+	+	−	−
7687-1B-3	−	−	+	S	+	+	+	+	−	−
7687-1B-2	−	−	−	I	−	−	−	+	−	−

[a] *S. flexneri* loci inherited by recipient *E. coli* K-12: *arg*, arginine; *rha*, rhamnose; *tnaA*, tryptophanase; *mtl*, mannitol; *his*, histidine. Clo, Transconjugants sensitive (S) or insensitive (I) to killing by cloacin DF13; 76K, presence (+) or absence (−) of the 76-kDa outer membrane protein observed on SDS-PAGE; *iut*, positive (+) or negative (−) signal on colony hybridization, using *iutA* gene probe; Aer, aerobactin production; LPS, presence (+) or absence (−) of O polysaccharide as indicated by SDS-PAGE and silver staining.

[b] Fifty strains were examined, but only examples of those exhibiting differences in the phenotypes studied are shown. The number of strains tested and found to be exhibiting properties identical with those of the indicated strains were as follows (number of strains in parentheses): 7300-1-5 (2); 7687-1A-2 (5); 7687-1A-3 (6); 7687-1A-7 (2); 7687-1C-1 (10); 7687-1C-3 (5); 7687-1B-3 (7); 7687-1B-2 (6).

SDS [2%, wt/vol], glycerol [10%, vol/vol], bromophenol blue [0.001%, wt/vol]). The mobility of the 74-kDa protein was not altered by the solubilization conditions, although antibodies could not be raised against protein solubilized at 100°C for 5 min. Outer membrane protein preparations (0.8- to 1-mg amounts) were applied to a single well extending the whole width of the gel, and electrophoresis was carried out for an extended period of time. SDS-PAGE of the isolated protein preparations showed only a single 74-kDa band when the gel was stained with Coomassie blue. However, on silver staining, traces of breakdown products were visible (data not shown). The antiserum was raised in a female Dutch rabbit by administering three injections of receptor protein, given subcutaneously in Freund incomplete adjuvant at intervals of 3 weeks. Thirteen days after the third injection the rabbit was bled and the serum was prepared in the usual way. Material obtained from three gels was used for each injection.

RESULTS

Sensitivity of *S. flexneri*, enteroinvasive *E. coli*, and *E. coli* K-12–*S. flexneri* transconjugants to cloacin DF13. *S. flexneri* and the four enteroinvasive strains of *E. coli* tested in our study (two serotype O124 and two serotype 0164) were insensitive to the action of cloacin DF13, even when grown under iron-restricted conditions and when expressing the 76-kDa outer membrane protein. However, the conjugal transfer of *Shigella* chromosomal sequences encoding the 76-kDa protein to *E. coli* K-12 was found to confer cloacin DF13 sensitivity on a large number of the recipients: 27 of the 50 transconjugants examined were sensitive to cloacin (examples are shown in Table 2). Some degree of cloacin DF13 sensitivity was found in the iron-replete transconjugants, but this increased markedly under iron-restricted growth conditions (data not shown). The presence or absence of the 76-kDa protein in the *E. coli* K-12–*S. flexneri* hybrids was confirmed by SDS-PAGE in each case; all cloacin DF13-sensitive transconjugants expressed this protein (Table 2).

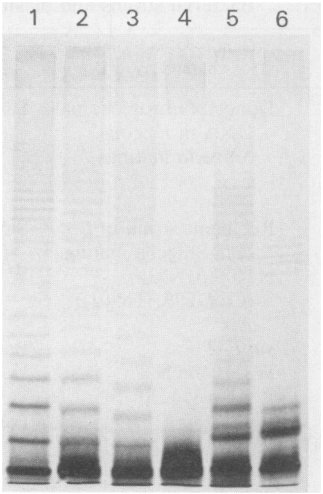

FIG. 1. SDS-PAGE profiles, stained with silver, of extracted LPS (3 μg per lane) from the indicated strains. Lane 1, *S. flexneri* 256; lanes 2 to 4, *E. coli* K-12–*S. flexneri* transconjugant strains 7300-1-5, 7185-2-2, and 7312-1-18, respectively; lanes 5 and 6, enteroinvasive strains of *E. coli* O164 (E12652/0) and O124 (E12632/0), respectively.

However, some of the *E. coli* K-12 transconjugants which expressed the 76-kDa protein were insensitive to cloacin DF13 (Table 2). All such insensitive transconjugants had also inherited the *his* locus of *S. flexneri*, a region known to be linked to the genes encoding the *Shigella* O antigen (Table 2) (27). The expression of O antigen by cloacin DF13-insensitive transconjugants was confirmed by SDS-PAGE and silver staining (see, for example, Fig. 1). Interestingly, these profiles showed minor variations in migration patterns between different strains. None of the profiles from the transconjugants examined was identical to the LPS profile exhibited by the *S. flexneri* parent strain, a point which has been noted previously (27). The enteroinvasive strains of *E. coli* also exhibited LPS patterns that differed from those of *S. flexneri* (Fig. 1). The transconjugants which were sensitive to the action of cloacin DF13 were found not to possess O-antigen chains, as judged by the absence of an LPS ladder profile. An example of such a strain is shown in Fig. 1, lane 4.

In addition to conferring cloacin DF13 sensitivity on *E. coli* K-12–*S. flexneri* transconjugants, the iron-regulated 76-kDa outer membrane protein reacted on immunoblotting with an antiserum raised in a rabbit to the purified 74-kDa iron-regulated aerobactin receptor protein encoded by pColV-K30; the results for two hybrid strains, 7687-1B-3, which expressed the 76-kDa protein, and 7687-1A-2, which did not, are shown in Fig. 2. The antiserum also reacted with the 76-kDa protein of enteroinvasive *E. coli* (Fig. 2). The distortion of the band in lane 2, together with its slightly reduced mobility compared with that in lane 3, was likely due to the presence in the outer membrane protein preparation of high-molecular-weight LPS. Attempts to raise an antiserum to the purified 76-kDa receptor protein itself have not yet been successful.

Screening transconjugants by using a combination of tech-

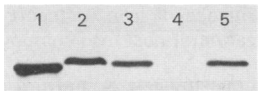

FIG. 2. Immunoblot showing the reaction of polyclonal antiserum raised to the isolated 74-kDa aerobactin receptor protein (20 μl per lane) with the separated outer membrane proteins (approximately 30 μg per lane) of the indicated strains. Lane 1, *E. coli* BZB1022(pABN1); lane 2, *S. flexneri* 256; lanes 3 and 4, *E. coli* K-12–*S. flexneri* transconjugant strains 7687-1B-3 and 7687-1A-2, respectively; lane 5, enteroinvasive *E. coli* O164 (E12652/0). The organisms were grown in Trypticase soy broth containing Desferal. Electrophoresis was carried out for an extended period of time, and only the region of the gel containing the iron-regulated outer membrane proteins is shown.

niques. Having established that the iron-regulated 76-kDa outer membrane protein cross-reacted immunologically with an antiserum raised to the 74-kDa ColV-encoded aerobactin receptor protein and functioned in *E. coli* K-12 in the absence of *Shigella* O antigen as a receptor for cloacin DF13, we proceeded to examine the ability of different analytical techniques to provide information about the various transconjugants. This analysis involved SDS-PAGE, assay for aerobactin production, cloacin DF13 sensitivity, and colony hybridization analysis using a *Bgl*II *iutA* gene probe derived from pColV-K30, which encodes the 74-kDa aerobactin receptor protein. The use of the *iutA* gene probe confirmed in each case the transfer of similar sequences to *E. coli* K-12–*S. flexneri* hybrids expressing the iron-regulated 76-kDa outer membrane protein. The results (Table 2) showed that expression of the 76-kDa protein, cloacin DF13 sensitivity, and a positive signal in colony hybridization analysis were characteristics preferentially coinherited with the genes for aerobactin biosynthesis and the *tnaA* locus. With the exception of strain 443-2-1E, all transconjugants which had inherited the *mtl* locus (81 min) but not the *tnaA* locus (83 min) failed to produce a 76-kDa outer membrane protein under conditions of iron restriction. These transconjugants were insensitive to cloacin DF13 and gave a negative result in the colony hybridization assay. Transconjugant 443-2-1E, although insensitive to cloacin DF13, was nevertheless shown to hybridize with the *iut* gene probe, to express a 76-kDa protein, and to synthesize aerobactin; this strain was insensitive to the action of cloacin DF13 due to the inheritance of the *his*-associated LPS locus from *S. flexneri*. In contrast, the cloacin DF13-sensitive transconjugant 7687-1A-6, which had inherited the *tnaA* locus but not the *mtl* locus, was found to be unable to produce aerobactin, although it had inherited the gene encoding the 76-kDa protein. Generally, strains which had inherited both the *tnaA* and *mtl* loci but not the *his* locus (44 min) were cloacin DF13 sensitive and produced aerobactin. Strain 7687-1A-15 was exceptional in that it had inherited the *tnaA* and *mtl* loci, expressed the 76-kDa protein, and was cloacin DF13 sensitive but was unable to produce aerobactin. Since positive selection was not made for the *mtl* locus, failure to incorporate large tracts of nonhomologous sequences into the recipient *E. coli* genome may have occurred, leading to the transfer of nonhomologous sequences encoding only the 76-kDa outer membrane protein.

DISCUSSION

This report presents the following four lines of evidence which strengthen the view that the iron-regulated 76-kDa outer membrane protein of *S. flexneri* is indeed the receptor for aerobactin: (i) like the aerobactin receptor of pColV, the protein functions as a receptor for cloacin DF13; (ii) it reacts on immunoblotting with an antibody raised to the purified 74-kDa aerobactin receptor encoded by pColV-K30; (iii) strains that express it contain sequences which hybridize with a gene probe obtained from the region encoding the aerobactin receptor protein of the ColV plasmid; (iv) these sequences are closely linked to other genes that specify the synthesis of a hydroxamate siderophore chemically characterized previously as aerobactin (11). The results show that the gene encoding the 76-kDa iron-regulated protein and those encoding aerobactin synthesis are preferentially cotransferred with the *tnaA* locus rather than with the *mtl* locus.

This work also shows that the presence of LPS O-antigenic chains interferes with the lethal action of cloacin DF13 on organisms expressing the 76-kDa protein; this most probably occurs by preventing access of the cloacin to the outer membrane receptor. The shielding of *E. coli* against the action of other bacteriocins by the O-antigenic chains of LPS has also been reported recently (32).

These results have important implications for the use of cloacin DF13 in the screening of clinical isolates of *E. coli* or *Shigella* or *Klebsiella* species for the presence of aerobactin receptor proteins. The screening of strains for susceptibility to cloacin DF13 has been used on several occasions recently to assess the expression of an aerobactin receptor (17, 20, 23). Clearly there is a need for caution in interpreting the results of such studies since most wild isolates of the family *Enterobacteriaceae* synthesize smooth, O antigen-carrying LPS which may shield the receptor and prevent binding of the cloacin DF13. Likewise, if susceptibility to the action of cloacin DF13 is the only test employed, the transfer of the ability to synthesize the O-antigenic chain of LPS from one organism to another could mask the cotransfer of genes encoding the synthesis of an aerobactin receptor protein, as found in the present study.

However, not all strains which produce smooth LPS are insensitive to the action of cloacin DF13. The enteroinvasive strains of *E. coli* studied by Marolda et al. (20), in contrast to those examined in this study, were reported to be sensitive to cloacin DF13, as were the *Klebsiella pneumoniae* strains examined by Nassif and Sansonetti (23) and some of the avian strains of *E. coli* studied by Lafont et al. (17), although several degrees of cloacin DF13 susceptibility were noted by the last group of investigators. Thus, it may be the nature of the O-antigenic chain present that is important in blocking cloacin action. It is also possible that, although the relevant structural gene for the aerobactin receptor is present, it may not be expressed. Linggood et al. (19) found that several *E. coli* strains of animal origin which were positive in colony hybridization tests for aerobactin genetic determinants were nevertheless negative in tests for siderophore synthesis. Thus, for the reasons given above, we believe that it is unwise to use cloacin DF13 sensitivity alone to screen organisms for the presence of an aerobactin receptor. Such studies need to be complemented by colony hybridization analysis using a gene probe specific for the *iutA* gene, as well as by examination of the outer membrane proteins by SDS-PAGE and immunoblotting using an antibody raised to an aerobactin receptor.

ACKNOWLEDGMENTS

We thank K. Hantke, W. J. Krone, and A. P. Pugsley for some of the bacterial strains and plasmids used in this study.

LITERATURE CITED

1. **Bachmann, B. J.** 1972. Pedigrees of some mutant strains of *Escherichia coli* K-12. Bacteriol. Rev. **36**:525–557.
2. **Bindereif, A., and J. B. Neilands.** 1983. Cloning of the aerobactin-mediated iron assimilation system of plasmid ColV. J. Bacteriol. **153**:1111–1113.
3. **Bywater, M., R. Bywater, and L. Hellmann.** 1983. A novel chromatographic procedure for purification of bacterial plasmids. Anal. Biochem. **132**:219–224.
4. **Chart, H., and E. Griffiths.** 1985. Antigenic and molecular homology of the ferric-enterobactin receptor protein of *Escherichia coli*. J. Gen. Microbiol. **131**:1503–1509.
5. **Chart, H., D. H. Shaw, E. E. Ishiguro, and T. J. Trust.** 1984. Structural and immunochemical homogeneity of *Aeromonas salmonicida* lipopolysaccharide. J. Bacteriol. **158**:16–22.
6. **de Graaf, F. K., and P. Klaasen Bloor.** 1977. Purification and characterization of a complex between cloacin and its immunity protein isolated from *Enterobacter cloacae* (Clo DF13): dissociation and reconstitution of the complex. Eur. J. Biochem. **73**:107–114.
7. **de Graaf, F. K., E. A. Spanjaert Speckman, and A. H. Stouthamer.** 1969. Mode of action of a bacteriocin produced by *Enterobacter cloacae* DF13. Antonie van Leeuwenhoek J. Microbiol. **35**:287–306.
8. **Feinberg, A. P., and B. Vogelstein.** 1984. A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. **137**:266–267.
9. **Formal, S. B., P. Gemski, Jr., L. S. Baron, and E. H. Labrec.** 1970. Genetic transfer of *Shigella flexneri* antigens to *Escherichia coli* K-12. Infect. Immun. **1**:279–287.
10. **Griffiths, E.** 1987. The iron-uptake systems of pathogenic bacteria, p. 69–137. *In* J. J. Bullen and E. Griffiths (ed.), Iron and infection: molecular, physiological and clinical aspects. John Wiley & Sons, Chichester, England.
11. **Griffiths, E., P. Stevenson, T. L. Hale, and S. B. Formal.** 1985. Synthesis of aerobactin and a 76,000-dalton iron-regulated outer membrane protein by *Escherichia coli* K-12-*Shigella flexneri* hybrids and by enteroinvasive strains of *Escherichia coli*. Infect. Immun. **49**:67–71.
12. **Griffiths, E., P. Stevenson, and P. Joyce.** 1983. Pathogenic *Escherichia coli* express new outer membrane proteins when growing *in vivo*. FEMS Microbiol. Lett. **16**:95–99.
13. **Gross, R., F. Engelbrecht, and V. Braun.** 1985. Identification of the genes and their polypeptide products responsible for aerobactin synthesis by pColV plasmids. Mol. Gen. Genet. **201**: 204–212.
14. **Hale, T. L., and S. B. Formal.** 1986. Genetics of virulence of Shigella. Microb. Pathog. **1**:511–518.
15. **Hale, T. L., P. J. Sansonetti, P. A. Schad, S. Austin, and S. B. Formal.** 1983. Characterization of virulence plasmids and plasmid-associated outer membrane proteins in *Shigella flexneri*, *Shigella sonnei*, and *Escherichia coli*. Infect. Immun. **40**:340–350.
16. **Hollifield, W. C., Jr., E. H. Fiss, and J. B. Neilands.** 1978. Modification of a ferric enterobactin receptor protein from the outer membrane of *Escherichia coli*. Biochem. Biophys. Res. Commun. **83**:739–746.
17. **Lafont, J. P., M. Dho, H. M. D'Hauteville, A. Bree, and P. J. Sansonetti.** 1987. Presence and expression of aerobactin genes in virulent avian strains of *Escherichia coli*. Infect. Immun. **55**: 193–197.
18. **Lawlor, K. M., P. A. Daskaleros, R. E. Robinson, and S. M. Payne.** 1987. Virulence of iron transport mutants of *Shigella flexneri* and utilization of host iron compounds. Infect. Immun. **55**:594–599.
19. **Linggood, M. A., M. Roberts, S. Ford, S. H. Parry, and P. H. Williams.** 1987. Incidence of the aerobactin iron uptake system among *Escherichia coli* isolates from infections of farm animals. J. Gen Microbiol. **133**:835–842.
20. **Marolda, C. L., M. A. Valvano, K. M. Lawlor, S. M. Payne, and J. H. Crosa.** 1987. Flanking and internal regions of chromosomal genes mediating aerobactin iron uptake systems in enteroinvasive *Escherichia coli* and *Shigella flexneri*. J. Gen. Microbiol. **133**:2269–2278.
21. **Monod, J., G. Cohen-Bajire, and M. Cohn.** 1951. Sur la biosynthese de la β-galactosidase (lactase) chez *Escherichia coli*. La specificite de l'induction. Biochim. Biophys. Acta **1**:585–599.
22. **Nassif, X., M.-C. Mazert, J. Mounier, and P. J. Sansonetti.** 1987. Evaluation with an *iuc*::Tn*10* mutant of the role of aerobactin production in the virulence of *Shigella flexneri*. Infect. Immun. **55**:1963–1969.
23. **Nassif, X., and P. J. Sansonetti.** 1986. Correlation of the virulence of *Klebsiella pneumonia* K1 and K2 with the presence of a plasmid encoding aerobactin. Infect. Immun. **54**:603–608.
24. **Payne, S. M., D. W. Niesel, S. S. Peixotto, and K. M. Lawlor.** 1983. Expression of hydroxamate and phenolate siderophores by *Shigella flexneri*. J. Bacteriol. **155**:949–955.
25. **Pugsley, A. P.** 1985. *Escherichia coli* K12 strains for use in the identification and characterization of colicins. J. Gen. Microbiol. **131**:369–376.
26. **Rogers, H. J.** 1973. Iron-binding catechols and virulence in *Escherichia coli*. Infect. Immun. **7**:445–456.
27. **Sansonetti, P. J., T. L. Hale, G. J. Dammin, C. Kapfer, H. H. Collins, Jr., and S. B. Formal.** 1983. Alterations in the pathogenicity of *Escherichia coli* K-12 after transfer of plasmid and chromosomal genes from *Shigella flexneri*. Infect. Immun. **39**:1392–1402.
28. **Simon, E. H., and I. Tessman.** 1963. Thymidine-requiring mutants of phage T4. Proc. Natl. Acad. Sci. USA **50**:526–532.
29. **Singh, L., and K. W. Jones.** 1984. The use of heparin as a simple cost-effective means of controlling background in nucleic acid hybridization procedures. Nucleic Acids Res. **12**:5627–5638.
30. **Stevenson, P., and E. Griffiths.** 1985. Detection of antibodies and antigens by immunoblotting, p. 457–464. *In* M. Sussman (ed.), The virulence of *Escherichia coli*. Society for General Microbiology special publication no. 13. Society for General Microbiology, London.
31. **Tsai, C. M., and C. E. Frasch.** 1982. A sensitive silver stain for detecting lipopolysaccharide in polyacrylamide gels. Anal. Biochem. **119**:115–119.
32. **van der Ley, P., P. de Graaff, and J. Tommassen.** 1986. Shielding of *Escherichia coli* outer membrane proteins as receptors for bacteriophages and colicins by O-antigenic chains of lipopolysaccharide. J. Bacteriol. **168**:449–451.
33. **Van Tiel-Menkveld, G. J., J. M. Mentjox-Vervuurt, B. Oudega, and F. K. de Graaf.** 1982. Siderophore production by *Enterobacter cloacae* and a common receptor protein for the uptake of aerobactin and cloacin DF13. J. Bacteriol. **150**:490–497.
34. **Wieslander, L.** 1979. A simple method to recover intact high molecular weight RNA and DNA after electrophoretic separation in low gelling temperature agarose gels. Anal. Biochem. **98**:305–309.
35. **Wray, W., T. Boulikas, P. Wray, and A. Hancock.** 1981. Silver staining of proteins in polyacrylamide gels. Anal. Biochem. **118**:197–203.