Genetic and Biochemical Characterization of *Citrobacter rodentium* sp. nov.

DAVID B. SCHAUER,^{1,2*} BRIAN A. ZABEL,¹ ISABEL F. PEDRAZA,¹ CAROLINE M. O'HARA,³
ARNOLD G. STEIGERWALT,⁴ AND DON J. BRENNER⁴

*Division of Toxicology*¹ and *Division of Comparative Medicine*,² *Massachusetts Institute of Technology, Cambridge, Massachusetts 02139*, and *Nosocomial Pathogens Laboratory Branch, Hospital Infections Program*,³ and *Emerging Bacterial and Mycotic Diseases Branch, Division of Bacterial and Mycotic Diseases*,⁴ *Centers for Disease Control and Prevention, Atlanta, Georgia 30333*

Received 3 March 1995/Returned for modification 14 April 1995/Accepted 1 May 1995

An unusual bacterial pathogen of laboratory mice has been previously classified as an atypical biotype of *Citrobacter freundii*. Designated *C. freundii* biotype 4280, this bacterium is the etiologic agent of transmissible murine colonic hyperplasia. An *eaeA* gene has been shown to be present in this organism and to be necessary for virulence in laboratory mice. However, other biotypes of *C. freundii* lack DNA homology with the *eaeA* gene. Because of the recent reclassification in which five named species and three unnamed species, all previously considered *C. freundii*, were described, we determined the taxonomic status of *C. freundii* biotype 4280. With a battery of biochemical tests and DNA relatedness studies, three isolates of *C. freundii* biotype 4280 were shown to be members of an unnamed *Citrobacter* species, designated species 9. In total, six isolates of *Citrobacter* species 9, but none of the type strains of the other eight named species or of the two remaining unnamed species of *Citrobacter*, were shown to possess DNA homology with both the *eaeA* and the *eaeB* genes. Species 9 was named *Citrobacter rodentium* sp. nov.

The genus *Citrobacter* has recently been reclassified on the basis of DNA relatedness to include eight named and three unnamed genomospecies (7). A genomospecies is a species defined by DNA relatedness (23), but for convenience, these 11 groups are simply referred to as species in this report. One of these unnamed groups, species 9, contains only strains isolated from rodents. The purpose of this study was to compare *Citrobacter* species 9 with a naturally occurring bacterial pathogen of laboratory mice previously classified as *Citrobacter freundii* biotype 4280 (2). The disease caused by this organism, known as transmissible murine colonic hyperplasia, is characterized by mucosal proliferation (3) and by the presence of histopathologic changes known as attaching and effacing lesions (16). These lesions are morphologically indistinguishable from the histopathologic changes caused by enteropathogenic *Escherichia coli* (EPEC). In EPEC, a chromosomal gene called *eaeA*, for *E. coli* attaching and effacing, is necessary for attaching and effacing lesion formation (15). *C. freundii* biotype 4280 has also been shown to possess an *eaeA* gene (20).

We report here that, on the basis of biochemical characteristics and DNA relatedness, *C. freundii* biotype 4280 is a member of *Citrobacter* species 9, which we have now named *Citrobacter rodentium* sp. nov. Furthermore, we have examined the six *Citrobacter* species 9 strains and the type strains from each of the other eight named *Citrobacter* species and from the two remaining unnamed *Citrobacter* species for DNA homology with *eaeA* and *eaeB* by Southern blot analysis. *eaeB* is a second chromosomal gene in EPEC which has been shown to be necessary for attaching and effacing lesion formation (10). The demonstration of *eaeA* and *eaeB* homology in *Citrobacter* species 9, but not in the other *Citrobacter* type strains, suggests that *Citrobacter* species 9 shares more than one virulence de-

terminant with EPEC and raises the possibility that horizontal transfer of multiple virulence genes has taken place.

MATERIALS AND METHODS

Strains and plasmids. Bacteria were stored at -80°C in Luria-Bertani broth containing 50% (vol/vol) glycerol (Massachusetts Institute of Technology) at -70°C or in sheep blood (Centers for Disease Control and Prevention [CDC]). Bacteria were grown at 37°C in Luria-Bertani broth or on Luria-Bertani agar (Massachusetts Institute of Technology) or on 5% sheep blood agar (CDC). For maintenance of recombinant plasmids, strains were grown in media supplemented with ampicillin (100 $\mu\text{g}/\text{ml}$). The strains and plasmids used in this study are described in Table 1. *C. freundii* biotype 4280 strains DBS125 and DBS126 were generously provided by Earl Steffen (University of Missouri College of Veterinary Medicine).

Biochemical tests. The methods used for biochemical testing were performed at the CDC essentially as described previously (11, 13).

DNA relatedness. To obtain cells for DNA extraction, *Citrobacter* strains were grown in brain heart infusion broth at $36 \pm 1^{\circ}\text{C}$ with shaking until they reached the late logarithmic phase. The methods used for extraction and purification of DNA have been described previously (8). DNA samples were labeled enzymatically in vitro with [^{32}P]dCTP by using a nick translation reagent kit (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) as directed by the manufacturer. Relatedness between pairs of bacterial DNA was determined by the hydroxyapatite method. DNA samples to be hybridized were incubated at 60°C ; thermal-elution profiles were obtained as previously described (8). Divergence within related DNA sequences was calculated to the nearest 0.5%.

PCR. To amplify an *eaeB* gene probe, oligonucleotides based on the published *E. coli eaeB* sequence (10) were synthesized. BAZ 104, corresponding to nucleotides 484 through 500 (5'-GGCGCGAGTGATGTCG-3'), and BAZ 105, complementary to nucleotides 1050 through 1066 (5'-AGCGAGCCGCTTGC CCC-3'), are predicted to amplify an internal 583-bp fragment from the *E. coli eaeB* gene. PCR was performed in a 100- μl volume with 20 pmol of each oligonucleotide and 125 pmol of each deoxynucleoside triphosphate in a final concentration of 50 mM KCl, 10 mM Tris (pH 8.3), and 1.5 mM MgCl_2 . Approximately 1 μg of JPN15 genomic DNA was used as a template, and 2.5 U of *Taq* DNA polymerase (Promega Corp., Madison, Wis.) was included in the reaction mixture. An overlay of sterile mineral oil was applied, and the samples were subjected to 30 cycles of 95°C for 1 min, 55°C for 1 min, and 72°C for 2 min in a DNA thermal cycler (Perkin-Elmer Corp., Norwalk, Conn.). After agarose gel electrophoresis, the PCR products were visualized in the presence of ethidium bromide with a UV light source.

Southern blot analysis. To obtain DNA for Southern blot analysis, plasmid DNA was isolated with QIAGEN-tips (QIAGEN Inc., Chatsworth, Calif.) according to the manufacturer's recommendations. Genomic DNA was isolated from

* Corresponding author. Mailing address: MIT, Room E18-564, Cambridge, MA 02139. Phone: (617) 253-8113. Fax: (617) 258-5424. Electronic mail address (Internet): schauer@mit.edu.

TABLE 1. Strains and plasmids used in this study

Strain or plasmid	Description	Reference(s) or source
<i>Citrobacter</i> species 9 strains		
CDC1843-73 ^T	ATCC 51116, species 9	7
CDC2643-76	ATCC 51637, species 9	7
CDC3311-75	ATCC 51638, species 9	7
DBS100	ATCC 51459, original biotype 4280	2, 20
DBS125	UMC 1136-89, ^a biotype 4280	This study
DBS126	UMC 2896-81, ^a biotype 4280	This study
Other <i>Citrobacter</i> type strains		
CDC621-64	ATCC 8090, <i>C. freundii</i>	7
CDC8132-86	ATCC 27028, <i>C. koseri</i>	7
CDC9020-77	ATCC 25407, <i>C. amalonaticus</i>	7
CDC2991-81	ATCC 51112, <i>C. farmeri</i>	7
CDC460-61	ATCC 29935, <i>C. youngae</i>	7
CDC80-58	ATCC 51113, <i>C. braakii</i>	7
CDC876-58	ATCC 51114, <i>C. werkmanii</i>	7
CDC4696-86	ATCC 51115, <i>C. sedlakii</i>	7
CDC4693-86	ATCC 51117, species 10	7
CDC2970-59	ATCC 51118, species 11	7
<i>E. coli</i> JPN15	Human EPEC strain	15
Plasmids		
pCVD434	<i>eaeA</i> gene probe	15
pDBS2	Cosmid containing DBS100 DNA	20

^a From the University of Missouri College of Veterinary Medicine.

bacteria as previously described (18). Genomic DNA was digested with restriction enzymes (New England Biolabs Inc., Beverly, Mass.), and the DNA fragments were separated by electrophoresis on a 0.75% agarose gel. The DNA fragments were transferred to a nylon membrane (Hybond-N+; Amersham Corp., Arlington Heights, Ill.) by the capillary method as previously described (19). To generate an *eaeA* gene probe, pCVD434 was digested with *Kpn*I and *Sal*I (New England Biolabs). The PCR-amplified *eaeB* gene probe was similarly isolated. The fragments of the *eaeA* gene from EPEC (15) was then separated by electrophoresis and isolated from a low-melting-point agarose gel (NuSieve GTG; FMC Corp., Rockland, Maine). The PCR-amplified *eaeB* gene probe was similarly isolated. These fragments were directly labeled with a chemiluminescence detection kit (ECL; Amersham Corp.) according to the recommendations of the manufacturer. After hybridization overnight at 42°C, the filters were washed twice for 20 min each time at 42°C in 6 M urea–0.4% sodium dodecyl sulfate–0.1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate); these washes were followed by two additional 5-min washes at room temperature in 20× SSC. For low-stringency washes, the final concentration of SSC in the primary wash solution was increased from 0.1× to 2×. The detection reagents included with the kit were used as directed, and positive signals were detected by exposure to radiographic film (Hyperfilm-ECL; Amersham Corp.).

RESULTS

Biochemical reactions. Biochemical tests were performed with three strains previously shown to belong to *Citrobacter* species 9 (7), with the strain originally designated *C. freundii* biotype 4280 (2), and with two additional *C. freundii* biotype 4280 isolates recovered in the 1980s from laboratory mice at the University of Missouri College of Veterinary Medicine. The biochemical reactions of all the strains were essentially identical (Table 2), which is consistent with the conclusion that the *C. freundii* biotype 4280 strains belong to *Citrobacter* species 9. This species can be differentiated from other *Citrobacter* species by the fact that it is negative in tests for indole production, citrate utilization (negative within 2 days), growth in KCN (originally reported to be positive for one strain [7]), arginine dihydrolase, and motility and the fact that it is positive in the test for ornithine decarboxylase. Also helpful in distin-

guishing *Citrobacter* species 9 from one or more *Citrobacter* species are its negative reactions for H₂S, sucrose, dulcitol, melibiose, and glycerol and its positive reaction for malonate.

DNA relatedness. Labeled DNA from the *Citrobacter* species 9 type strain 1843-73 was 92 to 97% related, with 0.5% divergence, to unlabeled DNA from the three *C. freundii* biotype 4280 strains.

DNA homology with *eaeA* and *eaeB*. Samples of genomic DNA from all six *Citrobacter* species 9 strains, as well as from each of the type strains of the other eight named *Citrobacter* species and the two remaining unnamed *Citrobacter* species, were examined for *eae* gene homology by Southern blot analysis. As controls, genomic DNA from the human EPEC strain JPN15 and cosmid DNA from pDBS2 were also examined by Southern blot analysis. pDBS2 contains a fragment of DBS100 chromosomal DNA, which has been shown to contain the entire *Citrobacter eaeA* gene (20). As expected, the *eaeA* gene probed hybridized to a single fragment of genomic DNA from the human EPEC strain JPN15 and to a single ~18-kb fragment of *Eco*RI-digested pDBS2 DNA (Fig. 1A). The *eaeA* gene probe was found to hybridize under conditions of high stringency to single 18-kb fragments of *Eco*RI-digested genomic DNA from all of the *Citrobacter* species 9 strains (Fig. 1A). In contrast, even under conditions of low stringency, the *eaeA* gene probe was not observed to hybridize to *Eco*RI-digested DNA from any of the other *Citrobacter* type strains (Fig. 2). The *eaeB* gene probe was also found to hybridize to an 18-kb fragment of *Citrobacter* species 9 DNA under conditions of high stringency (Fig. 1B). Under conditions of low stringency, no hybridization between the *eaeB* gene probe and any of the other *Citrobacter* type strains was detected (data not shown).

DISCUSSION

The three previously studied strains of *Citrobacter* species 9 were 93 to 96% related, with 0% divergence within related DNA sequences. The three *C. freundii* biotype 4280 strains were 92 to 97% related to *Citrobacter* species 9, with 0.5% divergence within related DNA sequences, leaving no doubt that they belong to *Citrobacter* species 9.

We have determined on the basis of biochemical reactions and DNA relatedness that three strains of *Citrobacter* species 9 and three strains of what was previously called *C. freundii* biotype 4280 together constitute a species of *Citrobacter*. We have named this species *Citrobacter rodentium* sp. nov. (see description below). We have also determined by Southern blot analysis that DNA from all six *C. rodentium* strains, but not DNA from the type strains of the other *Citrobacter* species, contains sequences homologous with the *eaeA* and *eaeB* genes of EPEC. The *eaeA* gene probe was an internal restriction fragment from a portion of the gene known to be conserved between EPEC and *Citrobacter* spp. (20). Hybridization studies with a probe derived from the 3' end of the gene, which is divergent between EPEC and *Citrobacter* spp. (20) and which has been shown to encode a domain with epithelial cell binding activity (12), have not yet been performed. The *eaeB* gene probe was an internal fragment of the EPEC *eaeB* gene. We are currently cloning and determining the nucleotide sequence of the *Citrobacter eaeB* gene.

Transmissible murine colonic hyperplasia is characterized by intimate bacterial adherence to colonic enterocytes and effacement of the microvillus brush border (16). In EPEC, both *eaeA* (15) and *eaeB* (10) are necessary for intimate adherence to cultured epithelial cells. In addition, inactivation of the *eaeA* gene in *C. rodentium* DBS100 results in the loss of the ability to

TABLE 2. Biochemical reactions of *Citrobacter* species 9 strains^a

Test or characteristic	CDC1843-73 ^T	CDC2643-76	CDC3311-75	DBS100	DBS125	DBS126
Indole	—	—	—	—	—	—
Methyl red	+	+	+	+	+	+
Voges-Proskauer	—	—	—	—	—	—
Citrate (Simmons)	5	5	6	4	—	—
H ₂ S (triple sugar-iron agar)	—	—	—	—	—	—
Urease (Christiansen)	+	+	+	+	7	+
Phenylalanine deaminase	—	—	—	—	—	—
Lysine (Moeller)	—	—	—	—	—	—
Arginine (Moeller)	—	—	—	—	—	—
Ornithine (Moeller)	+	+	+	+	+	+
Motility	—	—	—	4	4	—
Gelatin hydrolysis (22°C)	—	—	—	—	7	—
Growth in KCN	—	—	—	—	—	—
Malonate utilization	+	+	+	+	+	+
D-Glucose (gas)	+	+	+	+	+	+
Acid produced from:						
D-Glucose	+	+	+	+	+	+
Lactose	+	+	+	+	+	+
Sucrose	—	—	—	—	—	—
D-Mannitol	+	+	+	+	+	+
Dulcitol	—	—	—	—	—	—
Salicin	4	5	4	4	4	4
D-Adonitol	—	—	—	—	—	—
<i>i</i> -Inositol	—	—	—	—	—	—
D-Sorbitol	+	+	+	+	+	+
L-Arabinose	+	+	+	+	+	+
Raffinose	—	—	—	—	—	—
L-Rhamnose	+	+	+	+	+	+
Maltose	+	+	+	+	+	+
D-Xylose	+	+	+	+	+	+
Trehalose	+	+	+	+	+	+
Cellobiose	+	+	+	+	+	+
α -CH ₃ -glucoside	—	—	—	—	—	—
Erythritol	—	—	—	—	—	—
Melibiose	—	—	—	—	—	—
D-Arabitol	—	—	—	—	—	—
Glycerol	—	—	—	7	7	—
D-Mannose	+	+	+	+	+	+
Mucate (acid production)	+	+	+	+	+	+
Tartrate (Jordan)	+	+	+	+	+	+
Acetate utilization	7	3	7	—	—	—
Esculin hydrolysis	7	7	7	7	—	7
Lipase (corn oil)	—	—	—	—	—	—
DNase (25°C)	—	—	—	—	—	—
NO ₃ reduced to NO ₂	+	+	+	+	+	+
Oxidase	—	—	—	—	—	—
ONPG ^b	+	+	+	+	+	+

^a Preparations were incubated at 35 ± 1°C, except gelatin liquefaction (22°C) and DNase (25°C). Results for each test were read daily for 7 days of incubation, except those for the methyl red and Voges-Proskauer tests, which were read after 2 days of incubation. +, positive within 2 days; —, negative after 7 days. A number indicates that a delayed positive reaction was obtained on day *d*.

^b ONPG, *o*-nitrophenyl- β -D-galactopyranoside.

colonize the mouse colon (21). While it is clear that other genes are required for effacement (for a review, see reference 9), the presence of *eae* gene homology suggests that all six strains of *C. rodentium* can adhere intimately in the mouse colon and can cause transmissible murine colonic hyperplasia. Indeed, DBS100, DBS125, DBS126, and CDC3311-75 were each isolated from mice with this disease. CDC2643-76 was also isolated from a mouse, but in this case the presence of clinical signs of disease was not recorded. Disease susceptibility is known to be influenced by both the age and the genetic background of the host (4). Infection of adult, outbred mice with *C. rodentium* often does not result in clinical signs of disease (3). The type strain, CDC1843-73, was isolated by George Osbaldiston, Section of Laboratory Animal Services,

Yale University, New Haven, Conn. CDC records indicate that it was isolated from the ileum of a hamster. However, it is highly probable that the strain was actually isolated from a mouse, since studies of transmissible murine colonic hyperplasia were ongoing in that laboratory and no such strain was isolated from hamsters (1a). Furthermore, oral challenge of Syrian hamsters with DBS100 failed to result in intestinal colonization (4). We are currently testing each strain for virulence in mice and are attempting to characterize the true host range of *C. rodentium*.

The presence of *eae* genes homologous with those of EPEC in *C. rodentium* but not in the other *Citrobacter* species raises the question of where these genes originated. Genes homologous to *eaeA* have also been identified in enterohemorrhagic *E.*

FIG. 1. Southern blot analysis of *Citrobacter* species 9 for *eae* gene homology. Both autoradiographs are of the same filter, which was hybridized with the *eaeA* (A) and *eaeB* (B) gene probes and washed under conditions of high stringency. Lanes contain *Eco*RI-digested genomic DNA from EPEC strain JPN15, from the six isolates of *Citrobacter* species 9, from the *C. freundii* type strain, and from *Eco*RI-digested pDBS2, as indicated.

FIG. 2. Southern blot analysis of the type strains of 10 species of *Citrobacter* for *eaeA* homology. This filter was hybridized with the *eaeA* gene probe and washed under conditions of low stringency. Lanes contain *Eco*RI-digested genomic DNA from DBS100, from the type strains of eight named *Citrobacter* species, from the type strains of two unnamed *Citrobacter* species, and from *Eco*RI-digested pDBS2, as indicated.

coli (5, 24) and in some isolates of *Hafnia alvei* (1). More recently, it has been reported that both *eaeA* and *eaeB*, along with several other genes involved in effacement, are located on an ~35-kb fragment of chromosomal DNA in EPEC (17). This region has been called the locus for enterocyte effacement (LEE) and maps to 82 min on the *E. coli* chromosome (17). This is the same map location at which the retronphage ϕ R73 (14, 22) and the pathogenicity islands of uropathogenic *E. coli* (6) are known to insert. It may be that large clusters of virulence factors, including the LEE, undergo horizontal transfer. Such mobile genetic elements may, on occasion, even move between genera in the family *Enterobacteriaceae*. We speculate that *C. rodentium* strains are descendants of a recipient of just this sort of gene transfer.

McDaniel et al. reported that DNA homology between four separate fragments of the EPEC LEE and *C. rodentium* DBS100 exists (17). Since this was determined by colony hybridization, it is not known if all four loci are physically linked on the *C. rodentium* chromosome. By Southern blot analysis of pDBS2, we have demonstrated that both the *eaeA* and the *eaeB* gene probes hybridize to a single ~18-kb *Eco*RI fragment of *C. rodentium* chromosomal DNA. Furthermore, both of these gene probes hybridized to a single restriction fragment of pDBS2, indicating that these two loci are linked on the *C. rodentium* chromosome. It remains to be determined if the other loci also localize to this region and if *C. rodentium* has a LEE similar to that of EPEC. If so, the unusual biochemical reactions of this species may simply reflect the biotype of the original *Citrobacter* strain to acquire the LEE. We plan to test this hypothesis by introducing the putative *C. rodentium* LEE into other *Citrobacter* species. Such a study should help clarify the relationship between attaching and effacing *E. coli*, *C. rodentium*, and other *Citrobacter* species.

Description of *C. rodentium* sp. nov. *C. rodentium* (ro.den'ti.um. L. part. adj. used as a gen. n. *rodentium*, of gnawing animals [since it was isolated from mice]). Corresponds to *Citrobacter* species 9 (7). Negative for indole production, ci-

trate (within 2 days), growth in KCN, and arginine dihydrolase and is ornithine decarboxylase positive. Other characteristics useful for differentiation are lack of motility; inability to produce H₂S; inability to produce acid from melibiose, sucrose, dulcitol, or glycerol; positive malonate reaction; and ability to utilize malonate but not glycerol as a sole carbon source. A full list of biochemical results is shown in Table 2.

It should be noted that there are some minor differences between the biochemical results reported in Table 2 and those reported previously for the three strains of *Citrobacter* species 9 (7). Those results are as follows: 67% (previously 0%) delayed citrate positive, 83% (previously 100%) urease positive and 100% delayed urease positive, 0% (previously 33%) KCN positive, 83% (previously 33%) delayed esculin positive, and 33% (previously 0%) delayed glycerol positive.

This organism has been isolated only from rodents, and it includes isolates known to be pathogenic in laboratory mice, previously referred to as *C. freundii* biotype 4280 (2). The type strain is CDC1843-73 (ATCC 51116), which was in all probability isolated from a mouse in Connecticut (1a).

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant CA63112 from the National Cancer Institute to D.B.S.

We thank Earl Steffen for generously providing strains, James B. Kaper for sharing unpublished data, and C. A. Lee for a critical reading of the manuscript.

REFERENCES

- Albert, M. J., S. M. Faruque, M. Ansaruzzaman, M. M. Islam, K. Haider, K. Alam, I. Kabir, and R. Robins-Browne. 1992. Sharing of virulence-associated properties at the phenotypic and genetic levels between enteropathogenic *Escherichia coli* and *Hafnia alvei*. *J. Med. Microbiol.* **37**:310–314.
- Barthold, S. W. Personal communication.
- Barthold, S. W., G. L. Coleman, P. N. Blatt, G. W. Osbaldiston, and A. M. Jonas. 1976. The etiology of transmissible murine colonic hyperplasia. *Lab. Anim. Sci.* **26**:889–894.
- Barthold, S. W., G. L. Coleman, R. O. Jacoby, E. M. Livstone, and A. M. Jonas. 1978. Transmissible murine colonic hyperplasia. *Vet. Pathol.* **15**:223–236.
- Barthold, S. W., G. W. Osbaldiston, and A. M. Jonas. 1977. Dietary, bacterial, and host genetic interactions in the pathogenesis of transmissible murine colonic hyperplasia. *Lab. Anim. Sci.* **27**:938–945.
- Beebakhee, G., M. Louie, J. De Azavedo, and J. Brunton. 1992. Cloning and nucleotide sequence of the *eae* gene homologue from enterohemorrhagic *Escherichia coli* serotype O157:H7. *FEMS Microbiol. Lett.* **91**:63–68.
- Blum, G., M. Ott, A. Lischewski, A. Ritter, H. Imrich, H. Tschäpe, and J. Hacker. 1994. Excision of large DNA regions termed pathogenicity islands from tRNA-specific loci in the chromosome of an *Escherichia coli* wild-type pathogen. *Infect. Immun.* **62**:606–614.
- Brenner, D. J., P. A. D. Grimont, A. G. Steigerwalt, G. R. Fanning, E. Ageron, and C. F. Riddle. 1993. Classification of citrobacteria by DNA hybridization: designation of *Citrobacter farmeri* sp. nov., *Citrobacter youngae* sp. nov., *Citrobacter braakii* sp. nov., *Citrobacter werkmanii* sp. nov., *Citrobacter sedlakii* sp. nov., and three unnamed *Citrobacter* genomospecies. *Int. J. Syst. Bacteriol.* **43**:645–658.
- Brenner, D. J., A. C. McWhorter, J. K. Leete Knutson, and A. G. Steigerwalt. 1982. *Escherichia vulneris*: a new species of *Enterobacteriaceae* associated with human wounds. *J. Clin. Microbiol.* **15**:1133–1140.
- Donnenberg, M. S., and J. B. Kaper. 1992. Enteropathogenic *Escherichia coli*. *Infect. Immun.* **60**:3953–3961.
- Donnenberg, M. S., J. Yu, and J. B. Kaper. 1993. A second chromosomal gene necessary for intimate attachment of enteropathogenic *Escherichia coli* to epithelial cells. *J. Bacteriol.* **175**:4670–4680.
- Farmer, J. J., III, M. A. Asbury, F. W. Hickman, D. J. Brenner, and The *Enterobacteriaceae* Study Group. 1980. *Enterobacter sakazakii*: a new species of “*Enterobacteriaceae*” isolated from clinical specimens. *Int. J. Syst. Bacteriol.* **30**:569–584.
- Frankel, G., D. C. A. Candy, P. Everest, and G. Dougan. 1994. Characterization of the C-terminal domains of intimin-like proteins of enteropathogenic and enterohemorrhagic *Escherichia coli*, *Citrobacter freundii*, and *Hafnia alvei*. *Infect. Immun.* **62**:1835–1842.
- Hickman, F. W., and J. J. Farmer III. 1978. *Salmonella typhi*: identification, antibiograms, serology, and bacteriophage typing. *Am. J. Med. Technol.* **44**:1149–1159.
- Inouye, S., M. G. Sunshine, E. W. Six, and M. Inouye. 1991. Retronphage ϕ R73: an *E. coli* phage that contains a retroelement and integrates into a tRNA gene. *Science* **252**:969–971.
- Jerse, A. E., J. Yu, B. D. Tall, and J. B. Kaper. 1990. A genetic locus of enteropathogenic *Escherichia coli* necessary for the production of attaching and effacing lesions on tissue culture cells. *Proc. Natl. Acad. Sci. USA* **87**:7839–7843.
- Johnson, E., and S. W. Barthold. 1979. The ultrastructure of transmissible murine colonic hyperplasia. *Am. J. Pathol.* **97**:291–301.
- McDaniel, T. K., K. G. Jarvis, M. S. Donnenberg, and J. B. Kaper. 1995. A genetic locus of enterocyte effacement conserved among diverse enterobacterial pathogens. *Proc. Natl. Acad. Sci. USA* **92**:1664–1668.
- Mekalanos, J. J. 1983. Duplication and amplification of toxin genes in *Vibrio cholerae*. *Cell* **35**:253–263.
- Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- Schafer, D. B., and S. Falkow. 1993. Attaching and effacing locus of a *Citrobacter freundii* biotype that causes transmissible murine colonic hyperplasia. *Infect. Immun.* **61**:2486–2492.
- Schafer, D. B., and S. Falkow. 1993. The *eae* gene of *Citrobacter freundii* biotype 4280 is necessary for colonization in transmissible murine colonic hyperplasia. *Infect. Immun.* **61**:4654–4661.
- Sun, J., M. Inouye, and S. Inouye. 1991. Association of a retroelement with a P4-like cryptic prophage (retronphage ϕ R73) integrated into the selenocystyl tRNA gene of *Escherichia coli*. *J. Bacteriol.* **173**:4171–4181.
- Wayne, L. G., D. J. Brenner, R. R. Colwell, P. A. D. Grimont, O. Kandler, M. I. Krichevsky, L. H. Moore, W. E. C. Moore, R. G. E. Murray, E. Stackenbrandt, M. P. Starr, and H. G. Trüper. 1987. Report of the Ad Hoc Committee on Reconciliation of Approaches to Bacterial Systematics. *Int. J. Syst. Bacteriol.* **37**:463–464.
- Yu, J., and J. B. Kaper. 1992. Cloning and characterization of the *eae* gene of enterohemorrhagic *Escherichia coli* O157:H7. *Mol. Microbiol.* **6**:411–417.