JOURNAL OF BACTERIOLOGY, May 1983, p. 980–983
0021-9193/83/050980-04$02.00/0

Vol. 154, No. 2

ST:LT:CFA/II Plasmids in Enterotoxigenic *Escherichia coli* Belonging to Serogroups O6, O8, O80, O85, and O139

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Colonization factor antigen II-producing enterotoxigenic *Escherichia coli* serotypes possessed at least one large plasmid. Loss of colonization factor antigen II correlated with either complete or partial loss of the large plasmid. Complete loss of the plasmid always correlated with complete loss of enterotoxin production. Three of five deletion events also correlated with the loss of toxin production.

Enterotoxigenic *Escherichia coli* (ETEC) is a common cause of acute diarrhea in adults (6, 7, 9) and children (1, 12, 18). These microorganisms usually produce a heat-stable enterotoxin (ST) or a heat-labile enterotoxin (LT) or both (1, 5, 9, 12, 18). Colonization of the small intestine by ETEC is favored by the presence of external fimbrial proteins called colonization factor antigens (CFA), which mediate adherence of ETEC to the epithelial cells of the small intestine. Two antigenically different CFAs have been found on clinical isolates of ETEC from humans (6, 10). CFA/I is found predominantly in serogroups O25, O63, O78, and O128 (15, 17, 19), and CFA/II is found in serogroups O6, O8, O80, and O85 (6).

The production of enterotoxins and CFA have been shown in several instances to be determined by plasmids (5, 15–17, 19). The genes responsible for the production of CFA/I are generally present with the ST genes in a high-molecular-weight plasmid (ca. 60×10^6), whereas the LT genes are found in a separate plasmid (5, 15, 17, 19, 20). We have reported previously that the genes responsible for the production of CFA/II, ST, and LT are present in a single plasmid isolated from the CFA/II-producing strain PB-176, serotype O6:H16 (16). We have also observed that the loss of a single plasmid is accompanied by the loss of the three characteristics (ST, LT, and CFA/II) in two other ETEC strains of different serotypes (16). In the present work, we studied 23 CFA/II-producing strains of ETEC representing various well-recognized serotypes to determine the relationship between CFA/II, enterotoxin production, and plasmid content in these strains.

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The 23 CFA/II-producing strains of ETEC were isolated from individuals with diarrhea from five different geographical locations (Table 1). The strains were serotyped as described elsewhere (6) and stored in peptone slants at room temperature until further analysis. *E. coli* strains were analyzed for the production of ST by the suckling mouse assay (3). LT production was tested by the Y-1 adrenal cell tissue culture assay (4) as previously described (6). The presence of CFA/II fimbriae on ETEC strains was determined by hemagglutination with bovine erythrocytes in the presence of 1% mannose and by the hemagglutination typing assay previously described by Evans et al. (11). CFA/II production was confirmed by slide agglutination and by immunodiffusion with anti-CFA/II serum (6). After several months in storage, each strain was streaked on CFA agar (8) for isolation of single colonies. Those colonies that failed to agglutinate bovine and chicken erythrocytes and were also negative by slide agglutination with anti-CFA/II serum were considered CFA/II-negative derivatives and identified with a P after the original strain number (Table 2).

For plasmid DNA analysis, cleared lysates of each strain were prepared by the method of Hansen and Olsen (13), and the DNA samples were electrophoresed in agarose slab gels. The molecular weight of each plasmid was determined by electrophoretic mobility relative to plasmids of known molecular weight (14). For isolation and further purification of large quantities of plasmid DNA, 1 liter of cultured cells were lysed with a final concentration of 4% sodium dodecyl sulfate (13). The cleared lysate was further purified by phenol extractions and ethanol precipitation. Plasmid DNA was separated from chromosomal DNA by equilibrium

TABLE 1. Plasmid content of ST$^+$:LT$^+$:CFA/II$^+$ ETEC

Strain	Origin	Serotype	Plasmids (10^6 daltons)
PB-176	Mexico	O6:H16	60, 48, 10, 1.9
TD-415C	Mexico	O6:H16	94, 49, 25
TD-219C	Mexico	O6:H16	78, 54
TD-260C	Mexico	O6:H16	69
TD-407E	Mexico	O6:H16	56, 41, 36, 1.9
H-22743	Bangladesh	O6:H16	56, 4.0
24BP-3	Houston, Tex.	O6:H16	78, 45, 2.0
CL-9620	Crater Lake, Ore.	O6:H16	56, 24, 8, 7, 6.5, 3.0
TR-428-10	Brazil[a]	O6:H16	57, 42, 30, 22, 3.8
PB-166E	Mexico	O6:H$^-$	92, 37, 3.0
GV-50B	Virginia	O6:H$^-$	65, 47, 34, 1.6
RSJB-10	Japan	O6:H$^-$	60, 46
SM-1	Houston, Tex.	O6:H$^-$	65, 42, 25, 5.0
H-15697-3	Bangladesh	O8:H$^-$	60, 42
H-10400	Bangladesh	O8:H$^-$	70, 43, 1.6
H-18646	Bangladesh	O8:H$^-$	54, 37, 9, 1.6
H-19796[b]	Bangladesh	O8:H9	35, 33
H-15862-29	Bangladesh	O8:H9	50, 3.0
H-16160-2	Bangladesh	O8:H9	60, 37
PB-122B	Mexico	O80:H9	78, 54, 3.0
24BP-42	Houston, Tex.	O80:49	68, 34
H-19935	Bangladesh	O85:H7	78, 19.5
B-16-4	Brazil[a]	O139:H28	57, 30, 22

[a] Kindly supplied by L. R. Trabulsi and M. H. L. Reis.
[b] Nontoxigenic (CFA/II$^+$ ST$^-$ LT$^-$).

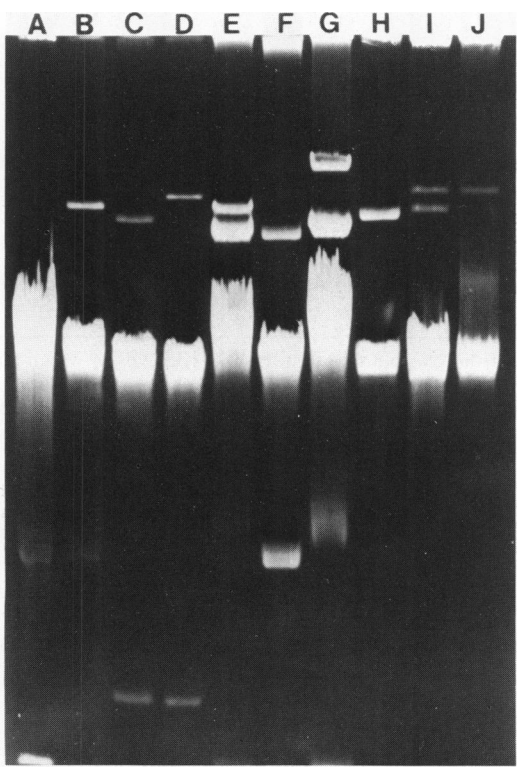

FIG. 1. Slab gel electrophoresis of plasmid DNA from CFA/II-producing ETEC strains and their negative derivatives (*P* strains). The clear lysates were prepared by the method of Hansen and Olsen (13) and run in a horizontal gel of 0.7% agarose in Tris-borate buffer (89 mM Tris, 89 mM boric acid, and 2.5 mM disodium EDTA) for 8 h at 150 V. Lane A, strain H-15862-29-P (ST$^-$ LT$^-$ CFA/II$^-$); lane B, H-15862-29 (ST$^+$ LT$^+$ CFA/II$^+$); lane C, H-22743-P (ST$^+$ LT$^+$ CFA/II$^-$); lane D, H-22743 (ST$^+$ LT$^+$ CFA/II$^+$); lane E, PB-166E-P2 (ST$^-$ LT$^-$ CFA/II$^-$); lane F, PB-166-P1 (ST$^-$ LT$^-$ CFA/II$^-$); lane G, PB-166E (ST$^+$ LT$^+$ CFA/II$^+$); lane H, PB-176-P1 (ST$^-$ LT$^-$ CFA/II$^-$); lane I, PB-176 (ST$^+$ LT$^+$ CFA/II$^+$); and lane J, *E. coli* K-12 RR-1 (ST$^+$ LT$^+$ CFA/II$^+$) transformed with the ST:LT:CFA/II plasmid from strain PB-176.

density centrifugation in a cesium chloride-ethidium bromide gradient (2).

The 23 strains studied belong to seven of the serotypes described for CFA/II-producing strains; the serotype of each strain is given in Table 1. The majority of the strains belong to four of the serotypes most commonly found, including O6:H16 (39%), O6:H$^-$ (17%), O8:H9 (13%), and O8:H$^-$ (13%). A total of 22 of the CFA/II-producing strains analyzed were ST and LT positive. Only one strain (H-19796) was negative for enterotoxin in the standard assays (Table 1).

The majority of the strains had from two to four plasmids, but some strains had up to five or six plasmids (Table 1). None of the strains seemed to have plasmids of common size. Even strains of the same serotype isolated in the same geographical area showed a totally different plasmid pattern.

TABLE 2. Plasmid content of CFA/II-negative derivative strains of ETEC

Strain	Serotype	CFA/II	ST	LT	Plasmids (10^6 daltons)[a]
PB-176	O6:H16	+	+	+	60, 48, 10, 1.9
PB-176-P1	O6:H16	−	−	−	48, 10, 1.9
PB-176-P2	O6:H16	−	+	+	(35) 48, 10, 1.9
TD-415C	O6:H16	+	+	+	94, 49
TD-415C-P	O6:H16	−	−	−	(25) 49
PB-407E	O6:H16	+	+	+	56, 41, 36, 1.9
PB-407E-P	O6:H16	−	−	−	41, 36, 1.9
H-22743	O6:H16	+	+	+	56, 4.0
H-22743-P	O6:H16	−	+	+	(37) 4.0
SM-1	O6:H$^-$	+	+	+	65, 42, 25, 5
SM-1-P	O6:H$^-$	−	−	−	42, 25, 5
GV-50B	O6:H$^-$	+	+	+	65, 47, 34, 1.6
GV-50B-P	O6:H$^-$	−	−	−	47, 34, 1.6
PB-166E	O6:H$^-$	+	+	+	92, 37, 3.0
PB-166E-P1	O6:H$^-$	−	−	−	37, 3.0
PB-166E-P2	O6:H$^-$	−	−	−	(48) 37, 3.0
H-19796	O8:H9	+	−	−	35, 33
H-19796-P	O8:H9	−	−	−	35, (20)
H-15862-29	O8:H9	+	+	+	50, 3.0
H-15862-29-P	O8:H9	−	−	−	3.0
H-16160-2	O8:H9	+	+	+	60, 37
H-16160-2-P	O8:H9	−	−	−	37
H-10400	O8:H$^-$	+	+	+	70, 43, 1.6
H-10400-P	O8:H$^-$	−	−	−	43, 1.6
H-18646	O8:H$^-$	+	+	+	54, 37, 9, 1.6
H-18646-P	O8:H$^-$	−	−	−	37, 9, 1.6
PB-122B	O80:H9	+	+	+	78, 54, 3.0
PB-122B-P	O80:H9	−	−	−	54, 3.0

[a] The number in parentheses corresponds to the size of the plasmid found in the P derivatives in which an apparent deletion of the original plasmid was found.

Of the 23 strains studied, 13 spontaneously lost the ability to produce CFA/II. The CFA/II-negative derivatives were retested for ST and LT production and for plasmid content. Strain H-19796 was nontoxigenic when isolated, as was its P derivative. Ten of the CFA/II-negative derivatives (P strains) were also negative for ST and LT production and had lost one plasmid (Table 2). In all 10 derivatives, the plasmid missing was the one with the greater molecular weight (Table 2), and these molecular weights ranged from 54×10^6 to 90×10^6. With five strains, the P derivative did not show complete loss of the CFA/II plasmid but appeared to have undergone a deletion resulting in a lower-molecular-weight plasmid (Fig. 1); the molecular size of the deleted plasmid is shown in parentheses under the size of the original plasmid in Table 2. Two of these five strains (PB-176 and PB-166E) demonstrated both phenomena, that is, either a complete loss of the CFA/II plasmid (P1 strains) or a deletion (P2 strains), whereas the other three strains (TD-415, H22743, and H19796) showed a deletion only (Fig. 1). Strains TD-415-P and PB-166E-P2 were also negative for ST and LT production. This was not the case for PB-176-P2 and H-22743-P, which still produced ST and LT. The finding of these CFA/II$^-$:ST$^+$:LT$^+$ deletion derivatives suggests that ST$^+$:LT$^+$ clinical isolates belonging to CFA/II-associated

serogroups, but not producing CFA, may represent derivatives of strains which have lost the ability to produce CFA before isolation.

The data presented here extend our previous observations of CFA/II plasmids to ETEC strains in serogroups O6 and O85 to include strains in serogroups O8, O80, and O139. Unlike CFA/I plasmids, which usually mediate production of ST but not LT (15, 17, 19, 20), CFA/II plasmids were associated with production of both ST and LT in all but one instance (i.e., strain H-19796). In this exception, the implicated CFA/II plasmid had a molecular weight of 33×10^6, indicating a possible deletion event, either before isolation or during storage before the present work. With the other strains which were originally CFA/II$^+$, ST$^+$, and LT$^+$, the plasmid which was lost or reduced in size ranged from molecular weights of 54×10^6 to 90×10^6. This is also in contrast to the observations with CFA/I plasmids, which usually have a narrower molecular-weight range of 54×10^6 to 72×10^6 (15, 19, 20), although with both CFAs, the implicated CFA plasmid is usually the largest one in the clinical isolate. Further studies are necessary to determine the molecular similarities and dissimilarities of these plasmids.

Technical assistance was provided by D. Johnson, V. Kadri, D. Mitchell, and L. Selvidge.

This investigation was supported in part by Public Health Service grants AI-15379 and AI-13385 from the National Institute for Allergy and Infectious Diseases.

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