INFECTION AND IMMUNITY, Jan. 1987, p. 193–197
0019-9567/87/010193-05$02.00/0

Vol. 55, No. 1

Presence and Expression of Aerobactin Genes in Virulent Avian Strains of *Escherichia coli*

JEAN-PIERRE LAFONT,[1]* MARYVONNE DHO,[1] HÉLÈNE M. D'HAUTEVILLE,[2] ANNIE BREE,[1] AND PHILIPPE J. SANSONETTI[2]

Station de Pathologie Aviaire et de Parasitologie, Centre de Recherches de Tours, Institut National de la Recherche Agronomique, Nouzilly, 37380 Monnaie,[1] and Centre de Référence des Shigelles, Service des Entérobactéries, Unité 199, Institut National de la Santé et de la Recherche Médicale, Institut Pasteur, 75724 Paris Cédex 15,[2] France

Received 16 June 1986/Accepted 7 October 1986

Virulent and nonvirulent isolates of avian *Escherichia coli* were tested for the presence of aerobactin genes by colony hybridization with a specific gene probe constructed from plasmid pABN1 (A. Bindereif and J. B. Neilands, J. Bacteriol. 153:1111–1113, 1983). Positive hybridization with the gene probe was highly correlated with virulence, as measured by the 50% lethal dose of the strains for chicks. Evidence for the expression of aerobactin genes in the virulent strains was obtained by demonstrating their susceptibility to cloacin DF13, which binds to the same receptor that binds aerobactin, and their ability to produce aerobactin, as revealed by cross-feeding the *E. coli* mutant WO987 (*aroB fepA iuc iut*$^+$), which is unable to synthesize but capable of taking up aerobactin. We suggest that the production of aerobactin is involved in the virulence of avian septicemic *E. coli*.

In domestic avian species, colibacillosis begins with a respiratory disease, which is followed by an invasive phase characterized by lesions of the internal organs: airsacculitis, pericarditis, perihepatitis, and septicemia (21). The determinants of virulence have been much less extensively studied in avian strains than in mammalian strains of *Escherichia coli*. Adhesiveness to the epithelial respiratory cells of chickens and turkeys is probably involved in the virulence of avian isolates (1, 2, 15, 16, 27, 28). The ability to grow under iron-limiting conditions is another frequent property of virulent microorganisms that provokes systemic infections (9, 17, 36). We have found that this physiological trait is highly correlated with virulence, as measured by the lethality for 1-day-old chicks, in avian strains of *E. coli* (16). High-affinity systems of iron acquisition can compete with transferrins, the physiological carriers of the cation in animals, and allow microorganisms to overcome growth limitation caused by insufficient amounts of free iron in the body fluids (36). Two such systems are known in *E. coli*. Both involve the production and excretion of a high-affinity iron ligand, or siderophore, and the synthesis of a specific membrane protein that acts as a receptor for the siderophore-iron complexes. The regulation and physiology of these systems have recently been reviewed (7, 12, 29).

The enterochelin system is chromosomally encoded and, although very efficient in vitro, seems less able to compete with transferrin in vivo (18, 22).

The aerobactin system is encoded by either plasmids or chromosomes in several species of enteric organisms (5, 11, 24, 26, 33, 35, 37, 38). It comprises genes responsible for the synthesis of the hydroxamate siderophore aerobactin (*iuc*) and for ferric aerobactin uptake (*iut*) (4, 10, 20). It can be considered to act efficiently in vivo and is involved in the invasive properties of human enteroinvasive *E. coli* and *Shigella* species (19, 24). The aerobactin genes have been cloned (4, 10, 20, 23, 30). By combining physiological experiments and colony hybridization with a specific gene probe, we demonstrated that this system is present and expressed in virulent avian isolates of *E. coli* and absent from most nonvirulent avian isolates.

MATERIALS AND METHODS

Bacterial strains. Sixty-four virulent and nonvirulent avian strains of *E. coli* were studied. They had been isolated from pathological products, from the internal organs of cull poults, and from the intestinal contents of healthy chickens (16). Their virulence was measured by a lethality test in 1-day-old chicks (16); the 50% lethal dose (LD_{50}) was assessed (13) after subcutaneous challenge. Their ability to grow in vitro under conditions of iron deprivation by transferrin had been previously investigated (16). Other *E. coli* derivatives used in various tests described below are listed in Table 1. The strains were stored as cell suspensions in fresh nutrient broth containing 20% glycerol (vol/vol) and frozen at −76°C. All cultures used in the present work were started from stock cultures on nutrient agar slopes covered with sterile liquid paraffin and held at 4°C.

DNA hybridization. Plasmid pABN1 DNA (4) was purified by cesium chloride-ethidium bromide ultracentrifugation, and a 6.8-kilobase-pair fragment was digested with restriction endonucleases *Hin*dIII and *Eco*RI (Genofit, Geneva, Switzerland) used in accordance with the recommendations of the manufacturer. The fragment was purified by electrophoresis in 0.7% low-melting-point agarose (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) by the method of Boileau et al. (6) and labeled by nick translation with [^{32}P]CTP (Amersham Corp., Arlington Heights, Ill.) as described by Rigby et al. (31). The specific activity of the labeled probe was 6×10^6 cpm/μg of DNA. Bacteria were grown overnight at 37°C in tryptic soy broth (Difco Laboratories, Detroit, Mich.), and drops of cultures were spotted on the surfaces of nitrocellulose filters (BA85; Schleicher & Schuell, Inc., Keene, NH) deposited on Drigalski agar plates (Institut Pasteur Production, Paris, France). After 4 h of

* Corresponding author.

TABLE 1. Reference strains used

Strain	Genotype	Relevant characteristics	Source or reference
E. coli N3024	*thr leu tonA lacY thi minA minB gal phX gyrA rpsL xyl mtl mal lam*	Resistant to cloacin DF13	Collection of the Free University of Amsterdam (Arnold J. Van Putten)
E. coli N5160	N3024(pJN60)	pCloDF13 *cop-3*::Tn*901*: Production of cloacin DF13	34
E. coli KH576	*rpsL gyrA*(pColV-K30)	pColV+: Production of aerobactin; susceptible to cloacin DF13	37
E. coli WO987	*araD lac aroB fepA rpsL thi iuc iut*$^+$ (pColV-EN41::Tn*10*)	Deficient in enterochelin synthesis and uptake, deficient in aerobactin synthesis but proficient in aerobactin uptake	*fepA* Derivative of strain EN41 (8, 31)
E. coli BM21	*gyrA*	Prototrophic K-12 strain	P. Courvalin, Institut Pasteur, Paris, France
Klebsiella edwardsii N4018	*bla*	Susceptible to cloacin DF13	Former S15 strain (14)

incubation at 37°C, the colonies were lysed, and the filters were dried, baked, and hybridized with the radioactive probe as described previously (6). Strains KH576 and BM21 were used as positive and negative controls, respectively (see Fig. 1).

Aerobactin production. The production of the iron hydroxamate chelator aerobactin was demonstrated by a cross-feeding method derived from Braun et al. (8) and Schoch and Lebek (32). Calf serum (GIBCO Laboratories, Grand Island, NY) was mixed in a ratio of 1/1 (vol/vol) with a sterile 3% solution of Noble agar (Difco). Aliquots (15 ml) of the solid medium thus obtained were kept at 50°C and added to 5 μl of an overnight broth culture of strain W0987 (unable to synthesize but fully capable of taking up aerobactin) (Table 1), and the mixture was poured into petri plates. After setting of the medium at room temperature, paper disks of 6-mm diameter were soaked with 20 μl of overnight cultures of the field strains in M9 medium (37) containing a 200 μM concentration of the iron chelator 2,2'-dipyridyl (Sigma Chemical Co., St. Louis, Mo.) and deposited on the agar surface. The plates were incubated for 24 h at 37°C, and satellite growth of the indicator strain W0987 around the disks was noted. Positive and negative controls were *E. coli* KH576 and BM21, respectively (Table 1).

Susceptibility to cloacin DF13. Cloacin DF13 is a bacteriocin that attaches to the same outer membrane protein that is involved in the transport of aerobactin, and cloacin sensitivity is indicative of the presence of a functional aerobactin transport system (3, 23). The method used to examine susceptibility to cloacin DF13 was derived from De Graaf et al. (14). Mitomycin C (Sigma) was added at 1 μg/ml to an exponentially growing culture of *E. coli* N5160 (Table 1) in brain heart infusion broth (Difco) incubated under aerated conditions for 10 min at 37°C. The cells were harvested by centrifugation, resuspended in half the original volume of pre-heated brain heart infusion broth, and incubated for 3 h at 37°C under static conditions. The supernatant was then collected by centrifugation, filtered on 0.22-μm-pore nitrocellulose membranes (Millipore Corp., Bedford, Mass.), and used as a cloacin DF13 preparation. Dried plates of L agar (25) containing 200 μg of 2,2'-dipyridyl (Sigma) per ml were seeded with approximately 10^5 cells of the field isolates grown overnight in M9 medium. The plates were dried for 15 min at 40°C, and 20 μl of the cloacin DF13 preparation was deposited on each plate. Growth in the cloacin DF13 spot was noted after 18 h at 37°C. Strains KH576, WO987, and N4018 (Table 1) were used as cloacin DF13-susceptible controls, and strain N3024 (Table 1) was used as a cloacin DF13-resistant control.

RESULTS

The molecular aerobactin probe hybridized with 44 of the colonies studied (Fig. 1). The presence of the aerobactin genes was highly correlated with virulence, as evidenced by positive hybridization of all of the 12 highly virulent strains (LD_{50}s, 10^3 to 10^6 organisms) and of only 1 of the 12 nonvirulent strains (Table 2). Among the isolates endowed with intermediate virulence properties, 19 of 20 of the strains with LD_{50}s of between 10^6 and 10^8 organisms hybridized with the probe, as did 11 of 19 of those with very high LD_{50}s ($>10^8$ organisms). The ability to provide exogenous aerobactin to strain WO987 was demonstrated by satellite growth of the indicator strain in a zone extending from 2 to 6 mm beyond the limits of the paper disk supporting the positive cross-feeding culture, whereas no growth appeared around the strains considered to be negative or around the negative controls (Table 3).

Several degrees of cloacin DF13 susceptibility were recorded, from a complete absence of growth in the cloacin DF13 spot to normal growth in the spot. Strains exhibiting any discernible reduction of growth in the cloacin DF13 spot were reported to be susceptible (Table 3).

Syntrophism of WO987 and susceptibility to cloacin DF13 were general properties of the isolates which hybridized with the aerobactin probe: 89% (39 strains) were positive for both these properties (Table 3). However, 5 (11%) of the hybrid-

TABLE 2. Hybridization with the aerobactin gene probe and virulence of the strains[a]

Hybridization	No. of strains with LD_{50} of:			No. of nonlethal strains
	10^3 to 10^6 organisms	10^6 to 10^8 organisms	10^8 to 10^{10} organisms	
Yes	12	19	11	1
No	0	1	8	11

[a] Significant differences were determined by the chi-square test ($P = 3 \times 10^{-7}$). The LD_{50} was determined after challenge of 1-day-old chicks by the subcutaneous route (16).

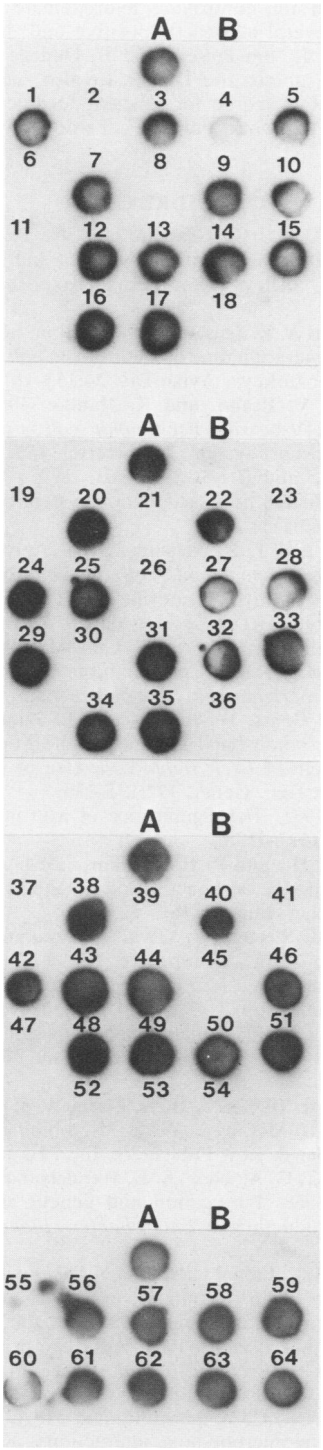

FIG. 1. Autoradiogram of the 64 avian strains studied after hybridization with the aerobactin gene probe. (A) Positive control strain KH576; (B) negative control strain BM21. Numbers 1 to 64 refer to the natural isolates (see also Table 3).

TABLE 3. Experimental results

Strain designation (same as in Fig. 1)	LD_{50} class[a]	Syntrophism of strain W0987	Susceptibility to cloacin[b]	Growth inhibition by transferrin[c]
1	1	+	S	NI
2	NL	−	R	I
3	1	+	S	NI
4	1	+	S	NI
5	1	+	S	NI
6	NL	−	R	I
7	NL	+	S	NI
8	NL	−	R	I
9	1	+	S	NI
10	1	+	S	NI
11	NL	−	R	I
12	2	+	S	NI
13	2	+	S	NI
14	2	+	S	I
15	2	+	S	NI
16	2	+	S	NI
17	2	+	S	NI
18	3	−	R	I
19	3	−	R	I
20	2	+	R	I
21	2	+	R	NI
22	3	+	S	NI
23	NL	+	R	I
24	1	+	R	NI
25	3	+	S	NI
26	3	−	R	I
27	1	+	S	NI
28	2	+	S	NI
29	3	+	S	NI
30	NL	−	R	I
31	2	+	S	NI
32	2	+	S	NI
33	3	+	S	NI
34	2	+	S	I
35	2	+	S	I
36	NL	−	R	I
37	NL	−	R	I
38	3	+	S	NI
39	3	−	R	I
40	2	+	R	NI
41	3	−	R	I
42	2	+	S	NI
43	2	+	S	NI
44	3	+	R	NI
45	3	−	R	I
46	3	+	S	I
47	3	−	R	I
48	2	+	S	NI
49	1	+	S	NI
50	3	+	S	I
51	1	+	R	NI
52	3	−	R	I
53	NL	−	R	I
54	NL	−	R	I
55	NL	−	R	I
56	1	+	S	NI
57	2	+	S	NI
58	2	+	S	NI
59	3	+	S	I
60	3	+	S	NI
61	3	+	S	I
62	1	+	S	I
63	2	+	S	I
64	NT[d]	+	S	NT

[a] 1, 10^3 to 10^6 organisms; 2, 10^6 to 10^8 organisms; 3, 10^8 to 10^{10} organisms; NL, nonlethal.
[b] S, Susceptible to cloacin; R, resistant to cloacin.
[c] I, Inhibited by transferrin in minimal medium; NI, not inhibited (16).
[d] NT, Not tested.

ization-positive isolates allowed satellite growth of WO987 in iron-limited medium but appeared to be resistant to cloacin DF13.

The 20 strains which were negative in the molecular hybridization experiment were resistant to cloacin DF13, and 18 (90%) were unable to complement WO987; strains 21 and 23, which were not susceptible to cloacin DF13, gave a paradoxical positive result in the cross-feeding experiment with WO987 (Table 3).

DISCUSSION

The presence of the genes coding for the aerobactin system of iron acquisition were demonstrated by specific DNA hybridization with an intragenic aerobactin probe in all of the most virulent isolates studied (LD_{50}, $<10^6$ organisms). By contrast, except in one case, these genes were absent from nonvirulent, nonlethal isolates (Fig. 1 and Table 2). A large majority (95%) of the strains which had intermediate virulence properties hybridized with the probe when their LD_{50}s were between 10^6 and 10^8 organisms, whereas the proportion of hybridization-positive strains was less (58%) for strains with high LD_{50}s ($>10^8$ organisms). Only one nonlethal isolate gave positive results in the hybridization assay (strain 7, Fig. 1 and Table 3). This was a rough strain isolated from the fecal flora of a healthy chicken. The *iuc* genes coding for the synthesis of the aerobactin molecule were apparently expressed in strains which harbored them, since all of these strains were able to supply aerobactin to strain WO987, deficient in aerobactin synthesis but able to use exogenous aerobactin, as evidenced by the results of syntrophism tests in iron-limited conditions (Table 3).

Moreover, the *iut* gene encoding the receptor for the iron-aerobactin complex was also expressed in a majority of the virulent strains. This receptor also functions as a receptor for cloacin DF13, a bacteriocin encoded by the CloDF13 plasmid (3, 23), and susceptibility to cloacin DF13 was used to assess the expression of *iut*. Most hybridization-positive and aerobactin-producing strains were susceptible to cloacin DF13, and all strains devoid of aerobactin genes were resistant to the bacteriocin (Fig. 1 and Table 3). Five strains appeared to be resistant to cloacin DF13 despite the presence of the aerobactin genes; it can be hypothesized that they were unable to express the *iut* gene in these experimental conditions. However, growth inhibition obtained with the cloacin DF13 treatment was not complete for many susceptible field isolates, whereas it was always complete for the rough K-12 control strains. We compared one apparently cloacin DF13-resistant, aerobactin-producing strain (strain 40, Fig. 1 and Table 3) with a rough variant derived from it (strain 64, Fig. 1 and Table 3) and observed that the rough derivative was susceptible to the bacteriocin. Thus, the presence of lipopolysaccharide O chains may impair the susceptibility of certain smooth *E. coli* strains to the lethal action of cloacin DF13.

A total of 77% (33 of 43) of the hybridization-positive strains tested were able to grow in minimal medium in the presence of transferrin, whereas 95% (19 of 20) of the hybridization-negative strains were inhibited by transferrin in these conditions (16). These results confirm that the method proposed by Williams (37) can be used to screen field isolates for the in vitro expression of the aerobactin system.

Our results represent evidence for the involvement of aerobactin production in the virulence of avian septicemic strains of *E. coli*.

ACKNOWLEDGMENTS

We thank J. B. Neilands for the gift of plasmid pABN1; A. J. Van Putten, P. Pohl, G. Schoch, and P. Courvalin for reference strains; M. Der Vartanian for controlling hydroxamate activity in the supernatants of several aerobactin-positive and -negative strains; and V. Braun, A. J. Van Putten, and B. Oudega for reading the manuscript before submission. Thanks are also due to C. Mouline, G. Brossé, and M. Lagorce for efficient technical assistance in various stages of the work and to F. Tardo-Dino for typing the manuscript.

LITERATURE CITED

1. **Arp, L. H., C. L. G. Graham, and N. F. Cheville.** 1979. Comparison of clearance rates of virulent and avirulent *Escherichia coli* in turkeys after aerosol exposure. Avian Dis. **23**:386–391.
2. **Arp, L. H., and A. E. Jensen.** 1980. Piliation, hemagglutination, motility and generation time of *Escherichia coli* that are virulent or avirulent for turkeys. Avian Dis. **24**:153–161.
3. **Bindereif, A., V. Braun, and K. Hantke.** 1982. The cloacin receptor of ColV-bearing *Escherichia coli* is part of the Fe^{3+} aerobactin transport system. J. Bacteriol. **150**:1472–1475.
4. **Bindereif, A., and J. B. Neilands.** 1983. Cloning of the aerobactin-mediated iron assimilation system of plasmid ColV. J. Bacteriol. **153**:1111–1113.
5. **Bindereif, A., and J. B. Neilands.** 1985. Aerobactin genes in clinical isolates of *Escherichia coli*. J. Bacteriol. **161**:727–735.
6. **Boileau, C. R., H. M. D'Hauteville, and P. J. Sansonetti.** 1984. DNA hybridization technique to detect *Shigella* species and enteroinvasive *Escherichia coli*. J. Clin. Microbiol. **20**:959–961.
7. **Braun, V.,** 1985. The unusual features of the iron transport systems of *Escherichia coli*. Trends Biochem. Sci. **6**:75–78.
8. **Braun, V., R. Gross, W. Koster, and L. Zimmermann.** 1983. Plasmid and chromosomal mutants of the iron(III)-aerobactin transport system of *Escherichia coli*. Use of streptonigrin for selection. Mol. Gen. Genet. **192**:131–139.
9. **Bullen, J. J.** 1981. The significance of iron in infection. Rev. Infect. Dis. **3**:1127–1138.
10. **Carbonetti, N. H., and P. H. Williams.** 1984. A cluster of five genes specifying the aerobactin iron uptake system of plasmid ColV-K30. Infect. Immun. **46**:7–12.
11. **Colonna, B., M. Nicoletti, P. Visca, M. Casalino, P. Valenti, and F. Maimone.** 1985. Composite IS*1* elements encoding hydroxamate-mediated iron uptake in FI*me* plasmids from epidemic *Salmonella* spp. J. Bacteriol. **162**:307–316.
12. **Crosa, J. H.** 1984. The relationship of plasmid-mediated iron transport and bacterial virulence. Annu. Rev. Microbiol. **38**:69–89.
13. **Davis, M. D., R. Dulbecco, H. N. Eisen, H. S. Ginsberg, W. B. Wood, and M. McCarty.** 1973. Microbiology, 2nd ed., p. 663–664. Harper & Row Publishers, Inc., Hagerstown, Md.
14. **De Graaf, F. K., G. A. Tieze, S. E. Wendelaar-Bonga, and A. H. Stouthamer.** 1968. Purification and genetic determination of bacteriocin production in *Enterobacter cloacae*. J. Bacteriol. **95**:631–640.
15. **Dho, M., and J. P. Lafont.** 1982. *Escherichia coli* colonization of the trachea in poultry: comparison of virulent and avirulent strains in gnotoxenic chickens. Avian Dis. **26**:787–797.
16. **Dho, M., and J. P. Lafont.** 1984. Adhesive properties and iron uptake ability in *Escherichia coli* lethal and non-lethal for chicks. Avian Dis. **8**:1016–1025.
17. **Finkelstein, R. A., C. V. Sciortino, and M. A. McIntosh.** 1983. Role of iron in microbe-host interactions. Rev. Infect. Dis. **5**(Suppl. 4):759–777.
18. **Griffiths, E.** 1985. Candidate virulence markers, p. 193–226. *In* M. Sussman (ed.), The virulence of *Escherichia coli*. Academic Press, Inc. (London), Ltd., London.
19. **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.

20. **Gross, R., F. Engelbrecht, and V. Braun.** 1984. Genetic and biochemical characterization of the aerobactin synthesis operon on pColV. Mol. Gen. Genet. **196**:74–80.
21. **Gross, W. B.** 1978. Colibacillosis, p. 321–330. *In* M. S. Hofstad, B. W. Calneck, C. F. Helmboldt, W. M. Reid, and H. W. Yoder (ed.), Diseases of poultry, 7th ed. Iowa State University Press, Ames.
22. **Konopka, K., A. Bindereif, and J. B. Neilands.** 1982. Aerobactin-mediated utilization of transferrin iron. Biochemistry **21**:6503–6508.
23. **Krone, W. J. A., B. Oudega, F. Stegehuis, and F. K. de Graaf.** 1983. Cloning and expression of the cloacin DF13/aerobactin receptor of *Escherichia coli*(ColV-K30). J. Bacteriol. **153**: 716–721.
24. **Lawlor, K. M., and S. M. Payne.** 1984. Aerobactin genes in *Shigella* spp. J. Bacteriol. **160**:266–272.
25. **Lennox, E. S.** 1955. Transduction of linked genetic characters of the host by bacteriophage P1. Virology **1**:190–206.
26. **McDougall, S., and J. B. Neilands.** 1984. Plasmid- and chromosome-coded aerobactin synthesis in enteric bacteria: insertion sequences flank operon in plasmid-mediated systems. J. Bacteriol. **159**:300–305.
27. **Nagaraja, K. V., D. A. Emery, and B. S. Pomeroy.** 1983. Identification and isolation of somatic pili from pathogenic *Escherichia coli* from turkeys. Am. J. Vet. Res. **44**:284–287.
28. **Naveh, M. W., T. Zusman, E. Skutelsky, and E. Z. Ron.** 1984. Adherence pili in avian strains of *Escherichia coli*: effect on pathogenicity. Avian Dis. **28**:651–661.
29. **Neilands, J. B., and K. Nakamura.** 1985. Regulation of iron assimilation in microorganisms. Nutr. Rev. **43**:193–197.
30. **Perez-Casal, J. F., and J. H. Crosa.** 1984. Aerobactin iron uptake sequences in plasmid ColV-K30 are flanked by inverted IS*1*-like elements and replication regions. J. Bacteriol. **160**:256–265.
31. **Rigby, P. W. J., M. Dieckmann, C. Rhodes, and P. Berg.** 1977. Labeling deoxyribonucleic acid to high specific activity *in vitro* by nick-translation with DNA polymerase I. J. Mol. Biol. **113**:237–251.
32. **Schoch, C., and G. Lebek.** 1984. Nachweis von plasmidisch kodierten Eisen-Transportmechanismen in Enterobacteriaceae. Hyg. Med. **9**:409–410.
33. **Valvano, M. A., and J. H. Crosa.** 1984. Aerobactin iron transport genes commonly encoded by certain ColV plasmids occur in the chromosome of a human invasive strain of *Escherichia coli* K1. Infect. Immun. **46**:159–167.
34. **Van Tiel-Menkveld, G. J., E. Veltkamp, and F. K. De Graaf.** 1981. Mitomycin C-induced synthesis of cloacin DF13 and lethality in cloacinogenic *Escherichia coli* cells. J. Bacteriol. **146**:41–48.
35. **Warner, P. J., P. H. Williams, A. Bindereif, and J. B. Neilands.** 1981. ColV plasmid-specified aerobactin synthesis by invasive strains of *Escherichia coli*. Infect. Immun. **33**:540–545.
36. **Weinberg, E. D.** 1984. Iron withholding: a defense against infection and neoplasia. Physiol. Rev. **64**:65–107.
37. **Williams, P. H.** 1979. Novel iron uptake system specified by ColV plasmids: an important component in the virulence of invasive strains of *Escherichia coli*. Infect. Immun. **26**:925–932.
38. **Williams, P. H., and P. J. Warner.** 1980. ColV plasmid-mediated colicin V-independant iron uptake system of invasive strains of *Escherichia coli*. Infect. Immun. **29**:411–416.