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Role of *crl* in Avian Pathogenic *Escherichia coli*: a Knockout Mutation of *crl* Does Not Affect Hemagglutination Activity, Fibronectin Binding, or Curli Production

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This study determined the role of *crl* in the production of curli by, the hemagglutination activity of, and fibronectin binding by avian pathogenic *Escherichia coli* χ7122. Curli, an extracellular structure that binds fibronectin, was recently described (A. Olsén, A. Jonsson, and S. Normark, Nature [London] 338:652–655, 1989). The *crl* gene product was hypothesized to be the subunit monomer of curli and to bind fibronectin. *E. coli* HB101, which does not contain *crl*, binds fibronectin and produces curli when harboring a plasmid containing the *crl* gene. We show that HB101 hemagglutinates chicken erythrocytes when harboring the *crl* gene and that χ7122 hemagglutinates chicken erythrocytes, binds fibronectin, and produces curli. Hemagglutination activity, fibronectin binding, and curli production are best expressed by χ7122 and HB101 harboring the *crl* gene when the bacteria are grown on colonization factor antigen agar at 26°C. The expression of hemagglutination activity, fibronectin binding, and curli production by both strains is decreased by growth on this agar at an increased temperature, of an increased osmolarity, or in an anaerobic atmosphere. This result indicates that the *crl* gene plays a role in the expression of the three phenotypes in HB101 and possibly in χ7122 as well. We inactivated *crl* in χ7122 by allele replacement in the expectation of abolishing hemagglutination activity, fibronectin binding, and curli production. The mutation was verified by Southern blot analysis and by a polymerase chain reaction, and there was no evidence of a second *crl* gene in χ7122. However, the mutant of χ7122 lacking *crl* hemagglutinated chicken erythrocytes, bound fibronectin, and produced curli at wild-type levels. This result indicates that *crl* plays a nonessential role in the expression of these three phenotypes in χ7122.

Pathogenic *Escherichia coli* is the causative agent of various diseases in humans and animals, including meningitis, bacteremia, and diarrhea, as well as infections of the urinary tract and respiratory tract. *E. coli* infection of the avian respiratory tract causes respiratory tract lesions and septicemia. This disease is referred to as air sacculitis, pneumonitis, septicemic colibacillosis, or colisepticemia. The majority of all *E. coli*-induced colisepticemias are caused by *E. coli* strains with one of three O antigens, O1, O2, or O78 (20). These infections are often secondary to primary infections with infectious bronchitis virus, Newcastle disease virus, and *Mycoplasma* spp. (11).

Transmission of colisepticemia is thought to occur via inhalation of feces-contaminated dust. The precise location of *E. coli* deposition within the respiratory tract that leads to disease is unknown. Investigators have shown that avian pathogenic *E. coli* isolates can bind to avian tracheal epithelial cells (29), suggesting that the binding of avian pathogenic *E. coli* to the avian trachea may be important in virulence. Recently, several avian pathogenic *E. coli* isolates were found to bind fibronectin (10). An extracellular structure produced by a bovine *E. coli* isolate binds fibronectin and has been designated curli (22). A gene, *crl*, has been cloned and hypothesized to encode the subunit monomer of curli, and curli has been hypothesized to bind fibronectin (22).

We were interested in identifying and analyzing determinants of avian pathogenic *E. coli* important in the colonization of host mucosal surfaces. An in vitro model of host-microbe interactions that is easily assayed is the hemagglutination of erythrocytes. Avian pathogenic *E. coli* strains examined to date do not hemagglutinate chicken erythrocytes (1, 7, 27). These tests were done with slide agglutination of erythrocytes to assay for hemagglutination (1, 7, 27). We used a more sensitive assay to characterize hemagglutination by a strain of avian pathogenic *E. coli*. This strain hemagglutinates chicken erythrocytes in the presence of mannose. This strain also binds fibronectin and produces curli. We present evidence that hemagglutination activity, fibronectin binding, and curli production are coexpressed in this avian pathogenic *E. coli* strain. These three phenotypes are also coexpressed in HB101 containing plasmid-encoded *crl* but not in HB101. These three phenotypes appear to require *crl* for expression in HB101. However, inactivation of *crl* in the avian pathogenic *E. coli* strain has no effect on hemagglutination activity, fibronectin binding, and curli production.

MATERIALS AND METHODS

Bacterial strains, plasmids, and media. Avian pathogenic *E. coli* EC1 (O78:K80:H9) was isolated from the liver of a diseased turkey and was received from L. H. Arp. We isolated a *gyrA* derivative of EC1, designated χ7122. AO12 is the bovine mastitis *E. coli* isolate from which *crl* was isolated (22). HB101 does not contain *crl* because of a deletion spanning the relevant region of the chromosome (2). pCRL10 (22) is pUC18 containing the *crl* gene flanked by 1.2 and 1.9 kb of DNA 5′ and 3′ to the gene, respectively; these DNAs also flank the gene in vivo. pCRL20 (22) is pUC18 containing *crl* on a 1.5-kb fragment subcloned from pCRL10. pUC4K contains the 1.4-kb kanamycin resistance (Km^r)

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cassette (*aphA*) used for in vitro gene interruption (28). pMAK705 is a temperature-sensitive plasmid derived from pSC101 and designed for allele replacement experiments (13).

L broth, L agar plates (18), phosphate-buffered (PB) agar plates (6), colonization factor antigen (CFA) agar plates (8), and CFA broth were prepared as described previously. CFA plates were supplemented as needed with 0.15 M NaCl. Plates were incubated at 37 or 42°C for 24 h and at 26°C for 48 h. Plates that were to be incubated anaerobically were placed in a GasPak jar containing a GasPak Plus gas generator envelope (BBL Microbiology Systems). Antibiotics were used at the following concentrations; ampicillin, 100 μg/ml; chloramphenicol, 30 μg/ml; and kanamycin, 30 μg/ml.

Hemagglutination assays. All hemagglutination assays were done in the presence of 0.5% α-methyl-D-mannopyranoside (Sigma), a nonmetabolizable mannose analog, to control for mannose-sensitive hemagglutination by type 1 fimbriae. Specific-pathogen-free White Leghorn chickens (SPAFAS, Inc.) were hatched in our animal facility. Blood was obtained from the wing vein of 3- to 6-week-old chickens by use of a heparinized syringe. The erythrocytes were washed three times in ice-cold 0.85% (wt/vol) NaCl and stored as a 3% (vol/vol) suspension of erythrocytes in 0.85% NaCl.

Hemagglutination activity was assayed by the microhemagglutination test (16). The starting concentration of the cells was approximately 10^{11}/ml, and 96-well round-bottom microtiter plates (Corning Glass Works) were used. Wells containing a small pellet of erythrocytes at the bottom after 1 h of incubation on ice were considered hemagglutination negative, and wells containing an even sheet of erythrocytes across the bottom were considered hemagglutination positive. The erythrocytes did not autoagglutinate under these conditions. The microhemagglutination test was also done with borosilicate culture tubes (10 by 75 mm; Fisher) to control for the adherence of bacteria to the plastic of the microtiter plate well which might appear to be hemagglutination (21). The hemagglutination titer was expressed as the reciprocal of the highest dilution of bacterial cells that resulted in positive hemagglutination.

Iodination of fibronectin and fibronectin binding by *E. coli*. Bovine plasma fibronectin (Calbiochem) was iodinated by the chloramine-T method as described by Thorell and Larson (26). Fibronectin binding by bacterial cells was accomplished by adding iodinated fibronectin to cells suspended in phosphate-buffered saline–0.1% Tween 80 (Sigma) and rotating the containers end over end for 1 h at room temperature (22). The cells and bound fibronectin were pelleted for 3 min at 13,000 × *g*, and the supernatant fluid, containing unbound fibronectin, was removed. The radioactivity associated with the pellet was determined in a gamma counter (Beckman Gamma 4000). The mean percentage of fibronectin bound in three assays was calculated. Cells were considered able to bind fibronectin if they bound at least 10% of the total radioactivity added and unable to bind fibronectin if they bound less than 10% of the total radioactivity added. Student's *t* test was used to determine the statistical significance of different fibronectin binding levels.

In vitro mutagenesis of *crl*. All enzymes used to manipulate DNA were purchased from Promega. Transformation was done by the technique of Cohen et al. (3). The temperature-sensitive plasmid containing the *crl* gene inactivated with a Km^r cassette, pYA3040, was constructed as follows (Fig. 1). The Km^r cassette was isolated from pUC4K, ligated to pCRL10 that had been partially digested with *Cla*I, and used to transform competent HB101. Plasmid DNA was isolated from Km^r transformants. One isolate, pYA3037, was digested to yield the fragment containing the inactivated *crl* gene and flanking DNA. This fragment was ligated to pMAK705 (13) and used to transform competent HB101. Km^r cells were selected after growth at 30°C. The temperature sensitivity and chloramphenicol resistance of the transformants and the restriction endonuclease digestion patterns of the transforming plasmids were determined to verify the construction. A plasmid with the desired restriction map and conferring the appropriate phenotype was chosen and designated pYA3040.

Allele replacement of *crl* in χ7122. Gene replacement of the wild-type *crl* gene with the insertionally inactivated *crl*::*aphA* gene was done by exploiting the temperature-sensitive replication of pYA3040. Km^r transformants of χ7122 were selected at 30°C. Cells that had integrated the plasmid into the chromosome were selected by growing individual transformants in L broth overnight at 30°C, plating them onto L agar containing kanamycin, and incubating them overnight at 42°C. The resulting Km^r isolates had the plasmid integrated into the chromosome.

After isolating cells that had integrated the plasmid into the chromosome, we enriched for cells in which the integrated plasmid had been excised from the chromosome by a second recombination event. Enrichment was accomplished by two serial passages of one Km^r chloramphenicol-resistant (Cm^r) colony isolated at 42°C. The serial passages were done with 100 ml of L broth and overnight incubation without selection at 30°C. Excision of the plasmid from the chromosome yielded a Cm^s phenotype at 42°C, while the loss of the excised plasmid from the cell yielded chloramphenicol sensitivity at both 30 and 42°C. Isolation of colonies that were stably Km^r and Cm^s during growth at both 30 and 42°C indicated that the integrated plasmid had been removed from the chromosome by recombination and that the plasmid had been lost during growth under nonselective conditions. Also, since the strains were still Km^r, the recombination event that had removed the plasmid from the chromosome resulted in excision of the wild-type *crl* gene with the other plasmid DNA sequences. The in vitro-constructed *crl*::*aphA* allele was left behind in the chromosome to cause the allele replacement event. One Km^r Cm^s colony was chosen for further analysis and designated χ7137.

Southern hybridization of χ7122 and χ7137 chromosomal DNAs. A 300-bp *crl*-specific DNA probe was isolated by digestion of pCRL20 with *Cla*I and *Hpa*I and gel purified. A Km^r cassette-specific DNA probe was isolated by digestion of pUC4K with *Pst*I and gel purification of the 1.4-kb fragment. These DNA fragments, as well as pMAK705, were labeled with [α-^{32}P]ATP (NEN Research Products) by use of a random priming DNA labeling kit (Boehringer Mannheim Biochemicals).

Chromosomal DNAs were isolated from χ7122 and χ7137 as described previously (14) and digested with *Sal*I or *Sma*I. DNA fragments were resolved on a 0.7% agarose gel and transferred to GeneScreen Plus membranes (New England Nuclear Corp.) in accordance with the manufacturer's instructions. DNA hybridizations were done as described previously (25). Hybridized membranes were washed under high-stringency (65°C) or low-stringency (42°C) conditions and exposed to Kodak X-Omat AR film at −70°C with an intensifying screen (Dupont Cronex Lightning-Plus).

Electron microscopy. Electron microscopy to visualize curli was done by growing bacteria on CFA plates at various

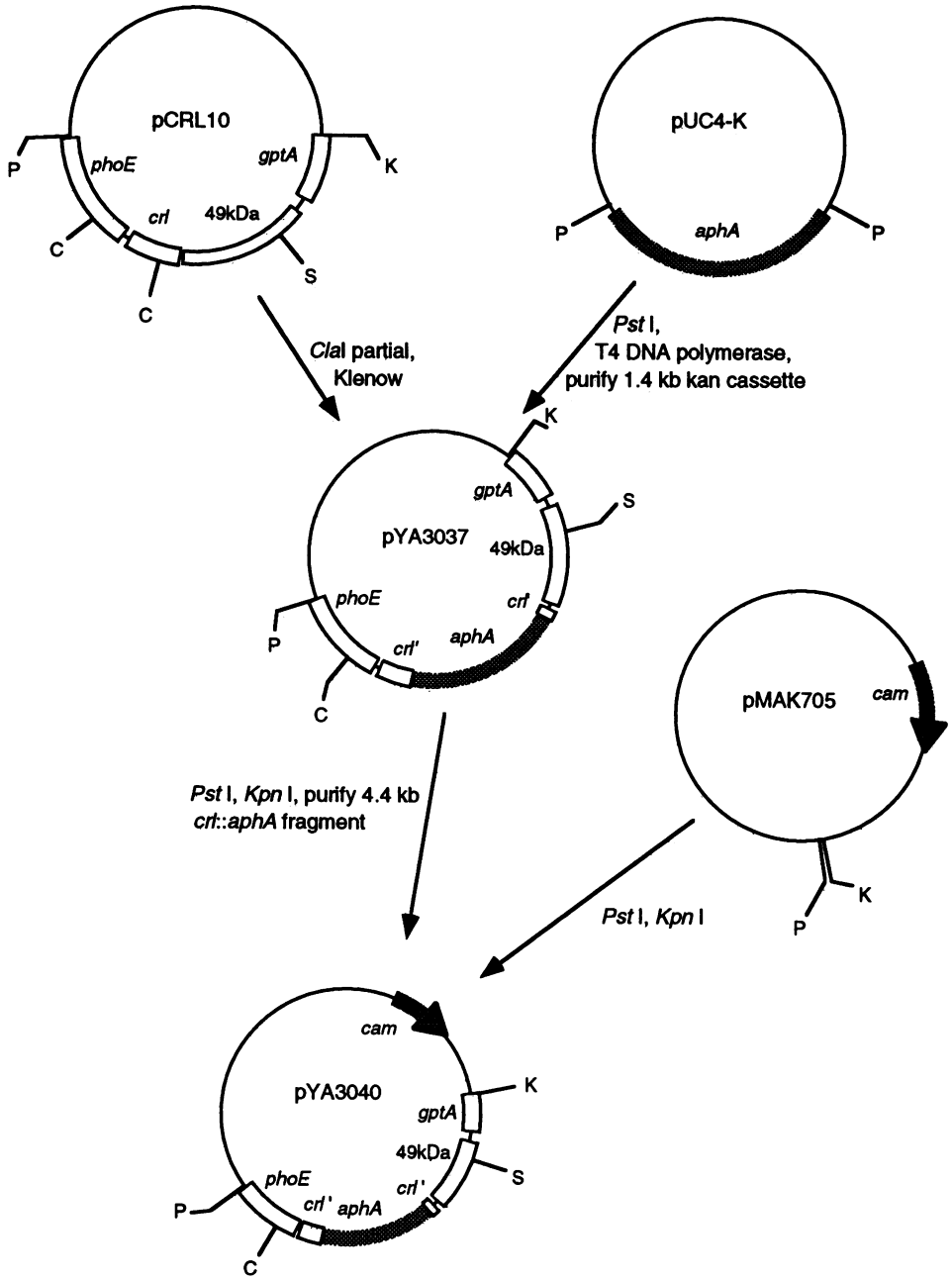

FIG. 1. Construction of pYA3040. Abbreviations: C, *Cla*I; K, *Kpn*I; P, *Pst*I; S, *Sph*I.

temperatures. The cells were removed from the plates and suspended in 0.85% NaCl, and the suspensions were placed on 400 mesh Butvar carbon-coated copper grids. The cell suspensions were rinsed with distilled water and negatively stained with 1% ammonium molybdate for 1 min. Electron microscopy was done with a Hitachi H-600 electron microscope.

RESULTS

Mannose-resistant hemagglutination by χ7122. χ7122 was grown on phosphate-buffered agar or CFA agar at 26°C and tested for hemagglutination activity in the presence of 0.5% α-methyl-D-mannopyranoside. χ7122 grown on CFA agar exhibited a hemagglutination titer of 64. The same hemagglutination titer resulted when the test was done with borosilicate culture tubes. Hemagglutination activity was found to be dependent on the incubation temperature; the hemagglutination titer was 8 after growth at 37°C, and there was no hemagglutination after growth at 42°C. χ7122 did not hemagglutinate chicken erythrocytes in the microhemagglutination test when grown on PB agar or when grown in CFA broth. This hemagglutination phenotype has been designated Tsh (temperature-sensitive hemagglutinin).

The expression of hemagglutination activity, fibronectin binding, and curli production is regulated by the same environmental conditions. The low-temperature-dependent in-

TABLE 1. Hemagglutination activity of and fibronectin binding and production of curli by χ7122 and HB101(pCRL20) as a function of incubation temperature[a]

Strain	Hemagglutination titer after growth at the following temp (°C):			Fibronectin binding[b] after growth at the following temp (°C):			Production of curli after growth at the following temp (°C):		
	26	37	42	26	37	42	26	37	42
χ7122	64	16	0	81.4 ± 0.19	64.0[c] ± 2.2	<10	Yes[d]	Yes[d]	No
HB101(pCRL20)	32	0	0	29	<10	<10	Yes	No	No
HB101	0	0	0	<10	<10	<10	No	No	No
HB101(pUC18)	0	0	0	<10	<10	<10	ND[e]	ND	ND

[a] All strains were grown on CFA agar at the indicated temperatures and tested for hemagglutination of chicken erythrocytes, fibronectin binding, and the presence of curli.
[b] reported as the mean ± standard deviation percentage of fibronectin bound in triplicate assays.
[c] The differences between percentages of fibronectin bound after growth at 26 and 37°C were statistically significant ($P < 0.001$).
[d] Although there is no quantitative assay for curli production, electron microscopy of χ7122 showed that cells grown at 26°C on CFA agar appeared to have more curli than cells grown at 37°C.
[e] ND, not determined.

duction of hemagglutination activity was similar to the effect of growth temperature on the expression of two other phenotypes in *E. coli* AO12. AO12 expresses fibronectin binding and the extracellular structure curli after growth at 26°C (22). The *crl* gene is the gene hypothesized to encode the structural monomer of curli, which is hypothesized to bind fibronectin (22). The *crl* gene has been cloned and, when it is present in HB101 on plasmid pCRL20, curli production and fibronectin binding are observed when the cells are grown at 26°C but not at 37°C (22). Thus, the hemagglutination activity of χ7122 and fibronectin binding and curli synthesis by HB101(pCRL20) appear to be regulated by temperature. This similar effect of temperature on the regulation of these phenotypes prompted us to determine whether χ7122 could bind fibronectin and produce curli and whether HB101(pCRL20) could hemagglutinate chicken erythrocytes. χ7122 and HB101(pCRL20) could each hemagglutinate chicken erythrocytes, bind fibronectin, and produce curli (Table 1 and Fig. 2). In each strain, these three phenotypes were best expressed after growth at lower temperatures (Table 1 and Fig. 2).

We determined whether there were culture conditions that would cause the expression of only hemagglutination activity or fibronectin binding. Previous work demonstrated that an *E. coli* diarrheal isolate lost the ability to bind fibronectin after growth on CFA agar containing 0.06 to 0.17 M NaCl (19) and that HB101(pCRL20) did not bind fibronectin or produce curli when grown at 26°C on CFA agar containing 0.15 M NaCl (23). We investigated hemagglutination activity and fibronectin binding after the growth of strains on higher-osmolarity media. When χ7122 was grown on CFA agar containing 0.15 M NaCl, the hemagglutination titer decreased from 64 to 0 and fibronectin binding decreased from 81.4% ± 0.19% to less than 10%. The hemagglutination titer decreased from 32 to 0 and fibronectin binding decreased from 26% ± 0.09% to less than 10% when HB101(pCRL20) was grown under the same high-osmolarity conditions. The growth of χ7122 and HB101(pCRL20) under anaerobic conditions also caused hemagglutination titers and fibronectin binding to decrease to 0 and less than 10%, respectively. These results show that (i) the expression of hemagglutination activity, fibronectin binding, and curli production in HB101 requires *crl*, (ii) the expression of these three phenotypes is coregulated by different culture conditions, and (iii) the expression of these three phenotypes behaves the same way in χ7122 as it does in HB101(pCRL20). These results led us to hypothesize that curli mediates hemagglutination activity and fibronectin binding and that *crl* has the same role in the expression of the three phenotypes in χ7122 as it does in HB101.

Inactivation of *crl* in pCRL10. To investigate the role of *crl* in χ7122, we mutagenized *crl* in vitro by ligating the Km^r cassette into the *crl* coding sequence in pCRL10 (see Materials and Methods and Fig. 1). The resulting mutagenized *crl* gene in pYA3037 (Fig. 1) was no longer able to cause HB101 to hemagglutinate or bind fibronectin (Table 2). The *crl* gene was first mutagenized by insertion of the Km^r cassette into the gene by Olsén et al. (22). They showed that a mutant *crl* no longer caused HB101 to bind fibronectin or produce curli (22). Our data indicate that *crl* is responsible for these three activities in HB101 and agree with the results of Olsén et al. (22).

Inactivation of *crl* in χ7122 by allele replacement. The hypothesis that curli mediated both hemagglutination activity and fibronectin binding in χ7122 was tested by inactivating *crl* in χ7122 to produce a strain that contained a mutant *crl* locus and then testing the phenotypes of the mutant *crl* strain. The inactivated *crl* gene was used to replace the wild-type *crl* gene in χ7122 as described in Materials and Methods. The resulting putative *crl*::*aphA* mutant of χ7122 was designated χ7137.

Southern blot analysis was used to verify that gene replacement had occurred in χ7137 (Fig. 3). A 300-bp *crl*-specific DNA probe hybridized with a 2.9-kb *Sal*I band in χ7122 and hybridized with a 4.3-kb *Sal*I band in χ7137. This increase in size of 1.4 kb is equal to the molecular size of the Km^r cassette. A Km^r cassette-specific DNA probe did not hybridize with χ7122 DNA but did hybridize with a band in χ7137 that migrated at the same molecular size as the 4.3-kb *Sal*I band that hybridized with the *crl*-specific DNA probe. The *crl*-specific DNA probe hybridized with a >12-kb *Sma*I band in χ7122 and hybridized with a *Sma*I band in χ7137 that migrated at 7.5 kb. The Km^r cassette-specific DNA probe hybridized with two *Sma*I bands in χ7137. One of these bands migrated at a molecular size similar to that of the *Sma*I band (7.5 kb) in χ7137 that hybridized with the *crl*-specific DNA probe. The 1.4-kb increase in the size of the band hybridizing with *crl* in χ7137 and the comigration of bands that hybridized with *crl* and the Km^r cassette in χ7137 suggested that chromosomal *crl* was inactivated by replacement of the wild-type gene with one that contained the Km^r cassette. Southern blot analysis did not indicate the presence of more than one DNA sequence homologous to *crl* in these *E. coli* strains. Polymerase chain reaction amplification of

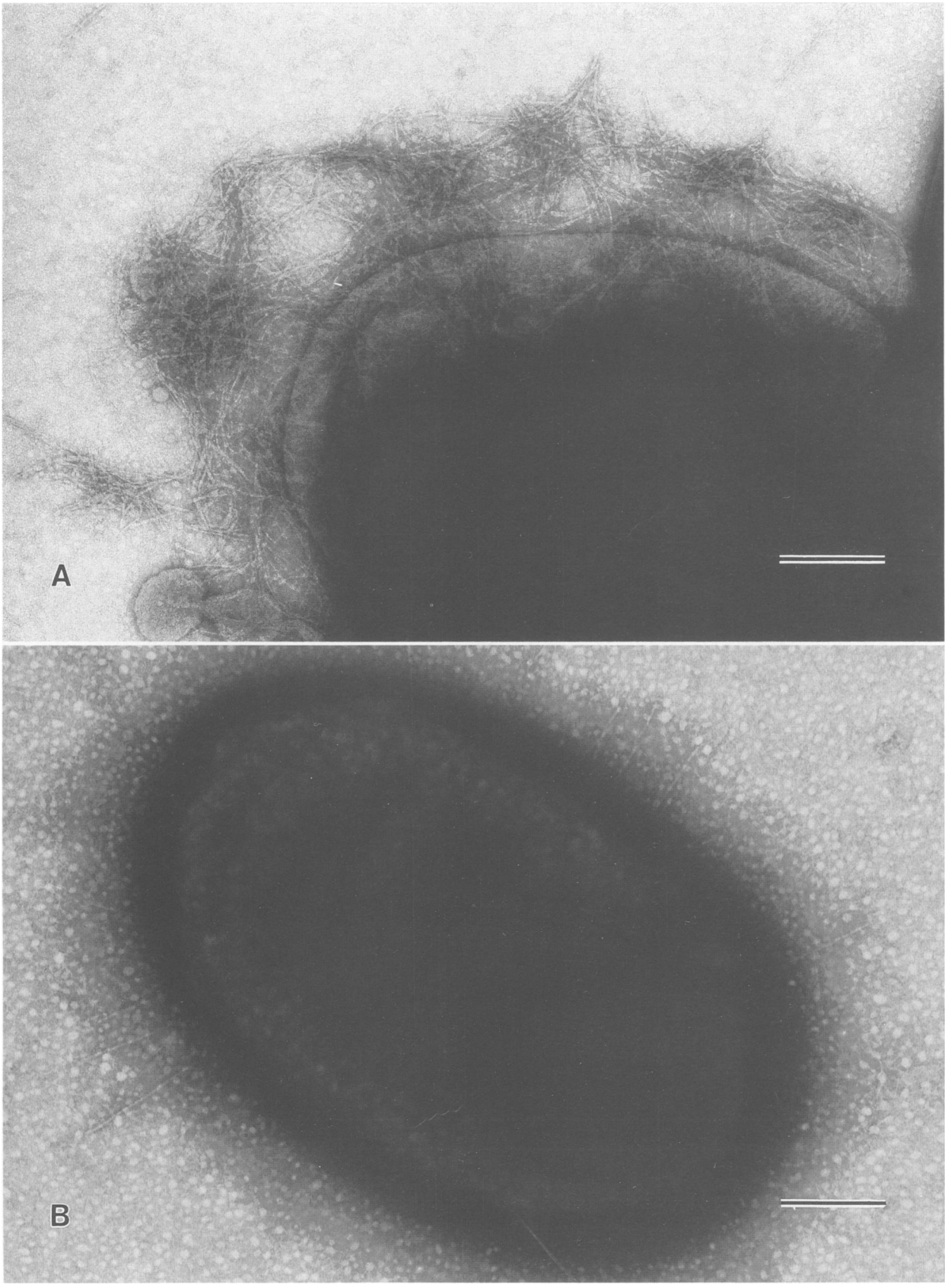
A
B

TABLE 2. Hemagglutination activity of and fibronectin binding and curli production by χ7122 and χ7137[a]

Strain	Hemagglutination titer	Fibronectin binding[b]	Curli production
HB101(pCRL20)	32	14 ± 1.7	Yes[c]
HB101(pYA3037)	0	<10	ND[d]
χ7122	64	89 ± 0.3	Yes
χ7137	64	89 ± 0.7	Yes

[a] Bacteria were grown on CFA agar at 26°C for 48 h and tested for hemagglutination of chicken erythrocytes, fibronectin binding, and curli production as described in Materials and Methods.
[b] Reported as the mean ± standard deviation percentage of fibronectin bound in triplicate assays.
[c] Determined by Olsén et al. (22).
[d] ND, not determined.

the *crl* gene in χ7122 and χ7137 also indicated that χ7137 contained a single copy of *crl* that had increased in size by the molecular size of the Km^r cassette (data not shown).

Phenotypes of χ7122 and χ7137. The effect of the *crl* mutation in χ7137 on hemagglutination activity, fibronectin binding, and curli production was determined. Inactivation of *crl* by allele replacement had no effect on any of the three phenotypes (Table 2).

DISCUSSION

The *crl* gene product, curlin, has been hypothesized to be the structural monomer of curli and to bind fibronectin (22). When *crl* is present in HB101, curli is produced and fibronectin is bound, and when *crl* is mutagenized or not present, HB101 no longer produces curli or binds fibronectin (22; Tables 1 and 2). The evidence that curlin binds fibronectin consists of a Western blot probed with iodinated fibronectin (22). HB101(pCRL20), grown to maximize curli production and fibronectin binding, bound the labeled fibronectin, while HB101 containing truncated *crl* did not. The fibronectin was localized to a single band that migrated at 17 kDa. The relative molecular mass of curlin is 17 kDa (22). Our data show that *crl* also causes HB101 to hemagglutinate. This hemagglutination activity is coexpressed with curli production and fibronectin binding in HB101 and χ7122 (Table 1), and different environmental conditions affect the expression of hemagglutination activity, fibronectin binding, and curli production in the same way.

The experiments described above constitute the first formal analysis of the structural role of the *crl* gene product in curli production. We believe that our data are incompatible with the hypothesis that the *crl* gene product is the structural monomer of curli and that it binds fibronectin. Curli production by, hemagglutination activity of, and fibronectin binding by χ7122 and by HB101(pCRL20) were expressed during growth on CFA agar and repressed during growth in the presence of high temperature, high osmolarity, and low oxygen tension (23; Table 1). The similar effects of these environmental conditions on the expression of curli production by, hemagglutination activity of, and fibronectin binding by χ7122 and HB101(pCRL20) had originally suggested to us that the *crl* gene product was the curli structural monomer, hemagglutinated erythrocytes, and bound fibronectin in both χ7122 and HB101(pCRL20). Mutagenesis of *crl* caused HB101 to no longer hemagglutinate or bind fibronectin (22; Table 2). Allele replacement of the wild-type *crl* in χ7122 with a mutagenized allele of *crl* had no effect on curli production, hemagglutination activity, or fibronectin binding. Southern blot and polymerase chain reaction analysis did not indicate the presence of a second copy of *crl* in χ7122 (Fig. 3).

The finding that *crl* mutant strain χ7137 still produces curli, hemagglutinates, and binds fibronectin suggests that the *crl* mutation in χ7137 may be complemented by another chromosomal gene(s). This possibility is unlikely, since a complementing gene would be expected to have some homology to *crl*. Our data indicate that there is no other gene in χ7122 with homology to *crl*. The data presented above do not rule out the possibility that χ7122 produces more than one type of structure that is morphologically similar to curli or that χ7122 is able to hemagglutinate or bind fibronectin via other genetically distinct means. Also, it should be noted that the *crl* gene used to replace *crl* in χ7122 was from a different isolate, AO12. Since the DNA flanking *crl* in AO12 may be different from the DNA flanking *crl* in χ7122, χ7122 and χ7137 are not, strictly speaking, isogenic.

An alternative explanation for the continued expression of hemagglutination activity of and fibronectin binding and curli production by χ7137 is that *crl* does not encode the structural subunit of curli. Two observations suggest this possible explanation. *crl* does not contain a cleavable signal sequence (22), yet a signal sequence would be expected if the *crl* gene product were exported. Also, if *crl* represented only one mechanism for producing curli, hemagglutinating erythrocytes, and binding fibronectin in χ7122, then the *crl* mutation would be expected to have some noticeable effect on the presence of curli, the hemagglutination titer, and the binding of fibronectin in χ7137. However, the amounts of curli produced, the hemagglutination titers, and the amounts of fibronectin bound were the same for χ7122 and χ7137 (Table 2). A structure that is morphologically similar to curli and that confers the ability to bind fibronectin was recently isolated from *Salmonella enteritidis* and diarrheagenic *E. coli* and characterized (4, 5). This structure appears to be composed of a 17- to 18-kDa subunit (4, 5). However, a comparison of the N-terminal sequence and total amino acid composition of the subunit protein with the deduced N-terminal sequence and total amino acid composition of curlin indicates that these two proteins are not related (4, 5).

We recently isolated a gene from χ7122 that allows HB101 to hemagglutinate and produce curli but not bind fibronectin (24). This gene does not hybridize to the *crl* gene. This result suggests that *crl* is not the only gene able to cause the expression of hemagglutination activity and curli production in HB101. Also, since curli was produced but fibronectin binding did not occur in HB101 containing this cloned gene, fibronectin binding may not be an intrinsic property of curli.

These results concerning the temperature regulation of fibronectin binding are in agreement with previous work on the binding of soluble fibronectin by avian *E. coli* (10). Fibronectin binding by other pathogenic *E. coli* strains has also been reported (see reference 15 for a review).

FIG. 2. Production of curli by χ7122. χ7122 was grown on CFA agar at 37°C (A) or 42°C (B). Cells were harvested and prepared as described in Materials and Methods to visualize curli. When grown at 26°C on CFA agar, χ7122 produces large amounts of curli that appear as a thick mat around the cells. Cells incubated at 37°C were used here to allow individual fibers to be more easily distinguished. Bars, 0.25 μm.

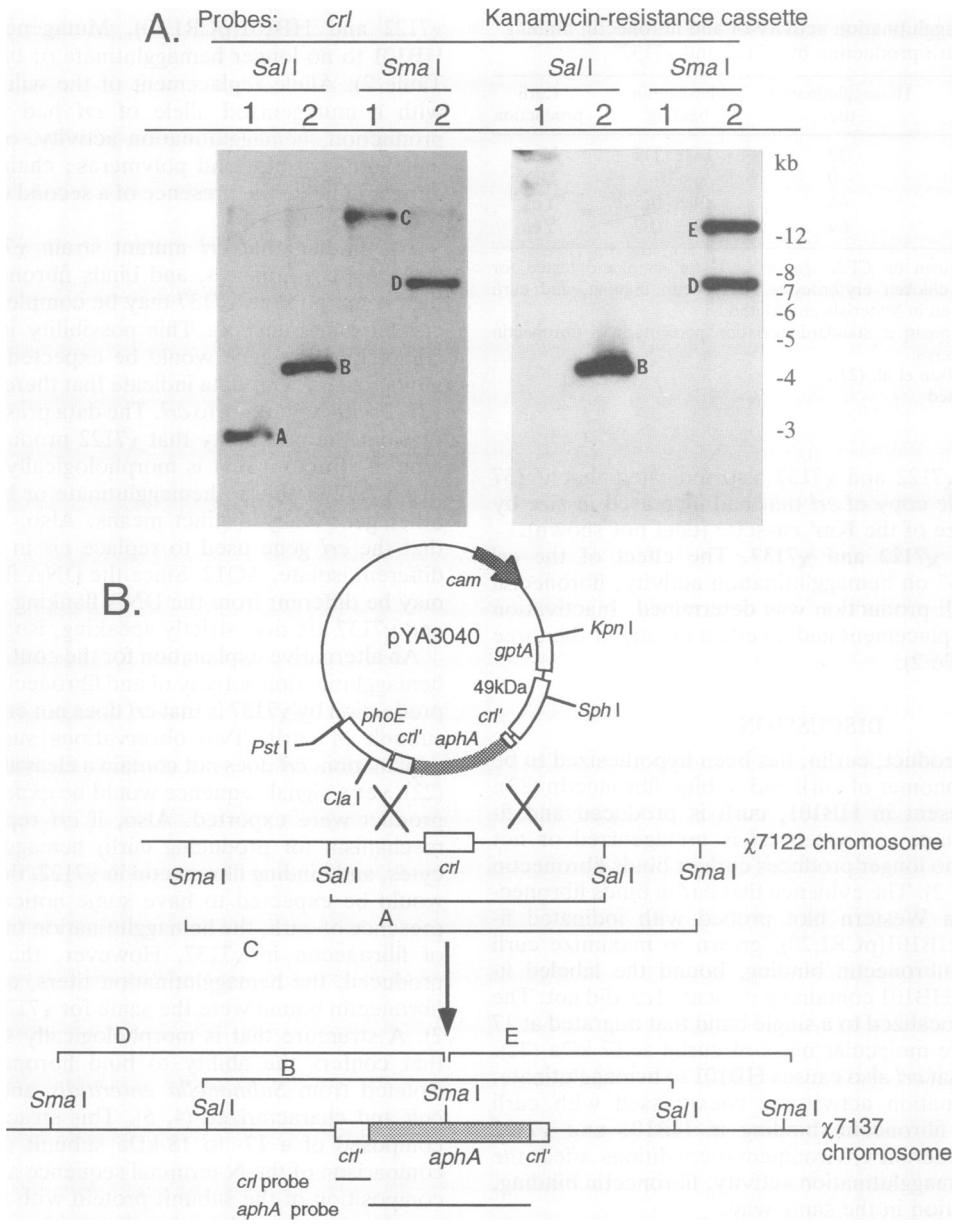

FIG. 3. Southern blot analysis of gene replacement in χ7122. (A) χ7122 (lane 1) and χ7137 (lane 2) whole-cell genomic DNAs were isolated, digested with *Sal*I or *Sma*I, electrophoretically resolved, and transferred to nylon membranes as described in Materials and Methods. The membranes were hybridized to a ^{32}P-labeled *crl*-specific or Km^r cassette-specific DNA probe and washed at 65°C. Letters indicate the origin of each band as indicated in panel B. (B) Schematic of double homologous recombination between pYA3040 and χ7122 and the resulting structure of the χ7137 chromosome. The DNA fragments resulting from *Sal*I or *Sma*I digestion of χ7122 and χ7137 are labeled A through E and correspond to the labeled bands in panel A.

Hemagglutination and fibronectin binding have long been recognized as virulence factors that may be important in the adherence of pathogens to host surfaces. The hemagglutination activity of enterotoxigenic *E. coli* is caused by several different types of fimbriae (9), and this activity is associated with the adherence of enterotoxigenic *E. coli* to intestinal epithilium, a crucial step in the pathogenesis of this organism. The expression of hemagglutination by uropathogenic *E. coli* is correlated with the ability to adhere to urinary tract epithelial cells (12, 17). A variety of pathogenic microbes are capable of binding fibronectin, and binding to fibronectin may be important in the adherence of bacteria to host tissues (see reference 15 for a review). It is unclear whether hemagglutination and fibronectin binding play a role in the virulence of avian pathogenic *E. coli*. The presence in χ7122 of the chicken erythrocyte adhesin described in this report represents a potentially relevant model of the interaction between a pathogen and its natural host. The avian model for colisepticemia also represents an experimental system exploiting the interaction between a pathogen and its natural host to test the role of fibronectin binding in the pathogenesis of this disease. Although a great deal of work has been done regarding the interaction of microbes with fibronectin, no experiments to conclusively evaluate the importance of

fibronectin binding in microbial pathogenesis have been done because of the lack of relevant animal models.

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