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Cloning and Characterization of the *Escherichia coli* K-12 *rfa-2* (*rfaC*) Gene, a Gene Required for Lipopolysaccharide Inner Core Synthesis

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A genetically defined mutation, designated *rfa-2*, results in altered lipopolysaccharide (LPS) biosynthesis. *rfa-2* mutants produce a core-defective LPS that contains lipid A and a single sugar moiety, 2-keto-3-deoxyoctulosonic acid, in the LPS core region. Such LPS core-defective or deep-rough (R) mutant structures were previously designated chemotype Re. Phenotypically, *rfa-2* mutants exhibit increased permeability to a number of hydrophilic and hydrophobic agents. By restriction analyses and complementation studies, we clearly defined the *rfa-2* gene on a 1,056-bp *Alu*I-*Dra*I fragment. The *rfa-2* gene and the flanking *rfa* locus regions were completely sequenced. Additionally, the location of the *rfa-2* gene on the physical map of the *Escherichia coli* chromosome was determined. The *rfa-2* gene encodes a 36,000-dalton polypeptide in an in vivo expression system. N-terminal analysis of the purified *rfa-2* gene product confirmed the first 24 amino acid residues as deduced from the nucleotide sequence of the *rfa-2* gene coding region. By interspecies complementation, a *Salmonella typhimurium rfaC* mutant (LPS chemotype Re) is transformed with the *E. coli rfa-2*$^+$ gene, and the transformant is characterized by wild-type sensitivity to novobiocin (i.e., uninhibited growth at 600 μg of novobiocin per ml) and restoration of the ability to synthesize wild-type LPS structures. On the basis of the identity and significant similarity of the *rfa-2* gene sequence and its product to the recently defined (D. M. Sirisena, K. A. Brozek, P. R. MacLachlan, K. E. Sanderson, and C. R. H. Raetz, J. Biol. Chem. 267:18874–18884, 1992), the *S. typhimurium rfaC* gene sequence and its product (heptosyltransferase 1), the *E. coli* K-12 *rfa-2* locus will be designated *rfaC*.

Lipopolysaccharide (LPS) is a major component of the outer membrane of gram-negative bacteria. The LPS is an important entity in determining outer membrane barrier function (12) and the virulence of gram-negative pathogens (21). LPS mutant strains, primarily of *Salmonella typhimurium* and *Escherichia coli* K-12 (8, 17–19), have been isolated, and their LPS structures have been defined in terms of polysaccharide content, i.e., LPS chemotypes. The various LPS chemotypes are defined in Fig. 1. The genetic determinants of the outer core region of LPS are essentially known. The five transferases required for assembly of this part of the core have been identified by mutations (15) of the corresponding genes, e.g., *rfaKJIGB* (see Fig. 1 for details). To elucidate the specific genes and gene products required for synthesis of LPS precursors or assembly of the LPS inner core region, we have previously searched LPS inner core biosynthetic mutants. We isolated LPS mutants (selected as novobiocin-hypersensitive derivatives) of *E. coli* that were phenotypically chemotype Re. Two nonallelic mutations (i.e., *rfaD* and *rfa-2*) in *E. coli* K-12 were precisely mapped at 81 min of the chromosome, between *cysE* and *pyrE* (5, 20). It should be noted that a similar region in *S. typhimurium*, where several LPS core genes are located, is appropriately called the *rfa* gene cluster (15). Recently, we reported (20) the cloning and sequencing of the *rfaD* gene, definition of the structure of this gene, and characterization of the gene product which is required for LPS core biosynthesis, specifically, biosynthesis of ADP-L-glycero-D-mannoheptose in *E. coli* K-12. Our results indicate that the heptose donor is ADP-L-glycero-D-mannoheptose and that the *rfaD* gene codes for ADP-L-glycero-D-mannoheptose 6-epimerase (4). In an earlier report (5), we described yet another *cysE-pyrE*-linked mutation, designated *rfa-2*, that resulted in a heptoseless LPS structure (chemotype Re). These mutants demonstrated increased permeability to hydrophobic and hydrophilic agents. The LPS of the Rfa-2 mutants consists of lipid A and 2-keto-3-deoxyoctulosonic acid.

Here we report the cloning of the *rfa-2* gene, its nucleotide sequence, flanking sequences from the *E. coli rfa* gene cluster, and the *rfa-2* gene's physical location on the *E. coli* chromosome. The *rfa-2* gene encodes a 36-kDa protein that has 90% similarity and 84% identity to the recently defined *rfaC* gene product of *S. typhimurium* (27). The DNA sequence of the *rfa-2* gene shares 76% identity with the reported DNA sequence of *S. typhimurium* (27). Additionally, by using the defined DNA sequence that complemented the Rfa-2 mutants, we successfully demonstrated interspecific complementation of a chemotype Re *S. typhimurium* LPS mutation, designated *rfaC* (2).

MATERIALS AND METHODS

Bacterial strains and plasmids, media and growth conditions, and bacteriological techniques. The bacterial strains and plasmids used are listed in Table 1. Strains were grown aerobically in LB medium (14) or in the minimal medium of Davis and Mingioli (6) at 37°C, except for strains used for thermal induction of specific proteins or lysogens. The antibiotics used were ampicillin (50 μg/ml), tetracycline (20

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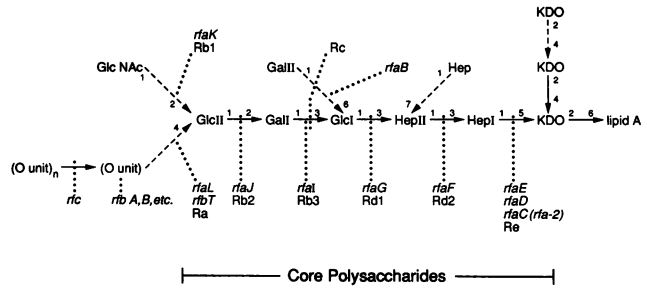

FIG. 1. Typical *S. typhimurium* LPS structure. LPS genetic determinants (*rfaBCDEFGIJK*) and the LPS structures (i.e., chemotypes Re, Rd2, Rd1, Rc, Rb3, Rb2, and Ra) of mutants blocked at various stages of LPS biosynthesis are indicated (4, 15, 17–19, 22, 27). The dotted lines indicate the defective LPS's termination points. KDO, 2-keto-3-deoxyoctulosonic acid; Hep, L-glycero-D-mannoheptose; Glc, glucose; Gal, galactose; GluNac, *N*-acetylglucosamine; $(O\ unit)_n$, number of O-antigen side chains. The structural genes presumed to be responsible for LPS core biosynthesis are as follows: *rfaE*, specific function unknown; *rfaC*, ADP-heptose:LPS heptosyltransferase 1; *rfaD*, ADP-L-glycero-D-mannoheptose 6-epimerase; *rfaF*, ADP-heptose:LPS heptosyltransferase 1; *rfaG*, UDP-glucose:LPS glycosyltransferase 1; *rfaB*, UDP-galactose:LPS α1, 6-galactosyltransferase; *rfaI*, UDP-galactose:LPS α1,3-galactosyltransferase; *rfaJ*, UDP-glucose:LPS glucosyltransferase 2; *rfaK*, UDP-*N*-acetylglucosamine:LPS glucosaminyltransferase.

μg/ml), kanamycin (15 μg/ml), novobiocin (30 μg/ml), and rifampin (200 μg/ml), unless indicated otherwise.

Complementation studies. We determined the feasibility and the effect of transformation of the Rfa-2 mutant of *E. coli* K-12 and the RfaC mutant of *S. typhimurium* with Rfa-2^+ plasmids. Transformants were selected on the basis of maximal growth temperature and LPS structural determination. Thus, these transformed Re strains were tested for complementation of the Rfa-2 and RfaC phenotypes, as indicated by maximal growth at 37 or 42°C on minimal or rich media containing 2.5 to 600 μg of novobiocin per ml or 0.1 to 0.4% sodium deoxycholate. Additionally, Rfa-2^+-transformed cells synthesize wild-type LPS.

Analytical and quantitative techniques. Plasmid isolation, transformation, gel electrophoresis, restriction endonuclease digestion, DNA ligation, and other cloning procedures were performed as previously described (16, 20). The procedures for sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis, the T7 RNA polymerase expression system, screening of lambda clones from the genomic library of Kohara et al. (11), P1*clr*100KM transduction, and N-terminal amino acid analysis were detailed previously (5, 20). LPSs were isolated from rough mutants and wild-type LPS bacterial strains by previously described extraction procedures (9, 31). Resolution and visualization of LPS structures were performed as described by Lesse et al. (13) and Tsai and Frasch (30).

Nucleotide sequence determination and sequence analysis. Inserts from the Clark-Carbon genomic bank were cloned into pBR322 or pUC19 for DNA sequence determination of both strands by the dideoxy-chain termination method (25). We modified the dideoxy-chain termination method for use with the Sequenase enzyme (29) and for double-stranded DNA sequencing as described by Kašpar et al. (10). The primers included commercially available universal primers, as well as oligonucleotide primers (17-mers) synthesized with a 381A DNA synthesizer (Applied Biosystems, Inc., Foster City, Calif.). Nucleotide and amino acid sequence alignments were done on the Convex facility at the National Institutes of Health with programs from the University of

TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Genotype or characteristic	Reference or source
E. coli K-12 strains		
DHα5		BRL[a]
K38	HfrC *phoA4 tonA22 garB10 ompF627 relA1 pit10 spo10* T2 (λ)	24
K38(pGP1-3)	Contains plasmid bearing T7 polymerase and Tet^r genes	28
JM15	F$^-$ *thi-1 cysE50 tfr-8*	20
CL605	HfrH *thi-1 galE28 relA1* λ$^-$ *spoT1 rpsL rfa-2*	20[b]
CL606	P1*clr*100KM lysogen of CL605	20
CL632	F$^-$ *thi-1 tfr-8 rfa-2*	This study
CL633	F$^-$ *thi-1 tfr-8*	This study
CL634(pCG17)	F$^-$ *thi-1 tfr-8* Rfa-2^+	This study
S. typhimurium LT2 Sa1377 strain	*rfaC630*	2
Plasmids		
pCG50	9.0-kbp *Pst*I fragment from pCK4 in pBR322; Amp^r Tet^r	5
pLC15-18	Colicin E1-*E. coli* DNA hybrid plasmid; Col^r	3
pCG14	6.0-kbp *Sma*I fragment from pLC15-18 in pUC19; Amp^r	This study
pCG15	9.0-kbp *Sma*I fragment from pLC15-18 in pUC19; Amp^r	This study
pCG17	2.8-kbp *Sma*I-*Eco*RV from pCG14 in pUC19; Amp^r	This study
pCG22	1,056-bp *Alu*I-*Dra*I fragment from pCG17 in pT7-5; Amp^r	This study
pCG26	1,056-bp *Alu*I-*Dra*I fragment from pCG17 in pUC8; Amp^r	This study
pGP1-3	T7 gene 1, λ promoter; *c*I857 Tet^r	28
pT7-5	Cloning and expression vector, T7 promoter; Amp^r	S. Tabor
pUC8	Cloning and expression vector; Amp^r	BRL
pUC19	Cloning and expression vector; Amp^r	BRL

[a] BRL, Bethesda Research Laboratories.

[b] It should be noted that the Rfa mutant (chemotype Rd1) recently described by Austin et al. (1), designated *rfa-2* by them, is distinct from the Rfa-2 mutant (chemotype Re) originally isolated in this laboratory in 1985 (5) and studied in the present investigation.

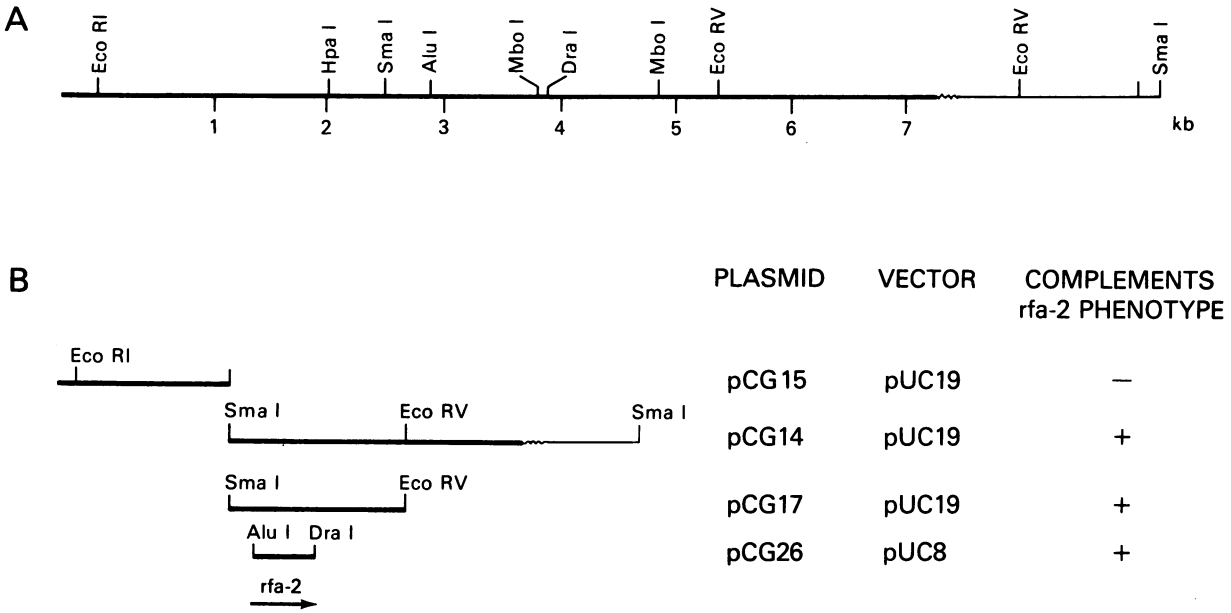

FIG. 2. Schematic illustration of restriction endonuclease sites of *rfa* sequences and *rfa-2* plasmid constructs. (A) The *Eco*RI-*Eco*RV DNA fragment is a composite of *E. coli* K-12 DNA cloned from Clark-Carbon genomic library plasmids pLC15-18 and pLC32-45. Relevant restriction sites of the Rfa$^+$ composite fragment are indicated. (B) Cloned fragments, vectors, and complementation phenotypes are shown. Only restriction sites relevant to plasmid construction are shown. Symbols: ▬, *E. coli* K-12 DNA; ——, colicin E1 DNA; ∿∿∿, dA-dT homopolymer. The arrow indicates the direction of transcription and translation of the *rfa-2* gene.

Wisconsin Genetics Computer Group, Inc., Madison, Wis. (7).

Nucleotide sequence accession number. The nucleotide sequence data reported here appear (since October 1991) in the EMBL and GenBank nucleotide sequence data bases under accession number X62530.

RESULTS

Cloning of the *rfa-2* gene. Restriction endonuclease sites of the Rfa-2$^+$ colicin E1-*E. coli* DNA hybrid plasmid pLC15-18 (3) are shown in Fig. 2. Restriction of this plasmid with *Sma*I resulted in two fragments of 6.0 and 9.0 kbp. Ligation of the 9.0- and the 6.0-kbp fragments to the *Sma*I-restricted vector pUC19 produced plasmids pCG14 and pCG15, respectively. The resultant plasmids, pCG14 and pCG15, were tested for the ability to complement Rfa-2 mutants. Complementation was observed with plasmid pCG14 only (Fig. 2B), and this determination was made on the basis of the LPS structure. Our previous results showed that the *rfa-2* mutation resulted in L-glycero-D-mannoheptose (heptose)-deficient LPS. Thus, the *rfa-2* mutant produces a truncated LPS (chemotype Re LPS) that is primarily a lipid A–2-keto-3-deoxyoctulosonic acid structure (2, 5, 12). The outer membranes of Re LPS mutants are characteristically more permeable to a number of hydrophobic agents (i.e., antibiotics, detergents, and bile salts). In addition to the structural difference of the mutant LPS, the maximal growth temperature of heptose-deficient strains is altered (for example, they grow slowly at 37°C but not at all at 42°C). We also took advantage of the maximal growth temperature characteristic in our selection of transformants.

Subcloning of pCG14 yielded pCG17. Additional restriction and complementation studies indicated that the Rfa-2$^-$ phenotype was abolished by plasmids containing a 1,056-bp *Alu*I-*Dra*I fragment from Rfa-2$^+$ plasmid pCG17 (Fig. 2B). This fragment was cloned into pUC8, and the resultant plasmid was designated pCG26. The overall results of the cloning studies definitively localized the *rfa-2* gene to a 1,056-bp fragment bounded by the *Alu*I and *Dra*I restriction

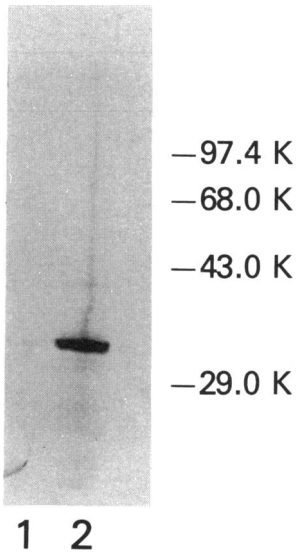

FIG. 3. Autoradiogram of the expression product ([^{35}S]methionine radiolabeled) of the plasmid-borne *rfa-2* gene in the T7 promoter-polymerase system. Lane 1 contained pT7-5, and lane 2 contained pCG22 (see Table 1 for plasmid descriptions). Following thermal induction at 42°C, cultures containing pCG22 or vector control pT7-5 were treated with rifampin and labeled with [^{35}S]methionine (28). The in vivo products were analyzed by SDS-polyacrylamide gel electrophoresis and autoradiography. Molecular weights were estimated with ^{14}C-labeled protein standards (M_r, 14,300 to 200,000; Bethesda Research Laboratories).

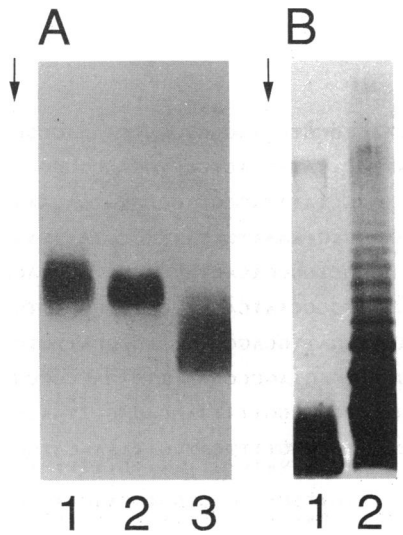

FIG. 4. Polyacrylamide gel electrophoresis of LPS from *rfa-2* mutant, *rfa-2*$^+$-complemented, and wild-type strains. LPS was prepared from 200- or 500-ml cultures as previously described (9, 16, 17). Samples of LPS were prepared in Laemmli sample buffer, applied to sample wells, and subjected to SDS-polyacrylamide gel electrophoresis at 30 mA per slab gel. The arrows indicate the direction of sample migration. The resolved gels were silver stained as described in Materials and Methods. Each sample well contained 1 μg of the indicated LPS. (A) *E. coli* K-12 LPS. Lanes: 1, LPS from *rfa-2* mutant strain CL632 transformed with an Rfa$^+$ plasmid; 2, wild-type LPS from Rfa-2$^+$ strain CL633; 3, *rfa-2* mutant strain CL632 LPS. (B) *S. typhimurium* LPS. Lanes: 1, LPS from *rfaC* mutant strain SA1377; 2, LPS from RfaC mutant strain SA1377 transformed with an Rfa-2$^+$ plasmid.

sites. The definition of the *rfa-2* gene was corroborated by results from complementation studies which consistently showed that this *Alu*I-*Dra*I fragment was capable of complementing the Rfa-2 phenotype in *rfa-2* mutants.

Identification of the *rfa-2* gene product. The *rfa-2* gene product, encoded by plasmids that complement the *rfa-2* mutation (Table 1 and Fig. 2B), was expressed in an in vivo expression system in the presence of [^{35}S]methionine. The in vivo expression system was described by Tabor and Richardson (28). The *rfa-2* gene, located on a 1,056-bp *Alu*I-*Dra*I fragment, was placed downstream of the T7ϕ10 promoter in vector PT7-5. This recombinant was designated pCG22. Cultures containing pCG22 or a vector control were induced at 42°C, treated with rifampin (200 μg/ml), and labeled with [^{35}S]methionine. Results of a representative experiment are shown in Fig. 3. Lane 2 shows the single protein band resulting from expression of *rfa-2*$^+$ plasmid pCG22. The estimated molecular weight of the radiolabeled protein band, based on electrophoretic mobility (Fig. 3), is 36,000. No protein was observed when vector PT7-5 was expressed as described above.

LPS structure of plasmid-complemented Rfa-2$^-$ and RfaC$^-$ strains. Our previous report (5) indicated that the LPS of *rfa-2* mutant strains migrated faster on polyacrylamide gel than that isolated from wild-type *E. coli* K-12. *E. coli* K-12 strain CL632 and its nearly isogenic pair CL633 (Table 1) were used to show the effect of the Rfa-2$^+$ plasmids on the LPS structure of an *rfa-2* mutant (Fig. 4A). The faster-migrating LPS structure from Rfa-2$^-$ strain CL632 is shown in lane 3. The more slowly migrating LPS structures are produced by plasmid-complemented Rfa-2$^-$ strain CL634 (lane 1), and the electrophoretic mobility is similar to that of nearly isogenic wild-type strain CL633. Rfa-2$^+$ plasmid pCG26 is known to contain the 1,056-bp *Alu*I-*Dra*I fragment from Rfa-2$^+$ plasmid pCG17 (Table 1). The finding that plasmid pCG26 restored the electrophoretic mobility of the mutant to that of the wild type further indicates that the *rfa-2* gene lies in the 1,056-bp *Alu*I-*Dra*I fragment.

An identical effect of Rfa-2$^+$ plasmid pCG26 (Fig. 4B) on LPS synthesis was observed with *S. typhimurium rfaC* mutants. The fast-migrating LPS structure (chemotype Re) characteristic of the *rfaC* mutant is shown in lane 1. The similarity in electrophoretic mobility between the LPS from the transformed *rfaC* strain (lane 2) and that of wild-type *S. typhimurium* LT2 (data not shown) indicates that the presence of the *rfa-2* gene is necessary and sufficient for wild-type LPS synthesis.

Nucleotide sequence and deduced amino acid sequence of the *rfa-2* gene product. The nucleotide sequence of a 3.2-kbp *Hpa*I-*Eco*RV fragment containing the *rfa-2* locus and flanking sequences was determined for both strands. The restriction map and the specific DNA fragments used in sequencing are shown in Fig. 2 and 5. Analyses of the DNA sequence (Fig. 6) indicated three major open reading frames (ORF) downstream of the *rfaD* gene. The ORF corresponding to the

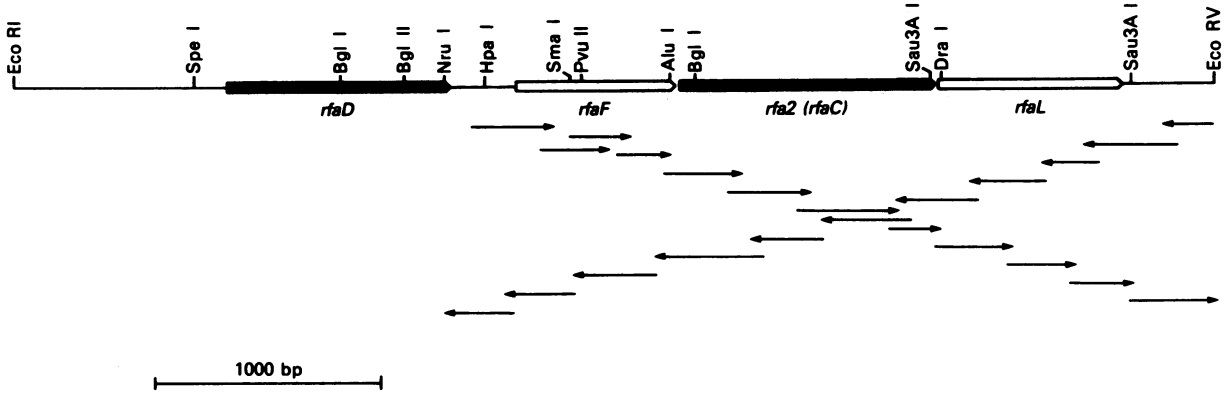

FIG. 5. Sequencing strategy for the *rfa-2* gene and the flanking *rfa* locus region. The 5,259-bp *rfa* DNA fragment and its restriction map are shown schematically. The *rfa-2* and *rfaD* coding regions are depicted as thick lines. The ORF indicated (open boxes) are tentatively designated the *rfaF* and *rfaL* gene sequences (see Discussion). The direction of transcription is indicated by the direction of the ORF arrows. The arrows below the diagram show primer sites, direction, sequence overlaps, and extent of sequencing.

FIG. 6. Nucleotide and deduced amino acid sequences of the *rfa-2* and associated reading frames. The DNA sequence of the sense strand of the *rfa-2* gene is shown. Nucleotides are numbered starting with the 5′ half of the *Hpa*I restriction site. The ORF and termination and relevant restriction endonuclease sites of the *rfa-2* gene are indicated.

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  1 MRVLIVKTSSMGDVLHTLPALTDAQQAIPGIKFDWVVEEGFAQIPSWHAA 50 ECORFAC
    ------------------------------Q---A----------S-    STYRFAC

 51 VERVIPVAIRRWRKAWFSAPIKAERKAFREALQAENYDAVIDAQGLVKSA 100
    -D----------------------T---R-VC-NQ---------------

101 ALVTRLAHGVKHGMDWQTAREPLASLFYNRKHHIAKQQHAVERTRELFAK 150
    ---------I------------------------------------------

151 SLGYSKPQTQGDYAIAQHFLTNLPTDAGEYAVFLHATTRDDKHWPEEHWR 200
    ----D---S-------K---HCQQAVSDP-----------------AN--

201 ELIGLLADSGIRIKLPWGAPHEEERAKRLAEGFAYVEVLPKMSLEGVARV 250
    -----VGNT-L-----------A---------D--D---R----E----

251 LAGAKFVVSVDTGLSHLTAALDRPNITVYGPTDPGLIGGYGKNQMVCRAP 300
    ------------------------------------------ACCS-

301 RENLINLNSQAVLEKL 316
    EQ--A--DATS-FG-I
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FIG. 7. Comparison of the deduced amino acid sequences of *E. coli* K-12 *rfa-2* (*rfaC* gene) and the *rfaC* gene (27) of *S. typhimurium*. A dash indicates identical amino acids at that position. The *E. coli* K-12 amino-terminal residues determined by microsequence analysis are residues 1 to 24.

region which expresses the 36,000-dalton protein capable of complementing the Rfa-2$^-$ phenotype begins with nucleotide 897 and ends at nucleotide 1856. No other ORFs, regardless of orientation, are apparent in this span of nucleotides. Further, microsequence analysis of the *rfa-2* (*rfaC*) gene product confirms the first 24 amino acids deduced for the sequence of the *rfa-2* ORF.

Homology of *E. coli* K-12 *rfa-2* to *S. typhimurium rfaC*. The nucleotide and deduced amino acid sequences of the *E. coli* K-12 *rfa-2* gene and the *S. typhimurium rfaC* gene (27) were compared. This comparison revealed extensive similarity and identity (76% in both cases) between the nucleotide sequences of the two genes. As shown in Fig. 7, the deduced amino acid sequences of the *E. coli* K-12 *rfa-2* and *S. typhimurium rfaC* genes are 90% similar and 84% identical.

Location of the *rfa-2* gene on the physical map of the *E. coli* chromosome. An adjunct of the sequence analysis was establishment of the chromosomal order of *rfa-2* (*rfaC*) relative to the *rfaD* and *cysE* loci. Schematic representations of these data are provided in Fig. 5 and 8. Figure 8 shows the results of hybridizing the 1,056-bp *Alu*I-*Dra*I Rfa-2$^+$ fragment to λ bacteriophage clones from the genomic library bank of Kohara et al. (11). On the basis of our previous chromosomal localization of the *rfa-2* (*rfaC*) locus by P1 transductional analysis (10), we chose library clones 572, 573, 574, 575, and 576. These clones span the 3880- to 3935-kbp region of the chromosome. Library clone 650, which contains the genomic region from 4450 to 4460 kbp, was used as a control. The results shown in Fig. 8 demonstrate hybridization of 574 and 575 clones with the *rfa-2* DNA probe. Clones 572, 573, and 576 and control clone 650 failed to hybridize with the *rfa-2* probe. These results are consistent with a chromosomal location of *rfa-2* between 3915 and 3916 kbp on the physical map of Kohara et al. (11).

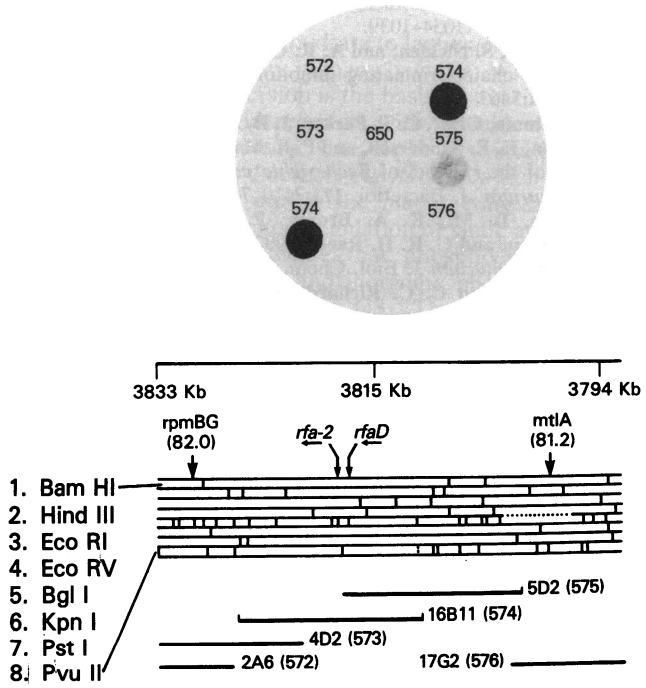

FIG. 8. Location of the *rfa-2* gene on the *E. coli* physical map. The upper portion shows the results of DNA-DNA hybridization of the 1,056-bp *Alu*I-*Dra*I fragment from pCG26 to selected λ *E. coli* clones (572 to 576) that contain the chromosome region from kbp pairs 3,781 to 3845. Clone 650 contains DNA from the chromosome region from kbp 4402 to 4417. The chromosomal kilobase pair regions indicated are based on information reported in reference 23. The diagram shows the physical map of the region of the *E. coli* map near the *rfa-2* locus and the chromosomal region analyzed by hybridization studies.

DISCUSSION

Previously, we reported the isolation of a core-defective *rfa-2* mutant of *E. coli* K-12 and demonstrated by P1 transduction that the mutation was located at 81 min on the genetic map of *E. coli* K-12. This is a region where other *rfa* genes, including *rfaD*, are located (5, 26). In this report, we describe the cloning and sequencing of the *rfa-2* gene and provide for the first time definitive evidence for the *rfa-2* gene product. The *rfa-2* gene was defined as 1,056 bp long and was located on the *E. coli* chromosome between 3814 and 3818 kbp by partial analysis of the genomic library of Kohara et al. (11).

The 3.2-kbp *Hpa*I-*Eco*RV Rfa-2$^+$ fragment contained the *rfa-2* ORF and two additional large ORF. In vivo expression studies of Rfa-2$^+$ plasmid pCG22 (Fig. 1B) demonstrated that the *rfa-2* gene encodes a 36,000-dalton polypeptide. The molecular weight is consistent with the span of the ORF, and microsequence analysis confirmed the first 24 amino acid residues. Studies are in progress in our laboratory to purify and characterize the physical and kinetic properties of the *E. coli* K-12 *rfaC* gene product.

Biochemical studies to support our assertion and others (26), based on ORF positions and reported interspecific-omplementation studies (26), that the undefined ORF reported here code for the *rfaF* and *rfaL* gene products are ongoing.

Transformation of the *rfaC* mutant *S. typhimurium* SA1377 with the *rfa-2* gene resulted in nearly wild-type LPS synthesis. This suggests that only a single genetic alteration in LPS synthesis in the *rfaC* mutant is responsible for the Rfa phenotype. These interspecific-complementation studies involving *rfa-2* clones and the significant similarity and identity of the reported (27) nucleotide and amino acid sequences of the *S. typhimurium rfaC* gene dictate that the *rfa-2* locus be designated *rfaC*.

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