Mutants of *Escherichia coli* O9:K30 with Altered Synthesis and Expression of the Capsular K30 Antigen

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Escherichia coli K30 produces a thermostable group I capsular polysaccharide. Two classes of mutants were isolated with defects in the synthesis or expression of capsule. The most common mutant phenotype was acapsular (K⁻), with no K-antigen synthesized. A second class of mutants, termed Ki or intermediate forms, produced colonies which were indistinguishable from those of acapsular forms yet K-antigenicity was expressed. Previous studies had demonstrated that *E. coli* strains that produce K30 antigen synthesize a lipopolysaccharide (LPS) fraction that is recognised by monoclonal antibodies against the K30 antigen. Synthesis of this LPS fraction was not affected in Ki forms. The results of morphological examination, LPS analysis and phage sensitivity studies are consistent with the interpretation that the defect in Ki strains results from an inability to polymerize the K30 antigen. Using plasmid pULB113 (RP4::mini-Mu), mutations resulting in both K⁻ and Ki phenotypes were localized near the *his* region of the chromosome.

INTRODUCTION

The capsular antigens, or K-antigens, of *Escherichia coli* consist of polysaccharides which can be subdivided into two groups based on chemical and physical properties (Jann & Jann, 1987).

Group II capsular polysaccharides (CPS) include those K-antigens originally designated thermolabile (Ørskov *et al.*, 1977). These polysaccharides structurally resemble the CPS of *Neisseria meningitidis* and *Haemophilus influenzae*. Some CPS from group II *E. coli* and *N. meningitidis* contain a phosphatidic acid residue at the reducing terminus (Gotschlich *et al.*, 1981; Schmidt & Jann, 1982) and this lipid may play a role in maintenance of the capsular layer at the cell surface, through hydrophobic interactions (Jann & Jann, 1987).

The group I CPS of *E. coli* differ fundamentally from group II in that they are thermostable and often closely resemble structures found in *Klebsiella* CPS. At least some group I CPS terminate in lipid A-core, indicating that these K-antigens may be expressed on the cell surface as an acidic lipopolysaccharide (LPS) (Jann & Jann, 1987). We have recently described an LPS fraction from *E. coli* K30 (group I CPS), which migrates near the lipid A-core in SDS-PAGE and appears to react with monoclonal antibodies directed against the K30 CPS (Homonylo *et al.*, 1988). These results are consistent with the interpretation that lipid A-core is involved in presenting at least some K-antigenicity at the cell surface in *E. coli* K30, and support previous reports of an LPS fraction consisting of a lipid A-core bearing a single repeating unit of K-antigen in *E. coli* K30 (Jann & Jann, 1977).

The genes for the group II *E. coli* CPS map at a locus termed *kps* (Silver *et al.*, 1984; Vimr *et al.*, 1989), formerly *kpsA* (Ørskov & Nyman, 1974), which is located near *serA* on the *E. coli* linkage map. In contrast, the genetic determinants for group I CPS have been reported to be

Abbreviations: CPS, capsular polysaccharide; LPS, lipopolysaccharide; KDO, 2-keto-3-deoxyoctulosonic acid (3-deoxy-D-manno-octulosonic acid); mAb, monoclonal antibody; MNNG, N-methyl-N'-nitro-N-nitrosoguanidine.

organized similarly to those for *Salmonella* LPS O-antigen (Mäkelä & Stocker, 1984). The products of CPS biosynthesis genes linked to *his* and *rfb* (O-antigen) are responsible for the synthesis and assembly of the CPS repeating unit (Schmidt *et al.*, 1977). A second locus, tentatively placed near *trp*, has been suggested to encode a putative CPS polymerase, analogous to the O-antigen polymerase (*rfc* gene) in *Salmonella* (Schmidt *et al.*, 1977). Mutations in the putative polymerase would yield intermediate or Ki forms which express K-antigenicity but lack the full capsular layer which masks underlying structures in wild-type bacteria. Studies in this laboratory have demonstrated that RP4 : mini-Mu mediated transfer of the *his* region from *E. coli* K30 to an unencapsulated *E. coli* K12 recipient was sufficient for synthesis of a 'full' K30 capsule (Laakso *et al.*, 1988).

We are interested in the synthesis and assembly of group I *E. coli* CPS using *E. coli* K30 as a prototype system. The objective of the studies presented here was to isolate and characterize mutants altered in the synthesis and expression of the K30 antigen, in an attempt to define the range of CPS mutant phenotypes and to resolve the apparently contradictory results regarding the distribution of K-antigen loci. To differentiate the group I CPS biosynthesis genes from those of group II (*kps*) in *E. coli*, we propose the designation *kst* (K-antigen, thermoSTable) for group I.

METHODS

Bacterial strains and growth conditions. The bacterial strains and plasmids used in this study are given in Table 1. *Escherichia coli* strains E69 and W6930 have been described elsewhere (Homonylo *et al.*, 1988). Bacterial strains were routinely grown in Luria broth (LB) medium supplemented with 1.5% (w/v) agar for plates. The antibiotics ampicillin (Ap; 100 µg ml⁻¹ final concentration), kanamycin (Km; 50 µg ml⁻¹), tetracycline (Tc; 15 µg ml⁻¹) and streptomycin (Str; 100 µg ml⁻¹) were obtained from Sigma. For conjugation experiments, M9 minimal medium (Gibco) was supplemented with 0.2% D-glucose and the appropriate antibiotics. Amino acids were obtained from Sigma and included at final concentrations of 40 µg whenever appropriate. All incubations were at 37 °C.

Determination of O9 and K30 antigen expression. The presence of capsule was determined by susceptibility to coliphage K30 (Whitfield & Lam, 1986), and by agglutination using K30-capsule-specific monoclonal antibody (mAb) 4-15A (Homonylo *et al.*, 1988). Similarly, bacteriophage O9-1 and Difco anti-O9 antibody provided tests for the presence of the O9 LPS (Homonylo *et al.*, 1988).

Isolation of mutants. The mutant strains reported in this study were obtained either as spontaneous mutants, or following mutagenesis with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) (Miller, 1972). The isolation of spontaneous mutants deficient in either K30 or O9 antigens, which occurred at relatively low frequency, was facilitated by the use of coliphages K30 and O9-1, respectively. LB plates were inoculated as lawns from overnight

Table 1. *Bacterial strains and plasmids used in this study*

Bacterial strain or plasmid	Relevant serotype	Genotype or relevant properties	Source or reference
<i>E. coli</i> E69	O9 : K30 : H12	Prototroph, reference strain for K30 antigen	Homonylo <i>et al.</i> (1988)
<i>E. coli</i> W6930	O9 : K30	E69 but His ⁻ Trp ⁻ Lac ⁻ Str ^R	Homonylo <i>et al.</i> (1988)
<i>E. coli</i> B192	O9 : K ⁻	W6930; <i>kst-1</i>	Spontaneous mutant selected using coliphage K30; this study
<i>E. coli</i> B198	O ⁻ : K ⁻	B192; defective in O-antigen expression	Spontaneous mutant selected using coliphage O9-1; this study
<i>E. coli</i> B70	O9 : K30i*	W6930; <i>kst-7</i>	MNNG mutagenesis; this study
<i>E. coli</i> B78	O ⁻ : K30i*	B70; defective in O-antigen expression	Spontaneous mutant selected using coliphage O9-1; this study
<i>Salmonella typhimurium</i> SGSC82		<i>rfc-497 metA22 trpC2</i> H1-b H1-e,n,x <i>fla-66 rpsL120</i>	K. E. Sanderson (Department of Biological Sciences, University of Calgary, Canada)
<i>Salmonella typhimurium</i> SA2876		[MA421(pULB113)]; <i>metA22 metE551 trpC2 ilv-452 rpsL120 xyl-404 galE436 hsdLT6</i> H1-b H2-e,n,x <i>flaA66</i> (pULB113)	Kadam <i>et al.</i> (1985)
pULB113		<i>tra⁺ bla⁺</i> (TEM-2) TnI tet ⁺ <i>aphA⁺</i> (Mu3A)	Van Gijsegem & Toussaint (1982)

* K30 intermediate form; see text.

broth cultures of the appropriate parent strain. Once dry, 0.05 ml spots of bacteriophage were applied. The plates were incubated at 37 °C. Mutants were picked as survivors from the zone of lysis. All survivors from lysates of coliphage K30 were acapsular (K^-). Similarly, survivors from coliphage O9-1 lysates had lost the LPS O-antigen. With the higher mutation frequencies obtained using MNNG mutagenesis, acapsular mutants were picked from dilution plates based on altered colonial morphology, and no bacteriophage selection was necessary. Each mutant was then tested for O9 and K30 antigen expression.

Isolation and fractionation of LPS from *E. coli* B78. LPS was extracted from bacterial cells using 45% (w/v) aqueous phenol at 65 °C (Westphal & Jann, 1965). The LPS was collected by ultracentrifugation (200000 *g* for 16 h), resuspended in 10 mM-MgCl₂, 50 mM-Tris/HCl, pH 7.2 and treated for 2 h at 37 °C with 5 µg RNAase and DNAase ml⁻¹ (Sigma). The sample was then treated with 5 µg proteinase K ml⁻¹ (Sigma) at 50 °C for 2 h. The LPS was recovered by ultracentrifugation and washed twice in distilled water prior to lyophilization.

LPS was fractionated using a modification of the method described by MacIntyre *et al.* (1986). The purified LPS was resuspended in 3% (w/v) sodium deoxycholate, 0.2 M-NaCl, 0.005 M-EDTA, 0.02 M-Tris/HCl, pH 8.3. Samples of LPS (8 mg in 1 ml buffer) were subjected to gel filtration on a column of Sephadex G50 Superfine (50 × 2.5 cm) (Pharmacia). Column equilibration and separation were done in a buffer containing 0.25% (w/v) sodium deoxycholate, 0.2 M-NaCl, 0.005 M-EDTA and 0.01 M-Tris/HCl, pH 8.0. Fractions (2.5 ml) were collected and analysed for 2-keto-3-deoxyoctulosonic acid (KDO) content (Karkhanis *et al.*, 1978). LPS-containing fractions were indicated by the presence of KDO and were examined by SDS-PAGE. Fractions containing homogeneous LPS species were pooled, exhaustively dialysed against distilled water and lyophilized.

LPS analysis. This was done by SDS-PAGE. Except where purified LPS was examined, routine samples were prepared by the SDS-proteinase K method (Hitchcock & Brown, 1983) and the SDS-PAGE system was that described by Darveau & Hancock (1983). The silver staining procedure of Tsai & Frasch (1982) was used to visualize electrophoretically separated LPS.

Immuno-electrophoresis. Rocket electrophoresis was done using 1% (w/v) agarose (Seakem ME; Mandel Scientific, Guelph, Ontario, Canada) gels and a buffer system containing 0.024 M-barbituric acid C IV, 0.00035 M-calcium lactate and 0.073 M Tris-base, pH 8.6 (Lam *et al.*, 1983). Ascites fluid containing mAb 4-15A was incorporated into the gel at 0.05 ml (ml gel)⁻¹. Electrophoresis was done at 10 V cm⁻¹ for 3 h. After electrophoresis, gels were washed, pressed, dried and stained with Coomassie brilliant blue as described by Weeke (1973).

Examination of bacterial morphology by freeze substitution and electron microscopy. The capsule morphology of *E. coli* mutants was examined using freeze substitution, a procedure which retains structural aspects of the highly hydrated CPS, without a requirement for antibody stabilization. A full description of the technique will be published subsequently. Briefly, bacteria were grown overnight in LB, harvested by centrifugation, resuspended and layered onto Millipore filters. The immobilized bacteria were frozen by plunging into liquid propane at -195 °C. Frozen samples were fixed and dehydrated at -75 °C, with 2% (w/v) osmium tetroxide and 2% (w/v) uranyl acetate in anhydrous acetone. After 2 d, samples were allowed to reach room temperature and were washed using anhydrous acetone. The samples were then infiltrated overnight with 1:1 (v/v) acetone/Epon 812 (Can EM) and embedded in Epon 812. Sections were post-stained with uranyl acetate and lead citrate before examination using a Philips EM300 electron microscope, operating at 60 kV.

Preparation of colloidal gold markers. Colloidal gold particles with an average diameter of 12.5 nm were coupled to diaminoethane-derivatized dextran as described by Lam *et al.* (1987). The colloidal gold was then coupled to Protein A (Boehringer Mannheim) using the method of Hicks & Molday (1986).

Colloidal gold labelling. Copper grids (100 mesh) were coated with carbon and Formvar. Prior to use, each grid was treated with 0.1% peptone as a wetting agent. Bacterial cells were scraped with a sterile toothpick from an overnight plate culture and resuspended in phosphate-buffered saline (PBS; Voller *et al.*, 1986). Bacteria in a drop of PBS were added to the grids and allowed to dry. The grids were then blocked for 15 min using BSA (3%, w/v, in PBS) and washed by inverting on successive drops of PBS. mAb 4-15A (0.05 ml) was added to the grid and incubated in a moist chamber for 30 min at room temperature. Excess antibody was removed by washing extensively over drops of PBS containing BSA (0.5%, w/v). Since mAb 4-15A is an IgM antibody, a second antibody (0.5 ml goat anti-mouse IgM; Cappel Worthington Biochemicals) was used to provide the IgG for protein A binding. After 30 min incubation at room temperature, the washing procedure was repeated. The protein-A-coupled gold marker (0.08 ml) was added and allowed to react at room temperature for 30 min. The grid was again extensively washed.

Negative staining and electron microscopy. Samples were negatively stained in 0.5% (w/v) ammonium molybdate, pH 7.0, by inverting the grids on a drop of stain for 5 s. Excess stain was removed by a filter paper wedge. The grids were examined in a Philips EM300 transmission electron microscope operating at 60 kV.

Conjugation experiments. Transfer of pULB113 (RP4::mini-Mu) was done as described by Kadam *et al.* (1985) and involved incubation on LB plates of 0.5 ml portions of overnight LB cultures of donor and recipient. After 18 h incubation at 37 °C, conjugation mixtures were collected, diluted in PBS, and spread on appropriate selective media. Strains carrying pULB113 were selected and maintained on media containing Ap, Tc and Km.

Table 2. *Phenotypes of cell surface polysaccharide mutants derived from E. coli O9:K30*

<i>E. coli</i> strain	Serotype	Phage sensitivity		Agglutination in specific antibody	
		K30	O9	K30	O9
W6930	K30:O9	+	—	+	—
B192	K ⁻ :O9	—	+	—	+
B198	K ⁻ :O ⁻	—	—	—	—
B70	Ki:O9	+	+	+	+
B78	Ki:O ⁻	+	—	+	—

RESULTS

Isolation of capsule-defective mutants

Spontaneous mutants were isolated from coliphage-K30-treated cultures of *E. coli* W6930 with frequencies in the order of 10^{-4} to 10^{-5} . These frequencies are similar to those reported for *E. coli* K1 CPS mutants (Vimr & Troy, 1985). Mutagenesis using MNNG increased this frequency by 10- to 100-fold. Approximately 250 independent capsule-defective mutants have been isolated and all were easily distinguished from the K30⁺ wild-type based on colony morphology; the wild-type strains produced larger, opaque colonies whereas colonies from capsule-defective mutants were smaller and transparent.

The mutants were examined for the presence of the O9 and K30 antigens. The majority of mutants (242 of 250), including all (180) spontaneous mutants isolated using coliphage K30, demonstrated typical acapsular (K⁻) phenotypes. In these strains, represented by *E. coli* B192, the LPS O9 antigen was exposed to anti-O9 antibody and coliphage O9-1 (Table 2). In *E. coli* W6930 (K30⁺), the capsule layer effectively masked the underlying LPS. Only six mutants simultaneously lost both O9 and K30 antigens.

Among 70 mutants isolated following MNNG mutagenesis, eight strains were identified with phenotypes expected of Ki, or intermediate forms (Schmidt *et al.*, 1977). This phenotype is represented by *E. coli* B70. Typically, these mutants reacted with probes directed against both O9 and K30 antigens (Table 2), indicating exposure of both antigens on the cell surface. Colonies of K⁻ and Ki forms could not be distinguished visibly.

Morphological examination of capsule-defective mutants

To confirm the absence of a capsule, mutant strains were freeze-substituted and examined by electron microscopy (Fig. 1). A fibrillar layer extending 50–100 nm from the surface of the outer membrane was evident in *E. coli* W6930 (K30⁺). No trace of a capsular layer was detected in *E. coli* B192 (K⁻) and *E. coli* B70 (Ki); the morphologies of these mutants were indistinguishable.

LPS profiles in capsule-defective mutants

Previously, we have shown the presence of a unique LPS fraction in K30⁺ strains which is recognized by mAb 4-15A, specific for K30 polysaccharide (Homonylo *et al.*, 1988). Results of SDS-PAGE analysis of capsule-defective strains (Fig. 2) illustrated that this LPS fraction (indicated by the arrow) was present in Ki strains (lane 4), and confirmed its absence in K⁻ strains (lane 3). This LPS fraction was also present in *E. coli* B78, a Ki derivative lacking the O9 antigen (lane 5).

For reference, the LPS from *E. coli* B198 (O⁻:K⁻) was included (lane 1) to illustrate the migration of rough lipid A-core. All the *E. coli* strains examined contained some rough lipid A-core. Lane 6 shows the migration of LPS from a *Salmonella typhimurium rfc* mutant; in this strain, the lipid A-core is substituted with a single repeating unit of O-antigen, due to a defect in the O-antigen polymerase (Mäkelä & Stocker, 1984).

Fig. 1. Morphological examination of *E. coli* K30 CPS mutants, using freeze substitution. (a) *E. coli* W6930 (O9:K30); (b) *E. coli* B192 (O9:K⁻), a typical acapsular strain; (c) *E. coli* B70 (O9:Ki), a Ki representative. Bar, 100 nm.

Immunoreactivity and surface expression of lipid A-core-associated K-antigen

The interaction of mAb 4-15A with the LPS-associated K-antigen fraction from the wild-type *E. coli* O9:K30 could not be demonstrated by Western immunoblotting (Homonylo *et al.*, 1988). This was also true for the material in the Ki mutant *E. coli* B78 (results not shown). The reasons for the lack of reaction remain unclear. To demonstrate that the unique fraction visualized in SDS-PAGE profiles of *E. coli* B78 (Ki) LPS was responsible for the reaction with mAb 4-15A, this fraction was purified to homogeneity by gel-filtration chromatography on Sephadex G50. The SDS-PAGE profile of the purified material is shown in Fig. 3(a). The samples were examined by rocket immunoelectrophoresis using mAb 4-15A (Fig. 3b). As found previously using the *E. coli* K30 parental strain, only the lipid A-core fraction unique to K and Ki strains reacted with the antibody. No reaction was obtained with other purified lipid A-core fractions. Also, no reaction was obtained with any column fraction lacking KDO (results not shown).

Surface expression of this K-antigen in Ki strains was indicated by the positive results of agglutination tests using mAb 4-15A. To directly confirm the surface exposure of the antigen, whole cells were labelled with colloidal gold markers coupled to protein A. As shown in Fig. 4(a), Ki mutant *E. coli* B78 showed extensive labelling. The colloidal gold probe was randomly

Fig. 2. SDS-PAGE analysis of LPS in *E. coli* K-antigen and O-antigen mutants. Lane 1, *E. coli* B198 (O⁻:K⁻); lane 2, *E. coli* W6930 (O9:K30); lane 3, *E. coli* B192 (O9:K⁻); lane 4, *E. coli* B70 (O9:Ki); lane 5, *E. coli* B78 (O⁻:Ki); lane 6, *S. typhimurium* SA2876, an *rfc* mutant. The arrow indicates migration of an LPS fraction present only in K⁺ and Ki strains.

distributed over the entire surface. Identical labelling was obtained using *E. coli* B70 (O9:Ki) and *E. coli* W6930 (O9:K30) (results not shown). The lack of any detectable differences between the labelling of Ki and authentic K30 strains probably reflects the collapse of the wild-type capsular layer present in the surface of the cell. This would result from dehydration prior to negative staining. No labelling occurred if either the first or second antibody steps were omitted. As a further control *E. coli* B192 (O9:K⁻) was used. As shown in Fig. 4(b), the acapsular strain was not labelled.

Preliminary mapping of E. coli K30 CPS mutations

To determine the approximate location of the K30 biosynthetic genes, we have previously used R-prime plasmids generated by pULB113 (Laakso *et al.*, 1988). Plasmid pULB113 contains a Mu3A (mini-Mu) which carries a functional transposase but lacks lethal functions. R-Prime plasmids are generated by random chromosomal integration and excision and can be transferred by conjugation (Van Gijsegem & Toussaint, 1982). Plasmid pULB113 was

Fig. 3. Rocket immunoelectrophoresis of the purified LPS fractions expressing K30 antigenicity in *E. coli* B78 (Ki). (a) SDS-PAGE profile of the K30-antigenic fractions: lane 1, *E. coli* B78 unfractionated LPS; lane 2, LPS-associated K-antigenic fraction. (b) Analysis of the reaction of the LPS samples with the K-antigen-specific mAb 4-15A: lane 1, control with purified high molecular mass K30 CPS; lane 2, *E. coli* B78 unfractionated LPS; lane 3, LPS-associated K-antigenic fraction. (10 µg of each antigen was analysed.)

Table 3. Mapping of K30 defects using R-prime plasmids

Figures in parentheses are percentage values

Recipient strain	Phenotype of His ⁺ transconjugants			Phenotype of Trp ⁺ transconjugants		
	Tested	Ki	K ⁺	Tested	Ki	K ⁺
B70 (Ki : O9)	289	15 (5)	274 (95)	249	249 (100)	0 (0)
B192 (K ⁻ : O9)	241	6 (2)	235 (98)	201	0 (0)	0 (0)

transferred to *E. coli* E69 to produce the source of R-prime plasmids. The R-primers were then transferred to either *E. coli* Ki or K⁻ mutants. Following selection for restoration of the His⁻ or Trp⁻ defects, transconjugants were tested for the presence of K30 capsule. Encapsulation was assessed by the masking of the LPS receptor for coliphage O9-1, by colony morphology, and was confirmed by electron microscopy. As shown in Table 3, both K⁻ and Ki defects were complemented in His⁺ transconjugants. No complementation occurred in Trp⁺ transconjugants. Only results obtained with *E. coli* B70 and *E. coli* B192 are shown; other strains with each phenotype gave identical results.

DISCUSSION

Two CPS mutant phenotypes were identified. The first and most prevalent was the true acapsular form with no capsular layer or K-antigen. The less frequent mutant class was the Ki strains which also produced no capsular layer but did express K-antigenicity. The Ki mutants were not detected among spontaneous mutants but approximately 10% (8 of 70) of the MNNG-derived mutants belonged to this class. In retrospect, this observation is easily explained. In

Fig. 4. Colloidal gold labelling of surface-exposed K-antigen in *E. coli* B78 (Kⁱ). (a) Surface labelling of *E. coli* B78; (b) *E. coli* B192 (K⁻) used as a negative control to demonstrate the specificity of the labelling procedure. Bar, 200 nm.

order to isolate spontaneous mutants at a frequency of 10^{-4} to 10^{-5} , coliphage K30 was used to lyse wild-type encapsulated bacteria and enrich for mutants in the population. Surprisingly, coliphage K30 also infected Ki strains, suggesting that the bacteriophage does not have an absolute requirement for a wild-type capsule as a primary receptor. As a result, the bacteriophage selection which was effective for isolation of K⁻ mutants removed any spontaneous Ki mutants from the population.

The K-antigenic fraction in Ki strains was surface expressed. However, morphological studies and exposure of LPS O-antigen demonstrated the lack of a wild-type capsular layer in Ki mutants. Other techniques commonly used to demonstrate capsules, such as cationized ferritin (Laakso *et al.*, 1988) or antibody stabilization (Whitfield *et al.*, 1984) also failed to show evidence of high molecular mass K-antigen (results not shown), as did the technique used to prepare high molecular mass K30 polysaccharide described by Hungerer *et al.* (1967). Based on rocket immunoelectrophoresis of LPS fractions from *E. coli* B78, it appears that the only source of K-antigenicity in the Ki strains is the LPS lipid-A-core-associated fraction. In addition to high molecular mass K30 antigen, wild-type strains also produced a low molecular mass LPS-associated K-antigen fraction (Homonylo *et al.*, 1988), with a migration in SDS-PAGE identical to that from Ki strains. This result suggests that synthesis of the K-antigenic fraction seen in isolation in Ki strains occurs under normal conditions, rather than resulting from an artifact induced by MNNG mutagenesis. A CPS polymerase defect, as proposed by Schmidt *et al.* (1977) would account for the Ki phenotype. The migration of the K30 antigenic LPS fraction in SDS-PAGE was consistent with this interpretation, using LPS from a *S. typhimurium rfc* mutant for comparison purposes.

It has not been possible to identify a lipid A-core moiety at the terminus of a high molecular mass K30 CPS chain. This may reflect the technical difficulties associated with isolating the small amount of lipid A-core present relative to the large mass of CPS. Little is known in general about the precise mechanism(s) involved in linkage of capsules to the bacterial cell surface. *E. coli* may simultaneously express both high and low molecular mass K30 antigens, with only the low molecular mass form being associated with lipid A-core.

The interrelationship between O- and K-antigen expression in strains bearing group I CPS remains unclear. Some enzymes may be shared between the two biosynthetic pathways. An example would be GDP-mannose pyrophosphorylase which is involved in the generation of GDP-mannose precursor for mannose residues in both O9 (Prehm *et al.*, 1976) and K30 antigen (Chakraborty *et al.*, 1980). We have isolated only six mutants (from a total of 250) which have simultaneously lost both O9 and K30 antigens. This would suggest that the number of shared biosynthetic enzymes is small. It has been proposed that synthesis of the group I CPS is determined by genes of the *rfb* cluster (Jann & Jann, 1987). Although the O- and K-antigens appear closely linked (Laakso *et al.*, 1988; Schmidt *et al.*, 1977), there is no direct evidence to suggest that they form a single gene cluster. Indeed, from the mutations described here, it is clear that both the O9 and K30 antigens can be expressed independently of one another. It is therefore reasonable to retain the *rfb* designation for genes involved in expression of the O9 antigen, which has been historically considered as an LPS O-antigen. To differentiate genes whose function leads specifically to the expression of the K30 antigen, we have suggested the designation *kst* (K-antigen, thermoSTable). This designation also distinguishes group I CPS from group II CPS (*kps*) loci.

Plasmid pULB113 (RP4::mini-Mu) provides a genetic tool for *E. coli* strains which are not amenable to other classical genetic approaches. In previous studies, we have demonstrated that transfer of the *his* region from *E. coli* O9:K30 donors to *E. coli* K12 recipients yielded transconjugants with capsules which were morphologically indistinguishable from that of the donor (Laakso *et al.*, 1988). These experiments did not rule out the possibility that some unlinked CPS functions (such as a putative polymerase function) were provided by the recipient *E. coli* strain. Complementation studies presented here extend those observations and support the conclusion that all the necessary genetic determinants for K30 CPS expression, defined by mutations, are linked to *his*. The number of gene defects required to give the Ki phenotype is

presently unknown. The differences obtained in these studies and the earlier results of Schmidt *et al.* (1977) are not clear.

The description of CPS phenotypes in capsule-defective mutants of *E. coli* K30 will now facilitate a detailed analysis of the K30 biosynthetic genes.

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